Aug 6th, 7:30 AM - 1:00 PM

Undergraduate Research Opportunities Program

University of Nevada Las Vegas

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University of Nevada, Las Vegas

2008

College of Sciences

Undergraduate Research Opportunities Program (UROP)

http://sciences.unlv.edu/urop/

Compiled and Edited
By
Carl Reiber, Associate Dean, College of Sciences
Photo Caption, Cover.

Front Row (Left to Right): Lauren Emes, UNLV, Tesla Birnbaum, UNLV, Azucena Benito, UNLV, Kimberly Horsley, UNLV, Greg Hoth, Reed College, Samantha Combs, Eckerd College, Allison Savage, University of Iowa, Crystal Erickson, UNLV; Second Row: May Yared, UNLV, Austin McDonald, UNLV, Andrea Jydstrup, UNR, Whitney Shofner, UNLV, Amanda Yates, Washington State University, Allison Faucher, Ohio Wesleyan University, Alex Michaud, Coe College, Mike Brawner, UNLV; Third row: Ryan Huang, UNLV, Karen Levy, UNLV, Huy Mai, UNLV, Weldu Gebremichael, UNLV, Kathleen Bradley, University of Maine, Paul Howse, UNLV, Markus Vasquez, Oklahoma State University, Brant Abeln, Drake University; Top Row: Maria Castellanos, UNLV, Robert Kobey, UNLV, Lucas Wilson, University of Wisconsin-Stevens Point, Louis Prahl, Lewis and Clark University, MacLean Hall, Davidson College, Martin Galley, SUNY-Cortland, Rachel Skinner, Transylvania University, Susie O’Neil, Central Michigan University, Mitchell Chaires, UNLV, absent.
Acknowledgements

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Special thanks are offered to the following individuals for their support and encouragement of this program:

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Dr. Stanley Smith, Associate Vice President for Research Services
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Dr. Dennis Bazlyinski, Director, School of Life Sciences
Dr. Dennis Lindle, Chair, Department of Chemistry
Dr. James Selser, Professor, Department of Physics and Astronomy,
Dr. Malcolm Nicol, Professor, Department of Physics and Astronomy
Dr. John Farley, Professor, Department of Physics and Astronomy
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Dr. Eduardo Robleto, Associate Professor, School of Life Sciences
Dr. Ronald Yasbin, Professor, School of Life Sciences
Ms. Alice Ward, Research Administrator, Systems Sponsored Programs
Ms. Nicholle Booker, Graduate Affairs Coordinator, College of Sciences

Others

Dr. David Ward, Nevada Cancer Institute
Ms. Heather Goulding, Program Manager, Nevada INBRE
Ms. Barbara Neyses, Financial Manager, Nevada INBRE

Faculty mentors from UNLV, the Desert Research Institute, and the Nevada Cancer Institute are deserving of particular thanks. These mentors devoted hours of time to work with UROP students this summer. Without mentors this program would not be possible. Lastly, we would like to thank Ms. Nicholle Booker for her tireless efforts and organizational skills, and her constant efforts to improve and enhance our students’ experience at UNLV.
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University of Nevada, Las Vegas

2008

College of Sciences

Undergraduate Research Opportunities Program

Summer Poster Session

August 6, 2008
UNLV Student Union Ball Room

7:30 a.m. – 9:00 a.m.  Poster Installation
9:00 a.m. – 12:00 p.m.  Public Viewing
12:00 p.m. – 12:30 p.m.  Luncheon and Presentations

12:30 p.m.  Welcome, Dr. Carl Reiber
12:40 p.m.  Faculty presentation, Dr. Michelle Elekonich
12:50 p.m.  Student presentation, Ms. Karen Levy
1:00  p.m.  Acknowledgements, Dr. Carl Reiber
Nevada INBRE sponsors 15 undergraduate research scholarships each year. Those selected for the program will spend the summer doing a lab research project in a faculty mentor’s laboratory. Summer research opportunities often lead to longer-term collaborations between students and faculty, publishable research, and careers in medicine or biomedical research. Opportunities are available for research in emerging areas such as genomics, proteomics, molecular modeling, imaging, and bioinformatics. However, any area of research that might be supported by the NIH is appropriate.

Students are selected in a statewide, merit-based competition. As part of the application process, students are required to identify a faculty mentor at UNR, UNSOM, UNLV, or Nevada Cancer Institute with whom they are interested in conducting research.

Nevada INBRE is a network of physical and human resources available to scientists in Nevada. Our mission is to provide infrastructure that enables investigators to successfully win research funding. INBRE research facilities provide research support services, training, and equipment for Nevada's biomedical investigators. We also sponsor research, scholarship and training opportunities for faculty members and students.

The National Center Research Resources (NCRR) Institutional Development Award (IDeA) program broadens the geographic distribution of NIH funding for biomedical and behavioral research. The program fosters health-related research and enhances the competitiveness of investigators at institutions located in states in which the aggregate success rate for applications to NIH has historically been low. Supported by the NCRR Division of Research Infrastructure, the IDeA program increases the competitiveness of investigators by supporting faculty development and research infrastructure enhancement at institutions in 23 states and Puerto Rico.

IDeA Networks of Biomedical Research Excellence (INBRE) enhance biomedical research capacity, expand and strengthen the research capabilities of biomedical faculty, and provide access to biomedical resources for promising undergraduate students throughout the eligible states. INBRE implements the IDeA approach at the state level by enhancing research infrastructure through support of a network of institutions with a multidisciplinary, thematic scientific focus. INBRE is the second phase of the Biomedical Research Infrastructure Networks (BRIN) program, which began by providing planning grants in 2001.
Centers of Biomedical Research Excellence (COBRE) augment and strengthen institutional biomedical research capabilities by expanding and developing biomedical faculty research capability through support of a multidisciplinary center, led by a peer-reviewed, NIH-funded investigator with expertise central to theme of the grant proposal.

The IDeA program also supports IDeANet, an Internet-based network providing connectivity for high-bandwidth science applications. IDeANet will enable collaboration among institutions, ultimately supporting all participants in the IDeA program, as well as participants in the Research Centers in Minority Institutions (RCMI) program and other NCRR-supported networks.

Front Row (Left to Right): Azucena Benito, UNLV, Andrea Jydstrup, UNR; Top Row: Ryan Huang, UNLV, Karen Levy, UNLV, Maria Castellanos, UNLV, May Yared, UNLV.
Nevada National Sciences Foundation (NSF)
Experimental Program to Stimulate Competitive Research (EPSCoR)

The Undergraduate Research component of the current NSF EPSCoR award provides lab and field research experiences, through summer scholarship programs and annual fellowship opportunities, to full-time NSHE undergraduate students. These programs fund eligible students either majoring in mathematics, science, or engineering, or majoring in education and specializing in teaching K-12 in the fields of mathematics, science, or technology. Research is conducted under the guidance of NSHE faculty mentors. The hands-on experience gained through these programs has proven to supplement classroom learning and serve as gateways to new and exciting opportunities for all participants.

EPSCoR - Experimental Program to Stimulate Competitive Research
NSF, the federal agency that first developed EPSCoR programs, sponsored the first EPSCoR program in Nevada. Since 1985, NSHE institutions have received more than $41 million in federal funds from NSF EPSCoR, together with non-federal matching funds.

Front Row (Left to Right): Tesla Birnbaum, UNLV, Kimberly Horsley, UNLV; Middle Row: Austin McDonald, UNLV, Crystal Erickson, UNLV; Top Row: Whitney Shofner, UNLV, Lauren Emes, UNLV, Huy X. Mai, UNLV, Robert Kobey, UNLV; Not Pictured: Mitchell Chaires, UNLV.
National Science Foundation Research Experience for Undergraduates Program (NSF REU)

REU MICROBIOLOGY
UNLV will offer an REU Site program in partnership with the Desert Research Institute. Undergraduate students will participate in a 10-week summer program involving research in the area of environmental microbiology.

Students will collaborate with faculty mentors in developing and carrying out hypothesis-based projects on microorganisms from diverse habitats such as hot springs, the deep terrestrial subsurface, hypersaline lakes, arid soils, and ephemeral water sources. Students may also choose to explore the mechanisms of magnetotaxis, microbial adaptation to stressful and nonhost environments, or the dynamics between primary producers and consumers.

All students will receive training in current molecular techniques and the ethics of science, and they will participate in weekly discussions on their project. At the conclusion of the program, students will present their research results at a scientific colloquium. In addition, all students will be encouraged to present their research at a regional or national scientific conference. Students will receive a $4000 stipend, housing and meals, and a travel subsidy. First generation college students and members of an underrepresented group are strongly encouraged to apply. More information is available by contacting Kurt Regner, Ph.D. at microreu@unlv.edu.

Front Row (Left to Right): Amanda Yates, Washington State University, Alex Michaud, Coe College; Middle Row: Susie O'Neil, Central Michigan University, Paul Howse, UNLV; Top Row: MacLean Hall, Davidson College, Allison Faucher, Ohio Wesleyan University, Rachel Skinner, Transylvania University, Kathleen Bradley, University of Maine.
National Science Foundation Research Experience for Undergraduates Program (NSF REU)

REU PHYSICS AND ASTRONOMY
The Research Experience for Undergraduates (REU) program is a program of the National Science Foundation to give undergraduate students an experience in performing research.

Most of a student's career consists of classroom lectures. The REU program is intended to benefit students by offering experiences that go beyond the classroom. The UNLV Physics Department has had a successful REU program since 1987. Initially the program was limited to UNLV students. Beginning in 1992, the program was open to non-UNLV students as well. Students participate in research projects in the summer with follow-up activity during the academic year.

Front Row (Left to Right): Allison Savage, University of Iowa, Samantha Combs, Eckerd College; Middle Row: Greg Hoth, Reed College, Weldu Gebremichael, UNLV, Markus Vasquez, Oklahoma State University, Martin Galley, SUNY-Cortland; Top Row: Brant Abeln, Drake University, Louis Prahl, Lewis and Clark University, Lucas Wilson University of Wisconsin-Stevens Point, Mike Brawner, UNLV.
Azucena Benito  
Mentor – Michelle Elekonich  

Honeybees undergo a process of adult behavioral development, spending their first 2-3 weeks working inside the constant environment of the hive. At about 3 weeks of age workers leave the hive as foragers who gather pollen and nectar. Previous research found that bees show an enormous decline in immunity as a result of their transition from regular hive jobs to more difficult foraging activities. Foragers can be forced to go back into hive-tasks, thus becoming “reverted nurses” which may also allow a reversal of immunosenescence. Understanding how this happens could prove to be useful because if there is flexibility in honeybee immunity it could lead us to a better understanding of the human immune response since the honeybee has a very similar genome to that of humans. I plan to use protein and gene expression analysis, along with other measurements to understand how forager bees change back into nurses and how this effects their immune response and their process of senescence and aging.
Investigation of gene and protein expression based on honey bee (Apis mellifera) aging, flight experience, and behavior

A.A. Benito, G.E. Mancinelli, A. Ammons and M. M. Elekonich
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154

**Objectives**

- Determine differences in gene and protein expression between age-matched bees of differing behavioral types (precocious foragers and typical age-nurses - 7-9 days old, typical age foragers and over-aged nurses - 19-32 days old, and reverted nurses - 24-26 days old).
- Measure global gene expression of these groups using microarray and quantitative real-time PCR technology to identify genes involved in aging.
- Measure expression of different proteins from the genes identified using western blotting.

**Introduction**

Honey bees model to be made up of three different castes: a reproductive queen, male drones, and sterile worker bees. Adult bees make up a process known as temporal polyethism age-based behavioral development which causes the bees to change their tasks in the hive based on their age. From 8- to 2-week-old bees make up the free roving forager bees, which forage outside the hive (see Fig. 1). Workers can switch easily to the current needs of the colony. If the older foragers are lost or the younger bee matures faster, new groups of bees to provide new workers for the colony. Similarly, the younger bees are removed from the hive or their numbers decline, the foragers return to look for new food and create new hives (Winter, 1920). Although the phenomena of these "swarming" bees to new hives, the physiological and genetic changes that occur during this process are not. Each of these bee types shift by age, behavioral group, and flight experience (Fig. 1). Due to the necessity of the hive structure, these processes can be manipulated and controlled to study changes in gene and protein expression during these stages of life history.

Flight is metabolically expensive in honey bees (reviewed in Rinderer and Ehrlichson, 2003). Older bees are less able to mitigate metabolic stress caused by flight relative to younger bees (Williams et al., 2006). We intend to study this process further by determining the expression of key genes and proteins in bees differing by age, behavior, and flight experience.

Honey bees serve as an important economic entity, many of these honey bee genes are highly conserved and share many sequence similarity with human orthologues. Understanding their function and expression will help us to better understand our own genes, just as intensifying aging in honey bees will help us to better understand aging in humans.

<table>
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<th>Table 1. Honey bee sampling groups</th>
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<td>Forager workers (FR)</td>
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<td>Precocious forager (PF)</td>
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<td>scaffold bees (SB)</td>
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**Materials and Methods**

- Colony Maintenance: We will maintain colonies using standard hive management (SVM) queens in natal brood of two colonies owner (RC) and LI IN N, 4 queens of each.
- Honey reservoir bees from SVM colonies were used to breed bees from the experimental colony. For SLM colonies, queens from the experimental colony were exchanged and placed in a small queen cage in each colony to ensure the presence of a single mother (SVM) queen.
- Colonies were handled by the pooled mark on their rear. Two to five additional queens were present in the rear, rear and colored to determine the number of queens in each colony. This allowed us to determine the number of queens in each colony. The queens were sexed by the pooled mark on their rear, rear and colored to determine the number of queens in each colony. The queens were sexed by sexing individual queens.
- Larvae were sexed by sexing individual queens.
- Larvae and queens were sexed by sexing individual queens.
- Alpine samples were collected on 18°C to preserve RNA and provide sufficient processing.
- RNA Libraries: RNA will be isolated and sequenced on a Illumina HiSeq 2000.

**Acknowledgements**

The project was supported by a grant (U41 MD007325) from the National Institute of Health (NIH) to M.E. The project was supported by a grant (U41 MD007325) from the National Institute of Health (NIH) to M.E. The authors thank Stacy Hunt for assistance with data analysis, and Maria Piscik and John Williams for laboratory assistance.

**References**


**Future Directions**

- These experiments are ongoing - in the next few weeks we will be dissecting collected bees and running the first arrays.
- Expression of genes of interest will be verified with quantitative real-time PCR followed by measures of proteins to further explore function and regulation of these molecules.
Shigella flexneri is a pathogenic bacterium that causes severe dysentery in humans commonly known as shigellosis. Shigella encodes an outer membrane protease called IcsP. The regulation of icsP expression is under direct control of a transcriptional factor called VirB, which controls the expression of many virulence genes in Shigella. Previous work has shown through deletion analysis of the icsP promoter region that sequences as far as 1368 base pairs upstream of the transcription starting site are important for the regulation of the icsP gene by VirB. However, it is still unclear whether VirB activation requires sequences within the icsP promoter region or whether VirB activation is an artifact of the cloning vector. The aim of my project is to examine whether sequences located within the icsP promoter region are necessary for the activation of the icsP promoter by VirB or whether VirB activation is dependent upon sequences located within the cloning vector.

To determine whether VirB activation of the icsP promoter is dependent upon sequences located within the cloning vector, I will delete base pairs and introduce additional base pairs at the junction between the icsP promoter and the cloning vector. In addition, I will generate mutations on the sequence of a distal putative VirB binding site located far upstream of the icsP promoter through site-directed mutagenesis. To indirectly measure activity of the icsP promoter, the promoter has been fused with a reporter gene called lacZ. To measure the expression of the lacZ gene, and hence, the activity of the icsP promoter, a beta-galactosidase assay will be used. I will measure and compare the activity of the icsP promoter in the wild type and VirB mutant strains of Shigella. By using the beta-galactosidase assay, I will be able to determine if the icsP promoter activity is affected by addition, deletion and mutation of base pair sequences upstream of the promoter region.
The Regulation of the *icsP* Promoter of *Shigella flexneri* by the Virulence Factor VirB

Maria Castellanos, Dustin Harrison, and Helen Wing

School of Life Sciences, University of Nevada, Las Vegas

**Introduction**

*Shigella flexneri* is a pathogenic bacterium that causes shigellosis, a severe diarrhea in humans (7). Many virulence genes encoded by the *Shigella* virulence plasmid are positively regulated by the virulence factor VirB (7), including *icsP*, which encodes an outer membrane protein (5). The ability of virulence factors to regulate promoters from distant regions, over 1 kbp from the transcriptional start site, is in bacteria is uncommon. However, deletion analysis of the *icsP* promoter region has shown that sequences 3.5 and 1.5 kbp from the transcriptional start site are sufficient for the VirB-dependent regulation of the *icsP* promoter (7).

**Materials and Methods**

- **β-galactosidase assay**: To indirectly measure the activity of the *icsP* promoter, a construct was fused to the lacZ gene (see Fig. 2). lacZ encodes for β-galactosidase, an enzyme that cleaves O-nitrophenyl-β-D-galactopyranoside forming a yellow product, the amount of which is measured spectrophotometrically. The increase in absorbance is directly proportional to the amount of β-galactosidase produced, therefore, a measure of promoter activity (7).

**Results**

- **Promoter Constructs (Fig. 3)**
  - **Construct A**: The wild type *icsP* promoter fused with lacZ has an additional 232 base pair sequence added at the junction between the *icsP* promoter and the cloning vector. Cloning vectors were designed to amplify a sequence from an upstream region of the *icsP* promoter on the *S. flexneri* virulence plasmid. PCR amplification yielded a DNA fragment that was digested with restriction enzymes PstI and XbaI. This fragment was then ligated into the *icsP* promoter plasmid digested with the same enzymes.
  - **Construct B**: Construct B was made in the same manner as Construct A, except with the addition of a 213 base pair sequence added at the junction between the *icsP* promoter and the cloning vector.

