

Photochemical stability of biomolecules in the experiments modelling Martian surface conditions

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Abstract: Life has probably existed on Earth for about 3.5 billion years and ever since people could wonder, they have tried to understand its origins. It is thought that organisms as complex as bacteria emerged within 0.5–1 billion years. Organic molecules, such as amino acids, organic acids and sugars have been observed in material from extraterrestrial sources in the solar system. But, to confirm the possibility of nucleic acid bases also being synthesized under these conditions, we have replicated the synthesis of monophosphates as dry pellets (5'-monophosphates, the predominant reaction products) under simulated Martian conditions. Our research task was to find out whether the main organic substances were able to survive in the absence of water. This reaction must proceed under solvent-free conditions and incident short ultraviolet radiation (UVC) in a vacuum. After 5 months of reaction time monophosphates could be identified in detectable quantities. These experiments utilized basalt and limonite as protectors and successfully shielded the products from decomposition by UVC. Our team has obtained more results concerning prebiotic synthesis of polypeptides and nucleotides in the presence of meteorite dust; these are the same kind of experiment, but using different extraterrestrial material.

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Introduction

Cosmic delivery of basic molecules to the Martian surface seems quite plausible for many reasons. The high percentage of organic components in carbonaceous chondrites provides biological building blocks brought by space carriers such as meteorites and cosmic dust. The amount of micrometeorites (from 50 to 500 μm in size) is from 50 to 100 tons daily, while the meteorite flux is equal to 0.03 tons per day (Maurette 1998). A certain energy supply is needed to start and sustain the chemical processing of molecular material. At the present time the Martian atmosphere consists mainly of CO_2 (95.3%), which reduces the short-wavelength ultraviolet (UVC, 190–280 nm) flux reaching the ground. Solar electromagnetic radiation with a wavelength below 190 nm no longer penetrates through the CO_2 atmosphere to the Martian surface (Cockell 2002). Only the present planetary conditions are considered, so one should bear in mind that the ancient solar luminosity and atmospheric carbon dioxide content may have caused a different response in protobiomolecules. Solar UV radiation has been found to be the most deleterious factor in space. The reason for this high biological efficiency is the highly energetic UVC that is directly absorbed by the genetic material of the cells, notably the DNA (Horneck *et al.* 1995). The magnetic field surrounding the Earth deflects this radiation, but Mars does not have such a field.

The UV flux on the surface of Mars is significant only in the near (300–400 nm) and far (200–300 nm) UV because of absorption and scattering in the Martian atmosphere. Short-wave radiation (200–280 nm) must be considered in any extraterrestrial situation when looking for the possibility of sustaining life (Patel *et al.* 2003). High-intensity UV environments, especially in combination with certain surface environments, can place restrictions on the survival of biological molecules, and can strongly influence their evolution. Radiation at a wavelength of 254 nm is capable of interacting directly with molecular structures. In biological systems UVC radiation causes temporary or permanent alterations that result from photochemical reactions of UVC with different biological target molecules, the so-called chromophores (Rettberg & Rothschild 2002). The absorbing parts of DNA are the bases, the purine derivatives adenine and guanine, and the pyrimidine derivatives thymine and cytosine. There is an absorption maximum at 260 nm and a rapid decline towards longer wavelengths.

High radiation levels suggest that any extraterrestrial life would have little chance of surviving unless it were shielded beneath the dusty, cold surface of the planet. This has resulted in the ability of biomolecules to resist degradation near the Martian surface. In the search for traces of life it is therefore sufficient to focus on and near the upper soil layer. These results agree with our previous experiments

