Abiotic Synthesis of Uracil from Carbon Monoxide, Nitrogen and Water by Proton Irradiation

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(Received June 9, 1997; CL-970437)

A mixture of ^{13}C-carbon monoxide, nitrogen, and water was irradiated with high energy protons. ^{13}C-Labelled uracil was identified in the product by GC/MS, resulting in confirmed abiotic formation of uracil, one of the RNA bases.

The discovery of ribozymes[1] has led to formulation of "the RNA world" hypothesis:[2] that RNA molecules played not only an informational, but also a catalytic role in the origin of life. The question then arises as to the origin of RNA molecules on the primitive earth. If RNA molecules could not be formed on primitive earth or on other bodies, the RNA world hypothesis would be analogous to a house built on sand. Numerous studies have been conducted regarding abiotic formation of constituents of proteins, *i.e.*, amino acids.[3] Although a number of attempts have been made to abiotically synthesize components of nucleic acids, whether or not these components could have been formed abiotically on the primitive earth is still unknown.

In the majority of previous studies on abiotic synthesis of RNA bases, intermediates possibly present on the primitive earth were used: *e.g.*, Adenine was formed from hydrogen cyanide,[4] and cytosine was formed from cyanoacethylene and cyanate[5] as well as from cyanoacetaldehyde and urea.[6] However, it has not been determined conclusively as to whether or not the intermediates used in those studies have been present in the primordial ocean at high concentration. Thus it is necessary to examine the possible formation of nucleic acid bases from possible components of primitive atmospheres. Several attempts have been made to demonstrate possible formation of bases from primitive atmospheres: Adenine was reported to be formed from a mixture of methane, ammonia, and water subjected to electron irradiation[7] or spark discharge;[8] and cytosine from a mixture of methane, nitrogen, ammonium ion, and water subjected to spark discharge.[9]

These results have been criticized from several points of view: (i) They used methane as a carbon source, but at present it is believed that earth's primitive atmosphere was less reducing than believed previously and that its major carbon compounds in it was carbon monoxide or carbon dioxide.[10] (ii) Indigenousness of nucleic acid bases in the products was not demonstrated conclusively, whereas that of amino acids could be confirmed by examining their D/L ratio. The present results convincingly demonstrate, for the first time, the possibility of forming one of the bases of RNA from a "mildly-reducing type" primitive atmosphere.

In the present experiment a mixture of carbon monoxide, nitrogen, and water was irradiated with high energy protons, a predominant consituents of cosmic rays, to examine possible abiotic formation of nucleic acid bases in simulated primitive earth environments. ^{13}C-Carbon monoxide was used as a source of carbon in order to discriminate abiotically-formed bases from those originating from contamination of the laboratory environments.

A gas mixture of carbon monoxide (350 Torr; UHP grade, Toho Sanso Co., Japan) and nitrogen (350 Torr; UHP grade, Toho Sanso Co., Japan) was enclosed in a glass tube containing liquid water (20 mL). In some iterations of the experiment, ^{13}C-enriched carbon monoxide (ISOTEC Co., U.S.A.; ^{13}C comprises 99.3% of total carbon on a mol basis), was used in place of ordinary carbon monoxide. Hereafter ordinary CO is referred to as ^{12}CO and ^{13}C-enriched CO is referred to as ^{13}CO. The gas mixture was irradiated with protons, each having an energy of 2.5—3.0 MeV, using a van de Graaff accelerator (Tokyo Institute of Technology) at 297 K. Irradiation was continued for 1—3 hours, during which time the gas mixture was irradiated with 1.3—7.6 × 10^{22}eV of total energy. A procedural blank was made with all the starting materials and all the experimental procedures but irradiation.

An aqueous solution of the irradiated product was separated into two fractions using cation-exchange high performance liquid chromatography (HPLC): Uracil and thymine are eluted around the void volume (Fraction A), and other bases are eluted later (Fraction B), if there are these bases in the sample. Each fraction was concentrated using a rotary evaporator, and then was separated by reversed-phase HPLC. A sharp peak appears at retention time corresponding to that of uracil, while no peak is found at the retention time corresponding to thymine. Fraction A of the procedural blank yielded neither uracil nor thymine. In the chromatogram of Fraction B, no peaks were found at retention times corresponding to adenine, guanine and cytosine. The eluent corresponding to uracil (hereafter referred to as Fraction U) was collected, desalted and dried.

In order to produce a derivative suitable for GC/MS analysis, a 2:1:1 mixture of bis(trimethylsilyl)trifluoroacetamide (BSTFA), acetonitrile, and dichloromethane was added to Fraction U.[11] Fraction U yielded a mass spectrum identical to that of natural $(TMS)_2$-uracil: When ^{12}CO was used as a source of carbon, m/z= 256 and 241 correspond to M^+ and $(M\text{-}CH_3)^+$ peaks of $(TMS)_2$-uracil, respectively as shown in Figure 1(a) and (b). Other fragment ions in both spectra also agreed each other. When ^{13}CO was used as a starting material, the peaks at m/z= 256 and 241 were diminished and peaks at m/z=260 and 245 appeared, as shown in Figure 1(c). In each case the difference of mass, 4, corresponds to the number of carbon atoms in uracil molecule. These results indicate the presence of indigenous uracil in the products of irradiation.

