ICARUS 114, 139–143 (1995)

Amino Acid Racemization on Mars: Implications for the Preservation of Biomolecules from an Extinct Martian Biota

JEFFREY L. BADA AND GENE D. MCDONALD*

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0212
E-mail: jbada@ucsd.edu

Received October 3, 1994; revised November 21, 1994

Using kinetic data, we have estimated the racemization half-lives and times for total racemization of amino acids under conditions relevant to the surface of Mars. Amino acids from an extinct martian biota maintained in a dry, cold (<250 K) environment would not have racemized significantly over the lifetime of the planet. Racemization would have taken place in environments where liquid water was present even for time periods of only a few million years following biotic extinction. The best preservation of both amino acid homochirality and nucleic acid genetic information associated with extinct martian life would be in the polar regions.

INTRODUCTION

The detection of biomolecules from an extinct martian biota is an important parameter in the context of future Mars lander/rover and sample return missions. During the early history of Mars, planetary conditions similar to those that gave rise to the origin of life on Earth may have existed (Pollack *et al.* 1987), although the chances any organisms still survive seems remote because of the present hostile surface conditions. The Viking lander gas chromatography/mass spectrometry experiments detected no indications of extant life, or any organic compounds above the part per billion level in the martian surface regolith (Klein *et al.* 1992). The failure to detect organics has been attributed to the intense surface ultraviolet flux on Mars and the presence of H_2O_2 or other oxidants in the soil. However, recent modeling of H_2O_2 diffusion in the martian regolith suggests that oxidizing conditions may extend downward only a few meters; below the oxidizing level the survival of organic molecules may be possible (Bullock *et al.* 1994).

If abiotic syntheses took place on Mars, amino acids would have been a likely product judging from their ease of synthesis using a variety of prebiotic conditions (Miller 1993). In addition, amino acids would have also been delivered to the surface of Mars by exogenous (cometary and meteoritic impacts) sources (Chyba and Sagan 1992) because these compounds have been found in a number of carbonaceous meteorites (Cronin and Pizzarello 1983). Kanavarioti and Mancinelli (1990) estimated the survival rate of amino acids derived from exogenous sources on Mars. Based on decomposition rates in aqueous solutions, they concluded that if amino acids were present 3.5 byr ago, some fraction of these amino acids should still be preserved today in the martian subsurface.

Amino acids would be a likely candidate for biomolecular components of an ancient martian biota considering their central role in terrestrial biochemistry. Terrestrial organisms, and presumably any martian organisms as well, use only one set of amino acid enantiomers in their proteins (the L-enantiomer in the terrestrial case). The functioning of enzymes is dependent on the folding of the protein chain into a highly ordered structure, which is not possible if the amino acids are racemic (e.g., equal amounts of both the D- and L-enantiomers). Thus, we assume that if proteins and enzymes were a component of ancient life on Mars, then amino acid homochirality would have been required. It should be emphasized that martian life could use either L- or D-amino acids in proteins because both pure enantiomers function equally well in enzymes (Milton *et al.* 1992). The detection of only D-amino acids in martian soils would provide convincing evidence that the origin of life had place taken on Mars. However, the detection of only L-amino acids would present a conundrum: did life originate independently on Mars, or was Mars inoculated with terrestrial organisms from material ejected from the Earth by bolide impacts (Melosh 1988)?

If amino acids can survive in the martian subsurface, their utility as indicators of an extinct martian biota is dependent on their rate of racemization under martian conditions. When an organism dies, its amino acids begin to racemize at a rate which is dependent on the particular amino acid, the temperature, and the chemical environment (Bada 1985, 1991). Racemization reactions are rapid on the terrestrial time scale, and even at deep ocean tem-

* Present address: Laboratory for Planetary Studies, Space Sciences Building, Cornell University, Ithaca, NY 14853.

00191-1035/95 $6.00

peratures of 2°C, amino acids are totally racemized (e.g., D/L = 1.0) in about 5–10 myr. Some amino acids are very resistant to racemization. For example, α-dialkyl amino acids with a chiral center, which are common in carbonaceous meteorites (Cronin and Pizzarello 1983), decompose much faster than they racemize (Bada 1991). However, these amino acids are not generally found in the proteins of terrestrial organisms. When biogenic amino acids are completely racemized, they would be indistinguishable from a chirality point-of-view from the racemic amino acids produced by abiotic organic synthesis or those derived from exogenous sources. An estimate of the preservation of amino acid homochirality associated with an extinct martian biota is thus crucial in assessing the possibility of detecting remnants of ancient life on Mars.