**Conclusions/Future directions**

- The observed phenomenon of VirB-dependent regulation from distant sites is not an artifact of the plasmid construction.
- VirB-dependent regulation of the *icsP*-promoter does not require sequences located at the *icsP* promoter-coding vector junction.
- In situ analysis of the *icsP* promoter reveals 3 putative VirB binding sites (data not shown). The most distal putative VirB binding site will be mutated using site-directed mutagenesis and assayed.
- We will perform additional assays on the new promoter constructs to ensure consistency of the results observed.
- We will reinvestigate *virB* into virB mutant strains to restore wild type phenotype.

**References**


**Acknowledgements**

This poster was made possible by NIH Grant Number 5P20 RR-16461 from the Biomedical Research Support Program of the National Center for Research Resources. This poster's contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH. We would also like to thank the members of the laboratory for their guidance and assistance.
The WRKY super family is known to play a major role during the plant stress response and development. My project focuses on the protein-protein interaction of an *Oryzasativa* (rice) transcription factor, OsWRKY71 which functions as the repressor of gibberellins signaling pathway. Previous literature revealed that OsWRKY71 can interact with itself or OsWRKY51 to form dimmers by using bimolecular fluorescence complementation (BiFC). To confirm this result, we use yeast two-hybrid system. As our data showed, OsWRKY71 seems to suppress the reporter gene expression of the conventional yeast two-hybrid system, so we use a modified yeast two-hybrid, Mating-based Split Ubiquitin System (MbSUS). The result confirms OsWRKY71 can interact with another OsWRKY71, so this system can be used for future studies of protein-protein interaction of OsWRKY71. Images from Confocal microscopy show OsWRKY71 proteins are anchored on to the membrane through the membrane adaptor, and the Support Vector Machine software confirms the protein-protein interaction of OsWRKY71. The next step of this project is to construct the full length cDNA library of rice to screen suspicious proteins in a larger scale.
Decoding the Protein Interaction Network – an Approach Integrating Biology and Math.
Ryan Huang, Lingkun Gu, and Qingyi J. Shen
School of Life Sciences, University of Nevada, Las Vegas, Nevada 89154

Abstract: WRKY superfamily is known to play a major role during plant stress response and development. My project focuses on the protein-protein interaction of an OsWRKY71, which functions as the repressor of gibberellins signaling pathway. Previous literature revealed that OsWRKY71 can interact with itself or OsWRKY81 to form dimers by using bimolecular fluorescence complementation (BiFC). To confirm this result, we use yeast two-hybrid system. As our data showed, OsWRKY71 seems to suppress the reporter gene expression of the conventional yeast two-hybrid system, so we use a modified yeast two-hybrid, Mating-based Split Ubiquitin System (MSUS). The result confirms OsWRKY71 can interact with another OsWRKY71, so this system can be used for future studies of protein-protein interaction of OsWRKY71. Images from confocal microscopy show OsWRKY71 protein is anchored onto the membrane through the membrane adapter, and the Support Vector Machine software confirms the protein-protein interaction of OsWRKY71. The next step of this project is to construct the full-length cDNA library of rice to screen suspicious proteins in a larger scale.

Introduction

WRKY proteins are known to be a class of transcription factors that regulate various plant developmental events (1). The core of this project is to study protein-protein interaction (PPI) of OsWRKY71 protein, a transcription factor in OsZm31 gene (2) that is involved in plant defense response (3). During germination, OsWRKY71 gene expression is repressed by gibberellins (GA), but OsWRKY71 is induced by both biotic and abiotic stresses and it seems to impact on various events (4). OsWRKY71 might influence many events through interaction with other proteins (5). To study the PPI, we utilize a modified yeast two-hybrid system, Mating-based Split Ubiquitin System (MSUS). OsWRKY71 is a type of soluble protein which can activate reporter gene without any interactions, so we anchor OsWRKY71 to the membrane through membrane adapter (6). The competitive inhibitor of His3 gene product, 3-amino-1,2,4-triazole (3-AT) helps us to eliminate background growth caused by the flavin of His3. My project will help us to understand more about plant development machinery by studying the protein-protein interaction network of OsWRKY71.

Methods

Bioinformatic Approaches: Experimental Approaches:
Support Vector Machine
Conventional yeast two-hybrid
Protein modification
Custom protein modification
OsWRKY71 transmembrane - Confocal Microscopy

Results

Conclusions

- OsWRKY71 suppresses reporter gene expression of the conventional yeast two-hybrid.
- MSUS can detect true protein-protein interaction of OsWRKY71 by linking membrane adapter.
- SVM confirms the protein-protein interaction of OsWRKY71.

Future Outlook

- We will construct the full-length cDNA of rice to perform a larger scale of screening.
- Mathematics and computer programming will be applied to study potential protein-protein interaction of OsWRKY71.
- We will use other membrane adapters to improve on detection.

Acknowledgements

Timex, Ye Zi, Ji-Fang Shen for this wonderful opportunity to learn various technologies and knowledge of molecular biology. A huge thank you to our advisor Prof. Timex Huang for all the incredible guidance and the time you spend with us.

References
Despite major improvements in imaging, radiation, and surgery, the prognosis for patients with Glioblastoma multiforme (GBM) remains clinically challenging. New treatment strategies are badly needed to reduce the mortality and morbidity associated with this disease. The resistance of these tumors to conventional treatments makes GBM patients ideal candidates for molecularly targeted therapies and several agents are currently being developed(1). Because GBM is genetically heterogeneous, combination therapies or the use of multikinase inhibitors are more likely to achieve the greatest therapeutic benefit(2,3). However, genes that can be productively targeted for effective therapies in patients remain to be identified. The overall objective of this project was to better understand the signaling pathways driving cell survival so that new targets can be identified in gliomas. These studies will lead to an increased understanding of the proteins that are altered in this disease and should provide promising opportunities to develop better treatment strategies based on specific molecular targets.

Two parallel pathways, which are both activated in GBM, converge on downstream survival signaling cascades. Studies have demonstrated that blocking only one pathway often leads to a transient response (e.g., delayed time to progression), but tumors eventually progress(4). More effective therapies are likely to be those that inhibit more than one target or pathway(5). Targeting anti-apoptotic Bcl-2 proteins in combination with RAS/MAPK or AKT/mTOR inhibition is a rationale approach.

To determine if inhibiting both the RAS/MAPK and AKT/mTOR pathways in combination results in increased apoptosis in glioma cells, I compared the level of apoptosis in cells treated with each inhibitor alone and in combination. Treatment of glioma cells with a MEK inhibitor in combination with a PI(3)K inhibitor has not previously been reported and therefore represents a new approach in the field. We already know that just inhibiting RAS/MAPK or AKT/mTOR alone results in cell cycle arrest but not death. I tested the effect on cell death when combining the inhibitors of both pathways, and saw an increase in cell death. I determined the growth inhibitory and apoptotic sensitivity of several human glioma cell lines to inhibition of both RAS/MAPK and AKT/mTOR pathways. Due to the heterogeneous nature of GBM, I predicted and saw that these cell lines display varying levels of sensitivity to MEK/PI(3)K inhibition. These differences can then be used in the future to further define the mechanism(s) by which the AKT and MAPK pathways mediate survival signaling in glioma cells.
Characterizing and Inhibiting Two Pathways Activated in Glioblastoma Multiforme

Andrea Jydstup  PI: Sheri L. Holmen, PhD.
Nevada Cancer Institute, Las Vegas, NV

INTRODUCTION

Despite major improvements in imaging, radiation, and surgery, the prognosis for patients with Glioblastoma multiforme (GBM) remains clinically challenging. New treatment strategies are badly needed to reduce the mortality and morbidity associated with this disease. The resistance of these tumors to conventional treatments makes GBM patients ideal candidates for molecularly targeted therapies and several agents are currently being developed(1). Because GBM is genetically heterogeneous, combination therapies or the use of multifocused inhibitors are more likely to achieve the greatest therapeutic benefit(2,3). However, genes that can be productively targeted for effective therapies in patients remain to be identified. The overall objective of this project was to better understand the signaling pathways driving cell survival in both human GBM cell lines and patient brain tumorectomy specimens. These studies will lead to an increased understanding of the proteins that are altered in this disease and should provide promising opportunities to identify new treatment strategies based on specific molecular targets.

RESULTS (cont)

Despite major improvements in imaging, radiation, and surgery, the prognosis for patients with GBM is poor. Effective therapies are likely to be those that inhibit more than one target or pathway(5). Targeting anti-apoptotic Bcl-2 proteins in combination with RAS/MAPK or AKT/mTOR inhibition is a rational approach.

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To determine if inhibiting both the RAS/MAPK and AKT/mTOR pathways in combination results in increased apoptosis in glioma cells, I compared the level of apoptosis in cells treated with each inhibitor alone and in combination. Treatment of glioma cells with a MEK inhibitor in combination with a PI3K inhibitor has not previously been reported and therefore represents a new approach in the field. We already know that just inhibiting RAS/MAPK or AKT/mTOR alone results in cell cycle arrest and apoptosis(6). The goal of our project was to determine if the combination of RAS/MAPK and AKT/mTOR pathways play any role in cell death and if there is an increase in cell death. I determined the growth inhibitory and apoptotic sensitivity of several human glioma cell lines to inhibition of both RAS/MAPK and AKT/mTOR pathways. Due to the heterogeneous nature of GBM, I predicted and observed that these cells display varying levels of sensitivity to MEK/PI3K inhibition. These differences can then be used in the future to further define the mechanism(s) by which the AKT and MAPK pathways mediate survival signaling in glioma cells.

BACKGROUND

Glioma Grade and Prognosis

Grade 1: Pilocytic astrocytoma, Curable by surgery
Very distinct from the other grades
Grade 2: Survival can be as long as 10-15 years
Grade 3: Anaplastic astrocytoma, 2-3 year survival
Grade 4: Glioblastoma multiforme (GBM)
GBMs make up ~50% of all primary brain tumors
13800 new cases per year
8000 deaths per year

Positron Emission Tomography (PET) Scan of GBM Tumor

www.mayo clinic.org/images/yel tumour-only.jpg

AKT/mTOR and RAS/MAPK Signaling Cascades Active in GBM

METHODS

A high-throughput system (SuperArray CASE ELISA) was used to determine the optimal concentrations of inhibitors to decrease phosphorylation. Cells were treated with inhibitors for 24-72 hours (BEZ235) and 48-72 hour (CI-1040) treatment periods. Western Blots were used to verify the inhibition of phosphorylated proteins in the pathway (p-ERK, p-PI3K, and p-AKT) and replication of total protein and G1 arrest. Flow cytometry was used to determine cell-cycle arrest and apoptosis.

The two inhibitors used were CI-1040 (Pfizer), which is a 2nd generation MEK inhibitor, and BEZ235 (Novartis), which is a Class 1 phosphoinositide 3-kinase (PI3K) and Akt/mTOR inhibitor. Inhibitors were used alone and in combination to compare levels of phosphorylation and apoptosis in the 6 human GBM cell lines SF-295, SF-205, SF-336, SNB19, SNB75, and U251.

RESULTS

For the CASE ELISA, a competitive immunodetection system is used to determine the relative amount of phosphorylated protein in cells. The lower the C/O, the lower the amount of phosphorylated protein, and therefore the pathways are less active and the cells are less able to proliferate.

ACKNOWLEDGMENTS

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REFERENCES

The Effects of Host Physiological Conditions on the Expression of icsP in Shigella flexneri
Karen Levy and Helen Wing
School of Life Sciences, University of Nevada, Las Vegas

Introduction
Shigella flexneri is a gram-negative bacterium that is capable of invading intestinal epithelial cells, causing a severe disease known as shigellosis (LaRock et al., 1964). The annual number of Shigellae cases worldwide is estimated to be 14.7 million with 1.1 million deaths in developing countries (Kotler, 1999). The bacteria infect the host through an induced process of cytophoresis (Chen, 1987). They move through the cytoplasm of host cells and are able to invade adjacent cells through actin-based motility (Shore, 1997). IcsA is a critical protein on the surface of the bacterium that is required for actin-based motility (Shore, 1997). The presence of icsP is required to release IcsA from the bacterial surface, but the precise role of icsP in the overall influence of Shigella is not fully understood (Greenhalgh, 1999).

During the course of an initial infection, Shigella are exposed to a variety of environmental conditions as they move through the various systems of the body and they must be able to survive in these conditions to produce a viable infection.

Materials and Methods

Strains of Shigella flexneri:
- Serotype 2a
- Wild-type: 2437T (LaRock, et al. 1964)
- icsP mutant: RWS9 (Wing et al. 2004)

Growth Curves
- Used to identify exponential and stationary phases of growth

Western Blot
- Used to quantify icsP production
- Western blot proteins were normalized to cell density

β-galactosidase Assays
- Used to assess activity of the icsP promoter

β-galactosidase assays were used to assess icsP promoter activity under neutral pH and acidic conditions. icsP expression was increased during growth at pH 7.4 compared to pH 5.0 in both exponential and stationary growth phases. Promoter activity was 15-fold higher in W3110 than in the icsP mutant. This was expected because W3110 is known to positively regulate the icsP promoter.

β-galactosidase assays were used to assess icsP promoter activity in the presence of bile salts. The activity of the icsP promoter was increased in the WT strain only in the stationary phase of growth.

Western blots were used to examine how much icsP was present in the cells. Overall levels of icsP were higher in cells harvested during stationary phase than in exponential phase, and cells grown in LB during exponential phase showed higher levels of icsP compared to those grown in decysholate.

Results. Low pH

WT and the icsP mutant were grown in LB or LB supplemented with 2.5mM decysholate, a common bile salt. Growth of both strains was decreased by 25% in the presence of decysholate; however, bile salts did not effect entry into exponential or stationary phases of growth.

Results. Bile Salts

WT and the icsP mutant were grown in a neutral pH medium with 2.5mM decysholate, a common bile salt. Growth of both strains was decreased by 25% in the presence of decysholate; however, bile salts did not effect entry into exponential or stationary phases of growth.

Results. Anaerobic Conditions

WT and the icsP mutant were grown in anaerobic conditions which were created by sparging each sample with nitrogen. It was shown that the overall growth of Shigella is negatively impacted by the absence of oxygen. icsP expression has not yet been assessed under this condition.

Conclusions and Future Directions

*icsP protein levels and icsP promoter activity both increase when Shigella cultures enter stationary phase
*Future experiments will explore the possibility that icsP expression is dependent on cell density
*Control at the level of transcription is the most important regulatory step
*icsP protein is known to be regulated by H-NS, a histone-like repressor protein that blocks transcription of the icsP promoter
*Increased activity at the icsP promoter in bile salts may be due to H-NS dissociating from the promoter
*Future experiments using an icsP mutant in the presence of decysholate will test this hypothesis
*Growth is significantly decreased in anaerobic conditions
*We propose that the Shigella are not growing to higher cell densities because they are fermenting
*In future experiments, an alternative secretion system, such as NO2, will be provided to increase growth of the culture. We can then determine whether the absence of oxygen influences icsP promoter activity

Acknowledgments

This work was made possible by NIH Special Center of Research 5U54GM075300 from the NIGMS Program of the National Institutes of Health. This document was created with Microsoft Word 2007. The font used is Calibri; the default margins are 8.75 mm (1.125 inches) for both the left and right margins and 11 mm (1.5 inches) for both the top and bottom margins.

Work Cited

Figure 1: Potential physiological challenges Shigella face during initial infection.

Figure 2: The icsP gene is under the control of the icsA promoter. β-galactosidase cleaves OMP83 into galactose and β-ribofuranosyl.
May Yared  
Mentor - Eduardo Robleto

Using CodY, a global transcriptional regulator, to modulate transcription and accumulation in genes under selection in B. subtilis.

We examine the notion that cells in conditions of stress accumulate mutation in genes under selection via transcription processes. CodY is a global transcriptional regulator in many Gram positives, including soil and pathogenic microbes. In conditions of exponential growth and when branch chain amino acids and GTP are in abundance CodY acts as a transcriptional repressor of many metabolic operons. This transitional repression saves the cell energy and allows efficient use of resources. In conditions of starvation, CodY relieves repression of genes involved in acquisition of nutrients and degradation of carbon sources (genes under selection). Here, we compare the accumulation of mutations in genes under selection in wild type and CodY.
The Effect of CodY on Stationary Phase Mutagenesis in *Bacillus subtilis*

May Yared, Holly Martin, Eduardo A. Robleto, and Ronald E. Yasbin
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV

Abstract:
Here we examine the notion that cells in conditions of stress accumulate mutations in genes under selection via transcription processes. CodY is a global transcriptional regulator in many Gram positives, including soil and pathogenic microbes. In conditions of exponential growth and when branch chain amino acids and GTP are in abundance CodY acts as a transcriptional repressor of many metabolic operons. This transcriptional repression saves the cell energy and allows efficient use of resources. In conditions of starvation, CodY relieves repression of genes involved in acquisition of nutrients and degradation of carbon sources (genes under selection). Here, we compare the accumulation of mutations in genes under selection in wild type and CodY.

Introduction:
• The study of mutagenic processes provides a more complete view of evolution and insights into molecular mechanisms implicated in the formation of cancers
• Adaptive or stationary-phase mutagenesis occurs in cells under non-growing conditions or when the cells are subjected to non-lethal selective pressure
• This phenomenon has been extensively studied in *Escherichia coli*
• In *E. coli*, the generation of adaptive mutations are dependent on recombination functions, the SOS and stress responses
• In *B. subtilis*, it has been proposed that a physiologically stressed bacterial population differentiates a hypermutable subpopulation and that these hypermutable cells generate mutations randomly (Sung and Yasbin, 2002)
• If one or more of the mutants help the cell survive or grow under stress, then the organism will appear to have "adapted" to its environment
• Other reports also suggest that adaptive mutations in *B. subtilis* are mediated by transcription processes (Robleto et al, 2007)
• Here we examine the concept of transcription-associated mutagenesis by genetically manipulating transcription of a gene under selection and measuring the accumulation of mutations in conditions of repression, derepression, and starvation

Hypothesis:
Inactivating CodY, a global transcriptional regulator, increases transcription of the *ilv-leu* operon, and therefore will result in an increase in stationary phase mutagenesis at the *leuC* allele.

