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Ubiquitylation of Proteins in the Frozen Wood Frog

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Abstract

Wood frogs (*Rana sylvatica*) are able to withstand freezing. Respiratory and cardiac activity ceases when frozen. Homeostatic functions like protein synthesis and degradation presumably must also be compromised. We investigated the fate of ubiquitin-dependent proteolysis in the freeze-thaw cycle and how that might give clues to wood frog survival. We performed western blots for ubiquitin conjugates.

Introduction

Rana sylvatica has the ability to remain frozen for as many as 11 days at -4°C with 100% survivorship. Few data are available as to the fate of major homeostatic processes like protein synthesis and degradation. Ubiquitin dependent proteolysis is responsible for as much as 80-90% of cytosolic protein degradation. Essentially, a small peptide modifier, ubiquitin, is conjugated to target proteins and marks those proteins for degradation. Ubiquitin dependent proteolysis is essential for the turnover of protein pools but is also critical in how a cell responds to cellular stress and protein damage. While there have been numerous predictions of how global climate change will affect temperatures, few studies have addressed the biological implications

Hypothesis

I hypothesize that when wood frogs (*Rana sylvatica*) recover from freezing, ubiquitin-conjugate concentrations will decrease.

Materials and Methods

Rana sylvatica were collected from the woods in Ottawa, Canada. Hind leg thigh muscle and liver tissue samples were collected from different points of the freeze-thaw cycle. Five individuals of each state were used (n = 5).

Control (C) wood frogs were acclimated 1-2 weeks at 5° C.

Frozen (F) wood frogs were exposed to -3° for 24 h.

Long thaw (LT) wood frogs were frozen 24 h @ -3° C and then thawed for 24 h.

Short thaw (ST) wood frogs were exposed to -3° C and then thawed for 8 h.

Sample Preparation-

Samples from the muscle and liver tissues were homogenized in 50 mM Tris-HCl, pH 8.3, 20% glycerol, 2% SDS, and 0.4 M 2-mercaptoethanol. Samples were centrifuged for 20 min @10,000 • g for 30 min, 4° C. Supernatants were disrupted using a 30 gauge needle and frozen until use.

A modified Lowry protein assay was performed to determine protein concentrations.

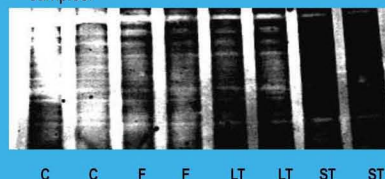
Western Blot

We are asking two questions in regards to the ubiquitylation of proteins. We asked if there were qualitative changes to which proteins were ubiquitylated as a function of state e.g. are there a few proteins that are preferentially ubiquitylated upon freezing? Question 2 was if there were quantitative changes to those proteins tagged for ubiquitylation upon freezing e.g. are more proteins tagged for degradation upon freezing?

We performed western blot analyses. Briefly, 40 µg proteins were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 9% gel. The proteins were electrotransferred to PVDF membrane. The membrane was treated with 0.5% glutaraldehyde for 20 min, then blocked with 5% nonfat dry milk. The blot was incubated with a monoclonal antibody to ubiquitin conjugates, washed, incubated with a HRP-linked secondary antibody, and visualized using ECL+ on a Typhoon imager.

Results

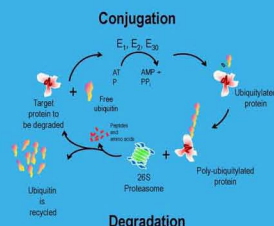
This is a western blot that was done for the liver homogenate tissue samples. See M&M, for key to samples.



Conclusion

The data demonstrate no qualitative differences in which liver proteins get ubiquitylated. The data do indicate quantitative differences where freezing and thawing is associated with increased ubiquitin conjugate concentrations. The muscle blot was similar in demonstrating no qualitative differences in ubiquitylated proteins (data not shown).

I will perform more quantitative dot blot analyses to determine precisely how much ubiquitylation occurs.



Wood frog (*Rana sylvatica*) thawed

Acknowledgements

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Wood frog (*Rana sylvatica*) frozen