ABIOTIC SYNTHESIS OF AMINO ACIDS AND IMIDAZOLE BY PROTON IRRADIATION OF SIMULATED PRIMITIVE EARTH ATMOSPHERES

KENSEI KOBAYASHI, MASAHIKO TSUCHIYA

*Department of Physical Chemistry, Faculty of Engineering, Yokohama National University,
Hodogaya-ku, Yokohama 240, Japan*

TAIRO OSHIMA

*Department of Life Science, Faculty of Science, Tokyo Institute of Technology,
Midori-ku, Yokohama 227, Japan*

and

HIROSHI YANAGAWA

Mitsubishi Kasei Institute of Life Sciences, Minamiooya, Machida, Tokyo 194, Japan

Abstract. Proton irradiation of simulated primitive earth atmosphere was performed, and amino acids and imidazole were analyzed. A mixture of carbon monoxide and nitrogen over water was irradiated by high energy protons (3 MeV, 0.6 μA) generated by a Van de Graaff accelerator for 2–5 h. Various kinds of proteinous and non-proteinous amino acids were detected in the irradiation products. Imidazole present in the irradiation products was also detected by high-performance liquid chromatography and mass spectrometry. The present results suggest that compounds of biological importance such as amino acids could be synthesized from primitive earth atmosphere by radiation of cosmic rays and/or solar flare particles.

1. Introduction

Since 1950's, a number of studies have been done on abiotic synthesis of molecules of biological importance under possible primitive earth conditions (Garrison *et al.*, 1951; Miller, 1953).

Various kinds of energy sources have been used to synthesize bioorganic compounds such as amino acids from simulated primitive earth atmospheres. They include spark discharges (Miller, 1953, Kobayashi and Ponnamperuma, 1985), ultraviolet lights from the sun (Sagan and Khare, 1971), heat from volcanoes (Harada and Fox, 1964), radioactivity from the earth crust (Ponnamperuma *et al.*, 1963), and shock waves by meteorite impacts (Bar-Nun *et al.*, 1970). A mixture of methane, ammonia (or nitrogen gas) and water were mainly used as starting materials.

In these days, however, primitive earth atmospheres are believed to be less reduced. It was proposed that a mixture of carbon dioxide, nitrogen gas and water was a more plausible starting mixture for prebiotic chemistry on the earth. In these couple of years, it is pointed out that carbon monoxide might have been a major carbon source in primitive earth when the ocean was formed (Abe, 1986, Holloway,

1988; Kasting, 1989). It was shown that bioorganic compounds such as amino acids were synthesized from the less reduced atmospheres much less than the conventional reduced atmospheres by spark discharges (Schlesinger and Miller, 1983). That fact became one of the biggest problems in prebiotic chemistry. We postulated that some other energy sources may produce amino acids effectively from less reduced ('very mildly reduced') atmospheres.

We are performing proton irradiation of various kind of gaseous mixtures (Kobayashi, *et al.*, 1989). Here we report the first abiotic synthesis of imidazole and various amino acids by proton irradiation of a mixture of carbon monoxide, nitrogen and water.

2. Materials and Method

2.1. REAGENTS

Methane, carbon monoxide, carbon dioxide and nitrogen, of UHP grade, were purchased from Taiyo Sanso Co. ^{13}C-labelled carbon monoxide (^{13}C purity $>$ 99.5%) used was prepared by Commissariat a L'Energie Atomique (CEA), France. Cation-exchange resin Bio-Rad AG-50WX8 (H^+ form, 20–50 mesh) was used to obtain an amino acid fraction. Modified sea waters used were 50 mM ammonium chloride-ammonia solution (pH 8.7) or 25 mM sodium hydrogencarbonate-HCl solution (pH 7.0), both with 1 mM each of magnesium chloride, calcium chloride, zinc chloride, ferrous ammonium sulfate and sodium molybdate. Reagents used for amino acid analysis were of 'amino acid analytical grade'. The other chemicals used were of analytical grade. Water was purified with a Milli-Q Labo^R after glass-distillation.

