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Rapid Communication

Stereo-Specific Glucose Consumption May Be Used to Distinguish Between Chemical and Biological Reactivity on Mars: A Preliminary Test on Earth

Henry J. Sun,[1] Vienna Saccomanno,[1] Brian Hedlund,[2] and Christopher P. McKay[3]

Abstract

Two alternative hypotheses explain the degradation of organics in the Viking Labeled Release experiment on Mars. Either martian soil contains live indigenous microorganisms or it is sterile but chemically reactive. These two possibilities could be distinguished by the use of pure preparations of glucose isomers. In the laboratory, selected eukaryotes, bacteria, and archaea consumed only D-glucose, not L-glucose, while permanganate oxidized both isomers. On Mars, selective consumption of either D- or L-glucose would constitute evidence for biological activity. Key Words: Biosignatures—Life detection—Mars—Microbe. Astrobiology 9, 443–446.

Introduction

A fundamental question regarding life on Mars concerns the planet's enigmatic soil reactivity, discovered by the labeled release experiment aboard the Viking landers (Klein *et al.*, 1976; Levin and Straat, 1976). At both landing sites, the labeled release test, which was designed to detect heterotrophic metabolism, was positive. Radioactive gas, presumably CO_2, evolved rapidly from soil following the addition of a nutrient broth that contained ^{14}C-labeled glycolate, formate, D,L-lactate, D,L-alanine, and glycine. Paradoxically, no native soil organic carbon was detected at the parts-per-billion level, which argues against the presence of a soil biota. Instead, it has been speculated that martian soil is chemically reactive, possibly due to the presence of inorganic oxidants such as peroxide and superoxide ions (Klein, 1977, 1978, 1979; Levin and Straat, 1981; Yen *et al.*, 2000).

In 1996, the Mars Oxidant experiment (MOx) science team made an attempt to clarify the nature of martian soil reactivity (McKay *et al.*, 1998). The MOx was to expose a series of redox-sensitive films, both organic and inorganic, for degradation on Mars. D- and L-cysteine were included to distinguish between chemical and biological activity. It was assumed that living microorganisms would consume only one of the two enantiomers, whereas oxidants would oxidize both. Unfortunately, the Russian Mars '96 mission, which carried the MOx, failed shortly after launch.

The idea behind the MOx has yet to be validated, however. First of all, there is no evidence that biological utilization of cysteine is stereo specific. Second, substrate stereo specificity/selectivity in microorganisms in general has not been well researched. Only two studies have looked into stereo selectivity of carbohydrates and amino acids in soils (Halpern *et al.*, 1966; Kelley *et al.*, 1975). Although a preference for L-amino acids (proline, glutamic acid, valine, serine, leucine, methionine, phenylalanine, tryptophan) and D-sugars (glucose, arabinose, fucose, mannose, xylose) over D-amino acids and L-sugars was observed, many critical issues remain. It is not clear, for instance, whether one, some, or all of the organisms present in the soils contributed to the results. In the case of sugars, because they were supplied in a mixture, it is also unclear as to whether the bias originated from one, some, or all of the substrates. Furthermore, these studies are complicated by the fact that desert soils can be both biologically active and chemically oxidizing (Navarro-González *et al.*, 2003; Quinn *et al.*, 2007).

The objective of the present study was to test the uptake of chiral substrates in pure cultures of microorganisms free of the complications associated with soils. Our study focused on glucose, but usage was assessed in representatives of all

[1]Division of Earth and Ecosystem Sciences, Desert Research Institute, Las Vegas, Nevada.
[2]School of Life Sciences, University of Nevada, Las Vegas, Nevada.
[3]Space Science Division, NASA Ames Research Center, Moffett Field, California.

three domains of life: Eukarya, Bacteria, and Archaea. In addition, the assumed absence of stereo specificity in abiotic redox processes was verified with potassium permanganate.

Materials and Methods

The organisms used in our study included *Penicillium expansum* (provided by Dr. C.L. Xiao of Washington State University), *Saccharomyces cerevisiae* (from the Agricultural Research Service Culture Collection in Peoria, Illinois), *E. coli* (DH10B from New England BioLabs), *Micrococcus luteus* (isolated from dust in Las Vegas), and archaea *Natronobacterium* sp. SL2.43 and *Halostagnicola* sp. SL1.19 [isolated from a playa in California (Navarro *et al*., 2009)]. The latter three isolates were identified by 16S rRNA phylogenetic analysis.

All biological experiments used late log-phase cultures. Bacteria were grown in Luria-Bertani medium, fungi in Malt Yeast Extract medium, and archaea in SL medium with 25% NaCl (Navarro *et al.*, 2009). The pH of all media was between 7.4 and 8.0. The archaea were grown at 40°C and the other organisms at 25°C. Cells were collected by centrifugation, washed twice in phosphate buffered saline (PBS) (10-fold strength for the archaea), re-suspended in PBS, and divided into two subcultures. After adding D- and L-glucose, incubation was resumed. After appropriate time intervals, aliquots were taken and assayed colorimetrically for glucose as described by Miller (1959).