RACEMIZATION RATES ON MARS

Schroeder (1974) measured rate constants for amino acid racemization in both desiccated (dry) and wet Lake Ontario sediments at temperatures between 100 and 150°C. For the epimerization of L-isoleucine to D-alloisoleucine (k_{iso}) in desiccated sediments

$$\log k_{iso}\,(\mathrm{hr}^{-1}) = 12.35 - 6688/T, \quad (1)$$

whereas for wet sediments

$$\log k_{iso}\,(\mathrm{hr}^{-1}) = 11.11 - 5774/T, \quad (2)$$

where T is the temperature in K. The isoleucine epimerization rates are significantly faster in wet than in dry sediments, which is consistent with the observations that amino acid racemization is greatly retarded in anhydrous environments (Bada *et al.* 1994). Isoleucine epimerization has one of the slowest amino acid racemization rates, with the rate of alanine racemization approximately 3× faster and that of aspartic acid about 7× faster (Bada and Schroeder 1975; Bada 1985, 1991).

Based on these data, we have calculated the racemization half-lives of aspartic acid in dry and wet sediments using the following equations. The value of k_{asp}, the rate of interconversion of the aspartic acid enantiomers, is given by

$$\ln Q = 2k_{asp}t \quad (3)$$

where $Q = (1 + \mathrm{D/L})(1 - \mathrm{D/L})^{-1}$ and t is time. The values of k_{asp} as a function of temperature were obtained using Eqs. (1) and (2) and the relationship $k_{asp} = 7 \times k_{iso}$. The half-life for aspartic acid racemization, e.g., the time to reach a D/L ratio of 0.33, is then calculated from

TABLE I
Estimated k_{asp} and Aspartic Acid Racemization Half-lives in Martian Sediments Maintained at Various Temperatures

	T (K)	k_{asp} (yr^{-1})	$t_{1/2}$ (yr)
Dry	150	4×10^{-28}	1×10^{27}
	215	1×10^{-14}	3×10^{13}
	252	4×10^{-10}	9×10^{8}
	273	4×10^{-8}	8×10^{6}
	300	7×10^{-6}	5×10^{4}
Wet	252	1×10^{-7}	4×10^{6}
	273	6×10^{-6}	6×10^{4}
	300	5×10^{-4}	8×10^{2}

Note. The minimum temperature in which "wet" conditions could exist was assumed to be 252 K, the freezing point of a eutectic NaCl solution (5.1 molal). We used the relationship $k_{asp} = 7 \times k_{iso}$ and Eqs. (1) and (2) given in the text for the calculations. The uncertainty of the values is roughly ± a factor of 2–4.

$$t_{1/2} = \ln 2(2k_{asp})^{-1}. \quad (4)$$

Calculations were carried out for temperatures at 150 (the present mean daily temperature at the martian poles), 215 (the present mean daily temperature at the martian equator), 252 (the freezing point of a eutectic NaCl solution), 273 (the freezing point of water), and 300 K (a warm martian surface). These temperatures were selected in order to represent the most extreme temperatures possible on Mars throughout its history. Values of k_{asp} and $t_{1/2}$ for aspartic acid at the various temperatures are shown in Table I.

If we use a value from D/L aspartic acid of 0.95 as the ratio equivalent for total racemization, then the time required for total racemization ($t_{\text{total racemization}}$) can be calculated from

$$t_{\text{total racemization}} = 1.8(k_{asp})^{-1} \quad (5)$$

A D/L value of 0.95 was used because the typical analytical uncertainty for D/L ratio measurements is about ±0.05. A racemic mixture would have an analytical uncertainty of 1.0 ± 0.05 and thus a D/L ratio of 0.95 would be indistinguishable statistically from a racemic mixture. The relationship of $t_{\text{total racemization}}$ as a function of temperature for aspartic acid over the 4.5-byr history of Mars is shown in Fig. 1 for both dry and wet environments.