2.2. INSTRUMENTS

An Irika automated amino acid analyzer Model A-5500 and a Shimadzu LC-6A high-performance liquid chromatograph (modified for an amino acid analyzer) were used to determine amino acids and ammonium ion. A Shimadzu gas chromatograph Model GC-9A and a Shimadzu gas chromatograph/mass spectrometer Model GCMS-QP1000 were used to identify amino acids with an optically-active capillary column 'Chirasil-Val'. A Gasukuro-Kogyo high-performance liquid chromatograph Model APUS-5 was used to separate imidazole with a cation exchange column Nucleosil 5 SA (4.6 mm i.d. × 150 mm). A Yanaco gas chromatograph Model G180 with a Unibeads C glass packed column (3 mm i.d. × 150 cm) was used to determine gaseous compounds. Liquid ionization mass spectrometry (LIMS) was performed with a Shimadzu mass spectrometer Model QP-300 and a JEOL mass spectrometer Model JMS-QH100; both were modified with liquid-ionization ion sources (Tsuchiya and Kuwabara, 1984).

2.3. PROTON IRRADIATION

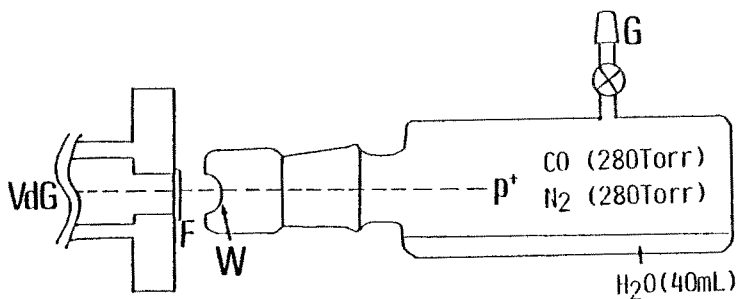Figure 1 shows a proton irradiation apparatus. In a 420-mL Pyrex glass tube, water

Fig. 1. Proton irradiation apparatus. VdG: Van de Graaff accelerator; F: Havar foil; W: concave end window; p$^+$: proton; G: gas inlet.

or modified sea water, and a gas mixture were introduced. In typical experiments, 280 Torr of carbon monoxide, 280 Torr of nitrogen and 40 mL of water were used. In some experiments, methane or carbon dioxide was used in place of carbon monoxide. Modified sea water containing carbonate (or ammonium ion) and metal ions was used in some runs to simulate primordial ocean (Kobayashi and Ponnamperuma, 1985). The gas mixture was irradiated with high energy protons (4.0 MeV, ca. 0.6 μA) generated by a Van de Graaff accelerator at room temperature. Energy of protons were decreased to ca. 3 MeV after passing through Havar foil, a concave window, and the air gap between them. After 2–5 hours' irradiation, the resulting gaseous product and aqueous solution was analyzed. A control experiment was performed with all the starting materials and procedures but proton irradiation.

2.4. ANALYSIS OF THE PRODUCTS

After irradiation, gaseous products were analyzed with a gas chromatograph equipped with a thermal conductivity detector (TCD). Nitrogen gas was used as a carrier to determine hydrogen, and helium was used to determine the other gases.

Resulting aqueous solution was filtered through a membrane filter (pore size: 0.2 μm) and a portion of it was used to determine ammonium ion by HPLC. The remainder of the solution was concentrated to dryness: A part of it was hydrolyzed with 6M hydrochloric acid at 105 °C for 24 h and dried-off by freeze-drying. The dried samples, both hydrolyzed and not hydrolyzed, were passed through an AG-50 column, and amino acids and imidazole were eluted out with 10% aqueous ammonia solution.

Amino acids were determined by high-performance liquid chromatography (HPLC) (using amino acid analyzers). They were also analyzed by gas chromatography (GC) and gas chromatograph/mass spectrometry (GC/MS) after derivatized with 2-propanol and trifluoroacetyl anhydride (TFAA).

Imidazole was analyzed by HPLC with a cation-exchange column using 25 mM phosphate buffer (pH 3.0) as a carrier.

Amino acids and imidazole were also identified with LIMS without any deri-vatization.

3. Results and Discussion

3.1. DETERMINATION OF MAJOR PRODUCTS

Table I shows analytical results of some gaseous compounds and ammonia in the products of proton irradiation. When carbon monoxide was used as a starting material, carbon dioxide was a major product. Either carbon monoxide or methane was used, a small amount of hydrogen was produced, and ammonia was found in the solution after irradiation. When carbon monoxide was used, however, concentration of hydrogen gas was much less than that of carbon dioxide, and very mildly reduced condition was found even at the end of the proton irradiation.