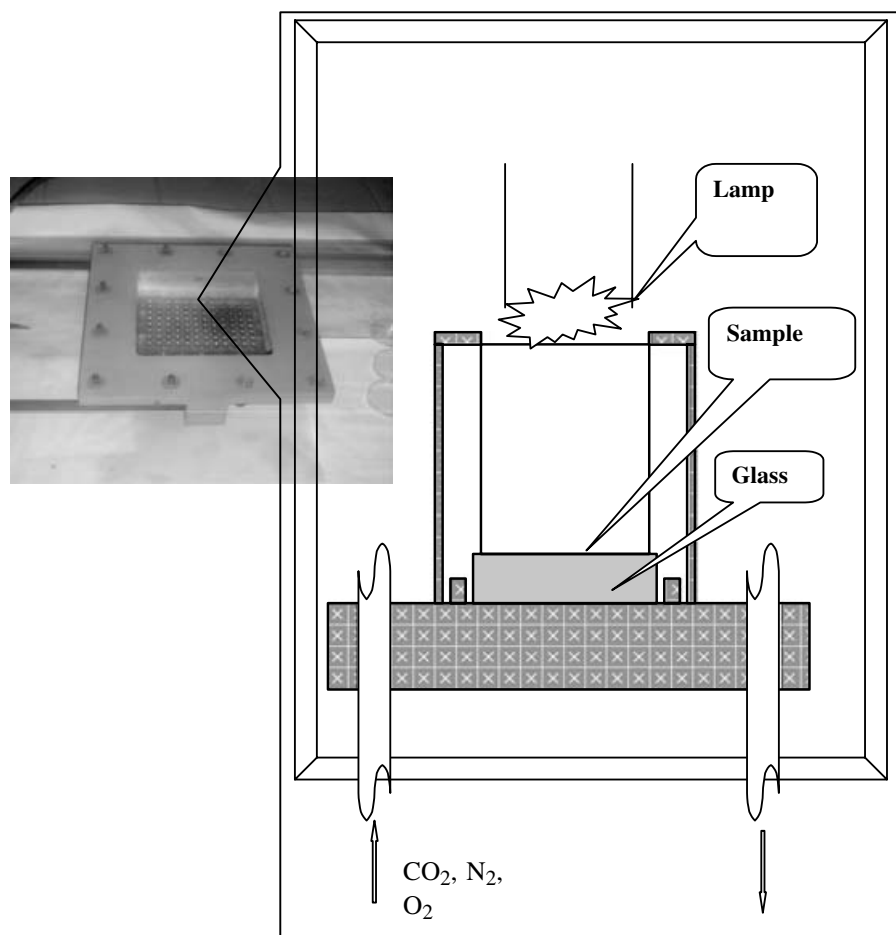


Fig. 1. Experimental chamber for the irradiation of dry samples containing water-free samples with and without mineral inclusions.

where minerals of extraterrestrial origin such as the Allende and Murchison meteorites as well as lunar soil were tested with respect to their decay and biological synthesis under similar conditions (Kuzicheva & Gontareva 2002).

Materials and methods

Our experimental dry pellets were exposed to the UVC (254 nm) radiation that is abundant on Mars at the present time. Mineral pellets impregnated with biological molecules were used to approach the model of Martian surroundings. Basalt and limonite were taken as an analogous material obtaining parameters similar to those of Martian soil. A mercury lamp having a capacity of 6 mW cm^{-2} ($2.16 \times 10^3 \text{ J m}^{-2} \text{ h}^{-1}$) was taken as a source of radiation. The irradiation flux was measured by a UVX digital radiometer. Samples containing $2 \mu\text{M}$ of nucleoside and phosphate were each loaded by dropping 300 μl of the stock sample solution on the cavity face and dried to afford a thin film. When a nucleoside was loaded with minerals, 150 μl of the stock sample solution was loaded, then covered by 3 mg of mineral powder and later the rest of the stock solution was added. Water solutions were evaporated to deposit dry pellicles on the experimental cavity surface. The optical density

of 1 cm^2 experimental samples was determined to obtain the full adsorption of the 254 nm wavelength radiation. Dry pellicles were placed into the experimental chamber with a CO₂-enriched atmosphere (see Fig. 1). After the irradiation, samples were retrieved in bi-distilled water (1 ml), filtrated to get rid of mineral contamination and analysed employing high-performance liquid chromatography (HPLC) and mass-spectrometry techniques.

HPLC using an Alliance instrument (Waters, Milford, MA) and a Symmetry C18 column ($3.9 \times 150 \text{ mm}^2$) was employed for the separation, identification and quantification of the products. The elution buffer for analysis consisted of 0.005 M KH₂PO₄ (pH 2.5). The reaction mixtures for adenine derivatives consisted of the following: a 95:5 (vol/vol) mixture of methanol and a 0.05 M KH₂PO₄ solution. The flow was set to 1 ml min^{-1} and the detector wavelength to 254 nm. The sample volume was 25 μl .

