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 Lancers were sent to Mars to look for evidence of alien life (Figures 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, glycinate, glycollate, D-amino acids, and D-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.

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 not only D-glucose, the natural eranomer, but ignore L-glucose (Figure 2a). In contrast, inorganic oxidizers do 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 were B. subtilis, Kocuria, and E. coli.

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 of D-histidine, D-lysine, and D-serine. Samples were taken hourly and assayed for enantiomeric levels. The samples were derivatized with o-phthalaldehyde thiocN-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 a bulky group to the amino group thereby making it easier to detect by the HPLC.

Discussion

This study showed that histidine, lysine, and serine 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 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.

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