SYNTHESIS OF BIOMOLECULES FROM N_2, CO, AND H_2O BY ELECTRIC DISCHARGE

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Abstract. A model primitive gas containing a mixture of N_2, CO and water vapor over a water pool (300 mL, ~ 37 °C) was subjected to electric discharges. The discharge vessel (7 L in volume) was equipped with a CO_2 absorber (The CO_2 being formed during the discharge), thus simulating possible absorption of CO_2 in the primitive ocean. The vessel also has a cold trap (~ −15 °C), which protects the primary products against the further decomposition in the discharge phase by enabling these products to adhere to the trap. Since the partial pressures of CO and N_2 decreased at rates of 1.5–1.7 cmHg day^{-1} and 0.1–0.2 cmHg day^{-1}, respectively, the gases were added at regular intervals. The solution was analyzed at regular intervals for HCN, HCHO and urea, and maximum concentrations of about 50, 2, and 140 mM were observed. The discharge phase was continued for 6 months. In the solution, glycine (5.6% yield based on the carbon), glycylglycine (0.64%), orotic acid (0.004%) and small amounts of the other amino acids were found.

1. Introduction

Since Miller (1953) demonstrated the synthesis of amino acids and other organic molecules by electric discharge through methane, ammonia and water, many experiments have been carried out on the prebiotic formation of biomolecules using various model primitive gases strongly or considerably reducing in total (Abelson, 1966; Keosian, 1968; Miller and Orgel, 1974; Chang *et al.*, 1983; Schlesinger and Miller, 1983a, b; Hattori *et al.*, 1984; Yuasa *et al.*, 1984a, b). However, according to present geochemical knowledge, the primitive atmosphere might not have been so reducing, but it was possibly mildly reducing (Rubey, 1955; Abelson, 1966; Keosian, 1968; Miller and Orgel, 1974; Matsuo, 1978; Shimizu, 1978, 1979; Folsome, 1979; Owen and Cess, 1979; Levine *et al.*, 1982; Chang *et al.*, 1983; Levine and Augustsson, 1985). We present here an experimental study for the production of bioorganic substances by electric discharges through a mixture of N_2, CO, and H_2O which was proposed as a model of the primitive atmosphere by Matsuo (1978) and Shimizu (1978, 1979).

2. Experimental Method

The discharge apparatus (Figure 1) was designed to include a CO_2 absorber (The CO_2 being produced by the action of the discharge on CO and H_2O). This simulates the condition that most of the CO_2 in the primitive gas must have been quickly

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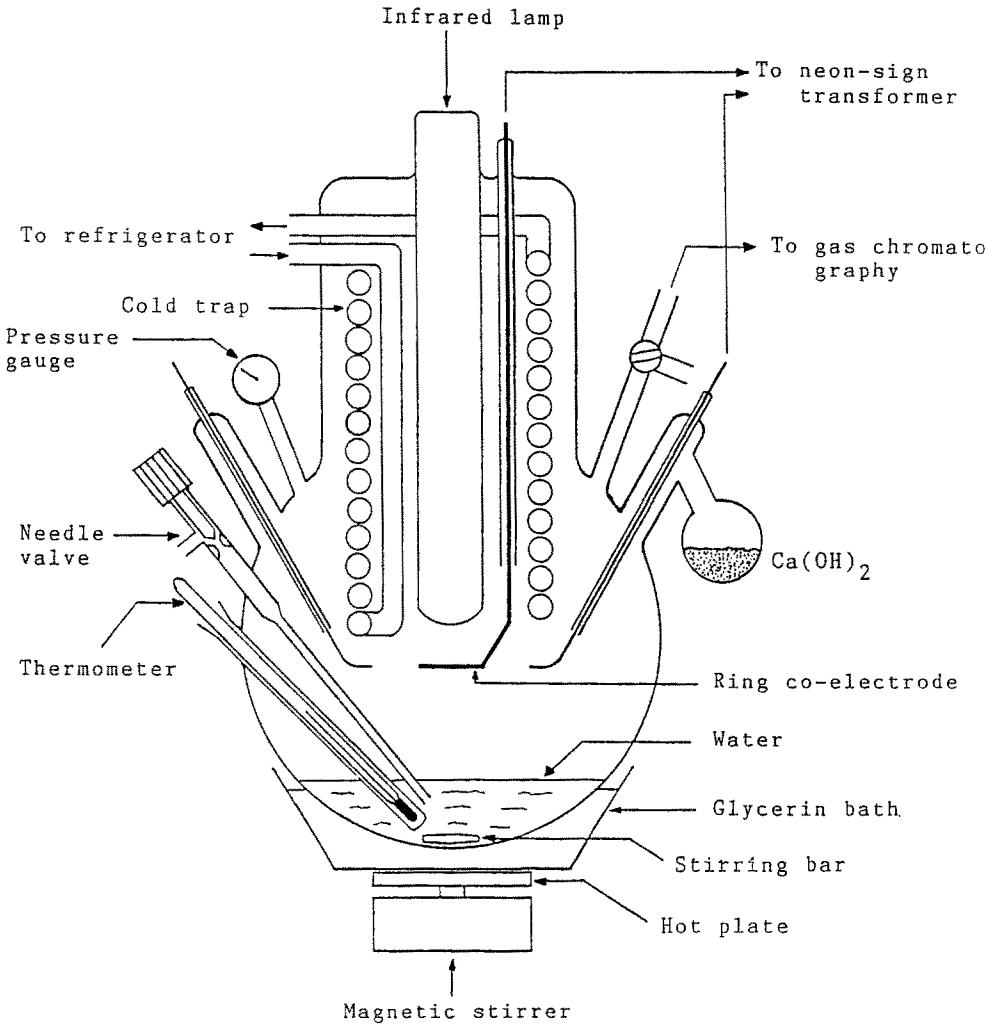