3.2. ANALYSIS OF AMINO ACIDS

Figure 2 shows ion-exchange chromatograms of amino acid fractions of the product after a mixture of carbon monoxide, nitrogen and water was irradiated for 2.5 h; (b) is a chromatogram before acid-hydrolysis, and (a) after hydrolysis. Without hydrolysis, a small amount of glycine and other amino acids were detected, together with unknown alkaline compounds. After hydrolysis, these unknown peaks were decreased, and much higher amount of amino acids were detected, including aspartic acid, threonine, serine, sarcosine, glycine, alanine, α-and γ-aminobutyric acid, and β-alanine. The following amino acids were tentatively identified only by ion-exchange chromatography: Glutamic acids, valine, isoleucine and leucine (Kobayashi et al., 1989). The following amino acids were also detected in some runs by ion-exchange chromatography: Proline, β-aminobutyric acid, β-aminoisobutyric acid and ornithine.

TABLE I

Analysis of the products of proton irradiation

System[a]	Irradiation energy	Gas[b]				Solution	
		CO_2	CO	CH_4	H_2	NH_3	pH
$CO-N_2-H_2O$	3.3 kJ	28	72	0	3.7	22 μmol	5.5
	8.4[c]	–	–	–	–	43	4.6
	16.8	52	48	0	5.2	63	4.6
$CH_4-N_2-H_2O$	16.8	0	0	100	26	209	8.7

[a] 5.7 mmol each of carbon monoxide (or methane) and nitrogen, and 40 mL of water were used as starting materials.
[b] Relative abundance ($CO_2 + CO + CH_4 = 100$).
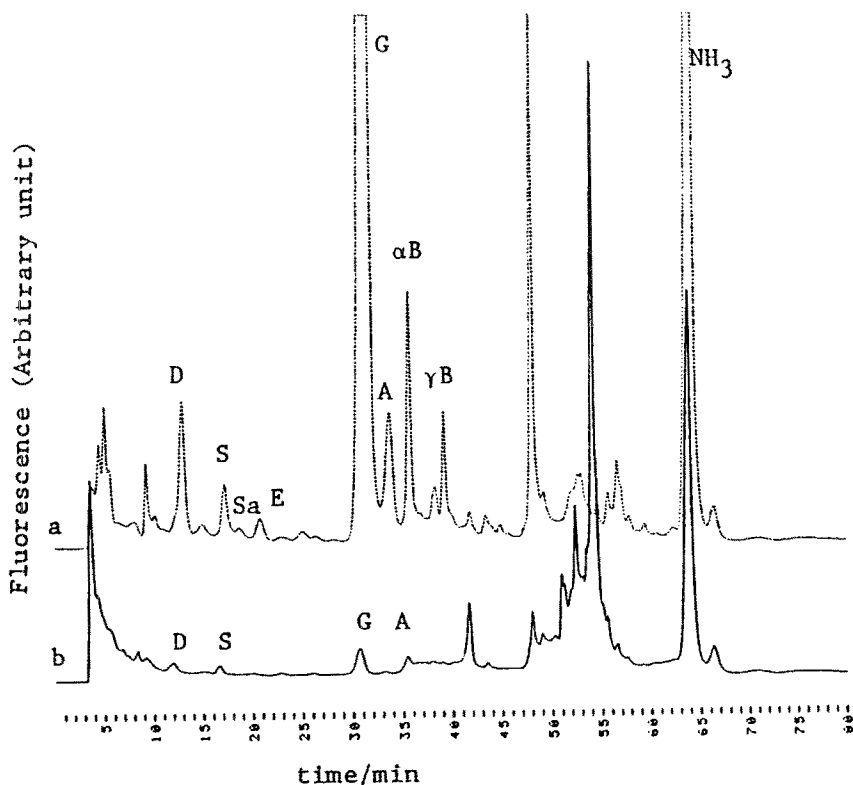[c] Gaseous composition was not measured.

Fig. 2. Ion-exchange chromatograms of amino acids produced by proton irradiation of a mixture of carbon monoxide, nitrogen and water. (a) After acid hydrolysis; (b) before acid hydrolysis; D: Aspartic acid; S: serine; Sa: sarcosine; E: glutamic acid; G: glycine; A: alanine; αB: α-aminobutyric acid; γB: γ-aminobutyric acid.