Because aspartic acid has the fastest racemization rate among the common terrestrial protein amino acids, its racemization represents a worst case scenario of the preservation of amino acid homochirality on Mars. Other amino acids, such as valine, could require as much as 10 times longer than aspartic acid to be completely racemized (Bada 1985), and their homochiral signature would thus

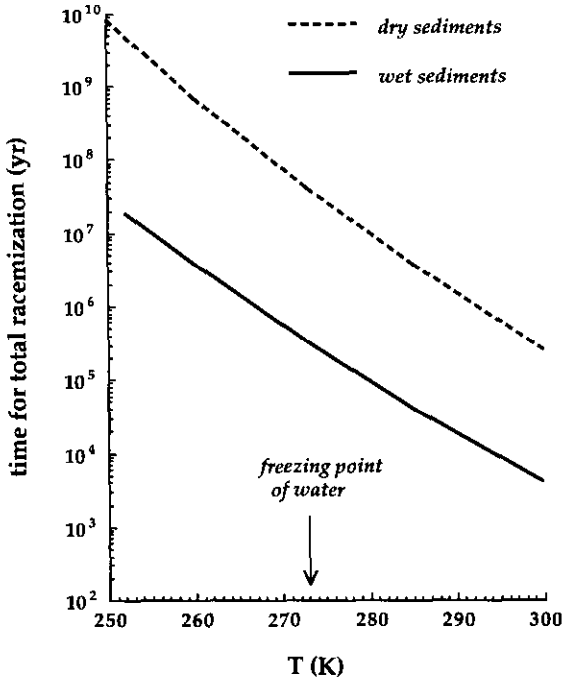

FIG. 1. Time (yr) for total racemiation (defined as $t_{\text{total racemization}}$ in the text) of aspartic acid in dry and wet sediments as a function of temperatures relevant to Mars throughout its history.

be preserved for a greater period of time. As mentioned previously, chiral α-dialkyl amino acids would preserve their homochirality indefinitely regardless of the exposure conditions.

DISCUSSION

Table 1 and Fig. 1 indicate that under dry conditions and temperatures <250 K, the time needed for an initially homochiral aspartic acid population to completely racemize is $\geq 10^{10}$ yr, a time span which is greater than the age of the Solar System. Thus, for amino acids from an extinct martian biota maintained under these conditions, no significant racemization should have taken place even if the extinction occurred >4 byr ago. However, this scenario requires that extinction, planetary desiccation, and cooling to <250 K all occur simultaneously. This particular case also presents a dilemma in that amino acids derived from extinct martian organisms would be indistinguishable from those from extant martian life which would also be homochiral.

Let us examine another scenario in which the putative martian biota became extinct, and amino acid racemization thus began, when the average planetary temperature on Mars dropped below 273 K, and all water on the martian surface froze. This scenario is probably too conservative with respect to when extinction would actually occur because there is evidence that terrestrial microorganisms can exist for some time (millions of years?) at subfreezing temperatures (McKay *et al.* 1992). Although liquid water microlayers 10–80 Å thick may exist on mineral surfaces at temperatures much below the freezing point of water (for example, see discussion in Rietmeijer (1985)), we will assume that the amount of amino acids dissolved in this small volume of water (for a 50-Å layer H_2O covering a 1-mm cubic crystal, the volume is only 3×10^{-8} ml) is negligible compared to the amino acids contained in the bulk frozen soils. Thus, we assume that in frozen soils the racemization rates are similar to those in dry sediments. In this case, if extinction occurred coincident with a drop in temperature to <273 K and the freezing of the planetary surface, followed by a rapid (10^6 to 10^7 years) cooling to 215 K, the modern equatorial mean temperature on Mars, the homochiral amino acid signature associated with the extinct martian biota would still be largely preserved today. This scenario should be discernible because the extent of racemization of the various detected amino acids should correspond to their relative racemization rates. Thus, aspartic acid would be one of the most highly racemized amino acids, alanine less racemized, and valine the least racemized. This scenario may be the most desirable because a partly racemized mixture of amino acids would make it easy to eliminate the possibility that any detected amino acids in martian soils were derived from extant life on Mars or from abiotic sources. In addition, if k_{asp} could be adequately modeled as a function of the temperature history of Mars since biotic extinction, then using Eq. (3), the time when life became extinct on Mars could be estimated (for an example of this type of calculation, see Bada and Schroeder (1975)).