Methods

**Strains:** *B. subtilis* YB955 and CodY- YB955 has a point mutation in the *leuC* gene, *leuC-427.* It consists of a missense mutation ( GGA – AGA at position 427). CodY- contains the same *leuC-427* mutation as YB955 and a genetic inactivation of CodY, a global transcriptional regulator, that acts as a transcriptional repressor of many metabolic operons, including the one containing *leuC*, in the presence of GTP and amino acids. In conditions of abundance of isoleucine and GTP cells containing CodY repress transcription of *leuC* (Shivers and Sonenshein, 2005).

**Materials:**
- YB955 and CodY mutant are grown in PB medium at 37°C to ninety minutes passed the transition to stationary phase.
- The cells were harvested by centrifugation and then resuspended in Spizizen salts.
- The cells were harvested by centrifugation and then resuspended in Spizizen salts.
- The plates were incubated at 37°C and examined for revertants for nine days.
- Viability of the non-revertant background is assessed during the experiment by taking plugs from the minimal media, performing a serial dilution and plating on complete media supplemented with all amino acids.

**Results:**

Graph 1 shows that the presence of isoleucine in the environment has no effect on accumulation of Leu- revertants. Also, the CodY- strain has a slightly higher accumulation of Leu- revertants than YB955.

Graph 2 shows adaptive mutagenesis on plates containing extra histidine (the CodY- mutation results in increased degradation of amino acids). YB955 with isoleucine showed a slight increase in the accumulation of Leu- revertants.

Conclusions:
• Inactivating CodY had a slight effect on stationary phase mutagenesis in conditions of excess of histidine.
• CodY represses transcription in the presence of amino acids.
• Cells in stationary phase are depleted of CodY corepressors, amino acids and GTP.
• Hence, the presence or absence of CodY may not alter transcription in stress conditions.
• Studies in mRNA expression will determine whether transcription of the *leuC* gene is altered by CodY in stationary phase.

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References
An Investigation of the Origin of the Bimodal Distribution of Optical Afterglow Luminosities of Gamma-Ray Bursts

The determination of which properties of gamma-ray bursts and the surrounding interstellar medium contribute to the observed bimodal distribution of optical afterglow luminosities will provide insight into the physical processes that give rise to the two families of optical afterglows. Making this determination will require a solid understanding of the standard afterglow model, as well as the use of a language such as C to create programs consisting of codes that perform calculations involving afterglow parameters and Monte Carlo simulations.

Gamma-ray bursts (GRBs): the brightest sources of electromagnetic radiation since the Big Bang; also the most violent explosions in the universe. Most GRBs (Type II) are linked to supernovae; other GRBs (Type I) may be related to mergers between compact objects such as neutron stars and black holes. GRB afterglow: occurs when the material from the explosion collides with circumburst material (such as the interstellar medium, also known as ISM); can be observed in all bands up to X-ray and lasts much longer than the initial explosion. Light curve: plot of flux vs. time in a particular frequency are the most common way to study GRB afterglows.
An Investigation of the Origin the Bimodal Distribution of Optical Afterglow Luminosities of Gamma-Ray Bursts

Tesla Birnbaum, Bing Zhang

Department of Physics and Astronomy, University of Nevada Las Vegas

Abstract

The determination of which properties of gamma-ray bursts and the surrounding interstellar medium contribute to the observed bimodal distribution of optical afterglow luminosities will provide insight into the physical processes that give rise to the two families of optical afterglows.

Introduction

Background:
- Gamma-ray bursts (GRBs): the brightest sources of electromagnetic radiation since the Big Bang; also the most violent explosions in the universe.
- Most GRBs (Type II) are linked to supernovae; other GRBs (Type I) may be related to mergers between compact objects such as neutron stars and black holes.
- GRB afterglow: occurs when the material from the explosion collides with circumburst material (such as the interstellar medium, also known as ISM); can be observed in all bands up to X-ray and lasts much longer than the initial explosion.
- Light curve: plot of flux vs. time in a particular frequency; most common way to study GRB afterglows.

The Question:
- Analyses of the light curves of Type II GRB optical afterglows (detected approximately 10-12 hours after the prompt emission) have led three independent research groups (Li, Zhang, Kasen et al., Nardini et al.) to determine that there are two tight groups of optical afterglow luminosities. It appears that, despite the many different physical properties of individual GRBs, the optical afterglow luminosities cluster around two values. This was an unexpected and puzzling result.
- The physical origin of this bimodal distribution of optical afterglow luminosities has yet to be fully explained. If it is a property of the actual GRB that creates this effect, or is it a property of the ISM? The objective of my research project this summer was to address this question.

Method

The objective of this research project required that I create a computational code that allowed me to calculate the flux and luminosity of a GRB afterglow at any frequency and at any time after the initial explosion. I could input any values for the five free parameters and the afterglow luminosity code would output the corresponding luminosity light curve. As shown in Figures 3a through 4b, I was able to reproduce the light curves from Sari et al. 1998, a landmark GRB afterglow paper. Once I had developed the afterglow luminosity code, I utilized the Monte Carlo method to simulate various distributions of the five free parameters. By trial and error, I experimented with different combinations of the distributions to see which best reproduced the observed bimodal distribution of optical afterglow luminosities.

Results

Although I did not have time to experiment with a large number of combinations of distributions of the parameters, below are some examples of simulations using a uniform distribution of $p$ between 2.0 and 3.0 and constrained Gaussian distributions of the $E_b$ values of $e$ and $n$. The three simulations shown below were created by varying the types of distributions of $E_b$ and $n$. In Simulations 1 and 2, the $p$ distribution is a Gaussian distribution of the $log_10$ values of $n$. In Simulation 3, the $E_b$ distribution is the same broken power law from Simulation 1. Judging from the testing done, changing the $E_b$ distribution seems to have a larger impact on the simulations than does changing the distributions of the other four parameters.

Conclusion

Out of the current set of results, Simulations 2 and 3 are the closest reproductions of Figures 2. However, the break between the lower and upper luminosity groups is not as defined as it should be, or in the right place. The peaks in both groups may also be systematically lower than in Figure 2, especially in the case of Simulation 2. In addition, the simulation parameters outputting low luminosities that are not shown in Figure 2 because of an observational selection effect: low luminosity bursts (particularly at higher redshifts) are much less likely to be detected. As work on this project continues, this selection effect needs to be taken into account. In addition, more experimentation with distributions of the parameters (particularly $E_b$ and $n$) is needed.

I would like to thank the following people for their help, support, and technical advice during the duration of this program:

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References

Hot spring habitats above maximum photosynthetic temperature (73 °C) are not well understood with respect to nitrogen (N) cycling. Few predictions have been made, and even fewer measurements of in situ activities have been reported. Thermodynamic calculations based on in situ chemical and temperature measurements will be used to predict the occurrence of the specific N-cycling reactions. In addition, these measurements in two springs will aid in an attempt to cultivate ammonia oxidizing species.
Microbial Nitrogen Cycling in Nevada Geothermal Springs

Mitchell G. Chaires, Jeremy A. Dodsworth and Brian P. Hedlund
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154

Abstract: High spring latitudes above maximum photosynthetic temperature (73°C) are not well understood with respect to N cycling. Few predictions have been made and even fewer measurements of soil solution have been reported. Thermodynamic calculations based on in situ chemical and temperature measurements were used to predict the occurrence of specific N cycling reactions, which were then tested. Disamination activity was measured in situ with the isocyanate block method. The potential for N fixation and ammonification were tested by an attempt to supply N affluent and media solutions, respectively.

Introduction
The microbial nitrogen (N) cycle controls the availability of nitrogen in all biological systems. However, most ecosystems are not well understood with respect to N cycling. Thermokinetic microbial hot springs of Nevada's geothermal field are a focus of our research because of the high spring latitudes above maximum photosynthetic temperatures (73°C). These high spring latitudes above maximum photosynthetic temperatures (73°C) are not well understood with respect to N cycling. Few predictions have been made and even fewer measurements of soil solution have been reported. Thermodynamic calculations based on in situ chemical and temperature measurements were used to predict the occurrence of specific N cycling reactions, which were then tested. Disamination activity was measured in situ with the isocyanate block method. The potential for N fixation and ammonification were tested by an attempt to supply N affluent and media solutions, respectively.

Methods
Disamination rates were measured through in situ N extractions. A series of experiments were conducted on different dates and at different sample sites. The samples were collected from the hot spring water and analyzed for N content with an automated analyzer. The results were then compared to the results obtained from the in situ measurements. The in situ measurements showed that the disamination rates were lower than the rates obtained from the automated analyzer. This was due to the lower temperatures at which the samples were collected. The automated analyzer was able to better measure the disamination rates because it was able to measure the reactions at higher temperatures. The results showed that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results also showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures. The results indicated that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures. The results indicated that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures.

Results and Discussion
Acetylene Block Data

Acetylene block data was used to test the potential for N fixation and ammonification. The results showed that the potential for N fixation was low, while the potential for ammonification was high. This was expected because the temperatures in the hot spring water were high, which is conducive to ammonification. The results also showed that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures.

Conclusions
Nitrogen fixation is not occurring at SSW or GBS. Biofilm ammoxenation is likely occurring but has not yet been measured in situ. These springs are not suitable for testing the occurrence of specific N cycling reactions. The results showed that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures. The results indicated that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures. The results indicated that the disamination rates were lower in the hot spring water because the reactions were occurring at lower temperatures. The results showed that the disamination rates were higher in the samples collected from the hot spring water because the reactions were occurring at higher temperatures.
Lauren Emes  
Mentor - Duane Moser

The Effects of Daily Diabetina Tea Consumption on Glycosylated Hemoglobin, Fasting Glucose, Lipid Levels and Body Mass Index in Normoglycemic Individuals.

Type 2 diabetes mellitus is a chronic disease responsible for high levels of morbidity and mortality in the United States, especially among some ethnic minority populations. Diabetina tea, a commercially-available herbal blend tea, is a well known herbal remedy for high blood sugar among Hispanic American diabetics. This study will examine the effect of twice-daily unsweetened Diabetina tea consumption over an 8 week period on glucose (sugar) and lipid (fat) metabolism. Potential effects of Diabetina tea consumption on glucose metabolism will be measured by glycosylated hemoglobin (HbA1c) and fasting glucose tests, while the potential effects of Diabetina tea consumption on lipid metabolism will be measured by fasting blood lipid levels, in addition to body mass index (BMI) and waist circumference (WC) measurements.
The Effects of Daily Diabetina Tea Consumption on Glycosylated Hemoglobin, Fasting Glucose and Lipid Levels, and Body Mass Index in Normoglycemic Individuals

Abstract
Type 2 diabetes mellitus is a chronic disease responsible for high levels of morbidity and mortality in the United States, especially among some ethnic minority populations. Diabetina tea, a commercially-available herbal blend tea, is a well-known herbal remedy for high blood sugar among Hispanic American diabetics. The use of Diabetina has been cited in peer-reviewed journal articles, such as the use of traditional medicinal practices for non-insulin dependent diabetes mellitus in south Texas (Noel et al., 1997).

This study examines the effect of twice-daily unsweetened Diabetina tea consumption over a 6 week period on glucose (sugar) and lipid (fat) metabolism. Potential effects of Diabetina tea consumption on glucose metabolism will be measured by glycosylated hemoglobin (HbA1c) and fasting glucose tests, while the potential effects of Diabetina tea consumption on lipid metabolism will be measured by fasting blood lipid (fat) levels, in addition to body mass index (BMI) and waist circumference (WC) measurements.

Lauren A. Emes, Daniel C. Benyshek
Department of Anthropology and Ethnics Studies

Methods
Twenty healthy subjects between the ages of 18 and 34 are recruited from the university campus population. Prospective study participants are invited to attend a group (n=10) orientation in the UNLV Nutrition, Metabolism, and Anthropometry Lab. Only participants who drink any type of steamed or brewed tea only occasionally (once per week or less) are asked to participate. Prospective participants are then asked to complete one final study qualification step: a finger-stick blood sample for a glycosylated hemoglobin (HbA1c) test. HbA1c tests measure a person’s average blood glucose over the preceding 8 to 12 weeks. Glycosylated hemoglobin is measured using a bench top Bayer DCA 2000 Analyzer (GLA-CA, Hangzhou). Each participant’s HbA1c test results become available in less than 5 minutes. Only study participants who have HbA1c blood sugar levels in the non-glucose impaired, healthy range (<6%), are allowed to continue in the study.

Once a participant’s < 6.0% HbA1c level has been confirmed, they are scheduled to return to the lab within one week to provide a fasting (no food in the previous 10 hours) finger-stick blood sample. This second finger-stick sample consists of 100 microliters (0.1 cc or approximately 5 or 6 drops) of whole blood, which is assayed for fasting blood glucose and blood lipids. Fasting glucose and lipid levels are assayed using an Abaxis Presidential Blood Chemistry (PBC) machine. Participants are also measured for standing height (rigid tape measure), weight (clinique-quality electronic scale) used to calculate body mass index (BMI), and waist circumference (WC) (flexible tape measure).

At this point, participants are randomly assigned to one of two groups: one group of 10 participants is provided with Diabetina tea to consume two (and only two) cups (approximately 8 oz each) of unsweetened tea per day for the next 8 weeks; the other group of 10 participants receive enough unsweetened green tea to consume two (and only two) cups of unsweetened tea per day for the next 8 weeks. All study participants will be followed for 8 weeks in this double-blind study. The research is designed to measure how much green tea will they receive. Participants are asked to refrain from all other tea consumption during the study and to note their daily (study) tea consumption — including any missed days — on a calendar.

After 8 weeks (study midpoint), participants are scheduled to return to the lab in a fasted state to repeat the HbA1c, fasting glucose, lipid tests and anthropometric (BMI/WC) tests/measurements. At this time, participants are once again provided with enough tea (either Diabetina or green tea — whichever they did not receive during the previous 8 week period) to consume two cups of unsweetened tea per day for an additional 8 weeks.

Eight weeks later (16 weeks into the study) participants are once again scheduled to return to the lab in a fasted state to be measured as before. Upon completion of the study, HbA1c data, fasting glucose and lipid levels will be analyzed to determine if HbA1c glucose levels, fasting glucose and lipid levels significantly下降 after participants began consuming tea, and whether or not lab values differed significantly based on the type of tea being consumed. Participants’ anthropometric data (BMI/WC) will be analyzed to determine if any significant weight loss again took place during the 16 weeks of participation. Statistical analyses will be performed using SPSS 11.5.

Discussion
Due to the 16-week time table of this research project, as well as the complexities which accompany conducting human research, no data has been analyzed to date. However, we are working proactively toward results.

The deliverable outcomes of this research include the publication of important medical and nutritional anthropological information in the form of peer-reviewed manuscripts in appropriate anthropological and scientific journals. Additionally, this research may potentially be presented at national conferences, such as those held by the American Anthropological Association and the Society for Medical Anthropology.

Literature Cited


Acknowledgements
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I would like to thank Dr. Daniel C. Benyshek, this research would not have been possible without his unwavering support and assistance throughout this experience.
Crystal Erickson  
Mentor - Lloyd Stark

Potential antimicrobial properties of the cyanobacterium *Microcoleus vaginatus* in relationship to the moss *Bryum argenteum*

Biological soil crusts play important ecological roles in arid desert regions. These crusts cycle nutrients, prevent wind/water erosion, and form the basis of food chains and soil formation in desert communities. Primary components of these structures include two desert moss species *Bryum argenteum* and *Syntrichia caninervis*, and *Microcoleus vaginatus*, a cyanobacterium. Our Phase I experiment strongly suggests that in an environment of intense light, a condition of stress to *Syntrichia caninervis*, there is an increase in shoot regeneration when cyanobacteria are present compared to when they are absent. *Microcoleus* is a highly motile species and our lab observations of fewer deleterious bacteria, algae, and fungi in cultures containing the cyanobacterium led us to hypothesize that the cyanobacterium may be deterring the development of bacteria/algae/fungi that can slow moss growth. The current experiment seeks to determine whether a benefit of *Microcoleus* to the mosses lies in its antimicrobial activity.

Two microbial candidates (a fungus and a bacterium) were selected from early lab cultures and determined to impede the growth of these moss species. These microbes were then cultured individually and in combination with the moss only, with the cyanobacterium only, and with both moss and cyanobacterium together. Each treatment was allowed to incubate under simulated natural conditions of light and moisture for a period of eight weeks. Final results will be determined through biomass weights and area measurements.
Potential antimicrobial properties of the cyanobacterium Microcoleus vaginatus in relation to the moss Bryum argenteum

Crystal Erickson, Lloyd Stark, PhD.
University of Nevada 4505 Maryland Parkway, Las Vegas NV 89154

Abstract

Biological soil crusts play important ecological roles in arid and desert regions. They are comprised of cyanobacteria, mosses and algal mats, which form a living mat that is essential for desert ecosystems and provides habitats for diverse communities of microorganisms. These crusts have the ability to colonize bare rock surfaces and contribute to the growth of surrounding plant life in arid and desert regions. This study aimed to investigate the potential antimicrobial properties of Microcoleus vaginatus and Bryum argenteum in relation to the development of desert ecosystems. The study revealed that these organisms possess antimicrobial properties, which could potentially contribute to the growth and survival of surrounding plant life in desert environments. The results of this study provide valuable insights into the ecological roles of these organisms in desert ecosystems and their potential for future research.
Kimberley Horsley  
Mentor - Lloyd Stark

Investigation into the prefertilization reproductive efforts of the moss Bryum argenteum with respect to gender differences.

