TRACE ELEMENTS IN CHEMICAL EVOLUTION

II: *Synthesis of Amino Acids Under Simulated Primitive Earth Conditions in the Presence of Trace Elements*

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Abstract. Electric discharge experiments have been performed in a plausible primitive earth atmosphere consisting of methane, nitrogen, and water over an aqueous phase of an ammonia-ammonium buffer solution. In some experiments, ions of metal elements, calcium, magnesium, zinc, iron and molybdenum were introduced. Gas phase products and amino acids in the liquid phase were analyzed by gas chromatography. With trace metal ions, less organic compounds in the gas phase and larger amounts of amino acids were obtained than without them. The results have shown the possible importance of trace elements in chemical evolution and the origin of life on the earth.

1. Introduction

Recent progress in analytical techniques has made it possible to recognize the importance of trace elements in biological systems. More than ten trace elements, such as fluorine, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, molybdenum, tin, and iodine, are known as 'essential trace elements' for higher animals. Among them, three elements, iron, zinc, and molybdenum, are believed to be essential for all living organisms including most primitive anaerobic procaryotes (Egami, 1975).

In addition, a large number of metalloenzymes – enzymes whose active center metal ions are essential for their enzymatic activity – have been discovered, and the roles of trace metal ions in these molecules are being studied. A new interdisciplinary field called bioinorganic chemistry and/or inorganic biochemistry, has been established (Eichhorn, 1973).

As the knowledge of the importance of trace elements in the present biological system is increasing, a number of studies on the roles of trace elements in the chemical evolution have been performed (Kobayashi and Ponnamperuma, 1985). For example, several investigators have pointed out that the more abundant trace elements in the present sea water are the more essential ones for living organisms, which supports the generally accepted view that life originated in the primitive sea water (McClendon, 1976; Egami, 1980).

There are several studies on the catalytic roles of trace elements in chemical evolution (Kobayashi and Ponnamperuma, 1985). Oró *et al.* reported that various kinds of amino acids were produced after heating paraformaldehyde and hydroxylamine solution, and that molybdic or vanadic acid could increase their yields (Oró *et al.*,

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1959). Orgel *et al.* studied the effects of metal ions (Zn^{2+}, Pb^{2+}, etc.) when oligonucleotides were formed from activated nucleotides and polynucleotidyl acids (Lohrmann *et al.*, 1980). Egami *et al.* found 'marigranules' – one of the protocell model – when heating amino acids dissolved in 'modified sea medium' including several trace metal ions such as Zn^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Co^{2+}, and MoO_4^{2-} (Yanagawa and Egami, 1980; Yanagawa *et al.*, 1980). These studies suggest that trace metal ions existing in the primitive sea water where life may have have originated should have important roles in the course of chemical evolution.

The studies presented here were done as electric discharge experiments under plausible primitive earth conditions. The role of trace metal ions dissolved in the acqeous phase were examined through analyses of the reaction products using gas chromatography combined with mass spectrometry.

2. Materials and Methods

Methane purchased from Matheson (UHP), and nitrogen from Air Products (UHP), were used as starting gases. All other chemicals used were analytical grade. Organic solvents, such as 2-propanol and dichloroethane, were purified by distillation. Hydrochloric acid (6N) was also purified by repeated azeotropic distillation. Water was de-ionized and glass distilled.

A Varian 2740–10 gas chromatography with an FID detector was used for analysis of gaseous samples and amino acids. The column for gas analysis was Porapak Q (1/8″ i.d. × 48′); that for amino acids was 0.65% EGA on 80/100 Chromosorb wAw (2 mm i.d. × 1.5 m). Peak areas were integrated using an Autolab 6300 Integrator. A Hewlett-Packard gas chromatograph-mass spectrometer (GC–MS) Type 5992–B was also used for identification of amino acids.

A Consolidated Electrodynamic 21–620A type mass spectrometer was used to analyze gaseous samples.