Chemical oxidation was studied in a respirometer. The system is closed and has a total internal volume of 477 ml. It consisted of a reaction chamber, two serially connected test tubes containing 1 *N* KOH for CO_2 scrubbing, a bypass, a peristaltic pump, and a Li850 gas analyzer. After adding 2 ml of 0.38 *M* potassium permanganate solution, the reaction chamber was closed, and the air was circulated through the KOH solutions. After CO_2 was depleted, air flow was routed to the bypass, and 2 ml of 28.8 m*M* glucose were added to the

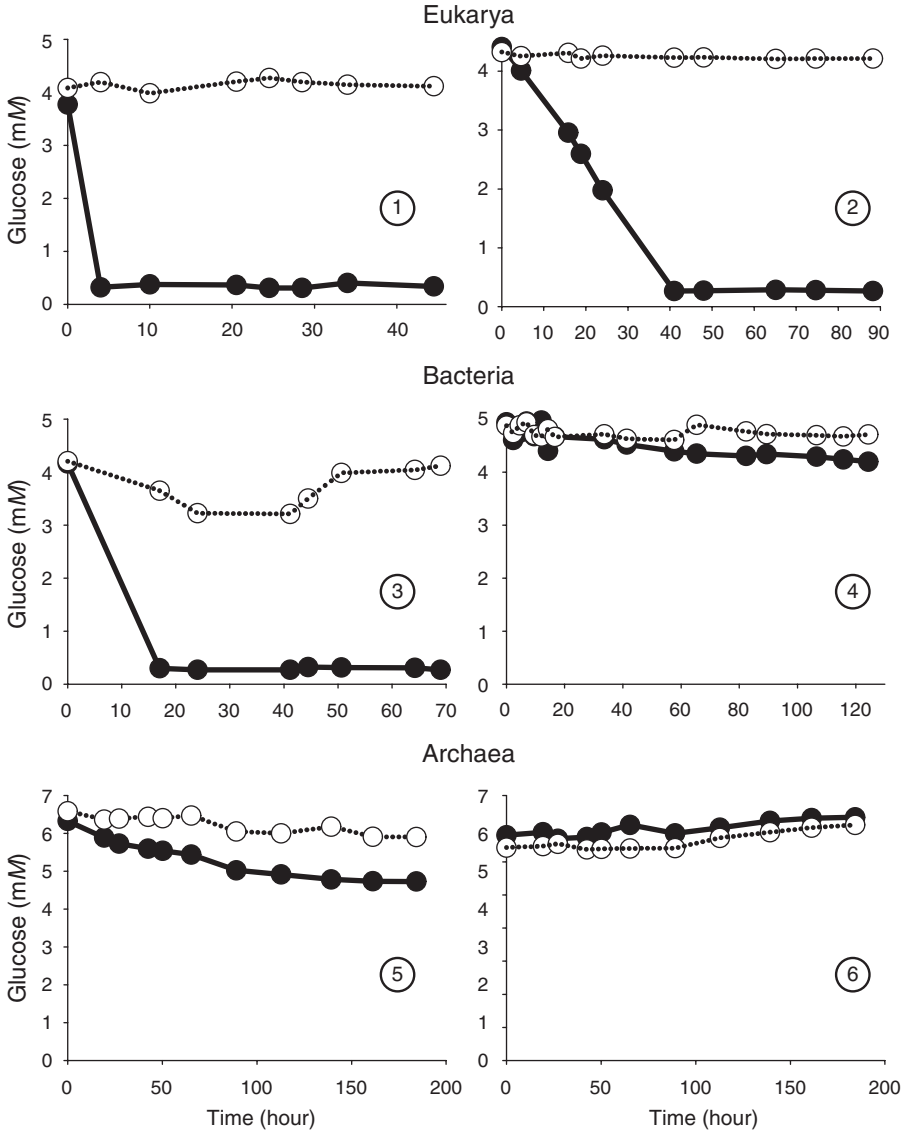

FIG. 1. Biological consumption of D-glucose (filled symbol) and L-glucose (open symbol) by (**1**) *Saccharomyces cerevisiae*, (**2**) *Penicilium expansum*, (**3**) *E. coli*, (**4**) *Micrococcus luteus*, (**5**) *Natronobacterium* sp., and (**6**) *Halostagnicola* sp.