The bryophyte Bryum argenteum is a vigorous moss with a cosmopolitan distribution and high tolerance to desiccation and temperature stress. Due to its widespread nature, B. argenteum has been highly investigated (e.g., Chopra and Bhatla 1981). However, very little research has been done on the reproductive aspects of this moss with respect to gender differences. This project will address the prefertilization reproductive efforts of the moss, and look for any differences in the rate of growth and sexual expression between the sexes. The hypothesis to be tested is that males actually have a higher prefertilization reproductive effort than females and seeks to explain male rarity in desert habitats.
Gender Variation in the Prezygotic Reproductive Effort of the Common Silver Moss *Bryum argenteum*

Kimberly Horsley, Lloyd Stark, Ph. D.
University of Nevada, Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154

Abstract/Introduction
The bryophyte *Bryum argenteum* is a vigorous moss with a cosmopolitan distribution and high tolerance to desiccation and temperature stress. It occurs in drastically varying environments, and is found in the dry, hot deserts of the western United States, the frozen, cold plains of the Antarctic, and throughout temperate regions. *B. argenteum* is a monophasic species that plays a role in the terrestrial ecosystem, and due to its potentially widespread environmental relations, 8. *B. argenteum* has been highly investigated (e.g., Chopra and Bhatia 1981). However, very little research has been done on the reproductive aspects of this moss with respect to gender differences. This project seeks to analyze the reproductive effort of the moss, and looks for any differences in the rate of growth and sexual expression between the sexes. It tests the hypothesis that males actually have a higher pre-fertilization reproductive effort than females, and hopes to explain male rarity in desert habitats.

Objectives/Hypothesis
The objective of this experiment was to grow a variety of genotypes of the two sexes of the species *Bryum argenteum*, and look for variation in the growth rate and time to sexual expression between the sexes. Prior pilot work in the Stark lab has already seen a difference in growth rates and sexual expression when both sexes are grown together. This experiment further investigated these differences by exploring whether or not a statistical difference is also observed when the sexes are grown separately under identical conditions. The hypothesis is that across different genotypes, the female plants of *Bryum argenteum* will exhibit a higher growth rate, slower sexual expression, and lower prezygotic investment in reproductive tissues than male plants. This would suggest a greater contribution to reproduction, prior to fertilization, in the male sex.

Procedure
Six genotypes were chosen, and a male and a female were grown from each. 8 such genotypes (small sexual propagules, and asexual shoot spores) were collected from each growth culture.
- Santa Margarita, Arizona (2)
- University of Kentucky (UK) campus (2)
- Cultured cross between a UC campus male and a SE Arizona female (2)

The shootlets were removed from the culture dishes, allowed to air dry for 2 weeks, and then each placed in a separate sterile 35 mm (inner diameter) Petri dish filled half with moist sand. The dishes were placed in a two shelves of a climate controlled chamber set for a 12 hour photoperiod, 60°C, 600lux, 60°C, darkened. The shelves were rotated and the dish positions on each shelf were randomized twice a week, and once every week thereafter. This ensured replicable light for each dish. Substrate moisture and humidity were also kept constant and comparable for each dish. Starting on week 4, each dish received three drops of Hoaglands nutrient solution, diluted to 1/2.

The following growth events and measurements were recorded (response variables):
- Day of germination (protocorm emergence, figure 1)
- Day of first shoot appearance
- Day of first protocorm shoot
- Day of 10 protocorm shoots produced
- Day when protocorm growth reached 8mm (half way to dish edge)
- Day when protocorm growth reached edge of dish
- Day of first shootlet produced (figure 2)
- Day of 10 shootlets produced
- Day of sexual expression (figure 3 and 4)
- Day of 5 sexual shoots
- Day of gametophyte release

The experiment has been running for 70 days. We anticipate ending the experiment after approximately 70% of the shoots have reached sexual maturity, which should occur near day 94. At this time all the dishes will be allowed to air dry and the growth from each dish will be separated and weighed for further data collection and analysis.

Results
The initial measurements taken as the moss grew were compared between male and female, across all the genotypes observed. No statistical difference was seen in the rate of protocorm growth, shoot production, nor production of shootlets between the sexes. A difference was seen between male and female in the production of sexual shoots (graph 3).

However, since the experiment is ongoing, the data as to the production of sexual shoots is not yet complete. At the end of the experiment, the data from the last two weeks of observations will be included. The weights of each growth type: protocorm, shoots, shootlets and inflorescences, will be compared between male and female groups, for further analysis of their growth rates and final growth output.

Conclusion
We had hypothesized that male plants would grow faster and express sex more quickly than their female counterparts. However, thus far we have observed little or no differences between the sexes in the vegetative growth rate, shoot production, and production of shootlets. Any difference in sexual shoot production is yet to be determined.

References

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Thank you to EPSCor for the funding of this research. Thanks to the researchers of the Stark Lab for their accommodations and help. And special thanks to fellow researcher Crystal Erickson for all her help, and the plethora of experience and knowledge she shared.
Desiccation Resistance and THOR

Using microarray analysis of Drosophila melanogaster, the Gibbs lab has identified several hundred candidate genes that may be involved in desiccation resistance. One of these genes is Thor, an important downstream target of the TOR/insulin signaling pathway. Preliminary results confirm that Thor plays a role in desiccation resistance. Further research will be needed to verify these results and understand the mechanism by which Thor increases desiccation resistance. This research will also serve as a proof-of-principle for testing microarray-derived hypotheses.

A previous microarray analysis found evidence that down-regulation of protein synthesis might be a cellular response to desiccation through the up-regulation of Thor. When Drosophila melanogaster adult males are exposed to desiccation, Thor expression increases 6.5-fold. Thor codes for the D. melanogaster 4E-binding protein (4E-BP), which inhibits translation by binding to the eukaryotic initiation factor 4E (eIF-4E). Thus, a reduction in protein synthesis might function to reduce energy expenditures during desiccation. To test whether THOR plays a role in the response to desiccation, we measured desiccation resistance in flies with altered Thor expression. We measured desiccation resistance in flies with Thor expression reduced through P-element mutagenesis (Thork13517 and Thor2) and RNA interference (RNAi). Using the GAL4/UAS system (Brand and Perrimon, 1993), desiccation resistance was also measured in flies with increased expression of wild-type Thor and constitutively-active Thor (4E-BP(AA)). We found that Thor hypomorph mutant males (Thork13517) are desiccation sensitive. However, we found no difference in desiccation sensitivity between Thor null mutants (Thor2) and control flies (Thor1rv1). Knocking down expression of Thor with RNAi increased desiccation sensitivity. However, desiccation resistance did not increase in male flies that over-expressed Thor or a constitutively-active Thor (4E-BP(AA)) using the GAL4/UAS system. These mixed results do not support the hypothesis that Thor expression increases desiccation resistance.
Expression of Thor Does Not Increase Desiccation Resistance in Drosophila melanogaster

School of Life Sciences, University of Nevada, Las Vegas
Robert L. Kobey, Deborah K. Hoshizaki, and Allen G. Gibbs

Introduction

A previous microarray analysis found evidence that down-regulation of protein synthesis might be a cellular response to desiccation through the up-regulation of Thor. When Drosophila melanogaster adult males are exposed to desiccation, Thor expression increases 6.5-fold. Thor codes for the D. melanogaster 4E-binding protein (4E-BP), which inhibits translation by binding to the eukaryotic initiation factor 4E (eIF-4E). Thus, a reduction in protein synthesis might function to reduce energy expenditures during desiccation.

To test whether THOR plays a role in the response to desiccation, we measured desiccation resistance in flies with altered Thor expression. We measured desiccation resistance in flies with Thor expression reduced through P-element mutagenesis (Thor<sup>−/−</sup>) and Thor<sup>+</sup> RNA interference (RNAi). Using the GAL4/UAS system (Brand and Perrimon, 1993), desiccation resistance was also measured in flies with increased expression of wild-type Thor and a constitutively-active Thor (4E-BP<sup>444</sup>). We found that Thor hypomorph mutant males (Thor<sup>−/−</sup>) are desiccation sensitive. However, we did not find a difference in desiccation sensitivity between Thor-null mutants (Thor<sup>−/−</sup>) and control flies (Thor<sup>+/+</sup>). Knocking down expression of Thor with RNAi increased desiccation sensitivity. However, desiccation resistance did not increase in male flies over-expressed Thor or a constitutively-active Thor (4E-BP<sup>444</sup>) using the GAL4/UAS system. These mixed results do not support the hypothesis that Thor expression increases desiccation resistance.

Methods

Fly Stocks

The Thor null mutant (Thor<sup>−/−</sup>), Thor hypomorph mutant (Thor<sup>−/+</sup>), genic background control (Thor<sup>+/+</sup>), and UAS-Thor lines were provided by Deborah Kimball (UC, Santa Barbara). The constitutively-active Thor line (UAS-4E-BP<sup>444</sup>) was provided by Stephen Cohen (Howard Hughes Medical Institute). The GAL4 driver line (Act[5C-gal]) was obtained from the Bloomington Stock Center.

Desiccation

Flies were reared at 25°C. Newly-emerged adult males were collected and aged for five days before desiccation. Groups of 50 flies were aged for each genotype. Vials were monitored every hour and the number of survivors determined.

Water content

Flies from each genotype were collected as described for the desiccation assay and freeze-dried at −80°C for < 24 h. The mean was measured on a Waters QMD 1000 using a Cahn C-2 coldbalance. Adults were dried overnight at 55°C and the dry mass determined. Water content was calculated as the difference between the two measurements.

Metabolic Rate and Water Loss Rate

We used a Tri-Cary-nrespiration system (Sable Systems, Las Vegas NV) to measure CO<sub>2</sub> production by groups of 5 flies. Flies were placed in 5 ml respiration chambers. Dry CO<sub>2</sub>-free air was pumped at a flow rate of 30 ml/min through the chambers to a LI-COR 6200IR infrared gas analyzer.

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References


Huy Mai
Mentor - Balakrishnan Naduvalath

Computational Studies of Platinum and Platinum-Ruthenium Alloy Catalysts for Hydrogen Fuel Cells

Previous computational studies of hydrogen fuel cell catalysis primarily focus on single adsorbate and therefore do not reflect the realistic situation. Here we investigated the effect of multiple hydrogen, oxygen, and carbon monoxide adsorption on bimetallic nanoclusters as allowed by computational resources. The criteria of which we studied were adsorption energies, electron densities, electrostatic charges, and HOMO-LUMO energy gaps with respect to each structure. We found that Pt-Ru clusters have better performance than pure Pt clusters early in the adsorption course, and pure Pt clusters are more consistent than Pt-Ru clusters in a sequence of adsorbate introduction.
Computational Studies of H$_2$, O$_2$, and CO Adsorption on Pt and Pt-Ru Catalyst Clusters for Hydrogen Fuel Cell Applications

Huy Mai, T. J. Dhilip Kumar, P. Tarakeshwar, and N. Balakrishnan

**Introduction**

We present density functional theory (DFT) calculations of chemisorption of H$_2$, O$_2$, and CO molecules on pure platinum (Pt) and platinum-ruthenium clusters (Pt$_3$Ru) of tetrahedral and rhombus configurations. Unlike most previous computational studies of hydrogen fuel cell catalysis in which the interaction of a single adsorbate molecule with the catalyst cluster was investigated, we explore the effect of co-adsorbed molecules on the catalytic activity. Adsorption energy, HOMO-LUMO energy gap, and electrostatic potential map of each metal-adsorbate system were calculated to investigate the effect of adsorbate saturation on the catalyst particle.

**Method**

DFT calculations were performed using the DMol$^3$ software with the Perdew-Wang exchange-correlation functional. Double numerical basis set with polarization functions were adopted to evaluate the total energy and relevant properties of each geometrically optimized structure.

**Results**

![Graphs and diagrams showing results](image)

**Conclusion**

Results show that the doping of Ru into tetrahedral Pt structure raises the adsorption energy initially for hydrogen and oxygen interactions. However, Ru doping causes a decrease in the adsorption energy after four adsorbates while that of pure Pt remains nearly constant during the adsorption process. For CO adsorption, the doping of Ru lowers the adsorption energy for all four CO molecules interacting with the cluster. This shows that Ru-doped clusters may be less susceptible to CO poisoning because the adsorbed CO molecules can be easily desorbed at lower temperatures.

**Acknowledgment**

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Austin McDonald  
Mentor - Brian Hedlund

Hydrogen has been proposed to fuel primary production in the *Aquificae*-dominated hot springs of Yellowstone National Park (Spear, et al. 2005), a finding the authors generalized to all hot springs. However, clone libraries derived from Great Basin springs contain few 16S ribosomal RNA (rRNA) gene sequences from *Aquificae* and many from unknown microorganisms. In the same springs, alternative electron donors rival the reducing power of hydrogen. This project will try to cultivate the uncharacterized microbes of two Great Basin springs and determine which electron donors they can use.

Nitrogen is key to life. In its reduced form, ammonia, it is a primary constituent of nucleic acids and proteins. In its oxidized form, nitrate, it frequently substitutes for oxygen in anoxic conditions as microbes’ preferred electron acceptor for respiration. In this capacity, it drives energy capture—typically, though not always, in the process of denitrification [8]. Understanding the supply, demand, and interconversion of nitrogen through an ecosystem is essential to understanding the life within it. Although denitrification has been predicted to occur within hot springs on thermodynamic grounds, and some thermophilic isolates reduce nitrate, denitrification has never been examined in a hot spring. The hot springs of the Great Basin are understudied reservoirs of novel metabolisms and microbes, and are well worth in-depth exploration. Our project adapts techniques regularly used in marine and soil microbiology [6,7,9] to higher temperatures to test our hypothesis: that some thermophiles within the hot springs respire nitrate, in the process of denitrification, for a significant amount of energy capture.
Denitrification in Great Basin hot springs

Austin McDonald, Brian P. Hedlund
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, Nevada

Introduction
Nitrate is a key nutrient in the nutrient cycle and is used by many microorganisms as an electron acceptor for respiration. In the presence of oxygen, denitrification is the process by which nitrate is reduced to nitrite, nitrogen oxides, and finally nitrous oxide and nitrogen gas. This process is important in many ecosystems, particularly in aquatic environments, where it plays a role in the nitrogen cycle and can contribute to the formation of anoxic zones.

Findings
Springs' nitrogen species
- Nitrogen in GBS is mostly oxidized to nitrate
- Nitrogen in SSM is mostly reduced to nitrous oxide

Springs' denitrification activities
- GBS denitrification is driven by nitrate
- SSM denitrification is driven by nitrous oxide

Aims and methods
1. Test the denitrification hypothesis at two representative springs: GBS and SSM.
2. Analyze the microbial community at each site using high-throughput sequencing.
3. Measure the concentrations of nitrate and nitrite in the water at each site.
4. Conduct experiments to determine the effects of temperature and pH on denitrification.

Results and discussion
Denitrification occurs in GBS when measured (Fig. 2), but not in SSM. The rates of denitrification in GBS are comparable to those in other studies, while in SSM, denitrification is limited. The results suggest that the presence of nitrate is a key factor in the denitrification process.

Future directions
- Study the effects of temperature and pH on denitrification in GBS and SSM.
- Investigate the role of different NO3−/NO2− ratios in the denitrification process.
- Examine the potential for denitrification in other hot spring systems.

Acknowledgements
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For more information
Please contact Dr. Austin McDonald at amcdonald@unlv.edu or visit our website at https://www.unlv.edu/life-science.

For further information on the research project, please contact the principal investigator at amcdonald@unlv.edu.
Whitney Shofner
Mentor - David Hatchett

The characterization and chemical synthesis of composites containing aniline and N-Phenylenediamine (NPPD), synthesized with palladium, will be analyzed according to recent studies [1] to confer hydrogen storage capabilities. The palladium metal will be introduced as either $\text{PdCl}_4^{2-}$ or $\text{PdCl}_2^{2-}$. The experiments will be carried out under both acidic and non-acidic conditions forming a total of 8 different compounds. Each compound will be reduced with $\text{NaBH}_4$ and analyzed using gas chromatography to measure hydrogen storage. Infrared spectroscopy and ultraviolet visible spectroscopy will also be used to gather data concerning each compound.
Influence of NaBH₄ Reduction on the Hydrogen Storage Properties of Aniline/Pd Composite Materials
Whitney Shofner and Dr. David W. Hatchett
Department of Chemistry
University of Nevada, Las Vegas

Abstract
The chemical synthesis and characterization of composites containing aniline and N-phenyl-N-phenylenediamine (NPPD) is examined [1]. The synthesis is achieved using palladium anions without additional oxidant. The palladium anions utilized in the synthesis were PdCl₂⁻ and Pd₄Cl₄²⁻. The influence of acid on the materials produced in the synthesis were examined. A total of eight different compounds were produced and evaluated for their hydrogen storage properties. In addition, each compound was further reduced with NaBH₄ and analyzed again using gas chromatography to measure the hydrogen storage properties after treatment. The goal is to produce materials that can reversibly sorb hydrogen for fuel applications.

Introduction
The use of conductive polymers in energy applications has increased recently due to the unique properties of the materials. The polymers behave like metals while retaining the properties of polymer materials improving processing and use in novel applications. For example, conductive polymers have been used in the development of hand prosthetics [2], earthworm-like robots [3], and in anti-fouling antifoul films. Polyaniline is one of the most environmentally stable polymers and is easily processed through chemical or electrochemical methods. It has been used for energy applications including fuel cells and batteries. More recent studies [1] have examined the hydrogen sorption properties of the pristine polymer with mixed results. In one study the sorption of hydrogen was estimated at 6% by weight. In contrast, a second study found the polymer sorbed no hydrogen. The discrepancies associated with hydrogen storage of polyaniline are not well understood and may well be influenced by the method of preparation, any secondary treatment of the material, or experimental conditions such as pressure and temperature used during hydrogen storage [4] [5]. Palladium has well-known hydrogen storage properties through the formation of metal-hydride bonds. Therefore, the incorporation of Pd into polyaniline should enhance the storage properties of the materials produced. The goal of this study was to produce composite materials with hydrogen storage properties using aniline or N-phenyl-N-phenylenediamine reacted with either PdCl₂⁻ or Pd₄Cl₄²⁻ in acidic and neutral solutions. In addition, the hydrogen sorption properties were measured using an in-house gas chromatograph equipped with a hydrogen sensor. In the event that Pd was not fully reduced in the synthesis, the composite materials were further reacted with reducing agents H₂ or NaBH₄. The treated samples were then compared to the untreated composite materials to determine the influence of Pd reduction on the hydrogen storage properties.

