

The effect of ionizing radiation on the preservation of amino acids on Mars

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Abstract

Amino acids are excellent biomarkers in the search for life on Mars because they are essential for biology as we know it and they are robust enough to survive for billions of years in the cold and dry Martian environment. However, amino acids and other organic compounds on Mars are exposed to the ionizing radiation from space and from the decay of radionuclides. This process and its role in the preservation of organic compounds has not been adequately addressed in the past. Based on measured radiolysis constants of amino acids and radiation dose estimates for Mars we show that the detection of an amino acid signature derived from an early Martian biosphere is not limited by its radiolytic decomposition as long as the amino acids are shielded adequately from space radiation. This indicates clearly the need to access the Martian subsurface in the search for molecular traces of an extinct Martian biosphere.

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1. Introduction

Mars today is a cold, dry desert planet. However, the climate on Mars is thought to have been more clement in its early history during the late Noachian and the early Hesperian periods dating to more than 3 billion years ago [1,2]. Mars at that time could have been like the early Earth, which suggests a realistic prospect for a second, independent, genesis.

If life ever developed on Mars it is uncertain whether it could have survived the dramatic environmental

changes in the transition period from the late Noachian to the early Hesperian or whether life was driven to extinction by these changes. Amino acids, which are essential molecules in biochemistry and readily synthesized in pre-biotic experiments, are of significance in this respect [3]. Their handedness or chirality can be used to determine whether any detected amino acids are of abiotic or biotic origin. Although amino acids are readily destroyed by the UV radiation on the surface of Mars [4], they can be preserved for billions of years in the subsurface at ambient Martian conditions [5,6]. Therefore, amino acids are excellent biomarkers for pre-biotic chemistry and any biology that might have developed on Mars.

One aspect, however, that has escaped wider attention in the past is the effect of ionizing radiation

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on the long term preservation of amino acids and other organic molecules on Mars. The Martian surface is exposed to the radiation from space, whereas the deeper subsurface is only exposed to the radioactive decay of radionuclides. We measured the radiolysis constant of several amino acids and determined the limits for their preservation in the Martian near- and subsurface depending on the exposure to these two radiation regimes. These results have implications for the search strategy for traces of life on Mars.

2. Materials and methods

2.1. Samples

The amino acids L-aspartic acid, L-glutamic acid, glycine, L-alanine and γ -amino-*n*-butyric acid (γ -ABA), as well as methylamine and ethylamine were obtained commercially from Sigma-Aldrich and used without further purification. All glassware used was washed with double distilled water (ddH₂O) and annealed at 500 °C for 3 h. Pyrex glass vials (inner diameter 4 mm, wall thickness 1 mm) were filled with weighed 20 mg aliquots of solid amino acid powder. Radiolytic products in the atmosphere above the sample (e.g., ozone) can potentially induce some indirect radiation effect through its chemical reactivity. Therefore, the glass vials were exposed to a nitrogen atmosphere overnight and flame sealed under constant nitrogen flow to avoid any adverse effect from ozonolysis.

2.2. Irradiation

The samples were irradiated with γ -rays from a ⁶⁰Co source at a rate of 2 Gray (Gy)/s, with a total dose of 0.5–5.42 × 10⁶ Gy (10⁶ Gy = 1 MGy) at the NASA Jet Propulsion Laboratory Radiation Effects Group. Samples were exposed to a radiation dose of 0.50, 1.00, 2.05, and 5.42 MGy.

2.3. Analysis

The irradiated samples, non-irradiated reference samples, and standards of γ -ABA, methylamine and ethylamine were analyzed in parallel. The irradiated vials were quickly cooled down in a liquid nitrogen bath before the top was scratched and cracked open with a graphite cutter. The cooling of the vials was necessary to reduce the pressure produced by the volatile products of the radiolysis in the glass vials. The amino acid powder of the individual vials was transferred to separate glass vials filled with 10 ml of double distilled water. All samples were diluted by a factor of 1:1000. Aliquots of 10 μ l were used to

analyze the amino acids and their enantiomeric ratios by high performance liquid chromatography (HPLC) using derivatization with *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) and UV-fluorescence detection [7]. The OPA/NAC solution was prepared by dissolving 4 mg of OPA in 300 μ l methanol, and then adding 250 μ l 0.4 M sodium borate buffer (pH 9.4), 435 μ l ddH₂O, and 15 μ l 1 M NAC. A 10 μ l aliquot of the amino acid sample or standard was mixed with 10 μ l 0.4 M sodium borate buffer and 5 μ l of the OPA/NAC reagent in an Eppendorf tube. The reaction was quenched with 475 μ l of 50 mM sodium acetate buffer (pH 5.5). Peaks were identified by comparison of retention times with known standards.