Fig. 1. Seven-liter electric discharge apparatus.

absorbed into the ocean, abundant in calcium and magnesium ions (Shimizu, 1978, 1979).

The apparatus was also designed to include a cold trap on which the primary products forming in the gas phase through discharges are captured together with the water vapor. Therefore, the products can escape from further discharges, which would cause decomposition of the molecules. The cylindrically coiled trap also acted as a chimney, giving a good circulation of the gas to carry the discharge products to the trap.

The discharge vessel (7 L in volume) was initially prepared with distilled water (300 mL), gases (N_2: 8 cmHg; CO: 16 cmHg) and the absorbent of CO_2 [$Ca(OH)_2$: 40 g]. Seven parallel discharges were induced in the gap (~ 3 cm) between a center

ring co-electrode and seven side electrodes, each connected to a 15 kV neon-sign transformer respectively. The primary voltage of the transformers was controlled with a slide voltage regulator so as to supply the lowest voltage necessary to induce the discharge, which gave us the highest yield of HCN. During the discharge, the water pool in the vessel was kept to a temperature range between 35 °C and 40 °C on a glycerine bath over a hot plate. The coiled trap was cooled with non-freezing water solution at between −15 °C and −20 °C.

Under these conditions, the water vapor evaporating from the pool in the vessel is gradually captured on the cold trap to form a frosty cylinder, and approximately half the water in the pool evaporates during a 3.5 hr run. At this stage, the electric power supply to the discharge electrodes and the refrigerator was turned off, and an infrared lamp at the center of the coiled trap was simultaneously turned on. Most of the frost on the trap was melted in half an hour by the infrared radiation. The discharge phase and the melting phase were switched using synchronized two time-switches.

The composition of gases in the vessel was regularly analyzed by gas chromatography. The apparatus was connected by a three-way valve to the discharge vessel.

The aqueous sample was withdrawn every few days for analysis and the same volume of water was supplied to maintain the volume of water in the pool at the initial value.

The withdrawn sample was analyzed by the Schilt method (1958) for HCN, by the Nash method (1953) for HCHO and by the Natelson *et al.* method (1951) for urea.

Amino acids and glycylglycine were analyzed by a HPLC-ninhydrin reaction system: Good coincidences with authentic samples were confirmed by the coinjection method. After the separation of the glycylglycine fraction and the hydrolysis in 5 N hydrochloric acid, the fraction gives a peak of glycine approximately twice as large as the original glycylglycine-like product in the amino acid analysis. This identifies the unknown product as glycylglycine.