From a mixture of methane, nitrogen and water, the same kinds of amino acids were produced and detected by ion-exchange chromatography.

Figure 3 shows a gas chromatogram of an amino acid fraction of the product, with an optically-active Chirasil-Val capillary column. Amino acids were shown to be a racemic mixture. Major amino acids (glycine, alanine, sarcosine, aspartic acid and β-alanine) were identified by GC/MS (for example, the mass spectrum of sarcosine was shown in Figure 4). When ^{13}C-carbon monoxide was used as a starting material, amino acids found in the products were labelled by ^{13}C; e.g., molecular weights of glycine and alanine found in the product were 77 and 92, respectively.

In control experiments, only trace amount of glycine and serine (less than 10 nmol each) were detected. The resulting amino acids were racemic mixtures, and could be labelled with stable isotopes in starting materials. These facts strongly showed that most of amino acids were actually synthesized from carbon monoxide, nitrogen and water by proton irradiation.
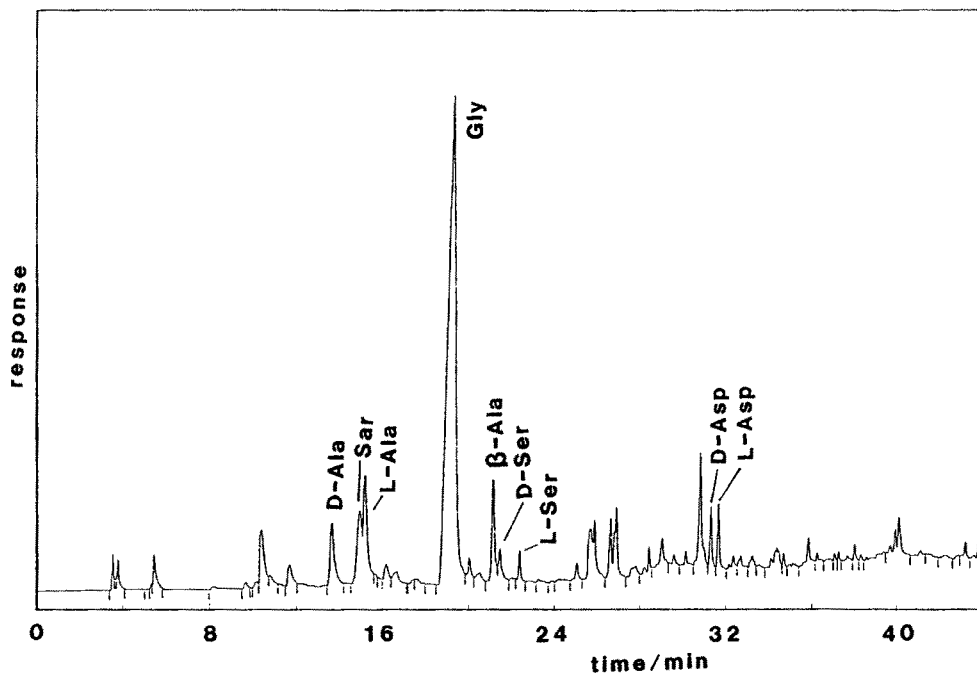
Fig. 3. Gas chromatogram of amino acids produced by proton irradiation of a mixture of carbon monoxide, nitrogen and water. Amino acids were injected after derivatization with trifluoroacetic anhydride and 2-propanol. Column: Chirasil-Val capillary column (25 m); detector: flame ionization detector; carrier: helium; oven temperature program: 80 °C (4 min) → 180 °C increasing at 3 °C min^{-1} → 180 °C; solvent cut: 2 min from the injection.
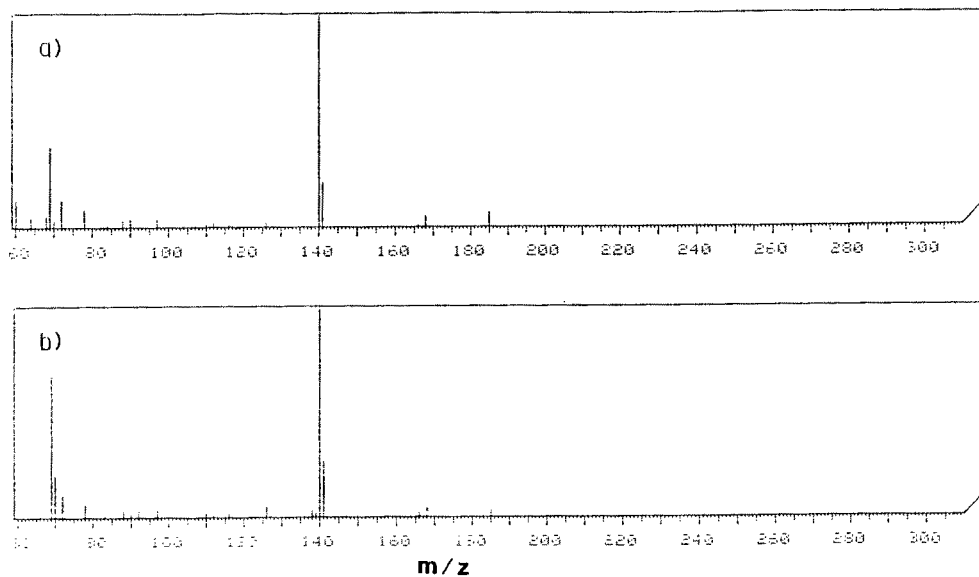