Conclusion
The data shows that composites reacted with sodium borohydride store more hydrogen after treatment. No hydrogen sorption is observed for the unreduced Pd (II) samples. However, sorption of hydrogen is observed for the samples after they were treated with sodium borohydride. The differences in sorbed vs. absorbed hydrogen peaks are represented as ratios for samples that were treated as well as those that were not treated. The Pd (II) aniline complexes with acid and without acid proved to be the most effective at hydrogen storage. The treated samples consist of Pd(II) aniline complexes showing little hydrogen sorption. The formation of Pd metal after reduction results in a significant enhancement of the hydrogen sorption properties.

Acknowledgements
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References:
Kathleen Bradley  
Mentor - Eduardo Robleto

The goal of our research is to determine whether the level of transcription of a gene is correlated with the level of mutation in that gene. One factor involved in the mutability of a transcribed gene is the ability of the single stranded DNA to form secondary stem loop structures (SLS), in the wake of the transcription bubble, that contain unpaired mutable bases. We are interested in correlating the levels of mutation with transcription in the thiF gene, which is predicted by bioinformatic analysis to be highly mutable. To achieve this goal, Kathleen will first construct a non-polar thiF genetic knockout using a chloramphenicol cassette. Then, she will test the phenotype of the ThiF^- strain. She will also build an IPTG-inducible construct containing thiF with a stop codon in the loop of a putative SLS. This will be introduced into ThiF^- Bacillus subtilis and assayed for the accumulation of Thy^+ mutations under starvation conditions, in the presence and absence of IPTG.
Construction of a thIF Genetic Disruption in Bacillus subtilis

Kathleen Bradley of the University of Maine
Christine Pybus, Ronald Yasbin, and Eduardo Robledo
School of Life Sciences, University of Las Vegas, NV

Abstract

The goal of our research is to determine whether the level of transcription of a gene is correlated with the level of mutation in that gene. We are interested in observing whether increasing expression of the thIF gene, which is predicted by bioinformatic analysis to be highly mutable, will result in increased reversion of a point mutation in this gene. To achieve this goal a non-polar thIF genetic knockout using a chloramphenicol cassette was constructed and introduced into Bacillus subtilis strain YB955. The phenotype of the ThIF strain will be tested. An inductive construct containing the mutated thIF gene will then be introduced into the YB955 thIF::cm strain. This will allow us to assess the correlation between levels of thIF mutation with levels of transcription.

Introduction

1. Stationary phase mutagenesis is the process by which non-dividing cells in a stressful environment may acquire adaptive mutations resulting in genetic diversity.

2. Previous experiments in E. coli and yeast have demonstrated that levels of transcription are correlated to levels of mutation of genes in growing cells (1,2).

3. Single-stranded DNA can form secondary loop structures in the transcription bubble (Fig. 1). The non-paired bases in these structures are predicted to be prone to higher mutability rates due to exposure to mutagenic substances (such as oxidative damage, lack of dinucleotide triphosphates, and conditions of low or no repair) in the cells' cytoplasm (3).

4. The thIF gene in Bacillus subtilis has been shown via bioinformatic analysis to be highly mutable.

5. We hypothesize that, in resting cells under non-lethal stress, increased transcription of genes induced under selective pressure results in a higher level of adaptive mutation of those genes.

Objective: Is there a correlation between the level of transcription of thIF and the level of adaptive mutation of that gene?

Goal One: Construct a thIF genetic disruption in B. subtilis strain YB955.

Goal Two: Integrate an expression vector containing a point-mutated thIF gene subcloned downstream of an IPTG-inducible promoter (Phytoparvus) into the amyE locus.

Goal Three: Assay the accumulation of mutations in Phs-thIF during starvation conditions in stationary cells in the presence and absence of inducer.

References


Methods

1. Amplify the thIF gene from the genome of B. subtilis strain YB955 using PCR.

2. Subclone thIF into the pGEMT vector. Cut pGEMT-thIF with restriction enzymes Clal and AfeI to remove a portion of thIF.

3. Amplify the chloramphenicol acetyltransferase cassette from the pMK4 vector by PCR.

4. Cut the cassette with Clal and AfeI and ligate into cut pGEMT-thIF. This results in a genetic disruption of thIF.

5. Transform this construct into B. subtilis strain YB955 to disrupt chromosomal thIF. Assay this strain for the presence of thIF::cam by PCR and by testing the strain for thiamin (vitamin B1) auxotrophy.

Future Directions

- A stop codon will be placed in a region of predicted high mutability in a putative stem loop structure of the thIF gene.
- This mutated gene will be subcloned downstream of an IPTG-inducible promoter.
- This construct will be introduced into strain YB955 and assayed for accumulation of ThIF mutations under starvation conditions, in the presence and absence of IPTG.

Acknowledgements

Funding was provided by the National Science Program (REU 0649267) and the NIH GM072554 Bob Marley for soothing my soul.

Figure 2. Experimental design.
The goal of our research is to determine whether the level of transcription of a gene is correlated with the level of mutation in that gene. One factor involved in the mutability of a transcribed gene is the ability of the single stranded DNA to form secondary stem loop structures (SLS), in the wake of the transcription bubble, that contain unpaired mutable bases. We are interested in correlating the levels of mutation with transcription in the \textit{argF} gene, which is predicted by bioinformatic analysis to be highly mutable. To achieve this goal, Allison will first construct a non-polar \textit{argF} genetic knockout using a kanamycin cassette. Then, she will test the phenotype of the ArgF\textsuperscript{-} strain. If a biochemical suppressor is present, she will disrupt the next possible genetic candidate. She will also build an IPTG-inducible construct containing \textit{argF} with a stop codon in the loop of a putative SLS. This will be introduced into ArgF\textsuperscript{-} \textit{Bacillus subtilis} and assayed for the accumulation of mutations under starvation conditions, in the presence and absence of IPTG.
Constructing an ArgF- strain of *Bacillus subtilis*

Allison Faucher¹*, Christine Pybus², Ronald E. Yasbin² and Eduardo A. Robleto²

¹Department of Botany and Microbiology, Ohio Wesleyan University
²School of Life Sciences, University of Nevada, Las Vegas

Abstract

The goal of our research is to determine whether and increase in the level of transcription of a gene results in an increased rate of mutation in that gene. The assay of the single stranded DNA to form secondary stems-loop structures (SLS) in the wake of transcription is one factor mediating mutations. A stable SLS has been predicted by bioinformatic analysis to be highly mutable. I am interested in testing whether there is a correlation between levels of transcription and accumulation of mutations in the argF gene. To achieve this goal, I constructed a non-polar argF genetic knockout using a kanamycin cassette. I will assay the phenotype of the ArgF- strain by plating on selective media to determine appropriate growth conditions for all future work. Also, an IPTG-inducible argF construct will be mutated by site directed mutagenesis to contain a stop codon produced by a single base mutation in the argF gene. These constructs once introduced into B. subtilis will be assayed for the accumulation of mutations under conditions of arginine deprivation and in the presence and absence of IPTG.

Methods

- The argF gene was amplified from *Bacillus subtilis* using primers designed with *Sac* I and *Eco* RI sites on the ends. (Figure 2)
- A kanamycin cassette was amplified from PDG780 with primers designed to add restriction sites for *Nco* I and *Nde* I to the ends of the cassette. This cassette was later used to knock out the argF gene (Figure 3)
- Once the plasmid had been dephosphorylated, argF was then inserted into pBluescript KS + II (pBSK). Successful ligation of the argF gene into the pBSK vector was identified by white colonies of *E. coli* into which the plasmid was transformed. (Figure 4)
- pBSK with the argF insert was cut with the restriction enzymes *Nco* I and *Nde* I, providing a ligation site for the kanamycin cassette, which disrupts the argF gene. (Figure 5 and 6)
- The plasmid was cut with *Sac* I to linearize the vector, and was transformed into B. subtilis YB955. The phenotype of the argF-kan mutant will be determined.

Discussion

- The first goal of this experiment was to produce an argF- allele in *Bacillus subtilis*. The argF- strain of *B. subtilis* is available for continuation of this project.
- From this experience I have learned numerous methods of restriction digesting as well as ligating and understand that different methods are successful under different circumstances.

Acknowledgement

I would like to thank Dr. Ronald E. Yasbin and Dr. Eduardo A. Robleto for this opportunity and guidance during my time at UNLV. Thank you also to Christy Pybus for her direction throughout my project as well as Katie Bradley, Holy Martin and Allesco Lunell for their suggestions and support in the laboratory. Dr. Helen Wing provided pBluescript KS + II for this project and Eun-Hae Kim suggested variations on methods of restriction digest. Funding for this research was provided by the National Science Program (REU 0649267) and NIH grants (P20RR016464) and (GM072954).
Bacillus anthracis and Bacillus cereus are both described as soil bacteria, but are almost exclusively found as spores within the soil. Soil is generally not a nutrient-rich environment and may lack the amino acids and nucleosides necessary for spore germination and vegetative reproduction. We aim to determine if soil alone can cause germination in these two species in order to produce vegetative cells that can reproduce. In addition, nematodes, decaying meat, maggots, and plant roots will be tested for their ability to cause germination in these species.
INTRODUCTION

Historically, Bacillus anthracis and Bacillus cereus have been described as saprophytic soil bacteria. However, recent studies have revealed that these bacteria can also cause disease in soil environments. B. anthracis, a pathogen specific to mammals, is typically believed to be dormant as a spore under adverse conditions. In contrast, B. cereus is a pathogen that can survive in the soil and has a close association with the plant rhizosphere, potentially functioning as an important source of infection for the host. While B. anthracis becomes active as the dominant agent of infection after a prolonged period of dormancy, B. cereus is known to produce a variety of toxins that are harmful to soil and can cause disease in soil environments.

SOIL RESULTS

Microorganisms were collected from each soil type and stored at 4°C for 7 days. Post-collection, the soil samples were divided into two groups: one was used for germination studies, and the other was used for germination inhibition studies. The soil samples were then subjected to various treatments to assess their effects on microbial growth and survival.

NEMATODE RESULTS CONTINUED

Figure 1: A. anthracis and B. cereus show differential germination in soil samples. The colony-forming units (CFU) of A. anthracis were significantly higher in the control group compared to the B. cereus group. The germination inhibition assay revealed that the addition of certain soil amendments can significantly reduce the germination rate of A. anthracis.

METHODS

Germination experiments were conducted using two soil types: soil A and soil B. Soil A was amended with Bacillus cereus spores, while soil B was amended with Bacillus anthracis spores. The soil samples were then incubated under controlled conditions to observe the germination rate of the microorganisms.

DISCUSSION

A. anthracis and B. cereus do not germinate in the soil received from TSB.

References

Magnetotactic bacteria is the categorical name for a group of prokaryotes that biomineralize magnetosomes which are intracellular, membrane-bounded magnetic iron mineral crystals. The focus of this study is on two magnetite-producing, magnetotactic sulfate-reducing bacteria (SRB), *Desulfovibrio magneticus* strain RS-1 and strain FH-1 which also belongs in the genus *Desulfovibrio* in the $\delta$-Proteobacteria. SRB utilize sulfate as a terminal electron acceptor under anaerobic conditions reducing sulfate to sulfide. A large number of organic compounds as well as some inorganic compounds have been shown to provide electrons for sulfate reduction. Traditionally, because no SRB have been shown to convincingly grow with O$_2$ as a terminal electron acceptor, they have been classified as obligate anaerobes.

In characterizing several magnetotactic SRB, we found that cells of *D. magneticus* and strain FH-1 utilized O$_2$ as an electron acceptor for growth. To prove this we grew cells of both strains in several different semi-solid growth media under air or N$_2$ gas. Cells of both strains grew as a microaerophilic band of cells at the oxic-anoxic interface (OAI) in media under air lacking sulfate (medium contained cysteine or cysteine with either Casamino Acids or Yeast Extract as a sulfur source). Sulfide (as FeS; high [Fe] was used as a trap for sulfide) was not produced in these tubes. Cells did not grow under anaerobic conditions (under N$_2$) in this medium unless sulfate was present. When sulfate was present in the growth medium, under air, initial growth of the strains was also as a microaerophilic band of cells at the OAI. However as time went on, the band of *D. magneticus* split into two. The band of FH-1 cells did not split into two bands and moved up the tube almost to the meniscus. The medium also turned dark indicating sulfide production. The results show that these magnetotactic SRB strains are capable of aerobic growth with O$_2$ as a terminal electron acceptor.
Aerobic Respiration by Two Sulfate Reducing Magnetotactic Bacteria, Strains RS-1 and FH-1

Paul Howse, Sabrina Schübbe, and Dennis A. Bazylinski
University of Nevada-Las Vegas, Las Vegas, Nevada 89154

Abstract
Magnetotactic bacteria are a group of prokaryotes that biomineralize magnetosomes, which are intracellular, membrane-bound magnetic iron mineral crystals. The focus of this study is on two magnetotactic bacteria isolated from a natural environment: Desulfobulbus magnesius strain RS-1 and strain FH-1. The authors investigate the mechanisms that allow these bacteria to use sulfite as an electron acceptor.

Results
The authors observed that cells of both strains were capable of using sulfite as an electron acceptor. They also found that the bacteria could survive in conditions where oxygen is limited, such as in anaerobic environments.

Conclusion
The study suggests that magnetotactic bacteria have the ability to use sulfite as an electron acceptor, which could have implications for understanding bacterial metabolism in environments where oxygen is limited.
Alex Michaud  
Mentor - Duane Moser

This research is focused on developing a better understanding of the physiological and phylogenetic diversity as well as environmental abundance of bacteria of the genus: Shewanella in selected desert ecosystems. Prior research from this laboratory has revealed that these bacteria are very abundant in sulfur- and organic-rich aquatics habitats. We have selected a number of habitats for detailed investigation (cultivation, molecular ecology and relevant environmental chemistry) including the Tropicana Wash, spring in Death Valley, the lower Virgin River and possibly Big Soda Lame, Nevada.
Microbial Ecology of Keane Wonder Spring, Death Valley National Park
Alexander B. Michaud1, Duane P. Moser2
1Coe College, Cedar Rapids, IA, 52402
2Desert Research Institute, Las Vegas, NV, 89119

Introduction
Heane Wonder Spring is located in Death Valley National Park, California. A desert spring such as this one is unique due to the large number of microbial communities supplied by a deeply sourced aquifer; also, more significant for this study, these springs are often "island" ecosystems which are surrounded by xerophyte-dominated desert (7). The spring water is thought to originate from the Amargosa Desert due to its similarity in composition to water sampled from three deep wells in the northwestern part of the Amargosa Desert (8). Metabolic activity of the spring also suggests that the water does not originate at the Funeral Mountains to the west, but rather comes from the Amargosa Desert (8). The high water temperatures indicates that flow through the Funeral Mountains is quite slow due to the depth of the water, which would need to travel about 1 km, in order to reach the temperature measured (9). The geology of this particular mountain range also supports this idea (8).

Members of the genus Shewanella are characterized by the ability to utilize a wide variety of terminal electron acceptors, whereas in Shewanella, the ability to oxidize inorganic substrates such as nitrate, nitrite, nitrous oxide, oxygen, sulfide, manganese, ferrous iron, and elemental sulfur (2-4). These species that belong to this genus are of interest to the U.S. Department of Energy for their ability to immobilize uranium and other radioactive elements (2).

The physiology of this organism and the composition of Heane Wonder Spring make it a unique location to find Shewanella. It has been found that Shewanella is present in the spring water, but a thorough investigation has not been conducted (10).

Objectives
- Identify and quantify the number and types of microorganisms in the water and sediment at three sites along the spring.
- Find new species of Shewanella that have previously been shown to occur in this environment.

Methods
Field Sampling
Samples were collected on July 7, 2008. Biological water samples and sediment samples were collected using a peristaltic pump (Cole-Parmer Instrument Company). Sediment samples were collected in 500 ml conical tubes by hand from the area around the pump that. Surface samples were taken in a sterile sealed syringe with force to the bottom. Gas samples were collected by passing water through a gas sampling bulb until all then, followed by liquid and gaseous samples in the sampling bulb. The bulb was shaken for one minute, and then the bulb was placed in a 50 ml autoclaved and evacuated serological vial. Physical measurements were collected with a TDS probe equipped with a 1000 µL TDS solution and a flow-through unit (YSI Corporation). O2 was collected by passing water through a blood oxygenator through an 18 mm tubing. This allowed us to pre-filter the water through a 100 µm filter and then a 0.2 µm filter.

Sample Preparation
Culture analysis of Heane Wonder Spring was a modified approach. We used RDA plates for plate counts of facultative heterotrophic organisms. Cultural media in the form of 14 µL of 14 µL of 14 µL of plates to 20 µL of 20 µL of plates which contains for sulfur reducing organisms. Kligar iron agar was used to select for organisms that reduce sulfates. All three plates were prepared using the spade-dip method. Liquid media included broth, which detects sulfate-reducing bacteria; Baclite, which detects iron-reducing bacteria; and RP4, which detects fermentative organisms. Liquid cultures were inoculated using a stab-wire technique. Flow cytometry was used to enumerate cells that autofluoresce, are viable, and total live or dead cells (Advanced Analytical).
Susan O’Neil  
Mentor - Kumud Acharaya

This project focuses on an assessment of coliform bacteria from point and non point sources in Las Vegas Wash. Correlations between land use related runoffs, nutrients and organic matter loading and total coliform will be studied. Data will be collected for both daily low and high flow events. Sampling locations will cover all major land use types such as golf course, hotels, hospitals, residential areas, etc., at both the main Wash and its tributaries.
Assessment of coliform bacteria from point and nonpoint sources in the Las Vegas Wash

O’Neill, Susan & Acharya, Kumud

Abstract

E. coli and total coliforms were measured in the Las Vegas Wash and its major tributary, Flamingo Tropicana Wash before and after rainfall to study the impact of urban land use and water reclamation activities on Lake Mead water quality. Results suggest that the Las Vegas Wash does have harmful fecal contaminants and does appear to be coming from wildlife and non-point source rather than the treatment plants.

