ABIOTIC PHOTOSYNTHESIS OF SUBSTANCES RELATIVE TO THE ORIGIN OF LIFE FROM AQUEOUS AMMONIUM CARBONATE SOLUTIONS

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Abstract - It has been demonstrated that amino acids and nucleic acid bases can be synthesized by UV irradiation even in such rather oxidizing atmosphere as aqueous solutions containing CO_2 , HCO_3^- and/or CO_3^{2-} in the absence of CH_4 or H_2 . For the production of amino acids and nucleic acid bases from ammonium carbonate solution or water to which CO_2 and NH_3 gases had been dissolved, UV shorter than 250 nm, temperature higher than 80 °C and the coexistence of Mg^{2+} were found to be effective. On the basis of some experimental evidences, oxalic acid and/or oxamic acid are assumed to be the possible intermediates for the synthesis.

Key words Photosynthesis, Amino acids, Nucleic acid bases, Ammonium carbonate, Intermediates

Since the synthesis of amino acids from CH_4 , NH_3 , H_2 and H_2O by Miller[1], wide variety of molecules of importance for the origin of life have been produced from mixtures of nonbiological substances with the aid of various energy sources. The mixtures adopted in most of previous works have been those which provide reducing atmosphere[2]. However, Judging from the oxidizing compositions of volcanic gases[3] and airs on Venus or Mars[4] and the geochemical estimations[5,6] based on the buffer actions of silicates or carbonates, it is fair to consider that the air on the primitive earth had been rather oxidizing and contained CO_2 instead of CH_4 .

In the present paper, the photosyntheses of amino acids and nucleic acid bases from aqueous ammonium carbonate solutions or water into which CO_2 and NH_3 had been dissolved are introduced and the processes for the syntheses are discussed. In connection with the present subject, Paschke et al.[7] synthesized glycine by α -ray irradiation of solid ammonium carbonate and Abelson[8] formed several kinds of amino acids in gaseous mixtures of CO_2 or CO , N_2 or NH_3 , H_2O and H_2 . Oro' et al.[9] reported the production of amino acids in a heated aqueous solution of formaldehyde and hydroxylamine and gave an interpretation of the synthesis. The group of Egami pointed out the important role of transition elements[10,11] in the synthesis of amino acids and peptides in the Oro's system, and found the formation of glycine and alanine from various sugars and ammonia in a modified sea water which contains various transition elements[12].

EXPERIMENTAL

A high pressure mercury arc lamp (H400-P, product of Toshiba Co. Ltd.) of which spectrum distributes between 230 and 600 nm was used as a light source. The quantity of light irradiated (dose-rate) was determined by a uranyl oxalate actinometer to be 2.4×10^{18} photons/s. Water used was distilled over permanganate followed by twice distillations. Although reagents of reagent grade were used without further purification, blank tests for amino acids and nucleic acids were carried out thoroughly for each reagents.

In a typical experiment, 35 ml of 10 % ammonium carbonate aqueous solution (pH = 8.9) was sealed in a quartz ampule and irradiated at 90 °C for 100 hr. The resulting solution was heated at 90 °C to dryness under the reducing pressure in order to remove the remaining ammonium carbonate and the residue (white) was hydrolyzed at 105 °C for 48 hr in 6 M HCl. After evaporating the HCl solution at 90 °C to dryness, the residue was dissolved with 35 ml of water. Amino acids in the solution were separated by HPLC (SCL-6A, product of Shimadzu Co.) equipped with a column of cation exchange resin (Shim-pack ISC-07/S1504Li, a product of Shimadzu Co.) and detected based on ninhydrin reaction[13]. Nucleic acid bases were separated by HPLC with an ODS reversed phase column (Shim-pack CLC-ODS, a product of Shimadzu Co.) and detected spectrophotometrically at 260 nm.

RESULTS AND DISCUSSION

When the sample obtained by the UV irradiation of 10 % ammonium carbonate solution for 100 hr at 90 °C had not been hydrolyzed with HCl before the HPLC analysis, small peaks corresponding to 1.8×10^{-6} M glycine and 4×10^{-7} M alanine appeared in the chromatogram. Upon hydrolysis of the same sample, however, 1.7×10^{-5} M glycine, 2.8×10^{-6} M alanine, 6.0×10^{-6} M serine and 1.4×10^{-6} M aspartic acid were detected. These results suggest that the irradiation product contained polymer(s) which is composed of several kinds of amino acids.

Tables 1 and 2 summarize the concentrations of amino acids in sample solutions obtained under various conditions.