It is obvious that under dry, cold (<250 K) conditions amino acid racemization would be largely prevented even over several billion years. However, a critical point for the preservation of a homochiral amino acid signature on Mars is how long the amino acids would be exposed to wet conditions (meaning liquid water is present) following the extinction event. Under the scenario of extinction at >273 K followed by slow cooling to present day martian surface temperatures, the initial wet conditions would result in significant racemization. If wet conditions and temperatures above 273 K persisted on Mars after extinction for even several million years, the amino acids associated with extinct life on Mars would be completely racemized. Even if homochiral amino acids were maintained in 252 K eutectic NaCl solutions for 10^7 to 10^8 years they would undergo significant and perhaps complete racemization. A cooling scenario over a period of 10^7 to 10^8 years may not be unreasonable because this is the time scale for the weathering removal of a 1-bar CO_2 atmosphere on Mars, and thus a loss of greenhouse warming (Pollack *et al.* 1987). In addition, if a major fraction of the amino acids in frozen martian soils were actually dissolved in

interfacial water, which can exist down to temperatures of roughly 200 K (Rietmeijer 1985), then significant racemization could have again taken place in $<10^8$ years even at the present day mean daily temperature (215 K) at the martian equator. Wet conditions persisting for a relatively short period of geologic time could thus essentially erase a homochiral signature of extinct martian life. This scenario would make it difficult to ascertain whether any amino acids detected by future Mars lander-based analyses were derived from extinct martian life or are abiotic in origin. A possible solution to this problem would be to evaluate the chirality of those amino acids not susceptible to racemization such as the α-dialkyl amino acids. However, as mentioned previously, these amino acids would be unlikely major components of an extinct martian biota based on their rarity in the proteins of terrestrial organisms. This problem might also be resolvable from the amino acid composition because the mixture of amino acids produced abiotically is distinctly different from that present in the proteins of terrestrial organisms and thus perhaps martian organisms as well.

Thus far, we have considered the preservation of a homochiral amino acid signature in very general terms with respect to the possible range of conditions on Mars. The times required for complete racemization of amino acids in the martian polar regions, where the present mean temperature is 150 K, would be much longer than for those near the equator. If the present temperature difference of 65 K between the poles and the equator is characteristic of that which has existed throughout most of the history of Mars, then even though the equatorial regions may have been wet at some time in the past, the poles would have been cold and dry, and thus amino acid homochirality preserved (again assuming frozen conditions are equivalent to dry conditions with respect to racemization). Although extinct martian life may have only existed in the wet equatorial areas, its characteristic homochiral amino acid signature could have been transferred to the polar regions by global dust storms (see Jones (1991)). Similar eolian transport processes today on Earth generates an amino acid signature derived from terrestrial life in polar ice (Bada *et al.*, in preparation). Although the ice caps on Mars may be fairly recent features, the subsurface soils in the polar regions would preserve the homochiral amino acid signature of extinct life on Mars even if extinction took place early in the history of the planet.

Besides the consideration of the preservation of homochiral amino acids derived from extinct martian life, amino acid racemization has another important implication. The rates of aspartic acid racemization and DNA depurination (the cleavage of the deoxyribose/adenine or guanine bond) have been shown to be very similar in aqueous solution at neutral pH (Bada *et al.* 1994). Depurination leads to DNA chain breakage and is thus a major mechanism for DNA degradation under geochemical conditions (Lindahl 1993). It has been recently suggested that the extent of racemization of amino acids can be used to assess geochemical DNA depurination rates and thus the degree of preservation of DNA in geologic environments (Bada *et al.* 1994). We can use our estimates of aspartic acid racemization on Mars to predict the survivability of DNA fragments of sufficient length to contain genetic information (>200 base pairs). The aspartic acid racemization results given in Table I and Fig. 1 indicate that in the dry, cold environments on Mars, where amino acid homochirality is best preserved, DNA preservation would also be optimal. The possible retrieval of genetic information from extinct martian life would have the best chance of success in samples from the polar regions.