3. Results and Discussions

The pressure in the vessel decreased during the discharges at a rate of 1.5–1.8 cmHg day^{-1}, which indicated the formation of water soluble substances. Approximately every 10 days, when the pressure decreased to about a half of the starting pressure, CO and N_2 gases were supplied, simulating a continuous supply of volcanic gas into the primitive atmosphere. So, the partial pressures of CO and N_2 during the discharge ranged approximately from 30 to 10 cmHg and from 10 to 5 cmHg, respectively. Gas chromatography showed that the partial pressure of CO rapidly decreased at the rate of 1.5–1.7 cmHg day^{-1}, whereas N_2 decreased at the slower rate of 0.1–0.2 cmHg day^{-1}. H_2 and CO_2 were present throughout the whole period of the discharge in pressure ranges of 0.2–0.6 and 3–4 cmHg, respectively. The existence of this steady state meant that balances were achieved between the

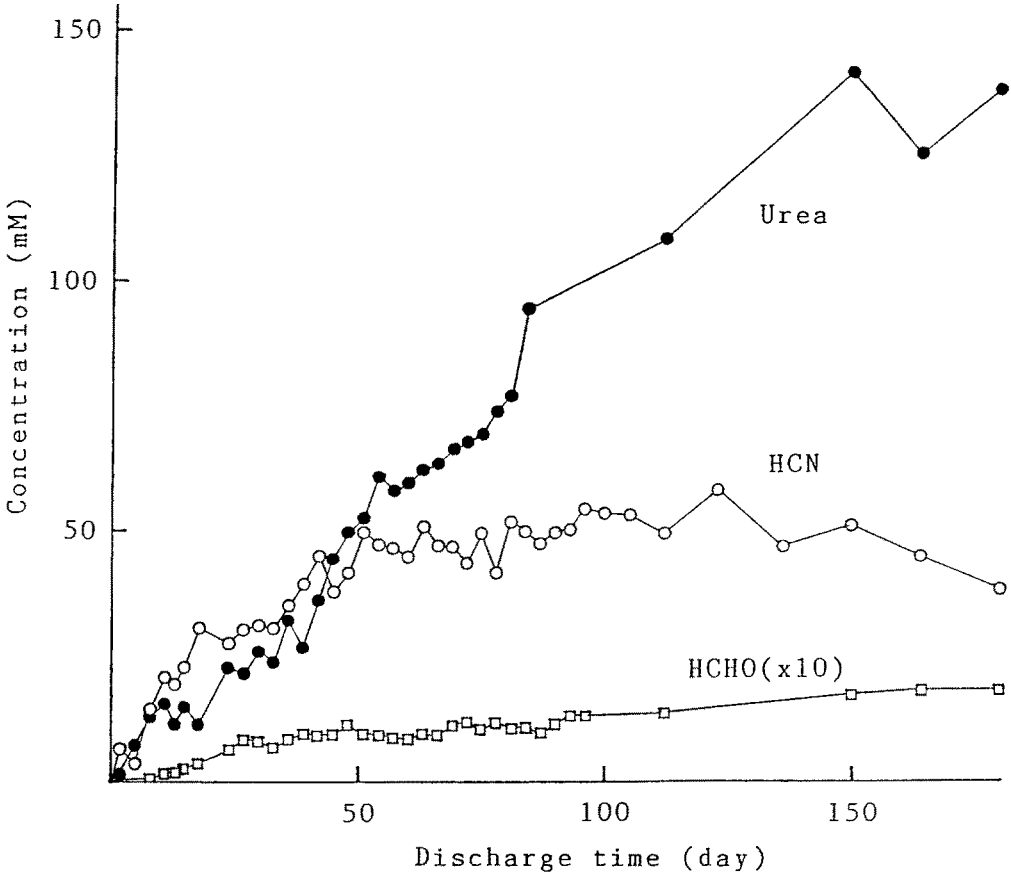


Fig. 2. Dependence of the concentration of HCN, HCHO and urea on the discharge time.

production of CO_2 molecules from CO and H_2O and the absorption of them by the absorber, and between the release of H_2 molecules from H_2O and the consumption of them by the production of hydrogen cyanide and formaldehyde etc.