A Finnegan MAT 95 XL spectrometer with electro-spray ionization and magneto-sector double-focus mass analyser was employed for obtaining mass spectra. Both irradiated samples and their individual HPLC fractions were analysed. Dried samples were dissolved in 50:50 (vol/vol) mixture of 0.2% CH₃COOH and acetonitrile. A sample volume of 20 μl with 1–5 $\mu\text{M ml}^{-1}$ concentration quantified by the peak

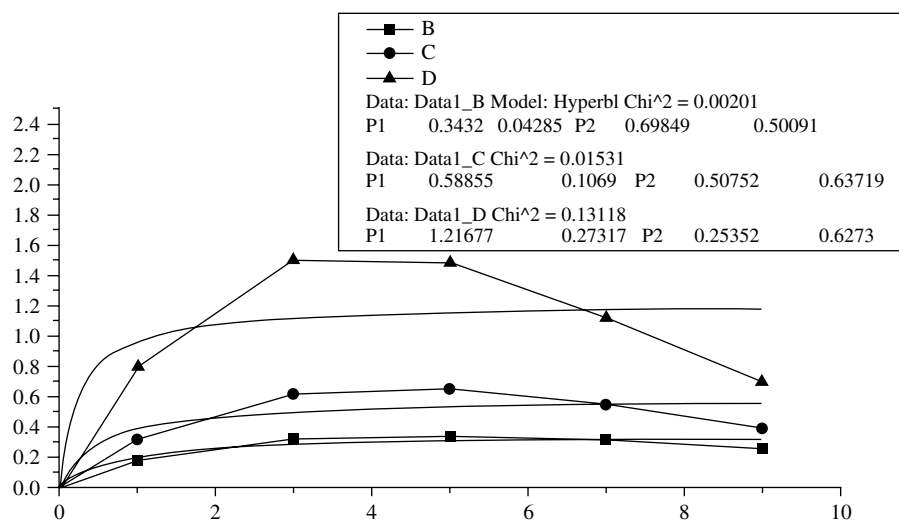


Fig. 2. Formation of 5' AMP from an adenosine + phosphate mixture after UV (254 nm) irradiation of dry pellicles containing limonite (D), basalt (C) or mineral-free samples (B). X-axis, irradiation time (months); Y-axis, amount of 5' AMP as a percentage of the initial amount of adenosine. The smooth curves reproduce the results of computer approximations using experimental data (hyperbolic).

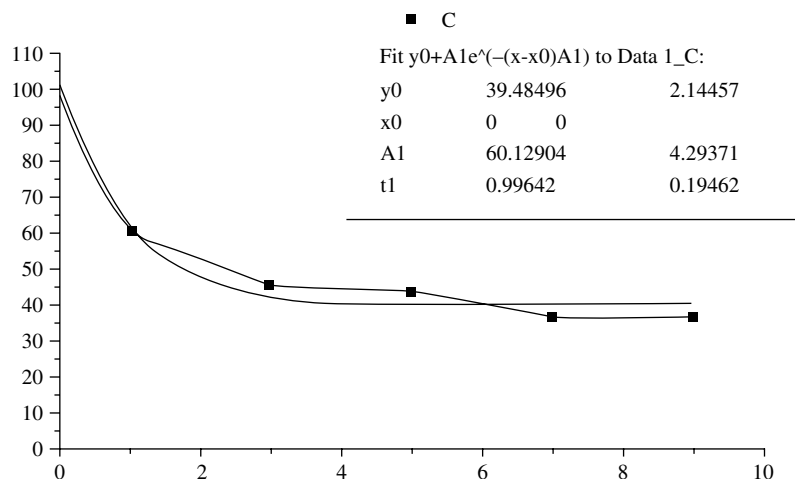


Fig. 3. Amount of adenosine remaining in the reaction mixture after irradiation by UV (254 nm) within 9 months. X-axis, irradiation time (months); Y-axis, percentage of the initial amount of adenosine remaining. The smooth curve reproduces the results of computer approximations using experimental data (exponential decay).

area was injected into the device at a rate of 1 ml min^{-1} . Monoisotopic m/z rates were measured with an accuracy of 0.1 %.