Fig. 4. Mass spectra of N-trifluoroacetyl-2-propyl sarcosine. (a) Authentic standard; (b) proton irradiation product (sample used was the same as shown in Figure 3).

TABLE II

Formation of amino acids by proton irradiation of gas mixtures

System	CO–N_2–H_2O			CH$_4$–N_2–H_2O		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Reaction condition						
Gas mixture[a]						
CO (Torr)	280	280	280	0	0	0
CH$_4$ (Torr)	0	0	0	280	280	280
N_2 (Torr)	280	280	280	280	280	280
Aqueous solution						
H_2O (mL)	40	40	40	40	40	40
NH$_4$Cl (mM)	0	0	0	50	50	0
Na_2CO_3 (mM)	50	50	0	0	0	0
Metal ions[b]	+	+	–	+	+	–
pH	8.7	8.7	–	7.0	7.0	–
Irradiation energy (kJ)	43	17	17	20	21	17
Amino acid produced (μmol)						
Aspartic acid	1.63	0.59	0.18	0.16	0.19	0.09
Threonine	0.18	0.15	0.01	0.74	0.88	0.07
Serine	0.81	0.43	0.06	0.73	0.95	0.67
Glycine	19.5	7.40	5.17	19.0	26.0	9.11
Alanine	7.86	1.23	0.30	3.84	5.69	2.43
α-Aminobutyric acid	0.40	0.29	0.27	0.18	0.62	0.22
β-Alanine	2.21	1.61	0.44	0.10	0.36	0.18
Total amount	32.6	11.7	6.43	24.8	34.7	12.77
Yield (%)[c]	1.4	0.50	0.26	0.92	1.4	0.52
G-value × 100	0.75	0.65	0.37	1.2	1.6	0.73

[a] Gas volume in a reaction vessel is 380 mL.
[b] +: 1 mM each of MgCl$_2$, CaCl$_2$, ZnCl$_2$, Fe(NH$_4$)$_2$, (SO$_4$)$_2$, and Na$_2$MoO$_4$ were added to aqueous solution; –: not added.
[c] Yield was calculated based on initial methane or carbon monoxide.

Yields of amino acids synthesized in the protonirradiation experiments were summarized in Table II. When methane was used as a carbon source, G-value for amino acids by proton irradiation was around 0.01, which was one of the highest values among G-values by various prebiotic energy sources. The yield of amino acids obtained from carbon monoxide was much the same as that from methane, though carbon monoxide gave much less amino acids than methane by spark discharges. When 'modified sea water' was used in place of distilled water, more amino acids were synthesized.