Electric discharges were performed using a 4.3 liter dumbbell shaped flask. The systems for electric discharges are summarized in Table I: Series N had no metal ions; series MS (modified sea) contained two major metal ions, Ca^{2+} and Mg^{2+}, and three of the most important trace metal ions, Zn^{2+}, Fe^{2+}, and MoO_4^{2-}. The initial pH of the aqueous phase was adjusted to 8.7 using 50 mM ammonia-ammonium buffer.

The discharge flask was prepared by washing with: (i) hot alkaline permanganate solution: (ii) 7M nitric acid: and (iii) de-ionized, distilled water successively. Chromic acid could not be used because it is very difficult to remove from glass surfaces (Thiers, 1957).

Discharges were performed as follows: Buffer solution was filtered through 0.2-μm membrane filters, bubbled with nitrogen gas to remove dissolved oxygen, and was put into the flask. The aqueous phase in the flask was degassed by freezing-evacuating-thawing cycles at least three times, and was flushed with nitrogen three times. Then the gas mixture of methane and nitrogen was introduced over the frozen, degassed buffer. The composition of all gas mixtures was monitored with a mass spectrometer.

TABLE I

Systems for electric discharge experiments

Series		N	MS
Gas phase	CH_4	200 torr	200 torr
	N_2	200	200
Water phase	H_2O	100 ml	100 ml
	$NH_4^+NH_3$	50 mM	50 mM
	Ca^{2+}	0	1
	Mg^{2+}	0	1
	Zn^{2+}	0	0.1
	Fe^{2+}	0	0.1
	MoO_4^{2-}	0	0.1
Temperature		60 °C	60 °C
pH		8.7	8.7
Discharge period		48 hr	48 hr

Electric discharge was performed using a tesla coil (Electro-Technic Products BD–50 High Frequency Generator); the spark gap between two tungsten electrodes was 7 mm, and its estimated peak voltage was *ca.* 15 000 V. The discharges were continued for 48 hr at approximately a 50 % duty cycle; a complete experiment therefore required 96 hr. The water phase was heated throughout the experiment to 60 °C with a heating mantle.

2.1. Analysis of Gas Phase Products

The gaseous product in the flask was sampled and analyzed by gas chromatography and mass spectrometry. Gas chromatographic conditions were as follows: Carrier (helium) flow rate: 30 ml min^{-1}, injection port temperature: 250 °C; detector temperature: 250 °C; oven temperature program: 30 °C (4 min) → increase by 4 °C min^{-1} → 200 °C.

2.2 Analysis of Amino Acids

After the electric discharge was over, the water phase in the flask was removed with a pipette, filtered through a membrane filter (pore size: 0.2 μm), then concentrated by a rotary evaporation. Amino acid analysis was performed by the procedures shown in Figure 1: The concentrated sample was divided into two portions. One portion (H) was acid-hydrolyzed with 6M hydrochloric acid at 105 °C for 24 hr, and the other portion (N) was not hydrolyzed. The samples were then derivatized for gas chromatography using 2-propanol and trifluoroacetic anhydride (Draganić *et al.*, 1978) after cation-exchange purification (Bio-Rad AG–50WX8, 20–50 mesh).