CONCLUSIONS

An amino acid analytical system landed on Mars should be able to detect the homochiral amino acid signature from an extinct martian biota, even if this biota became extinct in the very early history of the planet, as long as there was no exposure to liquid water at >250 K for extended periods of geologic time after extinction. The best environment on Mars for both amino acid homochirality and nucleic acid genetic information to survive essentially intact is in the polar regions, regardless of when extinction occurred in the past history of the planet. If the conditions under which the homochiral amino acid signature has been preserved are sufficiently well known and can be adequately modeled, it may even be possible to derive an estimate of the time of extinction of the martian biota based on the racemization rates of the various detected amino acids. The fact that a homochiral amino acid signature could still be preserved from an extinct martian biota underscores the need for thorough spacecraft decontamination in order to exclude terrestrial biogenic amino acids which could generate misleading results from any future amino acid enantiomeric measurements carried out on Mars.

ACKNOWLEDGMENTS

This work was supported by a grant for a NASA Specialized Center of Research and Training in Exobiology at UCSD. We thank K. Kvenvolden and C. Sagan for helpful comments and an anonymous reviewer for pointing out that liquid water microlayers can exist at temperatures much below 273 K.

REFERENCES

BADA, J. L. 1985. Racemization of amino acids. In *Chemistry and Biochemistry of the Amino Acids* (G. C. Barrett, Ed.), pp. 399–414. Chapman and Hall, London.

BADA, J. L. 1991. Amino acid cosmogeochemistry. *Phil. Trans R. Soc. London B* **333**, 349–358.

BADA, J. L., AND R. A. SCHROEDER 1975. Amino acid racemization reactions and their geochemical implications. *Naturwiss.* **62,** 71–79.

BADA, J. L., X. S. WANG, H. N. POINAR, S. PÄÄBO, AND G. O. POINAR 1994. Amino acid racemization in amber-entombed insects: Implications for DNA preservation. *Geochim. Cosmochim. Acta* **58,** 3131–3135.

BULLOCK, M. A., C. R. STOKER, C. P. MCKAY, AND A. P. ZENT 1994. A coupled soil–atmosphere model of H_2O_2 on Mars. *Icarus* **107,** 142–154.

CHYBA, C. F., AND C. SAGAN 1992. Endogenous production, exogenous delivery and impact-shock synthesis of organic molecules: An inventory for the origins of life. *Nature* **355,** 125–132.

CRONIN, J. R., AND S. PIZZARELLO 1983. Amino acids in meteorites, *Adv. Space Res.* **3,** 5–18.

JONES, H. G. 1991. The chemical profiles of polar ice on Earth and Mars: What we read from the first; what we could read from the second. *Space Sci. Rev.* **56,** 43–57.

KANAVARIOTI, A., AND R. L. MANCINELLI 1990. Could organic matter have been preserved on Mars for 3.5 billion years? *Icarus* **84,** 196–202.

KLEIN, H. P., N. H. HOROWITZ, AND K. BIEMANN 1992. The search for extant life on Mars. In *Mars* (H. H. Kiefer, *et al.*, Eds.), pp. 1221–1233. Univ. of Arizona Press, Tucson.

LINDAHL, T. 1993. Instability and decay of the primary structure of DNA. *Nature* **362,** 709–715.

MCKAY, C. P., R. L. MANCINELLI, C. R. STOKER, AND R. A. WHARTON, JR. 1992. The possibility of life on Mars during a water-rich past. In *Mars* (H. H. Kiefer, *et al.*, Eds.), pp. 1234–1248. Univ. of Arizona Press, Tuczon.

MELOSH, H. J. 1988. The rocky road to panspermia. *Nature* **332,** 687–688.

MILLER, S. L. 1993. The prebiotic synthesis of organic compounds on the early Earth. In *Organic Geochemistry* (M. H. Engle, and S. A. Macko, Eds.), pp. 625–637. Plenum Press, New York.

MILTON, R. C. DEL., S. C. F. MILTON, AND S. B. H. KENT 1992. Total chemical synthesis of a D-enzyme: The enantiomers of HIV-1 protease show demonstration of reciprocal chiral substrate specificity. *Science* **256,** 1445–1448.

POLLACK, J. B., J. F. KASTING, S. M. RICHARDSON, AND K. POLIAKOFF 1987. The case for a wet, warm climate on early Mars. *Icarus* **71,** 203–224.

RIETMEIJER, F. J. M. 1985. A model for diagenesis in proto-planetary bodies. *Nature* **313,** 293–294.

SCHROEDER, R. A. 1974. Kinetics, mechanism and geochemical applications of amino acid racemization of various fossils. Ph.D. thesis, University of California at San Diego.