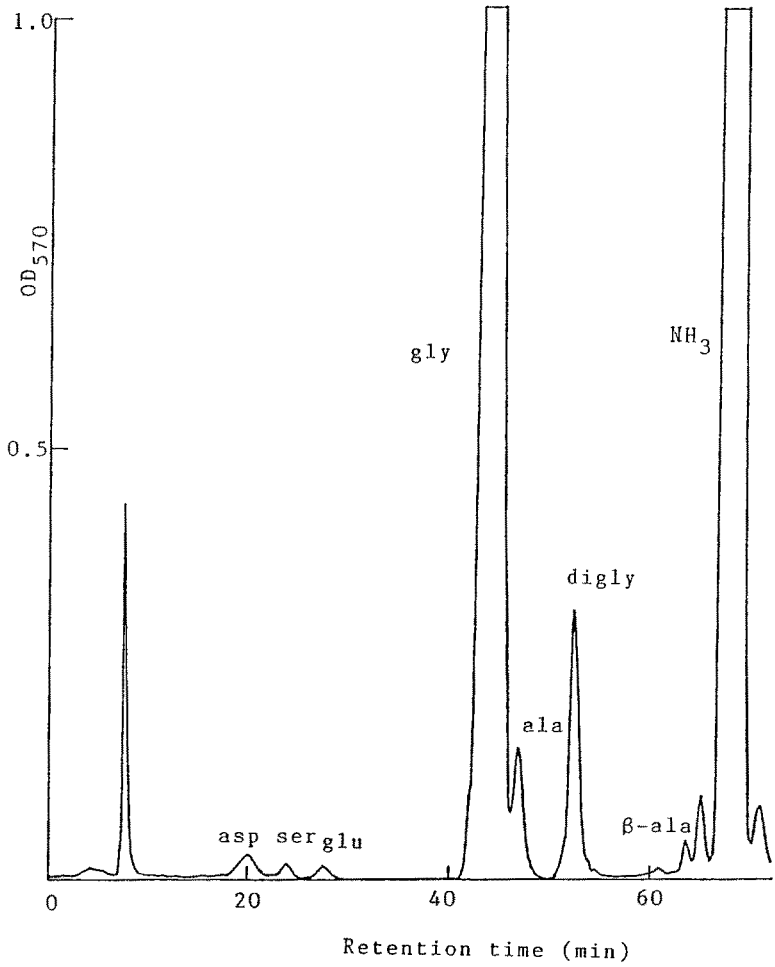
After two weeks of discharges, the water pool began to color light yellow, gradually turning dark brown. The concentration of HCN increased first at a rate of about 1.7 mM day^{-1} and then gradually attained a constant level, whereas those of HCHO and urea continued to increase (Figure 2). The former feature is considered to mean a balance between the production of HCN and its conversion to other molecules. The concentration of ammonia was approximately 60 mM, though it was not regularly analyzed. The pH of the solution was almost constant between 7.5 and 8.0 through the discharge period.

The experiment was stopped after 6 months (the aqueous sample at this stage is named Sample-A) when the partial pressure of CO_2 began to rapidly increase and the concentration of HCN began to decrease. Later it was recognized that at this time all the $Ca(OH)_2$ had been converted to $CaCO_3$. Total quantities of N_2 and CO supplied in the vessel were estimated to be 0.14 and 0.88 mole, respectively,

but 0.54 mole of CO, more than a half of the total supplied CO was fixed in the crystal as $CaCO_3$. The water pool in the vessel was boiled for a month (65–70 °C at a final pressure of about 25 cmHg) on a heating mantle to promote reaction of the remaining HCN and other unstable compounds under oxygen-free condition (this sample is named Sample-B). During the course of the heating, the color of the solution turned black, similar to the polymerization process of HCN. Parts of Sample-B were further heated in sealed tubes at 100 °C for various lengths of time. Amino acid analysis of these samples showed that the concentrations of amino acids continued to increase linearly with the heating time (the maximum heating time was 322 hr; this sample is named Sample-C), though that of glycylglycine increased up to approximately 220 hr (~ 6.5 mM) and then gradually decreased. The analysis for Sample-C is shown in Figure 3 with that for the acid hydrolysate (Sample-D: 6N HCl, 100 °C, 24 hr) of Sample-B: In the case of Sample-A, none of peaks were observed except that of ammonia, but in the case of Sample-B a chromatogram similar to the case of Sample-C was obtained with relatively smaller peaks.

Sephadex G-15 column was also used for the analysis (Voet and Schwartz, 1983) of the samples (Figure 4a, b, c). The elution volume at the first peak in Figure 4b correspond to the exclusion limit of the gel filtration. This suggests the formation of polymers probably from HCN. The polymerization of HCN might have been accelerated by formaldehyde in the solution (Schwartz and Goverde, 1982). This fraction was proved to afford several amino acids only after acid hydrolysis (Figure 4d).