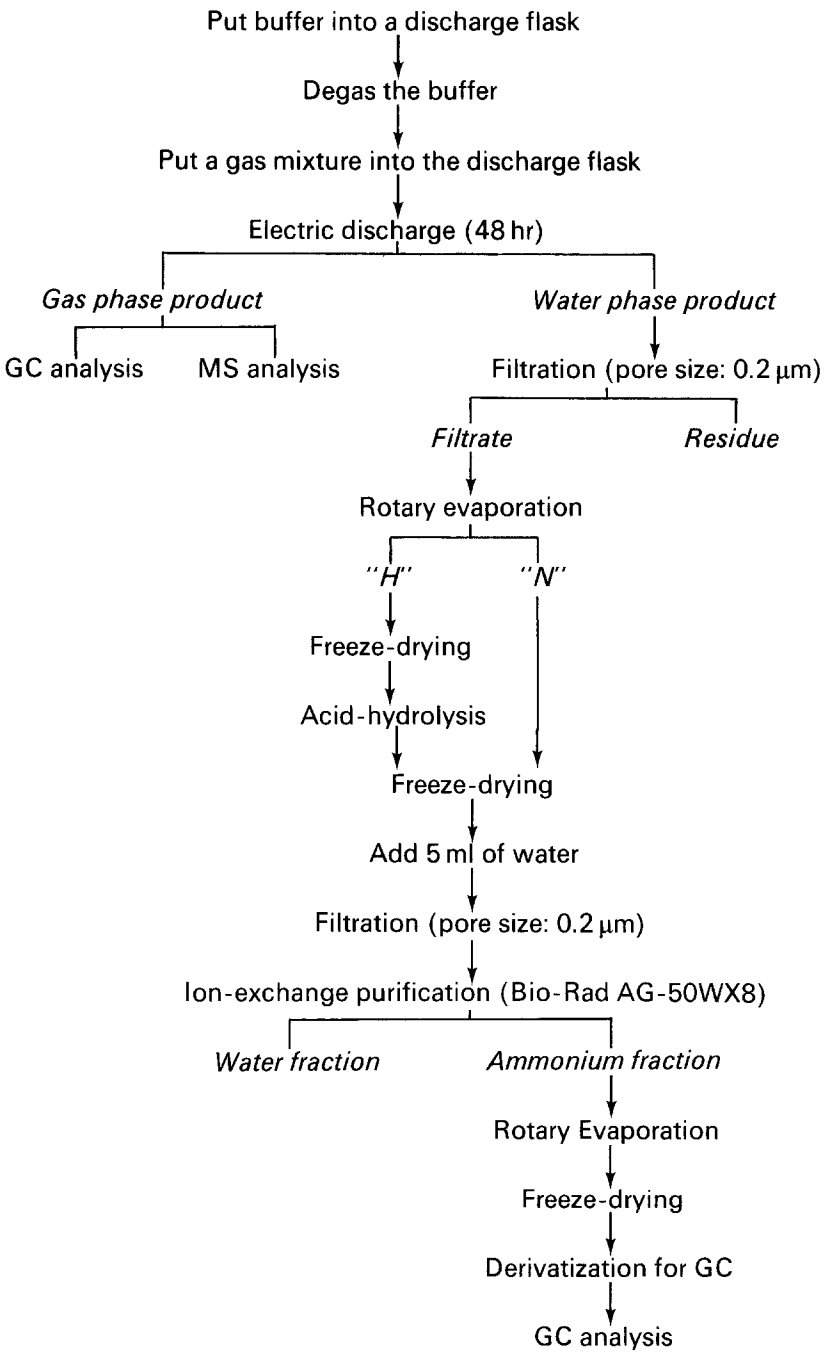

Fig. 1. Experimental procedures.

Gas chromatographic conditions were as follows: Carrier (helium) flow rate: 30 ml min^{-1}; injection port temperature: 250 °C; detector temperature: 250 °C; oven temperature program: 80 °C (4 min) → increase by 4 °C/min → 200 °C.

3. Results

In the course of the electric discharges, both with and without metal ions, the color of the product solution turned golden yellow, and the pH decreased to around 8.0. This was likely due to the consumption of ammonia. The results of gas chromatography and mass spectrometry showed that most of the methane was consumed: less than 10 % of the initial methane remained after 48 hr discharge. Besides the initial nitrogen and methane, hydrogen, acetylene, and carbon monoxide have been shown to be major constituents of the discharge products.