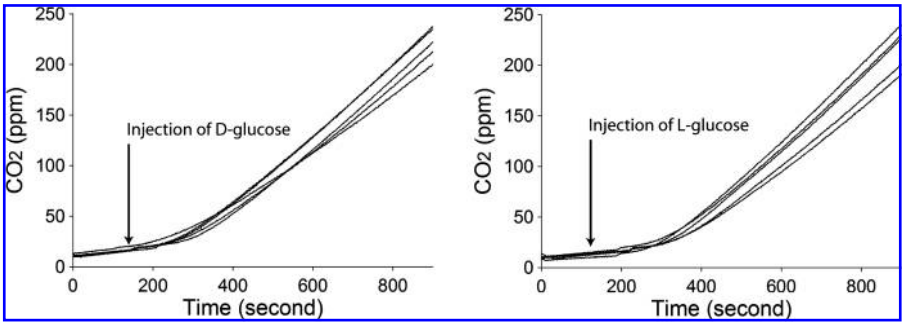

FIG. 2. Rates of chemical oxidation of D-glucose and L-glucose in the presence of potassium permanganate, measured in carbon dioxide release.

reaction chamber through a septum-lined injection port. The rate of subsequent CO_2 release was recorded by the gas analyzer.

Results

Archaeum *Halostagnicola* sp. did not utilize D- or L-glucose (Fig. 1). The other five organisms, including the eukaryotes *Saccharomyces cerevisiae* and *Penicilium expansum*, the bacteria *E. coli* and *Micrococcus luteus*, and the archaeum *Natronobacterium* sp., utilized D-glucose, not L-glucose. Glucose consumption rates varied considerably among the organisms. *S. cerevisiae* and *E. coli* had the highest rates, $\geq$17 and $\geq$7 pmol·cell^{-1}·h^{-1}, respectively, followed by *P. expansum* at 2 pmol·cell^{-1}·h^{-1}, *M. luteus* at 0.3 pmol·cell^{-1}·h^{-1}, and *Natronobacterium* sp. at 0.2 pmol·cell^{-1}·h^{-1}. In *S. cerevisiae*, *P. expansum*, and *E. coli*, no further consumption occurred after glucose dropped to about 0.28 m*M*. Such depletion was not reached in *M. luteus* or *Natronobacterium* sp. within the duration of our study.

Potassium permanganate oxidized both D- and L-glucose (Fig. 2). Reaction between 2 ml of 0.38 *M* potassium permanganate and 2 ml of 28.8 m*M* D-glucose released CO_2 at a rate of 0.32 μmol·s^{-1} ($\pm$0.03; $n = 5$). Under identical conditions, reaction with L-glucose yielded CO_2 at a rate of 0.34 μmol·s^{-1} ($\pm$0.03, $n = 5$). The difference between the two reaction rates was statistically insignificant ($p = 0.47$, Student's *t*-test).

Discussion

Stereo-specific glucose consumption appears to be a valid approach for distinguishing between biological and chemical reactivity. All the selected bacteria, archaea, and eukaryotes consumed only D-glucose, not L-glucose (Fig. 1). This result is consistent with an early report that *Bacterium coli communis* (syn. *Escherichia coli*) and *Bacterium aerogenes* grow only on D-glucose, not L-glucose (Rudney, 1940). In contrast, chemical oxidation by permanganate is indiscriminate, destroying D- and L-glucose at equal rate (Fig. 2). On Mars, life may or may not have the same sign of chiral preference as do terrestrial organisms. There, selective degradation of either D- or L-glucose, but not both, would constitute evidence for biological activity.

The fact that not all organisms metabolize glucose suggests that use of glucose alone will not capture all heterotrophic activity present in a soil. For some bacteria, glucose may even be toxic under certain conditions (Russell, 1992). Non-glucose-using saccharotrophs could be targeted, however, with other chiral sugars, such as arabinose, fucose, mannose, and xylose, as was suggested by Kelley *et al.* (1975). The stereo specificity of these sugars, however, still needs to be tested. If confirmed, these substrates could be used in combination with glucose in a life-detection experiment.

Sugars may be combined with other chiral substrates, such as amino acids, organic acids, and organic alcohols, to further increase the types of biological activity detected. Stereo-specific uptake of amino acids, too, must be tested individually before combining with sugars. Such a test is crucial because, unlike glucose, which is naturally present on Earth only as a D-isomer, amino acids are present in both enantiomeric forms. They occur not only as L-enantiomers in proteins but also as D-enantiomers in bacterial peptidoglycan cell walls (Rogers, 1974). The latter are recalcitrant and accumulate in soils and waters to high concentrations (Bada and Hoopes, 1979; Kimber *et al.*, 1990; McCarthy *et al.*, 1998; Pedersen *et al.*, 2001; Grutters *et al.*, 2002; Amelung, 2003). Bacteria and archaea appear to be capable of breaking down peptidoglycans and incorporating D-amino acids (Jørgensen *et al.*, 2003; Nagata *et al.*, 2003; Teira *et al.*, 2006). The extent of stereo specificity in amino acid uptake is, therefore, an open question and requires more study.

Acknowledgments

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Abbreviations

MOx, Mars Oxidant experiment; PBS, phosphate buffered saline.

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Address correspondence to:
Henry J. Sun
Division of Earth and Ecosystem Sciences
Desert Research Institute
Las Vegas, NV 89119

E-mail: henry.sun@dri.edu