TABLE 1. Amino acids formed by UV irradiation of aqueous ammonium carbonate (AC) solution (pH = 8.9) at 90 °C

Conc. of AC (%)	Irradiation time (hr)	Conc. of Amino acids formed ($\times 10^{-7}$ M) ^a			
		Glycine	Alanine	Serine	Aspartic acid
10	100	170	28	6	14
20		650	175	22	51
5		83	19	3	8
10	440	575	78	21	74
	20	22	3	N.D.	2
10 ^b	100	21	N.D.	N.D.	N.D.
10 ^c	1000	7	2	N.D.	N.D.
10 ^d	100	18	6	N.D.	N.D.
NH ₃ + CO ₂ ^e	100	148	21	N.D.	8

a; Results after hydrolysis of irradiation products in 6 M HCl. Results given are concentrations converted to those in irradiated solutions (35 ml). b; Irradiated with light of wave length longer than 250 nm. c; Not irradiated. d; Irradiated at 25 °C instead of 90 °C. e; Aqueous solution prepared bubbling CO₂ into 1 M NH₃ until pH = 8.9. N.D.; Not detected.

TABLE 2. Amino acids formed by UV irradiation (100 hr) of aqueous ammonium carbonate (AC) solution at 90 °C under various conditions

Conc. of AC (%)	Reagent coexisted	pH	Conc. of Amino acids formed ($\times 10^{-7}$ M) ^a			
			Glycine	Alanine	Serine	Aspartic acid
10	none	8.9	170	28	6	14
10	none	9.9	36	19	N.D.	5
		7.0	59	23	N.D.	N.D.
10	0.1 M MgCl ₂	8.9	270	120	21	29
	0.1 M MgSO ₄		293	131	24	32
	2 M NaCl		187	27	9	19
10	0.05 M oxalic acid	8.9	364	107	29	32
	0.05 M oxamic acid		431	86	23	28
10	0.1 M MgCl ₂ + 0.05 M oxalic acid	8.9	1200	1230	83	186
	0.1 M MgCl ₂ + 0.05 M oxamic acid		1430	1170	72	158
	0.1 M MgCl ₂ + 0.05 M oxamide		1900	1190	68	161

a; Results after hydrolysis of irradiation products in 6 M HCl. Results given are concentrations converted to those in irradiated solutions (35 ml). N.D.; Not detected.

Data given are those determined after the hydrolysis.

Yields of amino acids increased with the initial concentration of ammonium carbonate (more than proportional) and were nearly proportional to the irradiation time.

The effect of temperature of the solution during the irradiation on the formation of amino acids was remarkable; i.e., the irradiation at 25 °C yielded negligible amounts of amino acids.

Practically no amino acids were formed by heating for 1000 hr at 90 °C in the dark. Selecting the range of wave length of the light with the aid of color filter glasses, UV shorter than 250 nm was found to be effective for the production of amino acids.

In another experiment, CO₂ gas was bubbled into 35 ml of 1 M ammonium solution, which had been prepared from gaseous NH₃ and distilled water, until pH of the solution attained to 8.9, and the solution was irradiated for 100 hr at 90 °C. The yields of amino acids and nucleic acid bases were similar to those with ammonium carbonate solution as seen in Table 1. Effects of various conditions during the irradiation were also similar to those with ammonium carbonate solutions.

When ammonium carbonate solutions of which pH had been adjusted to be 9.9 or 7.0 by adding LiOH or HCl, respectively, were irradiated, the yields of amino acids were smaller than that at pH = 8.9.

The coexistence of MgCl₂ or MgSO₄ in the ammonium carbonate solution resulted in the increase of the yields of amino acids, especially alanine as can be seen in Table 2, though the presence of NaCl up to 2 M gave no effect.

TABLE 3. Nucleic acid bases formed by UV irradiation of aqueous ammonium carbonate (AC) solution of pH = 8.9 at 90 °C under various conditions

Conc. of AC (%)	Reagent coexisted	Irradiation time (hr)	Conc. of bases formed (x 10 ⁻⁷ M) ^a	
			Cytosine	Uracil
10	none	100	40	18
20			164	25
10	none	440	127	33
10 ^b	none	100	N.D.	N.D.
10 ^c	none	100	3	2
10	0.1 M MgCl ₂	100	68	26
	0.05 M oxalic acid		170	81
	0.05 M oxamic acid		265	178
	0.05 M oxamide		814	420

a; Results after hydrolysis of irradiation products in 6 M HCl. Results given are concentrations converted to those in irradiated solutions (35 ml). b; Not irradiated. c; Irradiation at 25 °C instead of 90 °C. N.D.; not detected.