3.1 Analysis of gaseous products

Figures 2 and 3 are the gas chromatograms (GC) of the gaseous products by electric discharge without and with metal ions. In both chromatograms, there are more than 20 peaks. Some of them have been identified using standard gases or solutions. For

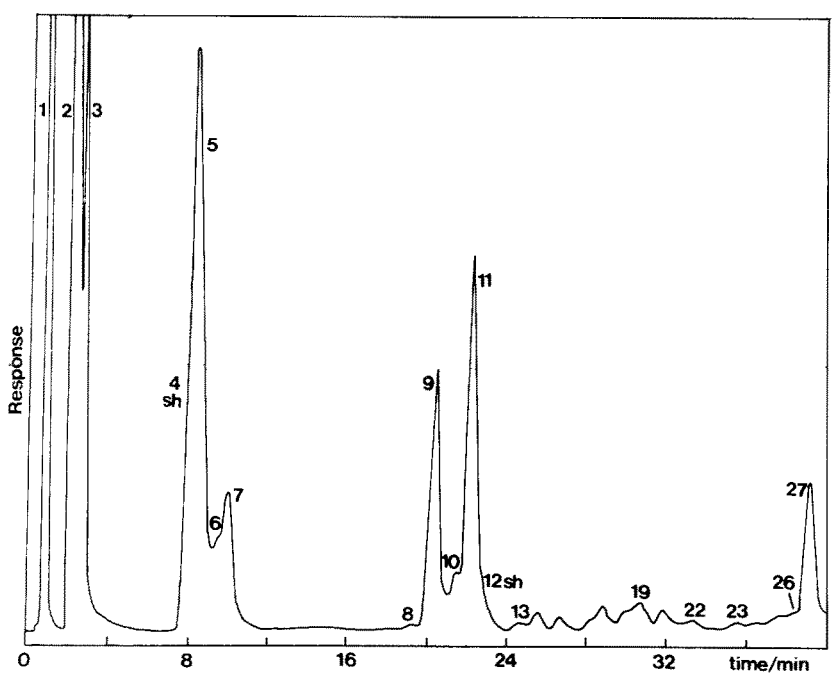

Fig. 2. Gas chromatogram of gaseous products of electric discharge reaction without metal ions (N–17). Column: Porapak Q; carrier: He, 30 ml min^{-1}. Identified peaks are as follows: 1: CH_4; 2: C_2H_2; 3: C_2H_6; 5: C_3H_8; 6: HCN; 10: n–C_4H_{10}; 12: CHCCN; 13: CH_3CN; 19: n–C_5H_{12}; 26: n–C_6H_{14}; 27: C_6H_6.

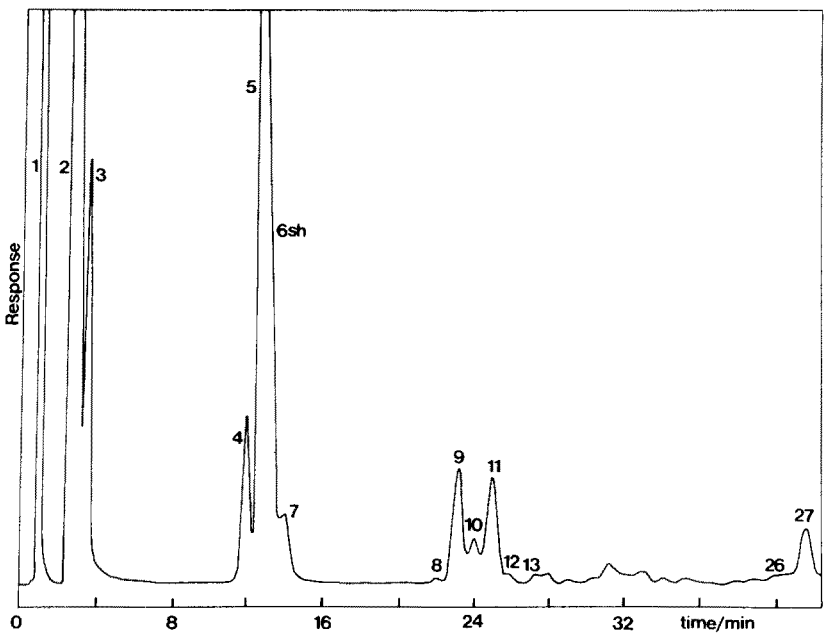

Fig. 3. Gas chromatogram of gaseous products of electric discharge reaction with metal ions (MS–3). Conditions and peak identification are shown in Figure 2.