Methods

- Grab samples taken at predetermined locations along Las Vegas Wash and its major tributary, Tropicana Flamingo Wash
- Locations were determined based on representative land use types, e.g., golf courses, waste treatment plants, hospitals, etc.
- YSI data collected on each site including for pH, temperature, specific conductivity, oxidation-reduction potential, and dissolved oxygen
- Samples diluted and vacuum filtered through a 0.45 mm nanofilter and grown on Colilert MF agar to distinguish coliforms. Plates incubated at 37.0°C
- Diluted samples were plated on LB agar for overall bacterial count
- Total cell counts verified by Flow Cytometer for a Las Vegas Wash sample
- Water samples were also analyzed for total Phosphorus, total nitrogen and total particulate organic loading (POC)

Results

- Main LV Wash
  - Site 2 has highest E. coli concentrations followed by Site 1, the rest decrease downstream
  - Site 1-2 have the highest individual coliforms, the remaining sites decrease downstream
  - Nitrate increases downstream from Site 1 to Site 9
  - Phosphorus is not significantly different from Site 1 to Site 5, but increases in Sites 6-9
  - Particulate Organic Carbon (POC) is highest in Site 1 and statistically similar for the remaining sites
  - In the main LV Wash, temperature increased by 4°C after Site 3
- Flamingo-Tropicana Tributary
  - E. coli and coliform counts are significantly lower than Sites 1-2 from the main LV Wash, but higher than Sites 3-9
  - E. coli and coliform counts increase post rainfall
  - Nitrate and Phosphorus levels do not change after rainfall
  - Small increase observed in POC for Sites 1-2

Discussion

- Main LV Wash
  - Increase in E. coli for Site 2 due to the presence of septic systems and increased wandt from the gold course upstreams
  - Decrease in E. coli and coliforms downstream due to dilution of water with outflows from wastewater treatment plants
  - Nitrate increased after Site 3 due to outflows from wastewater treatment plants
- Flamingo-Tropicana Tributary
  - E. coli and coliforms increase after rainfall from the watershed
  - Spikes in organic loading from the watershed: households, raves, farms, etc.

Conclusion

- Wastewater treatment plants did not contribute to E. coli and total coliform populations
- Bacteria increases due to wild animals and golf courses
- No significant correlations between nutrient concentrations and bacteria
- Las Vegas Wash and Tropicana Flamingo tributary showed an increase in bacteria with organic loading increased, especially after rainfall

References

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Acknowledgments:
Funding was provided by the National Science Program (REU 0649267). Thanks to Cindy Stalin, Abeer Adaileh, and Aman Lawn of Dr. Acharya’s lab.
Rachel Skinner  
Mentor - Brian Hedlund

Photosynthesis does not occur above 73°C, so organisms living above this temperature must obtain useable carbon by some other mechanism. It is generally assumed that carbon is fixed by thermophiles through the process of chemolithoautotrophy; however, primary production has never been demonstrated to occur in hot springs >73°C. We have shown that two organisms, Thermocrinis and Pyrobaculum, make up more than 90% of the cells in an 80°C Great Basin hot spring, Great Boiling Spring. We hypothesize that these organisms fix carbon in the hot spring via the reverse tricarboxylic acid (rTCA) cycle. To test this hypothesis we will: i) confirm that Thermocrinis and Pyrobaculum dominate in water from the spring; ii) determine whether key genes for the rTCA cycle, citryl co-A lyase (ccl), 2-oxoglutarate:ferredoxin oxidoreductase (korA), pyruvate:ferredoxin oxidoreductase (porA), are present and expressed in the spring; and iii) measure rates of carbon fixation in the spring. Linkage of the genetic data with carbon fixation rate data may help to provide an image of carbon fixation and cycling in Great Basin hot springs.
Genomic foundations of carbon fixation in bacteria living in hot springs

Rachel K. Skinner, Brian P. Hedlund, and Jeremy A. Dodsworth

Introduction

Results

DNA Amplification

- Gradient PCR of ec.
- G pneumoniae
- Gene products containing
- appropriate 500 base pairs
- highlighted in pieces of DNA
- obtained by PCR
- Lane 2 contains the
- positive control
- Lane 5 contains appropriate gene
- products amplified from water

Cetyl-CoA Lyase

Phylogeny

Figure 7. Phylogenetic tree of cetyl-CoA lyase genes from various sources. The tree was constructed using the Neighbor-Joining method.

Methods and Materials

References

Acknowledgments

Discussion and Further Directions

UNIV MIMUMOLOGY

In this study, we isolated and characterized several bacterial species from hot spring water samples. Our findings suggest that these bacteria have evolved unique mechanisms to survive and thrive in hot springs, which provide an ideal environment for carbon fixation.

We identified a novel cetyl-CoA lyase gene in one of the hot spring isolates. This enzyme is known to be involved in the degradation of cetyl-CoA, a key intermediate in fatty acid metabolism. Our results indicate that this lyase may play a crucial role in the carbon fixation process in these bacteria.

Our phylogenetic analysis of the cetyl-CoA lyase gene sequences revealed a close relationship with enzymes found in Thermoplasma acidophilum, a species known for its ability to fix carbon dioxide in hot springs.

In conclusion, our study highlights the unique adaptations of bacteria in hot springs and provides new insights into the carbon fixation processes that allow these organisms to thrive in such extreme environments.

References

Acknowledgments

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Determine whether Ompt expressed from new construct cleaves ICSA when expressed in shigella MBG34 (7csP-). I will be testing whether Ompt can protect shigella (and salmonella) from LL-37.

Omptins are outer membrane proteases found in gram negative bacteria that cause diseases in humans, such as pathogenic *Escherichia coli*, *Shigella flexneri*, *Salmonella typhimurium*, and *Yersinia pestis*. Bacterial species that express omptins cause diseases such as highly fatal plague and severe diarrhea and dysentery. The genes that encode these proteases are *ompT*, *icsP*, *pgtE*, and *pla*, respectively. These proteases are highly related in structure and share approximately 50% sequence identity. In *S. flexneri*, IcsP has been shown to cleave a key virulence determinant, IcsA (Egile *et al.*, 1997). IcsA recruits host actin and allows for intracellular movement within host cells (Steinhauer *et al.*, 1999). In *S. typhimurium*, PgtE has been shown to cleave a human α-helical cationic antimicrobial peptide (CAMP), LL-37 (Guina *et al.*, 2000). LL-37 is a major component of the innate immune defense system (Zasloff, 1992). It functions by permeabilizing the bacterial membrane, which ultimately results in bacterial cell lysis. PgtE cleaves LL-37, thereby protecting Salmonella against the bactericidal effects of CAMPs. In *E. coli*, OmpT has been shown to cleave protamine, an antimicrobial peptide that acts on the bacterial membrane and causes problems in cellular energy transduction and nutrient accumulation (Aspedon and Groisman, 1996) (Figure 2). Like PgtE, OmpT circumvents the immune defense by cleaving protamine into smaller fragments.
Characterization of the OmpT Protease, OmpT, in Escherichia coli
Amanda Yates, Eun-Hae Kim, Helen Wing
School of Life Sciences, University of Nevada, Las Vegas

INTRODUCTION
OmpT is an outer membrane protease found in gram-negative bacteria that causes diseases in humans, such as pathogenic Escherichia coli, Shigella flexneri, Salmonella typhimurium, and Pseudomonas aeruginosa. Bacterial species that express ompT cause diseases such as highly fatal plagues and severe diarrhea and dysentery. The genes that encode these proteases are ompT, icp, pgrl, and plg, respectively. These proteases are highly related in structure and share approximately 50% sequence identity (Figure 1).

- In S. flexneri, Icp has been shown to cleave a key virulence determinant, IcsA (Eagles et al., 1997). IcsA recruits host actin and allows for intracellular movement within host cells (Steinhauer et al., 1999).
- In S. typhimurium, Pgrl has been shown to cleave a human alpha-helical cationic antimicrobial peptide (CAMP, LL-37) (Quina et al., 2000). LL-37 is a major component of the innate immune defense system (Kirshen, 1992). It functions by permeabilizing the bacterial membrane, which is submembranous in intact bacterial cells. Pgrl leaves LL-37, thereby protecting Salmonella against the bactericidal effects of CAMPs.
- In E. coli, OmpT has been shown to cleave proteases, an antimicrobial peptide that acts on the bacterial membrane and causes problems in cellular energy transduction and nutrient accumulation (Apostolov and Gershman, 1996) (Figure 2). Like Pgrl, OmpT circumvents the immune defense by cleaving proteases into smaller fragments.

OBJECTIVES
Due to the high sequence identity at the amino acid level of the ompT proteases, we hypothesized that these proteins may share similar proteolytic activity and function.

- To characterize the proteolytic activity of OmpT,
  - Experiments were conducted to determine whether inducible OmpT recognized and cleaved one substrate, IcsA, in a similar manner to natively expressed IcsP.

- To determine whether the LPS environment surrounding the ompT influences its activity or site specificity.
  - OmpT switching experiments were conducted by introducing inducible OmpT into a Shigella background.

- To determine whether OmpT could promote resistance to the cationic antimicrobial peptide, LL-37 in E. coli.
  - Minimum inhibitory concentration (MIC) assays were conducted.

CONCLUSIONS
- Although the catalytic amino acids are completely conserved among IcsP and OmpT, it is clear that these proteases cleave IcsA differently, as judged by western blot analysis.
- The LPS background may affect the cleavage specificity of OmpT because the IcsA cleavage pattern in the ompT switching experiment.
- OmpT has a distinct structural activity and function to other members of the OmpT family.
- OmpT does not promote resistance to LL-37 in E. coli.

WORKS CITED

ACKNOWLEDGEMENTS
I would like to thank Eun-Hae Kim and Dr. Helen Wing for all of their help, support, and guidance with this project. Funding was provided by the National Science Program (NSF 0445297).
The compound YbB$_2$ has been reported to order anti-ferromagnetically at temperatures around 5.6 K. This transition was verified from heat capacity data taken in different magnetic fields by our Quantum Design PPMS system. X-ray diffraction measurements have been performed using this sample under different pressures at the APS facility in Argonne National Laboratory. A sudden change in resistivity measurements of the sample indicated a possible structural phase transition around 3 GPa. The data indicates the YbB$_2$ compound is not responsible for the anomaly.
Heat Capacity and High Pressure Exploration of YbB₂

Brant Abeln¹ and Andrew Cornelius²
¹ Drake University, Des Moines, Iowa 50311, USA
² High Pressure Science and Engineering Center and Department of Physics, University of Nevada Las Vegas, Las Vegas, Nevada 89154, USA

Abstract

The compound YbB₂ has been reported to order antiferromagnetically at temperatures around 5.6 K. This transition was verified from heat capacity data taken in different magnetic fields by our Quantum Design PPMS system. X-ray diffraction measurements have been performed using this sample under different pressures at the AP5 facility in Argonne National Laboratory. A sudden change in resistivity measurements of the sample indicated a possible structural phase transition around 3 GPa. The data indicates the YbB₂ compound is not responsible for the anomaly.

Experimental Details

**Heat Capacity:** First, an addenda measurement was taken using the Physical Property Measurement System (PPMS) Helium-4 puck with a small amount of N gas from Apleon. Then, the sample of YbB₂ was placed onto the puck. N gas was used to improve thermal coupling between the sample and the temperature stage on the puck. The sample was subjected to four different magnetic fields during the heat capacity measurements. A temperature rise of 2% was used for the measurements in the range of 8 K to 2 K. Once the system stabilized around a particular magnetic field, the system sends a heat pulse to the sample. The temperature of the sample is left to cool while the system measures the temperature drop. The PPMS uses a two-tau model to calculate the heat capacity of the sample and the heat capacity of the gas. This is then used to determine the heat capacity of the sample.

**X-ray Spectroscopy:** A Merill-Bassett type Diamond Anvil Cell (DAC) was loaded with a small amount of YbB₂, with sample also placed into a capillary tube for reference. The sample was in a stainless steel gasket that had a pre-indent of 50 μm filled with a 11 ml methanol-saline pressure medium and a ruby chip, for pressure determination. These samples were taken to the Advanced Photon Source (APS) facility at Argonne National Laboratory to the Sector 16 - bending magnet beamline D where x-ray diffraction data was taken. The sample was exposed to a beam that was 15 μm X 35 μm with a wavelength of 0.387451 Å. A MAA image plate was used to detect the x-ray patterns. The ruby chip allowed us to measure the pressure inside the cell by measuring the shift of the R₁ spectral line. Increasing the pressure of the diamonds allowed us to measure the structural data of the sample at over twenty different pressures between 0.26 GPa and 4.64 GPa.

Results

**Heat Capacity:** Figure 3 shows the data obtained from the heat capacity measurements in magnetic fields of 9T, 5T, 1T, and 0T. The data shows evidence for a second order magnetic phase transition. The temperature where the transition occurs decreases with increasing applied field. This is indicative of anti-ferromagnetic ordering.

**X-ray Spectroscopy:** Figure 2 shows an example x-ray powder diffraction pattern. The program FSDO was used to obtain spectral data from the pattern (Fig. 4). Next, the zero pressure peak positions were compared to the peak position of the pressurized data. Using Jade, a background was subtracted and the pattern used to compute the compressed unit cell size. As the pressure increased, the peaks shifted to the right. As can be seen in Figure 4, many impurities were in our sample. Identified impurities were Yb, Yb₂, Yb₃, YbO, and YbO. The pressures and corresponding unit cell sizes were then fit into EOSfit. The data was then fit to the third-order Birch-Murnaghan isothermal equation of state.

Figure 5 shows pressure versus unit cell size with the corresponding fit from EOSfit. Figure 6 shows the units of equation 1 for the fit in figure 5.

Conclusions

The compound YbB₂ orders antiferromagnetically around 5.5 K, with the ordering temperature decreasing with field. There is no structure phase transition at 3 GPa for YbB₂. The jump in resistivity is most likely from the many impurities embedded in the sample when the compound was pressurized.

References


Support from the Research Experience for Undergraduates (REU) program of the National Science Foundation is gratefully acknowledged under grant DMR-0552089.

The authors acknowledge the assistance of Daniel Antonio, Jason Baker, Matthew Jacobson, Patricia Kallta from UNLV and Peter Liefman from HPCAT.
Nuclear resonant inelastic x-ray scattering (NRIXS) of synchrotron radiation uses the energy transferred during the inelastic nuclear absorption of photons to determine phonon density of states for solid Mössbauer isotopes. This type of experiment can be conducted at ambient and high pressures with the use of a diamond anvil cell (DAC) and a rhenium gasket. Here, we are concerned with the phonon DOS of $\alpha$-FePt 10% at pressures up to 30 GPa, as well as FeAl 4.3%, 6.4%, and 27.1% at ambient pressures. The iron samples used are doped in order to increase the pressure at which the alpha to epsilon phase transition for iron occurs. As the most abundant element within Earth’s core, the study of iron is fundamental in geophysics and in terms of thermodynamic modeling.

$^{57}$Fe is the most common Mössbauer isotope, and its lattice dynamics have been greatly studied. The phase transition of magnetic $\alpha$-Fe, body-centered cubic structure, to nonmagnetic $\epsilon$-Fe, hexagonal close-packed structure, (see figure 1) occurs around 13 GPa [1]. We recently conducted experiments at the APS on beamline 16-IDD to determine how doping Fe samples with Pt and Al affects the Fe $\alpha$-$\epsilon$ transition. As iron is the most abundant element within Earth’s core, understanding how doping changes its transition is especially important in geophysics and in terms of thermodynamic modeling.
Phonon Density of States of Iron Solid Solutions at Ambient and High Pressures using Nuclear Inelastic X-ray Scattering (NRIXS)

Samantha L. Combs¹, Elizabeth A. Tani², Malcolm Nicol²
¹REU Physics, Eckerd College, St. Petersburg, FL 33711
²HIPSEC, Department of Physics, University of Nevada Las Vegas, Las Vegas, NV 89154

Background

²Fe is the most common Mössbauer isotope, and its lattice dynamics have been greatly studied. The phase transition of magnetic ³Fe, body-centered cubic structure, to nonmagnetic ³Fe, hexagonal close-packed structure, (see figure 1) occurs around 1.5 GPa [4]. We recently conducted experiments at the APS on beamline 16-ID-D to determine how doping Fe, sample, with Pt and Al affects the Fe₃O₄ transition. Al is the most abundant element within Earth's core, understanding how doping changes its transition is especially important in geophysics and in terms of thermodynamic modeling.

Figure 1: BCC structure of α-Fe and HCP structure of γ-Fe [2]

NRIXS

Figure 3 [3]. Fe has a nuclear resonant energy of 31.413 keV [1]. When not at this energy, a photon must either be created or annihilated to emit a photon and excite the nucleus.

Experimental Procedures

The NRIXS experiments were performed at ambient and high pressures using a Paderborn type diamond anvil cell (DAC), figure 1. Fe₃Pt₄ was loaded into the cell with a rhodium gasket [4], figure 5, along with several rubbers to measure the pressures and a methanol ethanol 4:1 liquid pressure transmitting medium. Solid solutions of Fe₃Al, 4.8%, 6.4%, and 27.1% were placed between two pieces of tape for ambient pressure measurement.

• sample: 16-ID-D
• High Resolution Monochromator: 3 bounce nested (Diamond (111))
• Focusing: K-B mirror (35 μm)
• Data Collection: 2 APD’s
• Energy Range Scanned: 800 eV in steps of 0.25 eV
• Pressures Tested: 0, 11, 16, 21, 30 GPa

Figure 2 [3]. Fe has a nuclear resonant energy of 31.413 keV [1]. When not at this energy, a photon must either be created or annihilated to emit a photon and excite the nucleus.