Uracil was determined using GC/MS after 10 nmol of interenal standard natural $^{12}C_4$-uracil was added to the proton irradiation product of a mixture of ^{13}CO, N_2 and H_2O. Based on the intensity ratio of m/z = 260 to m/z = 256, the total yield of uracil was calculated to be 18.3 ± 3.8 nmol, and the G-value (number of formed molecules per 100 eV) of uracil was determined to be 1.8 × 10^{-5}, which is about 1/1000 that of glycine. Concentrations of uracil[12] and glycine[13] found in the Murchison Meteorite were reported to be 0.56 nmol/g and 80

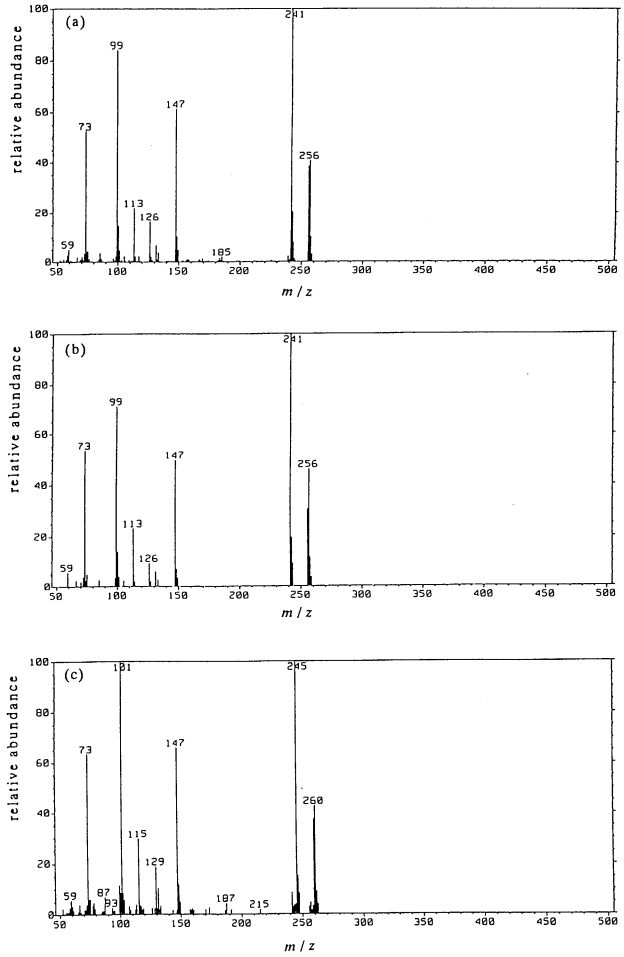

Figure 1. Mass spectra of uracil derivatives. (a) Natural $(TMS)_2$-$^{12}C_4$-uracil. (b) TMS derivatives of a "uracil fraction" of the proton irradiation product of ^{12}CO, N_2 and H_2O. (c) TMS derivatives of a "uracil fraction" of the proton irradiation product of ^{13}CO, N_2 and H_2O. Instrument: a JEOL JMS-AX 500 mass spectrometer with a Hewlett Packard HP5980A gas chromatograph. Column: Supelco SPB-1 (0.25 mm i.d. × 30 m).

nmol/g, respectively. It can be inferred that amino acids can be formed in greater abundance than nucleic acid bases in the course of chemical evolution - in simulation experiments or in parent bodies of meteorites.

The formation pathway of uracil in the present experiment was different from those proposed by Ferris et al.[5] nor Robertson and Miller[6], since (i) according to their proposed reactions, uracil was formed as a result of hydrolysis of cytosine; (ii) GC/MS did not indicate the presence of cyanoacethylene in the irradiation product of the present experiment, although its presence was detected in the product of a mixture of methane, nitrogen, and water subjected to spark discharge; (iii) only a trace amount of urea was detected in the irradiation product of the present experiment. Further studies are required in order to determine the reaction pathway of uracil formation.

Until high energy particles lose their kinetic energies they would induce the formation of organic compounds. This seems to be the major mechanism through which organic compounds such as amino acids can be formed much more efficiently using proton irradiation as opposed to other energy sources such as spark discharge or ultraviolet light.[14] It is suggested that not only amino acids, but also uracil could be formed from primitive terrestrial atmosphere by the action of cosmic ray.

The other RNA bases; cytosine, adenine and guanine, were not detected in the product. Uracil is the least reduced base (O /H ratio = 0.5) among the four RNA bases. Most promising idea would be that more reduced bases were formed in more reducing environments such as interstellar dust environments:[15] The possibility of abiotic formation of RNA bases under possible primitive terrestrial or extraterrestrial condition, a minimal condition for construction of the RNA world, constitutes a key subject of research.

The authors wish to express their gratitude to Mr. K. Kawasaki of Tokyo Institute of Technology for his kind assistance in operating of the accelerator. They are indebted to Mr. T. Kaneko and Dr. Y. Yokoyama of Yokohama National University for their kind assistance in analyzing reaction products. This work was supported in part by a Grant-In-Aid from the Ministry of Education, Science and Culture, Japan (No. 07640653).

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