TABLE II
Determination of gaseous products produced in electric discharge experiments

Group	Peak No.	Relative peak area*		
		Without metal ions (N–17)	With metal ions (MS–3)	Ratio $\left(\frac{\text{MS–3}}{\text{N–17}}\right)$
I	1	1386	984	0.71
II	2– 3	6695	6938	1.04
III	4– 7	627	886	1.41
IV	8–12	500	230	0.46
V	13–22	110	62	0.57
VI	23–27	160	65	0.40

Peak No.	Compound	Partial pressure (torr)		
		N–17	MS–3	Ratio $\left(\frac{\text{MS–3}}{\text{N–17}}\right)$
1	CH_4	20.1	14.3	0.71
2	C_2H_2	37.9	39.6	1.04
3	C_2H_6	1.3	1.1	0.85
5	C_3H_8	1.8	3.4	1.89
6	HCN	1.2	2.4	2.00

* Value given by an integrator.

example, peak No. 1 is methane, No. 2 is acetylene, No. 3 is ethane, No. 6 is hydrogen cyanide, and so on.

Table II shows some quantitative results after GC analysis, where peaks were grouped into 6, and named as C–1 to C–6 group, for convenience.

3.2. Analysis of Amino Acids

Some typical gas chromatograms of amino acids synthesized by electric discharge reactions are shown in Figure 4 (without metal ions) and Figure 5 (with metal ions). In every chromatogram, more than 60 peaks were found. Some major peaks, such as alanine, glycine, β-alanine and aspartic acid, have been identified by GC-MS.

Quantitative results are summarized in Table III.

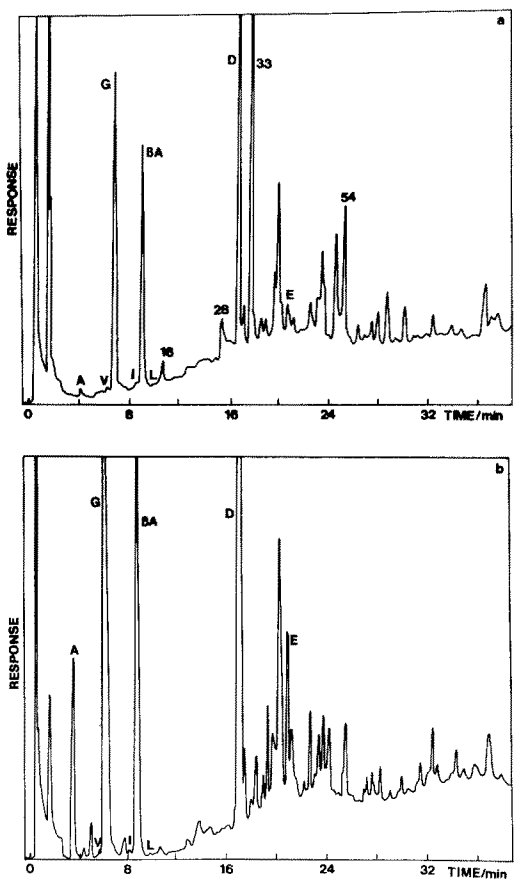

Fig. 4. Gas chromatograms of amino fraction from electric discharge reaction without metal ions (N–7). (a) Before acid-hydrolysis, (b) after acid-hydrolysis. Column: EGA column; carrier: He, 30 ml min^{-1}. Abbreviations for amino acids are as follows: A: alanine, G: glycine, V: valine, I: Isoleucine, βA: β-alanine, L: leucine, D: aspartic acid, E: glumatic acid.

