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Martian life detection with amino acid enantiomers

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The Viking mission showed that Martian soil can degrade a heterotrophic medium to carbon dioxide as if live microorganisms were present. The result is considered inconclusive, however, because abiotic oxidants, such as superoxides, may also exist on Mars and would explain the Viking result. One way to resolve this ambiguity is to repeat the Viking experiment with a isomerically pure medium. The consumption of one isomer, either D or L, would indicate biological activity. Indiscriminate destruction of both isomers would indicate abiotic redox processes. This idea was validated for glucose by REU research last summer (Sun *et al*. 2009). The objective of this project is to test this idea with amino acids. Specifically, the consumption rates of D- and L-enantiomers will be compared for histidine, lysine, and serine in selected bacteria, archaea, and eukaryotic fungi and yeasts. Results with *Bacillus* revealed that in histidine, only the L-isomer was consumed while for serine and lysine, both the D- and L-isomers were utilized. If confirmed in other microorganisms, these results indicate that histidine is a suitable substrate for Martian life detection but serine and lysine are not.

Martian Life Detection with Amino Acid Enantiomers

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The Viking LR: Biology or Chemistry?

In 1976 two Viking Landers were sent to Mars to look for evidence of alien life (Figure 1a,b). Each lander performed a series of soil tests including the Label Release (LR) experiment which tested for degradation of a nutrient broth containing formate, glycolate, glycine, D/L-alanine, and D/L-lactate. Each substrate was labeled with radioactive carbon-14. Upon injection, radioactive gases rapidly evolved from soil (Figure 1c) as if microorganisms were present. Paradoxically, no native organic carbon could be detected in the soil above the parts per billion level. To reconcile these results, most scientists hypothesized that the LR activity was caused by inorganic oxidants such as peroxides and superoxides instead of microorganisms. However, other scientists disagree, arguing that a biological cause for the LR result could not be ruled out.

1a

1b

1c

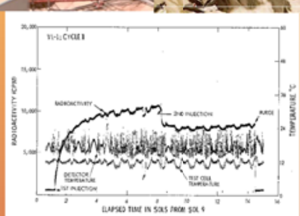


Figure 1a and **1b**: Photos of the two Viking Landers that traveled to Mars in 1976

Figure 1c: Results of the Viking Label release experiment showing that radioactive gas was emitted from the Martian soil upon injection of a heterotrophic nutrient broth. The graph is measured in CPM (counts per minute) vs Martian SOL (solar day). One Martian SOL equals twenty-four hours and forty minutes.

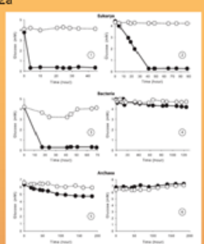
The Concept of Chiral LR

A concept for elucidating the Viking result has been validated by previous summer REU research (Sun *et al.* 2009). In the new scheme, biological and chemical reactivity are distinguished by stereo-selective degradation of chiral substrates. It was shown that on Earth known life forms recognized only D-glucose, the natural enantiomer, but ignore L-glucose (Figure 2a). In contrast, inorganic oxidizers does not have this enantiomeric bias, oxidizing both D- and L-glucose (Figure 2b).

This new approach is now being applied to amino acids alanine, glutamic acid, aspartic acid, and leucine by Drs. Gaosen Zhang and Henry Sun. Data collected so far showed that D- and L-enantiomers were consumed at equal rates. Clearly, not all amino acids are chiral selective.

The objective of this project is to determine whether or not other amino acids are stereo selective and therefore suitable for chiral LR. This study focused on histidine, lysine, and serine. The microorganisms used in or study included *Bacillus, Kocuria, and E. coli.*

2a



2b

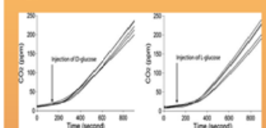


Figure 2a: The results from previous REU work showing that only the D-isomer of glucose is consumed, insinuating at the time that biological activity occurs stereo-specifically.

Figure 2b: Results from the same project showing that chemical oxidation is degrades D- and L-isomers of glucose at equal rates

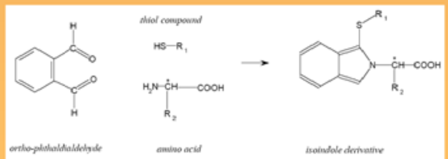
Hypothesis

Amino acids in which only one of their stereoisomers is consumed will serve as an optimal substrate to be used for a future life detection experiment on Mars, in addition to other substrates that are consumed in a stereoselective manner.

Experimental

Microorganisms were grown in LB media. Cells were collected via centrifugation, washed, and suspended in phosphate saline buffer (PBS). To the culture I added a mixture DL-histidine, DL-lysine, and DL-serine. Samples were taken hourly and assayed for enantiomeric levels. The samples were derivatized with o-pthaldialdehyde thiol N-acetyl L-cysteine, or OPA/NAC (Figure 3a). The derivatives were analyzed by the HPLC (Figure 3b). The OPA/NAC reaction is necessary prior to analysis because it adds steric bulk to the amine group thereby making it easier to be detected by the HPLC.

3a



3b

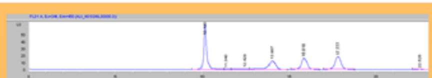
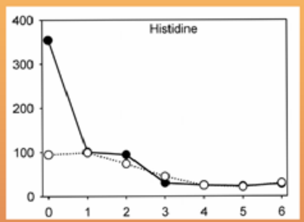


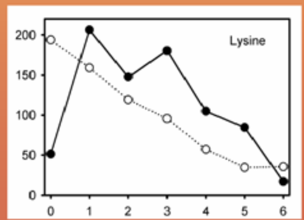
Figure 3a: The mechanism in which OPA/NAC reacts with amino acids by adding steric bulk to the amine group, thereby causing the amino acid to be more easily read by the HPLC machine.

Figure 3b: Example of a graph that is produced by the HPLC machine. The graph reads in fluorescence intensity vs time in minutes. At approximately ten, fifteen, and seventeen minutes are the retention times for serine, histidine, and lysine respectively.

4a



4b



4c

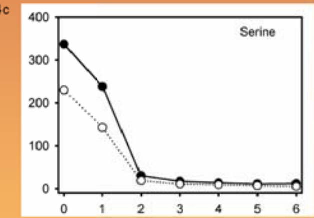


Figure 4a, **b**, and **c**: results showing the consumption patterns of Bacillus. Based on these trends serine and lysine would not be suitable for a future Mars mission because they are not consumed stereo-specifically. Histidine does show potential to be a suitable substrate but further research is required.

Discussion

This study showed that histidine is selective while serine and lysine are not. If confirmed in other life forms, this result suggests that histidine is suitable for chiral LR to clarify the nature of the Martian soil reactivity. If one isomer is consumed, this would illustrate biological activity. On the other hand, if both get consumed, it can be concluded that the reaction took place by chemical oxidation.

Acknowledgements

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