Results

The data obtained after applying mass-spectrometry and chromatography techniques on the samples revealed that the presence of basalt and limonite beds stabilized degradation processes that occurred within the radiation period and hence increased the reaction yield. Further calculations made to extrapolate the experimental results to a more extended time period suggested that organic molecules could possibly survive long-duration exposure to UVC radiation.

Both type of experiments (synthesis induced by UVC (145 nm) and UV (254 nm)) revealed the possibility for bioorganic molecules to survive a long-duration destructive environment and to form new derivatives significant for further evolution processing. Our results demonstrate the role of certain environmental factors in the process of nucleoside survival and nucleotide formation. Different types of gases were tested with respect to their influence on this process. An atmosphere enriched with carbon dioxide turned out to be the most favourable one for stability of initial molecules and their derivatives with the formation powered by UVC. The addition of mineral beads to the reaction mixture and further irradiation of mineral-containing pellicles resulted in higher stability of the initial substances and an increase of the derivative yield.

A set of products was obtained after irradiation of dry pellets containing adenosine and an inorganic phosphate mixture. Mineral beads were added in order to reveal a possible influence of basalt and limonite on the molecular stability and formation of the derivatives. Identification of the products was carried out primarily by HPLC analysis and by comparison of UV spectra with those of known values for 5' AMP, 3' AMP and 3'5' cAMP. A product with a UV spectrum typical for 5' AMP was obtained in most cases in amounts exceeding those from other derivatives. The equilibrium rates obtained within 9 months of extensive UVC irradiation and estimates from the reaction plot are given in Fig. 2.

The initial amount of adenosine in the post-reaction mixture was considerably in excess of the mineral-free samples. The protective effect was found to be dependent on the nature of the mineral. In most cases limonite contributed to the stability of adenosine more than basalt (see Fig. 3). The presence of basalt dust in the reaction pellicles did increase the yield as well, but to a smaller extent. The UVC radiation flow on Mars is $10^5 \text{ J m}^{-2} \text{ h}^{-1}$ (Young *et al.* 1965). The radiation flow of our lamp was of the same order of magnitude ($2.16 \times 10^5 \text{ J m}^{-2} \text{ h}^{-1}$). The energy of the radiation promotes both synthesis and decomposition of the organic material, delivered by cosmic carriers such as meteorites. For instance, the amount of amino acids is $1.14 \times 10^{-12} \text{ g m}^{-2} \text{ h}^{-1}$, corresponding to 0.001% of the total organic matter brought by interstellar dust and micro-meteorites (Maurette 1998).

Conclusion

Based on the averages obtained at the various landing sites of Viking Landers 1 and 2, Phobos 2 and Mars Pathfinder missions, the regolith composition is about 1.6 g cm^{-3} (De Angelis *et al.* 2003). The estimated average density of our experimental pellicles is 0.2 g cm^{-3} . For organic samples mixed with mineral powder, mechanical protection should play a significant role. A film of powder thicker than $5 \mu\text{m}$ ensures good sample protection against UV radiation (Barbier *et al.* 2001). In the case of our experimental pellets, its transverse dimensions vary from 5 to $20 \mu\text{m}$, which is enough for at least partial coverage of prebiotic material.

The catalysis of phosphodiester bond formation is ascribed to the orientation of adsorbed monomers and the acidity of the surface. It was shown that electrostatic interaction of the protonated base with the negatively charged surface of the clay is an important factor in the adsorption of adenine nucleotides to Na^+ montmorillonite (Ferris *et al.* 1989). The difference in catalysis observed with different clays can be related to the extent of binding to the clay and the

acidity of the surface. The bases are planar molecules that can tessellate a surface in two dimensions and are stabilized by the interaction with the underlying surface and by hydrogen bonds with adjacent molecules (Sowerby *et al.* 2001).

Although the minerals studied here may not be considered dominant prebiotic materials, uncharged mineral surfaces could facilitate the accretion of organic matter. It seems quite plausible that organic molecules associated with certain minerals are stable enough to survive in Martian conditions and thus might be found on the Martian surface. Since organic molecules are much more resistant than bacteria under strong radiation conditions, future missions to explore exobiology sites of interest can be also targeted in order to reveal possible biomarkers such as nucleosides and their main derivatives.

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