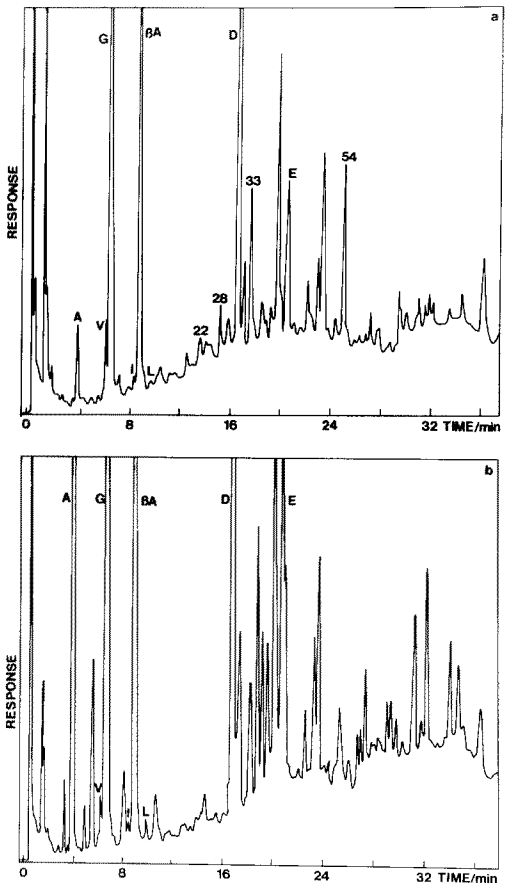

Fig. 5. Gas chromatograms of amino fraction from electric discharge reaction with metal ions (MS–1). (a) Before acid-hydrolysis, (b) after acid-hydrolysis. Column: EGA column; carrier: He, 30 ml min^{-1}. Abbreviations for amino acids are shown in the caption of Figure 4.

4. Discussion

4.1 Experimental conditions

The primary atmosphere of the earth was believed to be non-oxygenic, whose constituents were methane, ammonia and water (Miller, 1953). The primary atmosphere was probably replaced by a secondary atmosphere in which the gaseous ammonia was rapidly dissociated (Abelson, 1966).

We used a mixture of methane, nitrogen and water with ammonium ion as a basic system ('N') (Ring *et al.*, 1972). The initial pH of the aqueous phase was set at 8.7. The pH was decreased slightly during electric discharges to *ca.* 8.0, a plausible pH for the primitive and the present sea water.

TABLE III

Determination of amino acids synthesized in electric discharge experiments

	N-7		MS-1	
	N*	H**	N*	H**
Alanine	0.4 μmol	9.2 μmol	8.2 μmol	97 μmol
Valine***	0.06	0.10	2.6	1.2
Glycine	5.6	20	27	52
Isoleucine***	0.04	0.02	0.1	0.2
β-Alanine	2.8	6.0	13	23
Leucine	0.01	0.01	0.02	0.06
Aspartic acid	1.7	5.8	4.2	7.4
Glumatic acid	0.1	0.4	1.1	3.0
Total	11	42	56	184
Total as C	29	111	154	516
Yield		0.25%		1.1%

* Before hydrolysus.
** After hydrolysis.
*** Values have some uncertainty, because these peaks overlap a giant peak of glycine or β-alanine.

Egami *et al.* used Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, MoO_4^{2-}, Co^{2+}, and Mn^{2+} in their modified sea medium (Yanagawa and Egami, 1980). Here we simplified this mixture, and used only three of the most important trace elements, iron, zinc and molybdenum, with two major metal ions, Ca^{2+} and Mg^{2+}.

Zinc could have existed as Zn^{2+}, iron and molybdenum may have been in the form of Fe^{2+} and MoO_4^{2-}, because of their availability in water-soluble forms in the early stage of chemical evolution in sea water. Copper would be less important, because (i) it seems to have been water-insoluble Cu(I), and (ii) most primitive bacteria do not require copper (Ochiai, 1978).

4.2. Gas Phase Products

Figures 2 and 3 and Table III show that there was an apparent difference in gas products generated in the experiments with ('MS') and without ('N') metal ions. Particularly, the systems with metal ions gave smaller amounts of less volatile organic compounds, such as those in C-4-6 group, than the system without metal ions. On the other hand, the peaks of cyanoacetylene and hydrogen cyanide of the former were larger than those of the latter.