Figure 4: DAC

Figure 5: rhodium gasket

Figure 6: EDM

Figure 8: blue laser (481 nm)

References


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Yumin Xiao, Paul Choe and Guixin Shen, along with the rest of the staff at the APS.
We are studying how the mineral fayalite deforms under stress while it is subject to high pressures and temperatures. Specifically, we are analyzing x-ray diffraction spectra obtained from experiments with the D-DIA apparatus at Brookhaven national labs. By fitting peaks to the diffraction spectra, we can calculate the spacing between lattice planes of fayalite and so we can observe how this spacing changes over time as the crystal structure deforms. We hope to show that this deformation can be modeled using an Elastic Plastic Self Consistent model. In such a model, the material is treated as a cluster of independently oriented grains. When stress is applied to the material, deformation takes place because the lattice planes can slip by each other. A variety of slip systems are used to model the different ways these planes can move. The model allows us to calculate the aggregate properties of the material from the microscopic properties of the individual grains.

The goal of our project was to fit an Elastic Plastic Self Consistent (EPSC) model to experimental data on the deformation of the mineral fayalite (Fe$_2$SiO$_4$) which is the iron end member of olivine. In the past, Professor Burnley has successfully fit an EPSC model to experimental data on the deformation of quartz. If an EPSC model could be fit to data on fayalite, it would further support the use of these models to study the plastic deformation of minerals. Modeling the deformation of fayalite is of particular interest because olivine is an important component of the Earth’s mantle and studying how it deforms at high temperatures and pressures can help us understand how the mantle moves over time.

The first step in our project was to analyze the experimental data set. We also explored some of the parameter space of the EPSC model before trying to fit the model to the data. Unfortunately, our attempts to fit the model to the experimental data have been unsuccessful, and this suggests that there may be another deformation mechanism involved in the deformation of fayalite.
Modeling the deformation of Fayalite
Greg Hoth and Mike Breznar,
advised by Dr. Pamela Bunley

Abstract

The goal of our project was to fit an Elastic Plastic Softening (EPS) model to experimental data on the deformation of the mineral fayalite (Py920), which is the iron end member of olivine. In the past, Professor Bunley has used an EPS model to understand the deformation of olivine. As such, an EPS model could be fitted to the data on fayalite. This paper explores the use of the EPS model to study the plastic deformation of minerals. Modeling the deformation of fayalite is of particular interest because olivine is an important component of the Earth's mantle and studying how it deforms at high temperatures and pressures can help us understand how the mantle moves over time.

The first step in our project was to analyze the experimental data. We also explored some of the parameters of the EPS model before trying to fit the model to the data. Unfortunately, our attempts to fit the data to the experimental data has not been successful, and this suggests that there may be another deformation mechanism involved in the deformation of fayalite.

Analysis of the Experimental Data Set

The data set that we analyzed was produced using the D-EIA apparatus at Brookhaven National Laboratory. These experiments were done in sync with a deformation field. After deformation, the samples were removed from the oven. The deformed samples were examined by electron microscopy to understand the mechanisms of plastic deformation. The deformed samples were examined using electron microscopy to understand the mechanisms of plastic deformation. The deformed samples were examined using electron microscopy to understand the mechanisms of plastic deformation. The deformed samples were examined using electron microscopy to understand the mechanisms of plastic deformation.

By analyzing the spectra recorded by these detectors using the peak fitting program HitekG, we were able to calculate how the slopes of the lattice planes change at the sample was deformed. By measuring the position of the sample at high temperatures, we were able to calculate how the slope of the sample changed during the deformation. Our results are summarized in figure 1.

Exploring the Parameter Space of the EPS Model

Experiments have identified eight slip systems that may be involved in the plastic deformation of fayalite (O'Neill et al. 1995). We used an EPS model for this work by Curley, Tomin, and Tomin (1996). In the model, the behavior of each slip system is governed by two parameters, the critical resolved shear stress (CRSS) and the apparent frictional stress (APSS). These parameters are adjusted for each slip system to be consistent with the experimental data. Once these parameters have been specified, the model simulates the deformation of a material by performing an iterative calculation. The model compares lattice strains for planes perpendicular to three orthogonal directions.

Our analysis focused on determining how each slip system affected the model's output. We explored how changes in the parameters CRSS and APSS changed the behavior of each slip system and compared the slip systems to each other. To describe the behavior of each slip system quantitatively, we looked at the percent change in slope of the lattice planes relative to the unstrained state. The percent change was calculated for six planes and compared to the unstrained state. The percent change was calculated for six planes and compared to the unstrained state. The percent change was calculated for six planes and compared to the unstrained state. We estimated the yield point by finding the intersection of these two lines. Figure 2 provides a visualization of the method used to calculate both the percent change in slope and the yield point.

Fitting the Model to the Experimental Data

Figure 1A shows our best attempt to fit the EPS model to our data set. Clearly, we have not been able to fit the model to our data set. We are beginning to study how the model behaves with multiple slip systems activated in the hopes of obtaining a better fit, but the difficulty we have had in fitting the model to the data suggests that there may be other deformation mechanisms involved in the deformation of fayalite that are not incorporated into the model we have studied.

References


Figures

Fig 1. Plot of lattice strain vs. sample strain at the first few planes that were observed. For each plane, the lattice strain increases linearly at small sample strains. This linear behavior indicates that the lattice is deforming elastically. At the sample strains increase, the relationship breaks down because the lattice yields and begins deforming plastically. In this plot and all the plots on this page, negative strain corresponds to compression.

Fig 2. An example of the data produced by the EPS model showing the lattice strains for the six slip systems. We fit the model to the data to analyze the behavior of each slip system. From the results, we can calculate the percent change in slope and the yield point for each slip system. We can also estimate the percent change in slope and the yield point for each slip system. We can also estimate the percent change in slope and the yield point for each slip system. We can also estimate the percent change in slope and the yield point for each slip system.

Fig 3. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress decreases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 4. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 5. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 6. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 7. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 8. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 9. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.

Fig 10. These plots show how increasing CRSS affects the percent change in slope and the yield point for planes perpendicular to the compression direction when the [000]<011> slip system is activated with HAPSS. As the frictional stress increases, the percent change in slope and the yield point decreases almost linearly. These trends are generally representative of all the slip systems.
Martin Galley  
Mentor - Michael Pravica

We performed Raman spectroscopic studies of 1,3,5,7-cyclooctatetraene at elevated pressures up to 10 GPa with the aim of examining possible planarization of the molecule and further studying two prior-discovered phases of the solid with pressure. The Raman excitation source was a Krypton-ion laser operating at 674.1 nm (give wavelength).

1,3,5,7 Cyclooctatetraene has an octagonal formation however it is not aromatic or anti-aromatic (not a subject to the 4n+2 Hückel's rule) [1]. As a result, its adopts a somewhat reactive tub shape. Upon the addition or removal of one to two electrons under ambient conditions, the molecule planarizes and becomes aromatic [3-5]. Our goal was to determine if we can aromatize the COT molecule mechanically or via electron transfer at elevated pressure.
Raman Studies of 1,3,5,7 Cyclooctatetraene at High Pressure

Martin Galley, Ed Homann, Sergey Tkachev, Michael Pravica
High Pressure Science and Engineering Center, Department of Physics and Astronomy, UNLV, Las Vegas, Nevada USA, 09154-4002

Introduction

1,3,5,7 Cyclooctatetraene has an octagonal formation however it is not aromatic or anti-aromatic but is subject to the 4n+2 Hückel’s rule [1]. As a result, its adopts a somewhat reactive tub shape. Upon the addition or removal of one to two electrons under ambient conditions, the molecule planarizes and becomes aromatic [2-5]. Our goal was to determine if we can aromatize the COT molecule mechanically or via electron transfer at elevated pressure.

Abstract

We performed Raman spectroscopic studies of 1,3,5,7 cyclooctatetraene (COT) at elevated pressures, up to 11 GPa using a diamond anvil cell (DAC), with the aim of determining two previously discovered phases of the solid with pressure [6]. After reaching 11 GPa the pressure was decreased to ascertain the reversibility of high pressure effects on COT. The Raman excitation source was a Krypton ion laser operating at 647.1 nm and the spectrometer used was a Triax 550B with attached a CCD detector.

Procedure

We used a Merrill-Bassett diamond anvil cell with 500 micron occlusens inserted a lithium gasket to about 50 microns thick. We drilled a 2.10 micron hole through the gasket and then placed a 8-10 micron ruby chip as well as the liquid cyclooctatetraene into the gasket hole. Pressure was then applied until the first sign of the COT sample traversing a phase change towards a solid is reached. We then acquired the Raman spectra using a Krypton ion laser with wavelength of 647.17 nm, and stored data over four different Raman wavenumber ranges (200-800, 800-1500; 1350-2000; 2750-3300 cm⁻¹). This was performed for each pressure point until the sample began to show signs of reaction (possibly polymerization) around 11 GPa. Pressure was then decreased and Raman spectra were taken over the same wavenumber regions. The acquired data are displayed in the figures on the right.

Conclusion

We performed Raman spectroscopy on a COT sample as a function of pressure at ambient temperature. Raman spectra from 20 different pressure points were recorded from 0.05 GPa to 11.07 GPa. This study revealed two novel phase transitions which were not detected in this group using a 514.5-nm laser (Argon ion) but has better determined the pressure at which the phase transitions occur [8]. The sample was also studied in decompression to ascertain the reversibility of the sample with pressure. In leaving a sample under 11 GPa of pressure for over one week, partial reaction and/or polymerization had occurred. When COT had entered this amorphous state it becomes more sensitive to the effects of the laser, as black marks developed where the laser rested. These tests were inconclusive in determining if COT had aromatized while at high pressure. Further study will be needed.

Acknowledgments

We thank Michael Hruby for aid in the measurements and experiment process. Support from the Research Experience for Undergraduates (REU) program of the National Science Foundation is gratefully acknowledged under grant DMS-2685209.

We gratefully acknowledge support from the U.S. Department of Energy Cooperative Agreement No. DE-FG02-01ER15249 with the University of Nevada Las Vegas. We also wish to thank the NASA/Nevada Space Grant program for partially supporting this work.

References

Study of Thermoelectric Materials at High Pressure

It is of extreme importance to develop new potential energy sources to reduce dependence on fossil fuels. As a result of this, the study of thermoelectric materials, capable of changing heat into electrical energy, has become a field of great interest regarding fundamental properties. To help better understand these materials, facilities for the measurement of relevant properties at high pressure have been developed, but lack the ability to characterize the materials at high temperature and pressure. Therefore, this project has the goal of developing a heater arrangement to be used in conjunction with the high pressure capabilities already developed to fully characterize these materials.
Structural studies of CrSi₂ at high pressures and temperatures
Weldu Gabrimicel, Ravhi S. Kumar, Andrew Cornelius
Department of Physics and HIPSEC, University of Nevada Las Vegas, Las Vegas, Nevada 89154, USA

INTRODUCTION

Thermal and mechanical properties of CrSi₂ are of interest due to its potential use in high-temperature applications. The crystal structure of CrSi₂ is a hexagonal close-packed (HCP) structure, with a space group of R3m. It exhibits a high melting point and a high cohesive energy, making it a promising material for high-temperature applications. The study of the structural properties of CrSi₂ under high pressures is crucial for understanding its behavior under extreme conditions.

EXPERIMENTAL DETAILS

High-pressure x-ray diffraction experiments were performed at the high-pressure x-ray diffraction beamline at the National Synchrotron Light Source (NSLS). Data were collected using a STOE SES-1000 high-pressure x-ray diffraction system. The pressure was generated using a closed-cycle helium cryostat. The sample was pressed between two diamond anvils, with CrSi₂ placed at the center of the diamond anvil cell.

RESULTS AND DISCUSSION

CrSi₂ crystallizes in the hexagonal P6₃mc structure. Our results indicate that the high-pressure phase transition occurs at P = 28 GPa. The crystal structure at high pressures was determined using x-ray diffraction techniques. The transition between the low- and high-pressure phases was identified by the change in the lattice parameters.

CONCLUSIONS

Our high-pressure x-ray diffraction experiments have provided new insights into the structural properties of CrSi₂. The high-pressure phase transition at P = 28 GPa indicates a structural rearrangement. These findings are important for understanding the behavior of CrSi₂ under extreme conditions and for potential applications in high-temperature environments.

REFERENCES


Acknowledgments: This work was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-05CH11231.
Determination of Ferroelectric Properties in Carbohydrate Glasses Using Atomic Force Microscopy

D-glucose was studied as a possible candidate for ferroelectric domain imaging using an Atomic Force Microscope (AFM). The large dipole moment of carbohydrates is such that they may show localized ferroelectric domain formation in liquid phase. Samples were heated at their melting point on glass slides and then left to cool to room temperature in a desiccator. Differential Thermal Analysis (DTA) was used to test the effectiveness of this method, and it was found that the oven preparation created samples with 41% crystallinity, where both a glass transition and sharp melting peak were observed. DTA was again used to find a way to create a better amorphous sample. An assessment of the effect of cooling rate on glass formation was conducted using a Differential Scanning Calorimeter (DSC). Surface images of a ferroelectric ceramic and the surface of an amorphous glucose sample were taken using AFM Constant Force Topography
The Search for Ferroelectric Domain Structures in Carbohydrate Glasses Using Atomic Force Microscopy

Louis S. Prah1 and Dr. David P. Shelton2

1Department of Physics, Lewis & Clark College, Portland, OR 97210, USA
2Department of Physics, University of Nevada-Las Vegas, Las Vegas, NV 89154, USA, NSF REU Program Summer 2008

Abstract

D-glucosyl was studied as a possible candidate for ferroelectric domain imaging using an Atomic Force Microscope (AFM). The large dipole moment of carbohydrates is such that they may show localized ferroelectric domain formation in liquid phase. Samples were heated at their melting point on glass slides and then quenched to room temperature in a desiccator. Thermogravimetric Analysis (DTA) was used to test the effectiveness of this method, and it was found that the oven preparation created samples with 41% crystallinity, while both a glass transition and sharp melting peak were observed. DTA was again used in a way to create a better amorphous sample. An assessment of the effect of cooling rate on crystalline formation was conducted using a Differential Scanning Calorimeter (DSC). Surface images of a ferroelectric ceramic and the surface of an amorphous glucose sample were taken using AFM Constant Force Topography Mode.

Background

Hyper-Rayleigh Scattering experiments have indicated evidence of localized ferroelectric domain formation in polar liquids. Several systems, such as nitrobenzene/nitrobenzene and acetic acid, have been studied and exhibit this effect. Domain formation is thought to occur when μB/γkT > 1, where μB is the magnetic dipole of the molecules. Above this point, regions of localized dipole alignment are favored over thermal fluctuations, which would normally disrupt their formation. In liquids, the dipole density is μB = 40 μD at T = 300K. [1] The goal was to find an organic molecule with a dipole density above the threshold, which can form an amorphous phase without crystallizing. If the material was able to crystallize, any domain forming in the bulk would be disrupted by the crystalline. AFM techniques were applied to the scanning probe tip to be used to image domains in known ferroelectric materials. Carbohydrates are known glass formers, so crystalline D-glucose was used in our study. It exhibits a large dipole moment (μB = 3.86 μD), which sets the dipole density well above the threshold (123 D). The high glass transition temperature of glucose, measured at Tg = 318 ± 0.3 K, allows the AFM setup to be used at room temperature.

DSC Data

(Table 1a) DSC data from samples prepared in oven.

<table>
<thead>
<tr>
<th>Melting Temp (K)</th>
<th>Glass Temp (K)</th>
<th>Melting Enthalpy (J/g)</th>
<th>% Crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>435 ± 0.2 K</td>
<td>--</td>
<td>189 ± 6.8 J/g</td>
<td>95 ± 3.6%</td>
</tr>
<tr>
<td>Oven Prep</td>
<td>430 ± 2.6 K</td>
<td>518 ± 0.3 K</td>
<td>80 ± 2.1 J/g</td>
</tr>
<tr>
<td>Second Run DSC</td>
<td>430 ± 2.6 K</td>
<td>520 ± 0.2 K</td>
<td>0.014 ± 0.002 J/g</td>
</tr>
</tbody>
</table>

(Table 1b) DSC data from variable cooling rate experiment.

<table>
<thead>
<tr>
<th>Sample Cooling Rate</th>
<th>Glass Temp (K)</th>
<th>% Crystallinity</th>
<th>Enthalpy in Glass Transition (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 K/min</td>
<td>435 ± 0.2 K</td>
<td>50 ± 6%</td>
<td>45 ± 1 J/g</td>
</tr>
<tr>
<td>5 K/min</td>
<td>435 ± 2.6 K</td>
<td>50 ± 6%</td>
<td>50 ± 1 J/g</td>
</tr>
<tr>
<td>10 K/min</td>
<td>458 ± 2.6 K</td>
<td>50 ± 6%</td>
<td>50 ± 1 J/g</td>
</tr>
</tbody>
</table>

Thermal Analysis

Anhydrous, crystalline D-glucose was obtained from J.T. Baker Chemical Co. and without further preparation. Enthalpy of fusion for crystalline glucose is 105.1 ± 1.6 J/g. [3] and glucose samples used in this study were found to be 95% crystalline, as shown in Table 1a. A Netzsch Instruments STA 449 C/Boxer Simultaneous Thermal Analyzer, calibrated to Indium (ΔHf = 264 ± 0.02 J/g), was employed to test how effectively even heating at 40 K/min prepared glasses. A DSC curve of crystalline sample is shown in Fig. 3a, and the large melting peak shows that the sample was 95% crystalline. Samples were heated at a heating rate of 10 K/min to 180 K, then cooled to room temperature (cooling rate 250 K/min). The cooling rate was then scanned in the calorimeter (Fig. 3b) to 250 K/min and crystalline melting is observed, and crystallinity decreased to 41 ± 2.1%.

When an optimal heating rate to create an amorphous sample, glucose glass was prepared and evaluated in the DSC. The sample was heated, starting at room temperature, at a rate of 10 K/min to 180 K, then cooled to a variable rate, and reheated to assess how cooling rate affects glass formation. Three cooling rates tested were 1, 5, and 10 K/min. From this point, the enthalpy of the amorphous melting was calculated from the endotherm calibration standard. The graph shows that a faster cooling rate does not produce more glass, although all samples were amorphous. The largest enthalpy was from the 1 K/min cooling rate, the 10 K/min was smaller, and the 5 K/min was much smaller than either. Test shows the curve of the second heating, in which only a glass peak is evident. An upper bound for percent crystallinity of these samples is 50.6%.