In place of carbon monoxide, mixtures of carbon monoxide and carbon dioxide of various mixing ratio were used as carbon sources in the protonirradiation experiments. As shown in Figure 5, the yield of amino acids was related to the partial pressure of carbon monoxide, which showed that amino acids could be synthesized from carbon monoxide even if carbon dioxide was a major constituent
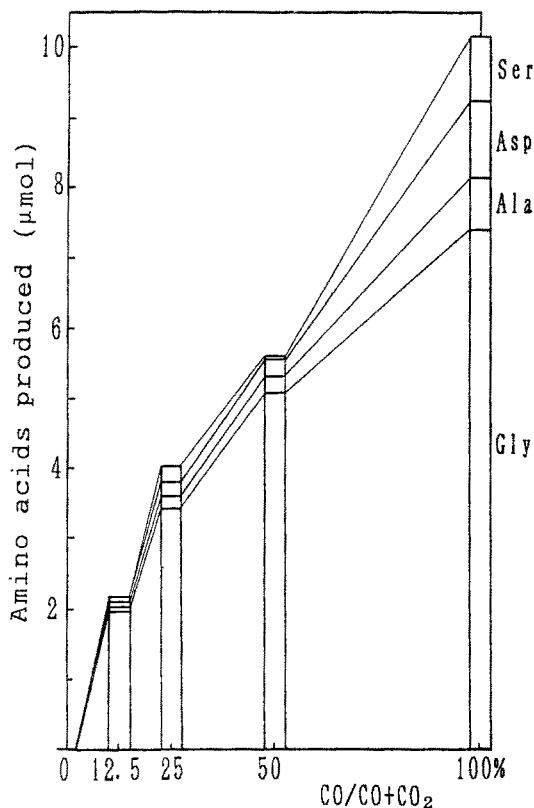
Fig. 5. Formation of amino acids by proton irradiation of a mixture of carbon monoxide, carbon dioxide, nitrogen and water. A mixture of carbon monoxide plus carbon dioxide (total 280 Torr) and nitrogen (280 Torr) was proton irradiated over 40 mL of modified sea water for 2.5 hr. Total energy deposited to the system was 17 kJ each. Amino acids were determined by ion-exchange chromatography.

of primitive earth atmosphere and carbon monoxide was a minor one.

3.3. IDENTIFICATION OF IMIDAZOLE

Figure 6(a) shows a HPLC chromatogram of the product by proton irradiation of carbon monoxide, nitrogen and water. The peak with an arrow has the same retention time as that of authentic imidazole. We fractionated this portion of the effluent, and analyzed by LIMS, where protonated molecules (MH$^+$) were detected. Mass spectra in Figure 6 (b) and (c) show that a molecular weight of the compound is 68 when carbon monoxide (mostly ^{12}CO) was used, and that is 71 when ^{13}C-labelled carbon monoxide was used. These results show that imidazole was abiotically synthesized from carbon monoxide, nitrogen and water. Yield (G-value) of imidazole by proton irradiation from a mixture of carbon monoxide, nitrogen and water was about 0.0001. Imidazole was also formed from a mixture of methane, nitrogen and water by proton irradiation.

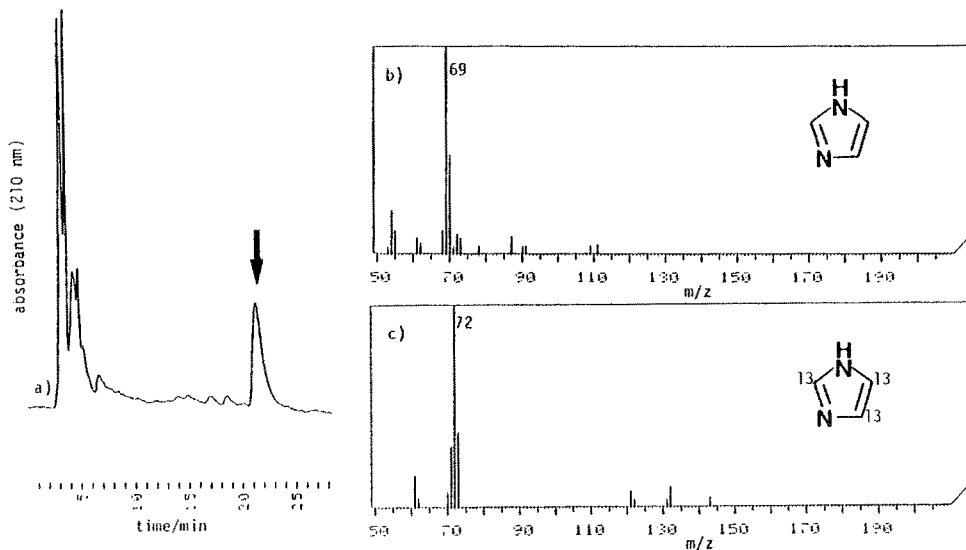Imidazole is a component of histidine, and imidazole itself has some catalytic

