

# Cytosine and Uracil Synthesis by Quenching with High-Temperature Plasma

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Received March 23, 1999

**Abstract:** Cytosine and uracil, which are components of RNA molecules, were obtained from complex organic matter produced by quenching a CO–N<sub>2</sub>–H<sub>2</sub>O high-temperature plasma. The carbon yields of cytosine and uracil were 0.01% and 0.08%, respectively, which were almost as much as those of amino acids. It was understood that processes of high activation and rapid quenching, which may be found in meteoritic impact or lightning, are important for the formation of bioorganic compounds from the CO–N<sub>2</sub>–H<sub>2</sub>O gas mixture.

It is generally believed that in the origin of life, bioorganic compounds were formed from the atmospheric CO, CO<sub>2</sub>, N<sub>2</sub>, and H<sub>2</sub>O. Miller<sup>1</sup> reported that amino acids such as glycine and alanine were synthesized from a spark discharge in a CH<sub>4</sub>–NH<sub>3</sub>–H<sub>2</sub>–H<sub>2</sub>O gas mixture. However, the amino acid yield from the spark discharge in CO–N<sub>2</sub>–H<sub>2</sub>O was much smaller,<sup>2</sup> because the N≡N and the C≡O strong triple bonds have to be broken. Even if these triple bonds are broken, the N and the C atoms are more likely to combine with the O atoms than the N and the C atoms, unless the atmospheric C:O ratio exceeds unity.<sup>3</sup> For this reason, then, the CO and the NO molecules are more likely to form than the C–C and the C–N bonds needed for bioorganic compounds.

The evolution of RNA molecules is generally believed to have played an important role in the origin of life, because some RNA molecules have both informational and catalytic functions.<sup>4</sup> Kobayashi and Tsuji<sup>5</sup> reported that uracil was synthesized from the proton irradiation in CO–N<sub>2</sub>–H<sub>2</sub>O. The N≡N and the C≡O bonds are likely to have been broken because of the collision of protons that had a high energy of 2.5–3.0 MeV. The yield of uracil, however, was 1/1000 that of amino acids.

In this study, we thought that nucleic acid bases might be formed by inputting a large enough amount of energy to break the N≡N and the C≡O bonds and by quenching the atoms and the radicals before the CO and the NO molecules were formed.

A magneto-plasma dynamic arc–jet (MPD arc–jet)<sup>6</sup> was utilized for producing a high-temperature plasma and for quenching it. The details of this apparatus are outlined in a previous report.<sup>7</sup> The starting material was an equimolar gas

mixture of CO and N<sub>2</sub>, containing H<sub>2</sub>O at vapor pressure. The molar ratio of H<sub>2</sub>O in the CO–N<sub>2</sub> mixture was ~1%. The CO–N<sub>2</sub>–H<sub>2</sub>O gas mixture was highly activated by instantaneously inputting a large amount of electric energy, 180 J/ms. This pulsed plasma was thoroughly dissociated and was quenched by colliding it against a glass tube,<sup>8</sup> on which the complex organic matter was formed. After this pulsed discharge was repeated 5000 times, the complex organic matter recovered from the glass tube surface was acid hydrolyzed with 6 M HCl at 110 °C for 24 h to liberate nucleic acid bases.

The sample was desalted and separated into two fractions using a cation-exchange resin (AG50W-X8): Uracil and thymine are eluted near the void volume (fraction A), and the other bases are eluted later (fraction B), if there are these bases in the sample. The fraction A was separated by a reversed-phase HPLC with an ultraviolet detector set at 260 nm. A sharp peak was observed at the retention time corresponding to that of the uracil (Figure 1Aa). The eluent corresponding to the uracil was collected (fraction U). The fraction B was separated by a cation-exchange HPLC with the ultraviolet detector set at 260 nm. A sharp peak was observed at the retention time corresponding to that of the cytosine (Figure 1Ba). The eluent corresponding to the cytosine was collected (fraction C). No peaks were observed at the retention times corresponding to those of the other bases.

After being heated with bis(trimethylsilyl)acetamide at 150 °C for 30 min, fractions C and U were subjected to a GC/MS. Cytosine and uracil were very clearly detected.

A <sup>13</sup>C-enriched mixture of CO (<sup>13</sup>C, 99 atom %; <sup>16</sup>O 99.9 atom %), N<sub>2</sub>, and H<sub>2</sub>O was used as the starting material to test for possible contamination. The GC/MS results (Figure 2) showed that the peaks shifted for the number of carbons from authentic standards, which means that the cytosine and the uracil detected were formed from a CO–N<sub>2</sub>–H<sub>2</sub>O gas mixture, not from contamination.