Nucleic acid bases in the sample which had been prepared irradiating 10 % ammonium carbonate for 100 hr at 90 °C were determined by HPLC. Three small peaks appeared in the chromatogram when the sample had not been hydrolyzed. After the hydrolysis with 6 M HCl, peaks at positions corresponding to cytosine and uracil grew remarkably and 4.0×10^{-6} M cytosine and 1.8×10^{-6} M uracil were found. Effects of various conditions during the irradiation on yields of the bases were similar to those observed for amino acids, as seen in Table 3.

Since oxalic acid, oxamic acid or oxamide were assumed to be the possible intermediates to form amino acids and/or nucleic acid bases from ammonium carbonate which is composed of ammonium carbamate and ammonium hydrogencarbonate, 0.05 M oxalic acid, oxamic acid or oxamide were added into solutions before the irradiation and the irradiation was carried out. Then, the yields of amino acids and nucleic acid bases in the irradiated solutions were investigated. As clearly seen in Tables 2 and 3, the presence of the intermediates facilitated the formation of amino acids and nucleic acid bases. The yields of amino acids were nearly proportional to concentrations of the intermediates. The yields of nucleic acid bases also increased with the increase of concentrations of the intermediates, though the reproducibility was fairly poor. When 0.05 M oxamic acid in the absence of ammonium carbonate was irradiated, amounts of amino acids and nucleic acid bases formed were negligible even in the presence of 0.1 M MgCl₂.

In order to confirm the formation of oxalic acid, oxamic acid and/or oxamide as the intermediates, a solution containing 10 % ammonium carbonate and 0.1 M MgCl₂ had been irradiated for 100 hr at 90 °C and, after the hydrolysis with 6 M HCl, the resulting solution was analyzed by HPLC equipped with an ODS reversed phase column (Shim-pack CLC-ODS) and spectrophotometer for the detection at 210 nm. The eluent employed was 0.01 M in tri-n-octylamine and 0.002 M in phosphoric acid (pH = 6.3, flow-rate; 0.8 ml min⁻¹). Oxalic acid, oxamic acid and oxamide found were 1.4×10^{-4} , 1.3×10^{-4} and less than 10^{-6} M, respectively. The voltammetric determination of oxalic acid and oxamic acid based on the oxidation waves at a stationary platinum electrode (cf. Fig. 2) supported the results by HPLC.

Fig. 1 Ammonium hydrogencarbonate, ammonium carbonate, oxalic acid, oxamic acid and oxamide.

Fig. 2 Voltammograms of oxalic acid, oxamic acid and the irradiated ammonium carbonate solution at a stationary platinum disk electrode (5 mm ϕ). Supporting electrolyte: 0.5 M Li_2SO_4 + 0.25 M KH_2PO_4 ; Scan-rate: 0.5 mVs^{-1} ; 1: 3×10^{-3} M oxalic acid; 2: 6×10^{-3} M oxamic acid; 3: the sample obtained after irradiation of 10% ammonium carbonate solution in the presence of 0.1 M MgCl_2 for 100 hr at 90 $^\circ\text{C}$ followed by 5 times concentration; : residual current.

Yanagawa et al.[12] reported that N-acylamino acids were formed from α -oxo acids and ammonia in aqueous medium by thermal energy and N-acylamino acids gave α -amino acids and carboxylic acids such as oxalic acid after 6 M HCl hydrolysis. In connection with the chemical evolution, they assumed the formation of formaldehyde at the initial stage from CO[14], CO_2 [15] and water by UV irradiation, or from CH_4 and water by electric discharge[16]. Formaldehyde forms α -oxo acids through aldol condensation.

In the present work, the formation of amino acids and nucleic acid bases from CO_2 and NH_3 in water by UV irradiation at high temperature has been clearly demonstrated, carboxylic acids such as oxalic acid or oxamic acid have been recognized as important intermediates, and Mg^{2+} has been proved to be one of effective catalysts for the formation of amino acids and nucleic acid bases.

Finally, as ammonium carbonate is composed of ammonium carbamate and ammonium hydrogen carbonate and CO_2 dissolved together with NH_3 in water exists as ammonium hydrogen carbonate at pH = 8 to 9, we consider that the investigation on photochemical reactions of hydrogen carbonate at high temperature may endow much information on the processes involved in the formation of amino acids and nucleic acid bases.

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