In the case of HCN, for example, we can consider the following pathway:

$$\begin{array}{l} CH_n + N \rightleftarrows (HCN)_{gas} \rightleftarrows (HCN)_{aq} \rightleftarrows [M(CN)_n] \\ \quad \Updownarrow \\ (C_mH_n)^{gas} \end{array}$$

where CH_n and N are radicals, $(HCN)_{gas}$ is hydrogen cyanide in the gas phase, $(HCN)_{aq}$ is hydrogen cyanide dissolved in water, $[M(CN)_n]$ is a cyanide complex, $(C_mH_n)_{gas}$ is a hydrocarbon in gas phase. Of course, the actual reactions are much more complicated than this.

Metal ions (M) can complex with cyanide ion, which then facilitates the transportation of hydrogen cyanide from the gas phase into the aqueous phase. As a result, more CH_n was transformed into HCN, and less hydrocarbons were formed.

Beck pointed out that HCN, $(CN)_2$, NH_3 and acetylene were the most important compounds for the complex-forming properties in primordial sea water (Beck, 1978). Acetylene was the most abundant product in electric discharge reactions. Metal ions must have had the role of fixing acetylene and other organic compounds in gas phase into aqueous solution to introduce further chemical evolution in water phase.

4.3. Yield of Amino Acids

As shown in Table IV, about 1 % of the initial methane was incorporated into amino acids. Figures 4 and 5 show that the yields of amino acids increased after acid hydrolysis. However, some peaks, such as Nos. 33 and 54, actually decreased after hydrolysis. This may indicate that there were peaks due to peptides. Only major amino acid peaks have been identified by GC–MS. The identification of the small peaks of amino acids (or peptides) is currently under way.

Based on comparisons between Figure 5 and Figure 6, the MS-system generally gave more amino acids than the N-system. The amounts of the simpler amino acids, for example, glycine, β-alanine and aspartic acid, were almost the same with and without metal ions; the differences in the amounts of the more complex amino acids (valine, glutamic acids, etc.) were, however, significant. The metal ions in primordial sea water might thus have catalyzed the formation of amino acids, especially the more complicated amino acids.

In preliminary experiments using only zinc ions, the total yield of amino acids was almost the same as those without any metal ions, but the chromatographic pattern of the former was different from that of the latter. This indicates that iron and molybdenum can facilitate the transfer of organic materials from the gas phase, and that zinc may have catalyzed reactions in the water phase.

5. Conclusion

We have shown that trace metal ions, such as Fe^{2+}, Zn^{2+}, and MoO_4^{2-}, existing in the primordial sea could have influenced the chemical reactions during the course of chemical evolution. Trace metal ions fixed some organic compounds in a gas phase into an aqueous phase, and gave larger amounts of amino acids. Systems with and without metal ions also gave the five naturally-occurring nucleic acid bases (Kobayashi *et al.*, 1986). Studies on the role of metal ions in base synthesis are currently under way.

To consider the role of trace metals in primordial sea water, it is important to examine the chemical forms of trace elements. In present-day sea water, it has been

demonstrated that some of the dissolved trace metals are in the form of metalloenzymes, such as zinc in alkaline phosphatase (Kobayashi *et al.*, 1983). The ratio of trace metal ions in metalloenzymes dissolved in sea water to total dissolved trace metal ions is very low, but their catalytic activity is quite significant and can influence the ecosystem.

In the processes of chemical evolution, the situation may have been the same. The most abundant metal complex could have been a cyano-complex. However, other less-abundant metal complexes might be equally important. As shown by Vallee and Williams (Vallee and Williams, 1968), less stable complexes work as better catalysts in present biosystems. The analysis of metal complexes in primordial soups may provide useful information of the catalytic role of trace metals in chemical evolution. Complexes of trace metal ions will also be key materials on the origin of autocatalyzation and self-organization (Calvin, 1959).

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