The fractions in the peaks of Figure 4a, b, c were investigated with HPLC (anion- and cation-exchange and inverse phase chromatography) to detect purines and pyrimidines and their precursers. In the retention time and the spectrum, none of the peaks except that orotic acid, corresponded to sixteen available authentic samples including nucleic acid bases. Inverse phase chromatography of the fractions at the elution volume of orotic acid in Figure 4c showed a single peak corresponding to orotic acid (Figure 4e), though the corresponding fractions in Figure 4b showed many other peaks including that of orotic acid. The chromatography in Figure 4e was repeated and the peak was separated and collected. After repeating the purification, the sample was analyzed with anion-exchange HPLC and silica gel TLC in two different solvent systems. The sample was also analyzed by GC/MS, which was carried out with the same method as that described in the preceding paper (Yamagata *et al.*, 1991). All the analysis showed good correlation with that of the authentic orotic acid. The concentration of the orotic acid in Sample-E was estimated to be about 30 μM (0.004% yield based on the carbon). Orotic acid has been reported as a product from some cyanide polymerizations (Ferris, *et al.*, 1978). The orotic acid in the present experiment might have also formed via a similar process. In that case, the absence of adenine (less than 1/20 as much as orotic acid) in our experiment could not be understood, since Ferris *et al.* (1978) has reported much higher yield of adenine, four times as much as orotic acid in



a)

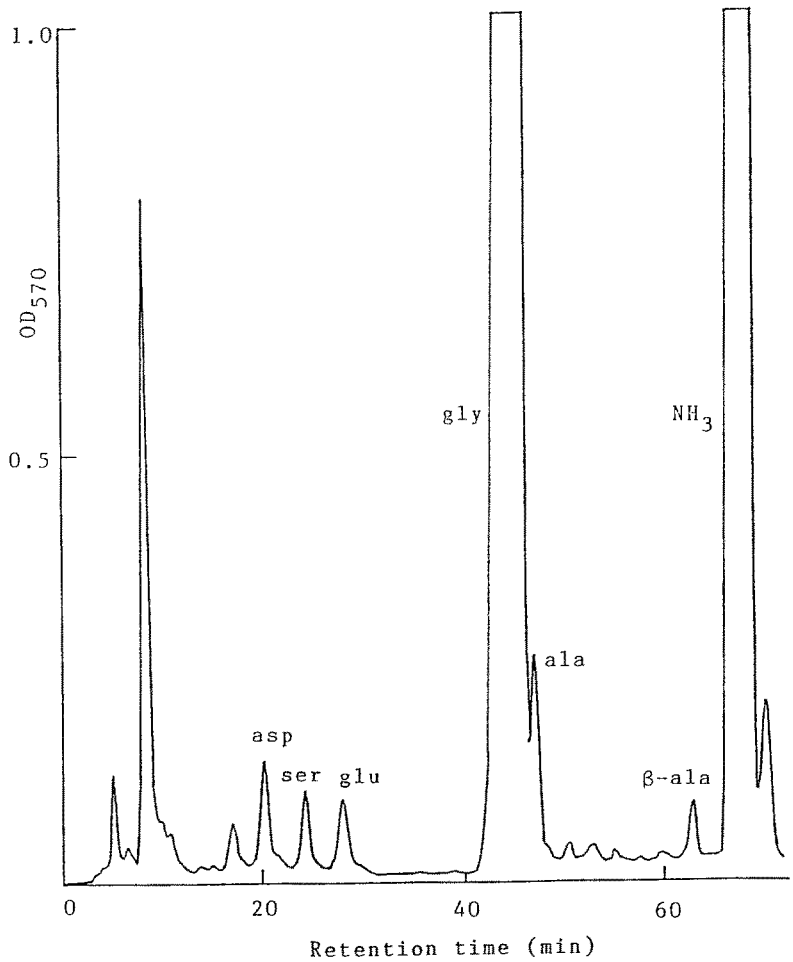
Fig. 3. HPLC chromatograms by ninhydrin reaction for amino acids analysis of the aqueous samples. (a) Sample-C; (b) Sample-D (see p. 477).

Concentrations (mM) and the percent yields based on the carbon (in parentheses) of amino acids.[a]

	gly	ala	asp	glu(?)[b]	ser	β-ala	gly-gly
Sample-C	100.0 (5.6%)	2.44	0.36	0.16(?)	0.12	0.15	5.6 (0.64%)
Sample-D	92.0 (5.2%)	1.28	0.72	0.48(?)	0.52	0.52	0.00

[a]) If it is taken into consideration that the aqueous sample (128 ml in total) was withdrawn in the course of the discharges, and was replaced by the same volume of water, above values should be revised approximately 20% higher. It is noted that the amino acid product ratios are similar to the CO experiments of Schlesinger and Miller (1983a).