Fig. 6. Identification of imidazole in the proton-irradiation product. A mixture of carbon monoxide, nitrogen and water was proton-irradiated for 2.5 hr. (a) Ion-exchange chromatogram. Chromatographic conditions are shown in the text. The peak with an arrow had the same retention time as authentic imidazole. (b) Liquid-ionization mass spectrum of the imidazole. Instrument: Shimadzu QP-300; m/z of protonated imidazole (ImH$^+$) is 69. (c) Liquid-ionization mass spectrum of the imidazole when ^{13}CO was used. m/z of protonated ^{13}C$_3$-imidazole is 72.

activities for the hydrolysis of esters. Imidazole is also very important compounds since imidazolides of nucleotides are very useful starting materials to synthesize oligonucleotides (Sawai, 1976; Inoue and Orgel, 1983; Kanaya and Yanagawa, 1986). There have been some reports on abiotic synthesis of imidazole or its derivatives under prebiotic conditions (Sanchez et al., 1967; Ferris et al., 1979, Oró et al., 1984), but there have not reported that imidazole are synthesized abiotically from plausible earth atmospheres. The present results show that imidazole could be formed from 'very mildly reduced atmosphere' by proton irradiation.

3.4. PREBIOTIC FORMATION OF AMINO ACIDS AND IMIDAZOLE ON PRIMITIVE EARTH BY COSMIC RAYS

From 1950's through 1960's, it was believed that primitive earth atmosphere was strongly reduced, and its major components were methane, ammonia, hydrogen and water. From 1970's through mid-1980's, however, many investigators, preferred the idea that primitive earth atmosphere were less reduced, and that carbon dioxide, nitrogen and water should have been major carbon/nitrogen compounds in it (Walker, 1986). Recent geophysical studies on the formation of planets have suggested that high-velocity impacts of planetesimals onto a growing planet resulted in the impact-degassing of volatiles (Matsui and Abe, 1986a, b). This scenario suggests that the surface of the primitive earth was melted and that the formation of an impact-induced atmosphere contains carbon monoxide as a major carbon source

after interaction with metal iron (Abe, 1986; Matsui, 1989). Photochemical model calculation also suggested that carbon monoxide was the dominant carbon compounds rather than carbon dioxide (Kasting, 1989). Therefore, it is plausible that the primitive earth atmosphere may have been very mildly reduced, containing carbon monoxide, nitrogen and water. From carbon dioxide-dominated atmosphere, no successful abiotic synthesis of amino acids have been so far reported. Even from carbon monoxide-dominated atmosphere (very mildly reduced atmospheres), it was believed to be very difficult to obtain bioorganic compounds in high yield by conventional energy sources such as spark discharges (Schlesinger and Miller, 1983): Only when high concentration of hydrogen and/or ammonia was incorporated into the gas mixtures, a considerable amount of amino acids was obtained by spark discharges (Abelson, 1956) or heat (Yoshino, *et al.*, 1971). It was reported that some organic compounds are formed after a mixture of carbon monoxide and water was irradiated by vacuum ultraviolet (Bar-Nun and Chang, 1983), but the formation of bioorganic compounds such as amino acids has not been examined yet.

The present results suggested that amino acids were formed from very mildly reduced atmosphere efficiently by proton irradiation if there was carbon monoxide in the primitive earth atmosphere; even if carbon dioxide was coexisted with carbon monoxide, amino acids were produced when the yield of amino acids were proportional to the partial pressure of carbon monoxide.

Major components of cosmic rays are high energy protons. Among various energy sources for prebiotic synthesis of amino acids on the earth, cosmic rays (galactic cosmic rays) have been regarded to be negligibly lower than other energy sources such as spark discharges and ultraviolet light (Miller and Urey, 1959). Considering the effectiveness on prebiotic synthesis, the roles of cosmic rays was shown not to be negligible, particularly not methane but carbon monoxide was a major carbon source. In addition to that, solar flare particles (solar cosmis rays) should be taken into account. Though the flux of solar flare particles are not continuous, the flux of solar flare particles (average energy: 10 MeV) is sometimes as high as 10^4 protons cm^{-2} s^{-1} when the solar activity is maximum (Fichtel *et al.*, 1963, Sakurai, 1974). Such events occur even in these days, and probably occurred more frequently when the sun was young (Sakurai, 1984). On such an occasion, it gave about 1 J hr^{-1} m^{-2} of energy to the upper earth atmosphere at high latitude areas, which can synthesize more than 10^6 mole of amino acids from carbon monoxide and nitrogen per hour at the Antarctic and Arctic Circles of the earth.