3. Results

3.1. Amino acid experiments

We measured the radiolytic decomposition of dry amino acids after irradiation with γ -rays. Radiolysis can be described by a simple exponential function:

$$\ln(N/N_0) = -kD$$

where N is the amino acid abundance after radiation, N_0 the amino acid abundance prior to radiation, k the radiolysis constant in MGy⁻¹, and D the radiation dose in MGy. The radiolysis constants for the individual amino acids were obtained from the slope of a semi-log plot of N/N_0 versus D at four different radiation dose levels, inducing a maximum decomposition of 30–60%. Table 1 shows the calculated radiolysis constant and the D_{10} dose ($N/N_0=0.1$) for L-aspartic acid, L-glutamic acid, glycine, and L-alanine.

The data in Fig. 1 indicate that the radiolysis constant scales with the molecular weight of the various amino acids. A similar dependence is used to determine the volume and molecular weight of enzymes from radiation inactivation using target theory [8,9]. The two scales of amino acids and enzymes do not match because of the more complex energy dissipation in larger molecules [10].

γ -ABA and ethylamine, decarboxylation products of glutamic acid and alanine, were identified in samples

Table 1
Amino acid radiolysis constant k (10⁶ Gray⁻¹ = MGy⁻¹)

Amino acid	Molecular weight [g/mol]	k [MGy ⁻¹]	D_{10} [MGy]
L-aspartic	133.12	0.160 ± 0.043	14
L-glutamic acid	147.15	0.1723 ± 0.0118	13
Glycine	75.08	0.0673 ± 0.0172	34
L-alanine	89.11	0.1127 ± 0.0087	20

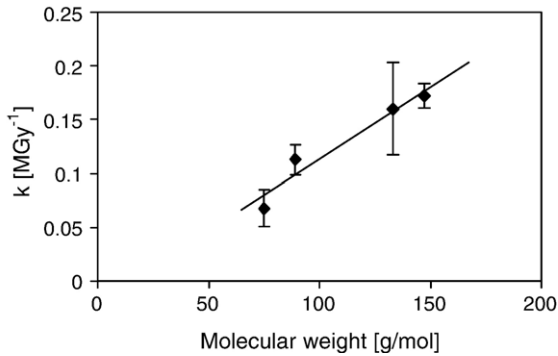


Fig. 1. Linear dependence of the radiolysis constant of amino acids from their molecular weight ($y=0.0014x-0.0223$).

after irradiation with 5.42 MGy. No methylamine, a decarboxylation product of glycine, was detected. Most of the decomposition products are volatiles. No effort was made to detect these volatile products.

3.2. Radiation conditions on Mars

To put the measured radiolysis constants of amino acids in the right context, we have to describe the radiation environment on Mars, starting with radiation from radioactive decay.

The abundance of potassium, thorium and uranium on Mars is about two orders of magnitude smaller than on Earth. This reflects the lower degree of differentiation of the Martian crust. Based on the SPB (Shergotti Parent Body) model, the abundance of potassium, thorium and uranium is 315, 5.6×10^{-2} and 1.6×10^{-2} ppm respectively [11]. The isotopic abundance today is 0.0117% for ^{40}K , 100% for ^{232}Th , 0.7200% for ^{235}U and 99.2745% for ^{238}U . The decay energy corrected for neutrino loss is 0.71 MeV for ^{40}K , 39.9 MeV for ^{232}Th , 47.1 MeV for ^{235}U and 44.2 MeV for ^{238}U [12]. The dose rate is proportional to the concentration of the radionuclide, the decay energy and the energy release per decay:

$$D[\text{Gy/yr}] = \frac{c[\text{ppm}] \times E[\text{MeV/decay}] \times \lambda[\text{yr}^{-1}] \times 6.022 \times 10^{23}}{A \times 6.24181 \times 10^{15}}$$

where D is the dose rate, c is the concentration of radionuclides, E is the energy per decay corrected for neutrino loss in beta particle decay, λ is the decay constant, 6.022×10^{23} Avogadro's constant in mol^{-1} , A is the atomic mass, and the conversion factor for Gray is 6.24181×10^{15} eV/g. Taking into account the changing isotopic abundance from radioactive decay over time, the dose rates ranges from 350 $\mu\text{Gy/year}$ 3 billion years ago, to 130 $\mu\text{Gy/year}$ today. The total accumulated dose

over the last 3 billion years in the Martian subsurface is close to 740×10^3 Gy (740 kGy).

In addition to the omnipresent radioactivity on Mars, the surface is exposed to ionizing radiation from the Solar Energetic Particles (SEP) and the Galactic Cosmic Radiation (GCR). The surface of the Earth is partially protected from this kind of ionizing radiation by its magnetic field and especially by its atmosphere with a shielding depth of 1000 g/cm^2 . Mars has no substantial magnetic field [13], and its current atmosphere with a shielding depth of 16 g/cm^2 , compared to the penetration depth of most GCR protons of $\sim 160 \text{ g/cm}^2$, does not offer much of a protection from the high energetic particle radiation from space [14]. The SEP particles can only penetrate several centimeters into the Martian regolith. This depth of several centimeters is equivalent to the erosion horizon of Martian net deflation areas over several billion years [15]. Therefore, we focus on the GCR particle radiation, which has energies up to GeV and can penetrate to depths of several meters. The GCR field on Mars is derived from results of theoretical calculations describing the free space radiation attenuation of the GCR behind Martian regolith [16]. With a regolith density of 2.6 g/cm^3 [17], the depth dependent dose rates range from 200 mGy/year at the surface to 0.6 mGy/year at 3 m depth. The accumulated dose over 3 billion years within the first meter is on the order of 200–800 MGy.

4. Discussion

The maximum radiation dose used in our experiment was chosen because it represents the expected radiation dose in the Martian subsurface, and it is on the same order of magnitude as the D_{10} value. The radiation model for Mars provides the physical radiation dose taking into account the fragmentation of ions and the electromagnetic cascade. Therefore, the radiolysis constants determined with sparsely ionizing radiation can be used to evaluate the radiolysis in the mixed radiation environment on Mars. The specific amino acids were chosen because they can be analyzed with the required sensitivity and represent a good fraction of all amino acids that can either be produced in pre-biotic experiments or are a major component of living organisms.

Bacterial cells contain about 50% amino acids by dry weight. We estimate an amino acid concentration of 1.15×10^{-13} – 13 g/cell based on an average dry cell weight of $2.3 \times 10^{-13} \text{ g}$ [18]. Comparing the accumulated dose from the ionizing radiation of radionuclides in the Martian subsurface since the Hesperian, about 3 billion years ago, with the measured radiolysis constant of dry glutamic acid of 0.1723 MGy^{-1} results in a 12% decomposition.

Glutamic acid being one of the largest amino acids in bacterial cells, represents a conservative scenario. Therefore, it would be possible to detect the amino acid composition derived from the equivalent of 10^3 cells/g of soil assuming a detection limit for individual amino acids of 0.01 ppb [19]. For comparison, the concentration of cells in terrestrial permafrost regions is in the range of 10^2 – 10^8 cells/g [20]. Thus, the preservation of amino acids in the Martian subsurface is practically not limited by its radiolytic decomposition caused by natural radionuclides.

The situation, however, is very different for the uppermost meters of the Martian subsurface, which are dominated by the effect of space radiation. Any newly deposited material on the surface of Mars is basically dust that has been produced by erosion during the Noachian and to a lesser extent Hesperian. This material is extensively processed in the atmosphere by UV-radiation and the SEP during redistribution processes and is therefore of little interest in the search for chemical traces of Martian life. Coincidentally, the old surfaces that are accessible by in situ exploration have been exposed to the GCR for the better part of Martian history. Notable exceptions are previously buried and recently excavated old surfaces.