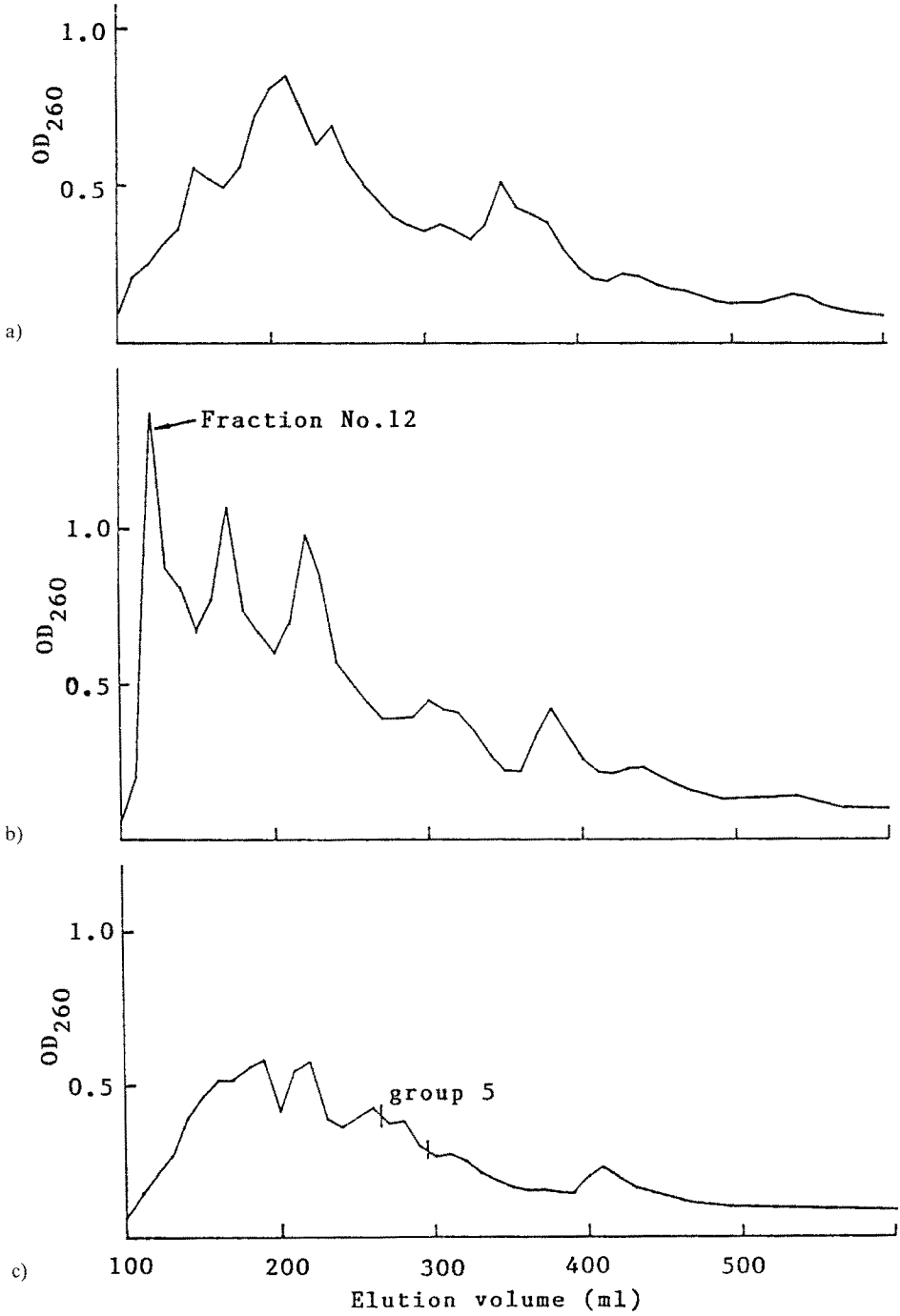
[b]) The glu(?) means glu and/or sarcosine. The HPLC peak of glu is coincident with that of sarcosine.