Solar wind may have had some roles in prebiotic synthesis since major components of solar wind is also proton. In order to evaluate its role in prebiotic synthesis, protons of lower energy level should be used in proton irradiation of simulated atmospheres, because the energy of solar wind is much lower than that of solar flare particles.

The present results suggest that amino acids and imidazole could be abiotically synthesized on the primitive earth, not only from strongly reduced atmosphere, but also from as very mildly reduced atmospheres such as a mixture of carbon

monoxide, nitrogen and water, if cosmic rays and solar flare particles are considered as one of energy sources for prebiotic synthesis.

Acknowledgments

References

Abe, Y.: 1986, Ph. D. Dissertation, University of Tokyo, Tokyo.

Abelson, P. H.: 1956, *Carnegie Institute of Washington Year Book* **55**, 171.

Bar-Nun, A. and Chang, S.: 1983, *J. Geophys. Res.* **88**, 6662.

Bar-Nun, A., Bar-Nun, N., Bauer, S. H., and Sagan, C.: 1970, *Science* **168**, 470.

Ferris, J. P., Narang, R. S., Newton, T. A., and Rao, V. R.: 1979, *J. Org. Chem.* **44**, 1273.

Fichtel, C. E., Guss, D. E., and Ogilvie, K. W.: 1963, *Solar Proton Manual*, NASA R-169, McDonald, F. B. (ed.), p. 19.

Garrison, W. M., Morrison, D. C., Hamilton, J. G., Benson, A. A., and Calvin, M.: 1951, *Science* **114**, 416.

Harada, K. and Fox, S. W.: 1964, *Nature* **201**, 335.

Holloway, J. R.: 1988, *Lunar and Planetary Science* (Lunar and Planetary Institute, Houston), No. 19, pp. 499–500.

Inoue, T. and Orgel, L. E.: 1983, *Science* **219**, 859.

Kanaya, E. and Yanagawa, H.: 1986, *Biochemistry* **25**, 7423.

Kasting, J.: 1989, *Origins of Life* **19**, 225.

Kobayashi, K. and Ponnamperuma, C.: 1985, *Origins of Life* **16**, 57.

Kobayashi, K., Oshima, T., and Yanagawa, H.: 1989, *Chem. Lett.* **1989**, 1527.

Matsui, T.: 1989, *Kagaku (Science)*, **59**, 357.

Matsui, T. and Abe, Y.: 1986a, *Nature* **319**, 303.

Matsui, T. and Abe, Y.: 1986b, *Nature* **322**, 526.

Miller, S. L.: 1953, *Science* **117**, 528.

Miller, S. L. and Urey, H. C.: 1959, *Science* **130**, 245.

Ponnamperuma, C., Lemmon, R. M., Mariner, R., and Calvin, M.: 1963, *Proc. Nat. Acad. Sci. U.S.A.* **49**, 737.

Oró, J., Basile, B., Cortes, S., Shen, S., and Yamrom, T.: 1984, *Origins of Life* **14**, 237.

Sagan, C. and Khare, B. N.: 1971, *Science* **173**, 417.

Sakurai, K.: 1974, *Astrophys. Space Sci.* **28**, 375.

Sakurai, K.: 1984, *Space Sci. Rev.* **38**, 243.

Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1967, *J. Mol. Evol.* **30**, 223.

Sawai, H.: 1976, *J. Am. Chem. Soc.* **98**, 7037.

Schlesinger, G. and Miller, S. L.: 1983, *J. Mol. Evol.* **19**, 376.

Tsuchiya, M. and Kuwabara, H.: 1984, *Anal. Chem.* **56**, 14.

Walker, J. C. G.: 1986, *Origins of Life*, **16**, 117.

Yoshino, D., Hayatsu, R., and Anders, E.: 1971, *Geochim. Cosmochim. Acta* **35**, 927.