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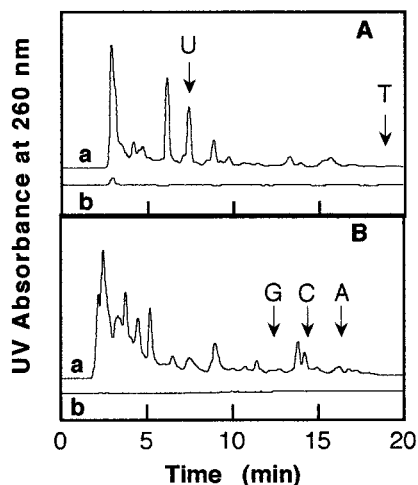
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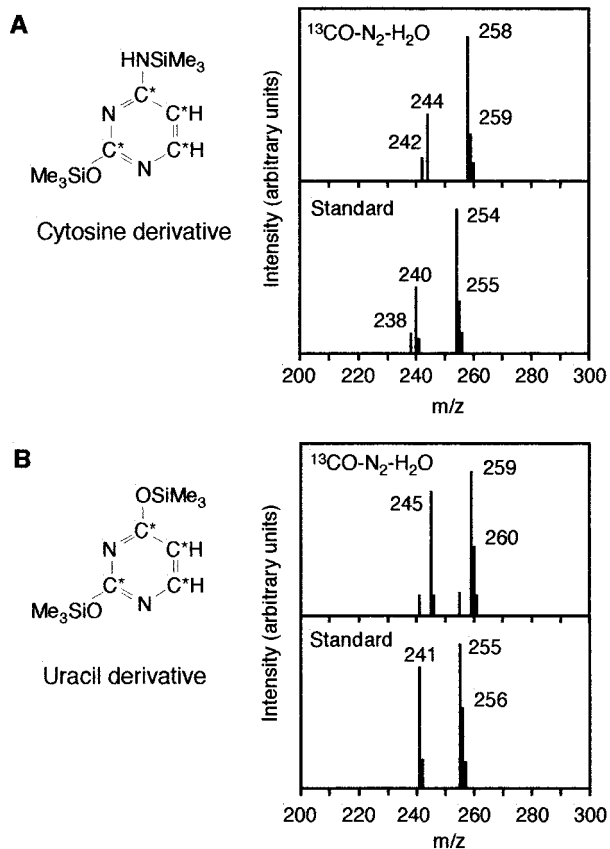
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**Figure 1.** HPLC chromatograms of (A) fraction A and (B) fraction B. Spectrum a is of the sample of the MPD arc-jet experiment. Spectrum b is of the sample of the spark discharge experiment. U, T, G, C, and A indicate the retention times corresponding to that of uracil, thymine, guanine, cytosine, and adenine, respectively. UV absorption at 260 nm was measured.



**Figure 2.** Mass spectra of (A) cytosine derivative and (B) uracil derivative. The upper values are for the  $^{13}\text{CO-N}_2\text{-H}_2\text{O}$  gas mixture and the lower values are for standard samples. Original carbons in cytosine and uracil are labeled with asterisks (\*). Masses of molecular ions of cytosine and uracil derivatives are 255 and 256, respectively. Me is the methyl group ( $-\text{CH}_3$ ).

Solid carbon yields of the cytosine and the uracil were 0.01% and 0.08%, respectively: The solid carbon yield is defined as

the percentage of carbon in products based on carbon in complex organic matter which was quantified by elementary analysis. Glycine, aspartic acid, alanine, and  $\beta$ -alanine were also detected in the hydrolysate of the complex organic matter examined.<sup>8</sup> Solid carbon yields of these amino acids were 0.1%, 0.03%, 0.007%, and 0.01%, respectively. Therefore, 0.09% of nucleic acid base precursors and 0.1% of amino acid precursors were contained in the complex organic matter.

A spark discharge experiment was also conducted. The input energy was 0.011 J/ms, which is  $10^{-4}$  times that of 180 J/ms in the MPD arc-jet experiment. Effective dissociation of the  $\text{N}\equiv\text{N}$  and the  $\text{C}\equiv\text{O}$  bonds and rapid quenching do not occur in the spark discharge experiment.<sup>8</sup> A gas mixture of CO (350 Torr) and  $\text{N}_2$  (350 Torr) was enclosed in a flask (710 mL) containing liquid water (20 mL). The gas mixture was subjected to spark discharge for 12 h. The total input energy was 500 kJ which is similar to that of 900 kJ in the MPD arc-jet experiment. After the discharge period, the aqueous solution was analyzed following the same procedures as detailed above.

No nucleic acid bases were detected in the spark discharge experiment. The HPLC chromatograms showed flat profiles, as shown in parts Ab and Bb of Figure 2. The yield of amino acids in the spark discharge experiment was less than 1/1000 that found in the MPD arc-jet experiment.<sup>8</sup>

Cytosine can be synthesized by the reaction of cyanoacetylene and cyanate<sup>9</sup> or that of cyanoacetaldehyde and urea.<sup>10</sup> Uracil is obtained by hydrolysis of cytosine.<sup>11</sup> Cyanoacetylene<sup>9</sup> and urea<sup>12</sup> are synthesized by the spark discharge. Cyanoacetaldehyde is formed by hydrolysis of cyanoacetylene.<sup>11</sup> Thus, cytosine and uracil had been expected to be synthesized by spark discharge, but no cytosine and uracil were actually detected in the spark discharge experiment.

In the present study, cytosine and uracil were formed from the  $\text{CO-N}_2\text{-H}_2\text{O}$  gas mixture by introducing sufficient energy to break the  $\text{N}\equiv\text{N}$  and the  $\text{C}\equiv\text{O}$  bonds and by quenching the atoms and the radicals to efficiently form the C-C and the C-N bonds before the CO and the NO molecules were formed. This suggests that there seems to be a mechanism of pyrimidine formation excluding intermediary molecules such as cyanoacetylene and urea. We have proposed the mechanism of amino acid formation without involving the reaction among hydrogen cyanide, formaldehyde, and ammonia: The amino acids are formed by adding water to amorphous substance produced by quenching a  $\text{CO-N}_2$  high-temperature plasma.<sup>13</sup> It is understood that processes of high activation and rapid quenching which may be found in meteoritic impact or lightning are important for the formation of bioorganic compounds from the  $\text{CO-N}_2\text{-H}_2\text{O}$  gas mixture.

**Acknowledgment.** The author (S. M.) is supported by JSPS Research Fellowships for Young Scientists.

JA990961H

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