Fig. 2 shows the survival fraction of amino acids derived from bacterial cells in the Martian subsurface as a function of depth with time. An average amino acid radiolysis constant of 0.113 MGy^{-1} was used for better illustration. This average radiolysis constant was calculated based on the measured amino acid radiolysis constants and the distribution of amino acids in a typical bacterial cell of 15–30% for major amino acids. To detect the amino acid signature of life that became extinct in the Hesperian, about 3 billion years ago, it is necessary to drill to a depth of 1.5 to 2 m (equivalent to a shielding depth of 400 to 500 g/cm^2) depending on the initial abundance. To be able to detect any remnants in the uppermost half meter of the Martian subsurface, the extinction or excavation event would have to be younger than 100–500 million years. This is in agreement with a more general statement for the radiolysis of organic molecules [21].

A denser atmosphere on early Mars would have provided better shielding of the surface from space radiation. At the same time it would have allowed the presence of liquid water. If wet conditions at any time after the extinction of life persisted for only millions of years, the amino acids associated with life would be destroyed independent of any radiation effect [22]. Therefore, all the arguments presented above are based on the assumption that thermal decomposition of amino acids can be considered as negligible. This seems to be a reasonable assumption as it is unlikely that life became extinct before the permanent disappearance of liquid

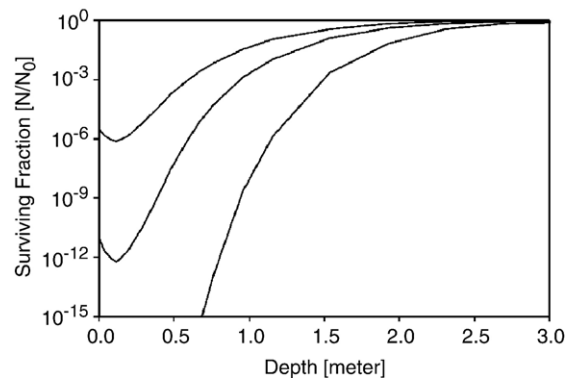


Fig. 2. Depth dependent surviving fraction of amino acids after being exposed half (top), one (middle), and three billion years (lower curve) to the ionizing radiation in the Martian subsurface. The current detection limit of 0.01 ppb per amino acid would allow a 2 to 6-log reduction until any amino acid signature of life became undetectable.

water, considering the endurance of cryptoendolithic microbial ecosystems here on Earth [20].

There might also be a certain meteoritic amino acid contribution to the Martian soil [23,24]. The organic molecules in the fine interplanetary dust contribution is effectively destroyed by the UV radiation [4], while in larger pieces they are exposed to the harsh SEP [24]. This SEP induces a radiation dose of 0.6 to 0.7 Gy/yr [21], in addition to the GCR of about 0.2 Gy/yr [16] on centimeters sized objects on the surface. Assuming an average carbonaceous chondrite density of 2.5 g/cm^3 [25], an individual amino acid abundance in the range of 1 ppm [26,27], and an average amino acid radiolysis constant of 0.1205 MGy^{-1} for carbonaceous chondrites allows for a decomposition in the range of 10^{-5} until reaching the detection limit of 0.01 ppb. Although the chemical and physical weathering processes on Mars are likely in the range of billions of years [24], it is concluded that the radiolytic decomposition of the organic constituents of carbonaceous chondrites exposed on the Martian surface takes place on a timescale of 100 million years. Thus, any meteorites on the Martian surface older than this would likely be devoid of detectable amino acids. Only those meteorites mixed into the regolith to depths of greater than a few meters would still have preserved amino acids.

5. Conclusions

Ionizing radiation from space affects the long-term preservation of amino acids associated with extinct Martian life or meteorites. This is particularly severe in the first meter of the Martian subsurface. However, below a shielding depth from space radiation of 400–

500 g/cm², amino acids will not be degraded substantially due to the relatively low radiation dose from radioactive decay. These results show clearly the need to access the Martian subsurface in the range of meters in the search for biomarkers of extinct Martian life.

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