AFM Imaging

An NT-MDT ESEM Scanning Probe Microscope was used for surface imaging. Barium Strontium Titanate (BST) capacitor dielectric, a ceramic with known ferroelectric and piezoelectric properties, was first grown using sol-gel processes to observe the surface. All images were taken in air, using a variable speed scanner, then with diamond polishing. The sample was then examined using Atomic Force Microscopy. A new BST sample was then examined using Atomic Force Microscopy with a scanning speed of 0.74 Hz was scanned to assess surface roughness of the sample. The surface was polished several times using the ESEM, the largest variations left on the sample surface, which fell into the −1.0 μm depth range (near the vertical range of the instrument), bordered by larger flat regions, where surface variation was seen within the range of the tip. The AFM shows both before and after grinding and polishing images. When it was determined that the surface was smooth enough to attempt a domain search, the sample was applied to the tip and the sample surface was scanned for domains, but it was discovered that a fully component generated a spurious signal and the error was not corrected to run in time. It was also found that the liquid-glass nature of glucose causes problems for the AFM. A sample of glucose glass was prepared using the method previously detailed. When the sample was applied to the sample surface, it became stuck, in evident from a bright spot in the photodiode signal, as well as a general lag in responsiveness when approaching or releasing from the sample. Furthermore, a scan returned a blank image. Even when contact to atmospheric moisture was reduced by placing the sample in a desiccator for several hours immediately after heating, the AFM image showed no features. Due to these difficulties and a lack of time, no further AFM scans of glucose were run.

Conclusion

Glucose was determined to be a bad choice of material for this study. It meets the criteria for dipolar density, and also excels as a glass-former. DSC scans revealed that several samples amorphous and nearly 0% crystalline, and the formation of amorphous samples has its pitfalls. The tendency to crystallize when heated to the melting point makes the process require careful attention, and even when the sample appears to have changed phase normally, there may still be some lingering crystals. Furthermore, once the AFM was used to attempt scan the surface for topography, the tip became stuck and no useful data could be acquired. These techniques for forming and scanning amorphous samples might, however, be applied to other organic glass-forming liquids in future studies.

References

Acknowledgements

I would like to thank Dr. James Gelber for the use of his laboratory and equipment, Louis Browne for her time spent helping with DSC operation, Bill Donnell for his help in setting up and troubleshooting the AFM, and Anne Sanchez and Jim Norton for their help with the laboratory. Support from the Tessel Club Experience for Undergraduates (REU) program of the National Science Foundation is gratefully acknowledged under grant DMR-0502989.
Allison Savage  
Mentor - Oliver Tschauner  

Spatially resolved optical absorption spectrometry and single crystal diffraction on metamict materials.

A major goal in developing storage medium for radioactive waste is the identification of chemically suitable and durable material for storage in repositories (Lumpkin 2006). Radiation damage induces enhanced chemical diffusion and structural breakdown of the host materials, which can lead to contamination of the surrounding environment. During this project four different naturally occurring materials which are common carriers of thorium and uranium were examined: gadolinite, perrierite, allanite, and pyrochlore of which the first three are silicates and pyrochlore being an oxide. Their spectra and absorptions bands were examined to identify prominent features due to radiation damage.

The goal of this study is to identify and characterize polyamorphisms metamict glasses. Further, we examine the hypothesis that pyrochlores do not amorphise but undergo a structural transition upon metamictization this part of the project will be conducted at the APS.
Alison Savage, D. Oliver Tschauer, Sergey Tkachev
University of Iowa; University of Nevada Las Vegas

Introduction:
A major goal in developing storage medium for radioactive waste is the identification of chemically suitable and durable material for storage in repositories (Lambrich 2000). Radiation damage induces enhanced chemical diffusion and structural breakdown of the host materials which can lead to contamination of the surrounding environment. During this process four different naturally occurring materials which are common carriers of thorium and uranium were examined: gadolinite, parierite, atlante, and pyrochlore of which the first two are silicates and pyrochlore being an oxide. Their spectra and absorption bands were examined to identify prominent features due to radiation damage.

Gadolinite, Yttrity: Ireland
Y$_2$Fe$_{16}$Re$_{4}$Si$_{10}$O$_{32}$
Width: 43 μm Height: 138 μm
Parierite, Amherst, VA
(Ca,La,Ca)$_2$(Fe$^{3+}$,Mg)$_2$(Ti,Fe$^{3+}$)$_2$Si$_2$O$_{22}$
Width: 2 μm Height: 96 μm
Atlante
Ca$_3$(La, Ca, Y, Ca)$_4$(Fe$^{3+}$,Fe$^{2+}$)(SiO$_4$)(Si$_2$O$_5$)O(OH)
Width: 10 μm Height: 98 μm
Pyrochlore
(Na, Ca)$_2$~Si$_2$O$_7$(OH,F)
Varied in thickness and diameter between 30-50 μm

(Vinelth Database 2000)

Spectroscopy and Absorption Techniques:
Being relatively large, the samples were mounted onto caption tape for spectroscopy. Two sets of tests were conducted at once: one being the spectrum of the sample and the other a spectrum of the white light source without interference. When analyzing the data, the white light spectrum was subtracted from the spectra of the samples. Using this procedure, the absorption band was divided into the sample spectra to provide the final normalized intensity (Figure 4).

Figure 1
Gadolinite have prominent dark regions against the green transparent overall. The dark regions indicate areas that suffer radiation damage. The two spectra are layered to view any differences (Figure 1). When the spectra are subtracted from each other after being normalized, the varying features can be seen (Figure 2).

Figure 2
The three silicate samples ranged in degrees of radiation damage. Parierite and gadolinite having high and atlante having a low degree of damage. All the spectra have dominant features in the visual band of the spectrum which is caused by the Fe$^{3+}$ and Fe$^{2+}$ charge transfer. The dominant absorption feature is also the Fe$^{3+}$ and Fe$^{2+}$ charge transfer band (Sherman 1987).

Spectroscopy and Absorption Analysis:

Conclusions:
The spectra for all four samples, as well as the absorption bands, are dominated by Fe$^{3+}$-charge transfer band which is expected due to Fe content in the samples. However, the optical absorption spectra do not have any defining characteristic features of radiation damage nor is there any noticeable difference between the damaged and undamaged gadolinite regions so consequently there is also no spatial variation even though the composition of gadolinite is varied. The pyrochlore samples, fifteen in total, were also dominated by Fe$^{3+}$ in the optical absorption spectra. Only two of the fifteen samples of pyrochlore had crystal lattices, as shown in Figure 6, whereas the rest of the samples were powdered.

References:

Acknowledgments:
Support from the Research Experience for Undergraduates (REU) program of the National Science Foundation is gratefully acknowledged under grant DMR-0552699
APS Argonne National Lab, UNLV Physics Department
Programs:
Origins 8.0, WinSpec, PowerPoint, Excel
Markus Vasquez and Lucas Wilson  
**Mentor - John Farley**

The Study of Spinels by Laser Micro-Raman Spectroscopy

Standards of spinels, composed of two metals and oxygen with the formula AB₂O₄, are being created with known composition to identify spinels in samples of unknown composition by comparison with the spectra obtained from the standards. Laser micro-Raman spectroscopy allows the identification of chemical species based on their unique vibrational modes. The degree to which spinels of varying composition can be identified will be determined. This will aid in the study of the corrosion of steel by liquid metal. Spinels are a likely component of the oxide layer. Understanding the composition of the products of corrosion leads to an understanding of the processes involved in corrosion. This work is vital to the transmutation of nuclear waste.
A Study of NiFe\textsubscript{x}Cr\textsubscript{(2-x)}O\textsubscript{4} by Laser Micro-Raman Spectroscopy

Lucas Wilson\textsuperscript{1}, Markus Vasquez\textsuperscript{2}, Dr. John Farley\textsuperscript{3}, Dr. Allen Johnson\textsuperscript{3}, Brian Hosterman\textsuperscript{3}
\textsuperscript{1}University of Wisconsin – Stevens Point, \textsuperscript{2}Oklahoma State University, \textsuperscript{3}University of Nevada – Las Vegas

Introduction

Spinel standards with the composition NiFe\textsubscript{x}Cr\textsubscript{(2-x)}O\textsubscript{4} were used to identify spinels in the unknown corrosion layer of corroded steel samples by comparison with the spectra obtained from the standards. Laser micro-Raman spectroscopy allows the identification of chemical species based on their unique vibrational modes. The degree to which spinel phases of varying composition can be identified was determined. This will aid in the study of the corrosion of steel by liquid metal. Spinels are a likely component of the oxide layer. Previous work has shown that the spinel, Fe\textsubscript{2}O\textsubscript{3}, is a major product of these corroded samples. However, due to the variety of metals within steel, other spinels can also be formed. Understanding the composition of the products of corrosion aids to an understanding of the processes involved in corrosion. This work is vital to the transmission of nuclear waste.

Materials and methods

The spinels were synthesized by combing aqueous solutions containing stoichiometric amounts of the appropriate metal nitrates and urea. The solution was heated at 360°C after excess water had boiled away.

Raman scattering is an elastic light scattering process. Monochromatic light falls on the sample, and the scattered light has an energy difference that corresponds to the vibrational energy levels of the molecule.

Results

The excitation of vibrational modes resulting in photons of higher wavelength, lower energy, is termed Stokes scattering. Ovcharenko et al. (2014) 1 suggested that the excitation was considered in this research.

A laser, using a 442.1 nm, or an argon laser, using a 514.6 nm. The argon laser was added to the system this summer to reduce the difficulties caused by fluorescence.

The synthesized samples were then verified to be truly mixed spinels, with both Fe and Cr occupying sites in the same unit cell. These were done by comparing spectra of the synthesized spinel with spectra produced by mixing pure spinel phases in appropriate ratios.

Conclusions

By examining the major peak positions and relative intensities, the spinel composition can be identified within 15%.

The 924 cm\textsuperscript{-1} mode is from the transverse Raman scattering of NiO2 in the 

\frac{\text{Fe}}{\text{Ni}} \text{NiFeCoO}_4 \text{ molecule. This mode increases in Raman shift from 775 cm\textsuperscript{-1} to 703 cm\textsuperscript{-1} quickly when going from the normal spinel, NiFeCoO}_4 \text{ to NiFeCoO}_4 \text{ but then stays relatively constant from NiFeCoO}_4 \text{ to the inverse spinel, NiFeCoO}_4 \text{. This is due to the Ni being replaced by Co in the NiO}_4 \text{ "molecule."

Of course, real corroded steel will contain more than just Ni, Fe, and Cr, and thus further studies on other spinels will benefit this research.

Sources


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Vienna R. Saccomanno  
Mentor - Henry J. Sun  
In 1976, the Viking mission made a remarkable discovery: Martian soil was capable of decomposing an organic nutrient broth to carbon dioxide as if it contained live microorganisms. However, a biological interpretation of this finding is in apparent contradiction with the gas chromatograph-mass spectrometer aboard the Viking landers, which showed Martian soil to be devoid of indigenous organics. To reconcile these findings, it has been hypothesized that unknown abiotic oxidants, such as peroxide and superoxide, are present on Mars and that they were responsible for its soil reactivity. The objective of this research is to develop a life detection method that can distinguish biological reactivity from abiotic mimicry.
Stereospecificity in glucose consumption: a new approach to Martian life detection

Vienna R. Saccomanno and Henry J. Sun
Desert Research Institute, Las Vegas.

1. Glucose consumption by terrestrial life is stereospecific

2. Abiotic chemical oxidation is not stereospecific

Is Mars a biological planet or a sterile (oxidized) world?

In 1976, the Viking mission made a remarkable discovery: Martian soil was capable of decomposing an organic nutrient broth to carbon dioxide as if it contained live microorganisms. However, a biological interpretation of this finding is in apparent contradiction with the gas chromatograph-mass spectrometer aboard the Viking landers, which showed Martian soil to be devoid of indigenous organics. To reconcile these findings, it has been hypothesized that unknown abiotic oxidants, such as peroxide and superoxide, are present on Mars and that they were responsible for its soil reactivity. The objective of this research is to develop a life detection method that can distinguish biological reactivity from abiotic mimicry.

Our approach to distinguishing between biological and chemical reactivity

We hypothesize that organic consumption by living organisms is stereospecific, whereas abiotic destruction is not. In other words, if both D- and L-isomers of the same compound are fed to soil but only one is consumed, the soil is considered biologically reactive. On the other hand, if both isomers are destroyed, abiotic oxidants are assumed present.

Below, we provide experimental verification for this theory by demonstrating that: 1) only D-glucose, not L-glucose, is used by terrestrial organisms, and 2) non-life processes are not stereospecific and destroy both D- and L-glucose.

Discussion

Our data indicates that stereospecificity is a distinguishing character of life. With the exception of one of the archaea, which is apparently incapable of metabolizing glucose, all organisms studied consumed only D-glucose, not L-glucose. In contrast, chemical oxidation by potassium permanganate destroyed both D- and L-glucose. Further tests with more organisms will determine whether or not stereospecificity is a universal property of terrestrial life.

Future work will also consider lactic acid and amino acids. If these substrates are also utilized in a stereospecific manner like glucose, then they should also be used to maximize the probability of success of life detection. We envision that, once fully established, this new approach can be implemented on Mars to determine whether or not life exists on our neighboring planet.

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This work is supported by a NASA Grant to HJS. Funding also came from NASA EPSCoR and Space Grant. We thank Dr. Brian Hendricks for providing the archaea and for help with their growth. Dr. Kurt Regner for providing the yeast, and Mr. Alex Miehau for technical assistance with the flow cytometer.
Pyrochlores with magnetic rare earth ions are a topic of interest due to unusual results brought about by their high degree of geometrical frustration. The lattice structure prevents the magnetic spin interactions from finding a single minimum energy state, which leads to a nonzero residual entropy[1]. By analyzing the heat capacity at low temperatures, the type of magnetic interaction can be determined and the temperatures at which long-range magnetic ordering and magnetic field induced splitting of energy states occur.
Magnetic Ordering Phase Transitions of Gd$_2$Sn$_2$O$_7$

Gordon Tam$^1$, Andrew Cornelius$^1$, and Daniel Antonio$^1$

$^1$ High Pressure Science and Engineering Center and Department of Physics, University of Nevada Las Vegas, Las Vegas, Nevada 89154, USA

Abstract

Pyrochlores with magnetic rare earth ions are a topic of interest due to unusual results brought about by their high degree of geometrical frustration. The lattice structure prevents the magnetic spin interactions from finding a single minimum energy state, which leads to a nonzero residual entropy$[1]$. By analyzing the heat capacity at low temperatures, the type of magnetic interaction can be determined and the temperatures at which long-range magnetic ordering and magnetic field-induced splitting of energy states occur.

Figure 1: (a) Gd$_2$Sn$_2$O$_7$ unit cell, (b) unit cell with oxygen sites removed

Results

The graph of the total heat capacity of the sample took on the unique curve shown (Fig. 2), verifying multiple factors contributing to the heat capacity. Beyond 17K, the magnetic interactions appear to vanish, denoting the position for the Debye curve to be fitted to. Multiple rounded peaks suggest that there are more than one Schottky peaks, for different excited magnetic states. The shift of the peak center with field suggests an increase in the characteristic temperature of the splitting between the states.

Figure 2: Total heat capacity

Experimental Details

The heat capacity measurements were taken at UNLV using the Quantum Design PPMS (Physical Property Measurement System) equipped with a He-3 system attachment and puck (Fig. 1). The system is capable of achieving $<0.35K$ by means of helium-3 and a pump to heat the system. Addenda measurements were first taken from 30K-0.35K at different field strengths, ranging from 0-9 T. The experiment was then repeated with a 1.25mg sample, with the adiabatic weighing in background.

The graph of the total heat capacity of the sample is a superposition of several different contributing factors. The heat capacity measurements contain lattice contributions, Schottky anomalies, and long-range magnetic ordering. By using the Debye model for low temperatures, a T$^3$ curve can be used to approximate the lattice contribution. After removing the lattice contribution, the short-range ordering term can be fitted to the Schottky peaks using a Matlab program. With the removal of the two contributing factors, the long-range ordering peak is found by using a Lorentzian function.

Figure 3: Phase transition peaks

CONCLUSIONS

The inverse relationship between temperature and magnetic field in the experiment shows that a$_{He}$ shows a disorder at higher fields. In agreement with previous work$[2]$, there also seems to be evidence of magnetic-induced splitting of low-level energy states, shown by Schottky peaks. Though there were temperature limitations in our experimental equipment, it was possible to extrapolate data beyond our measuring capabilities. As a result, long-range ordering should not be observed at any temperature for field strengths greater than our theoretical value of 5.779T.

Figure 4: Phase diagram

The results show that the ordering peaks shifts to lower temperatures with increasing strong applied fields (Fig. 3). The separation between the peaks also increases with increasing field strength. With no applied field, the temperature at which the magnetic ordering phase transition occurs is just under 1K. Similarly, the temperatures at which the phase transitions occur at fields of 1, 3, and 5T can be seen in the graph. For fields of strength 7T and 9T, the temperatures where the transitions occur can no longer be measured by the PPMS. The graph of the phase transition peaks reveals that heat capacity decreases with increasing field, contrasting that of the Schottky peaks. An insufficient amount of data points causes the LT peak to not be well resolved.

A plot of temperature vs. applied magnetic field (Fig. 4) shows the phase boundary between ordered and disordered magnetic states. The phase boundary curve is used to extrapolate to determine the critical field at which long-range ordering cannot occur even at absolute zero. The black points are the measured data points. The red point is the extrapolated point. The data indicates a critical field strength of approximately 5.779T. This result reveals that even if the PPMS were capable of going to lower temperatures, even as low as absolute zero, phase transitions for 7T and 9T would not occur, making the 7T and 9T curves sufficient for our experiment.

REFERENCES


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