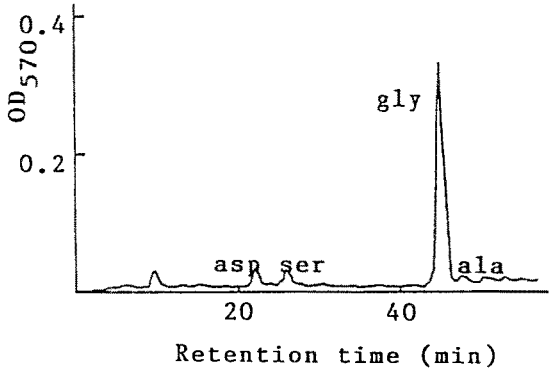


b)

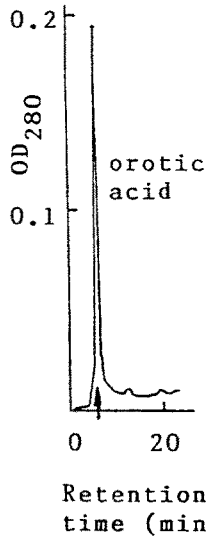
his experiment.

The observed glycylglycine would probably formed through the condensation reaction of glycines by cyanate and urea in the solution. Cyanate as a possible prebiotic dehydration condensing agent has been demonstrated to form in the similar experiments and the formation of urea which forms spontaneously from cyanate and ammonia has been also reported (Yamagata *et al.*, 1979; Yamagata *et al.*, 1981; Yamagata and Mohri, 1982). The formation of glycylglycine from glycine and urea in aqueous solution was demonstrated by Sakurai and Yanagawa (1984) and Yamagata *et al.* (1984). Recently, oligoglycines up to tetraglycine have been reported to form in aqueous solution of glycine and urea (Nagayama *et al.*, 1990). The mechanism for the glycylglycine formation proposed by Sakurai and Yanagawa (1984) starts with the formation of carbamylglycine which is known to form easily from glycine and urea or from glycine and cyanate in aqueous solution. Thus,


1.0
0.5
OD_{260}
a)
Fraction No.12
1.0
0.5
OD_{260}
b)
1.0
0.5
OD_{260}
group 5
c)
100
200
300
400
500
Elution volume (ml)



d)



e)

Fig. 4. Sephadex G-15 chromatograms (a, b, c (p. 478)) of aqueous samples and HPLC analysis of some fractions (d, e (above)). (a) Sample-A, (b) Sample-B, (c) Sample-E: Acid hydrolysate (1N, HCl, 100 °C, 27 h) of Sample-B; column size: 2.64 × 59 cm, eluant: 0.05 M NH_4HCO_3. (d) amino acid analysis after hydrolysis of fraction No. 12 in Figure 4b. (e) HPLC analysis of group 5 in Figure 4c; column: ODS-5 μm, 4 × 250 mm; eluant: (A) 0.02 M NH_4HCO_3, (B) 25% CH_3CN, gradient elution. Arrow shows position of orotic acid.

the Sakurai and Yanagawa's mechanism would be applied to the formation of glycylglycine in our experiment.

The present experiments demonstrated the possibility that early chemical evolution could have proceeded effectively even in a less reducing primitive atmosphere of N_2-CO-H_2O including CO_2 at a partial pressure approximately ten times higher than that of H_2. Notably the synthesis of HCN and its polymerization would have been very effective in clouds where plenty of water and lightning were available, and the products could have been concentrated as ice particles in clouds forming

the eutectic phase (Sanchez *et al.*, 1966, 1967), immediately after lightning passed through clouds.

In conclusion, the experimental results reported here were confirmed to be reproducible by repeating a few months discharge experiment several times. An essential point of our experiment was to remove CO_2 from the gas phase. This has made possible the continuous addition of the gases into the vessel and the persistence of a mildly reduced gas composition including H_2 during the experiment. Thus, it has allowed the constant production of HCN and H_2CO, followed by the accumulation of organic materials. It should be noted that lightning occurs usually in clouds of the troposphere and the molecular hydrogen produced in the clouds can hardly escape from the Earth's gravitational field. Molecular hydrogen in the troposphere can escape from the Earth only after the molecules arrive at a high altitude of the stratosphere through the troposphere, where most of atmospheric gas exist. However, the transfer of the molecular hydrogen from the troposphere to the stratosphere would be very difficult, since the rapid convection of the air in the troposphere would obstruct the separation between light and heavy molecules of the air due to the buoyancy, as shown by the fact that the present atmosphere has a constant gas composition independent of the altitude in the troposphere.

