

COMMUNICATION

Abiotic Photosynthesis of Substances Relative to the Origin of Life from Aqueous Ammonium Carbonate Solutions

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Since the synthesis of amino acids from CH_4 , NH_3 , H_2 and H_2O by Miller¹⁾, 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 atmosphere²⁾. Judging from the oxidizing compositions of volcanic gases³⁾ and airs on Venus or Mars⁴⁾ and the geochemical estimations^{5,6)} based on the buffer actions of silicates or carbonates, however, 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 communication, 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 briefly. In connection with the present subject, Paschke et al.⁷⁾ synthesized glycine by γ -ray irradiation of solid ammonium carbonate and Abelson⁸⁾ 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 elements¹²⁾.

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 purifica-

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tion, 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 was separated by HPLC (SCL-6A, product of Shimadzu Co.) equipped with a column of cation exchange resin (Shim-pack ISC-07/S1504Li, product of Shimadzu Co.) and detected based on ninhydrin reaction¹³).

When the sample had not been hydrolyzed with HCl, 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 asparatic acid were detected. The results given in the following are those determined after hydrolysis.

Table 1. Amino Acids Formed by UV Irradiation of Aqueous Ammonium Carbonate (AC) Solution at 90°C under Various Conditions.

Conc. of AC (%)	Reagent coexisted	pH	Irradiation time (hr)	Conc. of Amino acids formed ($\times 10^{-7}$ M) ^a			
				Glycine	Alanine	Serine	Asparatic acid
10	none	8.9	100	170	28	6	14
20				650	175	22	51
5				83	19	3	8
10	none	8.9	440	575	78	21	74
			20	22	3	N.D. ^b	2
10 ^c	none	8.9	100	21	N.D.	N.D.	N.D.
10	none	9.9	100	36	19	N.D.	5
		7.0		59	23	N.D.	N.D.
10	0.1 M MgCl ₂	8.9	100	270	120	21	29
	0.1 M MgSO ₄			293	131	24	32
	2 M NaCl			187	27	9	19
10	0.1 M MgCl ₂	8.9	100	1200	1230	83	186
	0.05 M oxalic acid						
	0.1 M MgCl ₂			1430	1170	72	158
	0.05 M oxamic acid						
	0.1 M MgCl ₂			1900	1190	68	161
	0.05 M oxamide						
NH ₃ +CO ₂ ^d	none	8.9	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; N.D.; Not detected. c; Irradiated with light of wave length longer than 250 nm. d; Aqueous solution prepared bubbling CO₂ into 1 M NH₃ until pH=8.9.

As summarized in Table 1, concentrations of amino acids produced 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. In this regard, 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 colour filter glasses, UV shorter than 250 nm was found to be effective for the production of amino acids. When pH of the ammonium carbonate solution was adjusted to be 9.9 or 7.0 by adding LiOH or HCl, respectively, the yield of amino acids was 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, though the presence of NaCl up to 2 M gave no effect.

Bases in nucleic acids in the irradiated ammonium carbonate solution were also examined by HPLC with an ODS reversed phase column (Shim-pack CLC-ODS, product of Shimadzu Co.) and spectrophotometric detection at 260 nm, after the treatment of the sample with the procedure same as that for amino acids. In the chromatogram of sample provided by the irradiation of 10% ammonium carbonate for 100 hr at 90°C, three small peaks appeared when the sample had not been hydrolyzed. With the hydrolysis in 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 on yields of the bases were similar to those observed with amino acids, as seen in Table 2.

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. Concerning with the yields of amino acid and nucleic acid bases and various effects on them, similar results to those with ammonium carbonate were obtained.

Since we assumed oxalic acid, oxamic acid or oxamide as the intermediates to form

Table 2. 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 ($\times 10^{-7}$ M) ^a	
			Cytosine	Uracil
10	none	100	40	18
20			164	25
10	none	440	127	33
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).

amino acids and/or nucleic acid bases from ammonium carbonate, the irradiation of solutions containing 10% ammonium carbonate and 0.05 M oxalic acid, oxamic acid or oxamide was carried out in the presence or absence of 0.1 M MgCl_2 .

As clearly seen in Tables 1 and 2, 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 increases 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 MgCl_2 .

In order to confirm the formation of oxalic acid, oxamic acid and/or oxamide as the intermediates, the product in the solution containing 10% ammonium carbonate and 0.1 M MgCl_2 irradiated for 100 hr at 90°C was analyzed after hydrolysis in 6 M HCl by HPLC equipped with an ODS reversed phase column (Shim-pack CLC-ODS) and spectrophotometer for the detection at 210 nm. 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 platinum electrode supported the results by HPLC.

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 $\text{CO}^{14)}$, $\text{CO}_2^{15)}$ and water by UV irradiation, or from CH_4 and water by electric discharge¹⁶⁾. Formaldehyde reacts by aldolcondensation to form α -oxo acids. 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 $\text{pH}=8$ to 9, we consider that the investigation on photochemical reactions of hydrogen carbonate at high temperature may endow much information on the process involved in the formation of amino acids and nucleic acid bases.

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