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References

Abelson, P. H.: 1966, *Proc. N.A.S.* **55**, 1365–1372.

Chang, S., DesMarais, D., Mack, R., Miller, S. L., and Strathearn, G. E.: 1983, in J. W. Schopf (ed.), *Earth's Earliest Biosphere, Its Origin and Evolution*, Princeton University Press (Princeton, New Yersey), Chap. 4, pp. 53–92.

Ferris, J. P., Joshi, P. C., Edelson, E. H. , and Lawless, J. G.: 1978, *J. Mol. Evol.* **11**, 293–311.

Folsome, C. E.: 1979, *The Origin of Life: A Warm Little Pond*, Freeman and Company, San Francisco and London.

Hattori, Y., Kinjo, M., Ishigami, M., and Nagano, K.: 1984, *Origins of Life* **14**, 145–150.

Keosian, J.: 1968, *The Origin of Life*, 2nd ed., Reinhold, New York.

Levine, J. S. , Augustsson, T. R, and Natarajan, M.: 1982, *Origins of Life* **12**, 245–259.

Levine, J. S. and Augustsson, T. R.: 1985, *Origins of Life* **15**, 299–318.

Matsuo, S.: 1978, in H. Noda (ed.), *Origin of Life*, Proc. 2nd ISSOL Meet. and 5th ICOL Meet., Japan Sci. Soc. Press, pp. 21–27.

Miller, S. L.: 1953, *Science* **117**, 528–529.

Miller, S. L. and Orgel, L. E.: 1974, *The Origins of Life on the Earth*, Prentice-Hall, Englewood Cliffs.

Nagayama, M., Inomata, K. and Yamagata, Y.: 1990, *Origins of Life* **20**, 249–257.

Nash, T.: 1953, *Biochem. J.* **55**, 416–421.

Natelson, S., Scott, M. L., and Beffa, C.: 1951, *Am. J. Clin. Path.* **21**, 275–281.

Owen, T. and Cess, T. D.: 1979, *Nature* **277**, 640–642.

Rubey, W. W.: 1955, Geol. Soc. *Am. Special Paper* **62**, 631–650.

Sakurai, M. and Yanagawa, H.: 1984, *Origins of Life* **14**, 171–176.

Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1966, *Science* **153**, 72–73.

Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1967, *J. Mol. Biol.* **30**, 223–253.
Schilt, A. A.: 1958, *Analyt. Chem.* **30**, 1409–1411.
Schlesinger, G. and Miller, S. L.: 1983a, *J. Mol. Evol.* **19**, 376–382.
Schlesinger, G. and Miller, S. L.: 1983b, *J. Mol. Evol.* **19**, 383–390.
Schwartz, A. W. and Goverde, M.: 1982, *J. Mol. Evol.* **18**, 351–353.
Shimizu, M.: 1978, in H. Noda (ed.), *Origin of Life*, Proc. 2nd ISSOL Meet. and 5th ICOL Meet., Japan Sci. Soc. Press, pp. 35–38.
Shimizu, M.: 1979, *Precambrian Res.* **9**, 311–324.
Voet, A. B. and Schwartz, A. W.: 1983, *Bioorganic Chem.* **12**, 8–17.
Yamagata, Y., Matsukawa, T., Mohri, T. and Inomata, K.: 1979, *Nature* **282**, 284–286.
Yamagata, Y., Mohri, T., Yamakoshi, M., and Inomata, K.: 1981, *Origins of Life* **11**, 233–235.
Yamagata, Y. and Mohri, T.: 1982, *Origins of Life* **12**, 41–44.
Yamagata, Y., Yamanaka, J., and Namba, T.: 1984, Oral presentation at the 9th annual meeting of The Society for the Study of the Origin and Evolution of Life in Japan.
Yamagata, Y., Sasaki, K., Takaoka, O., Sano, S., Inomata, K., Kanemitsu, K., Inoue, Y., and Matsumoto, I.: 1990, *Origin of Life* **20**, 389–399.
Yuasa, S., Flory, D., Basile, B., and Oro, J.: 1984a, *J. Mol. Evol.* **20**, 52–58.
Yuasa, S., Flory, D., Basile, B., and Oro, J.: 1984b, *J. Mol. Evol.* **21**, 76–80.