The Formation of Amino Acids by the Reactions of Singlet NH with Several Carboxylic Acids

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The photolysis of hydrogen azide was studied in liquid acetic acid, propionic acid, and isobutyric acid at room temperature. The formation of amino acids was confirmed by the color reactions with ninhydrin and with chromotropic acid and by the NMR spectra. From the propionic acid, α- and β-alanine were formed in the ratio of 1.5. In the case of isobutyric acid, α- and β-aminoisobutyric acids were formed in the ratio of about 5. These results are explained by the insertion reaction of NH($a^1\Delta$) into the C–H bonds of carboxylic acids.

The formation of NH($a^1\Delta$) has been observed in the photolysis of hydrogen azide with light of wavelengths between 250 and 320 nm.[1] Since NH radicals are isoelectronic with O or CH_2 radicals, it is expected that the reactions of the singlet NH radicals are similar to those of O(^1D) and CH_2($^1A_1$). Recently, we photolyzed hydrogen azide in liquid paraffin at the temperature of Dry Ice–methanol and found the formation of amines: ethylamine from the ethane solution, propyl- and isopropylamine from the propane solution, and isobutyl- and *t*-butylamine from the isobutane solution.[2,3] These amine formations were explained by the insertion reaction of the singlet NH into the C–H bonds of paraffins.

If the singlet NH inserts into the C–H bonds of hydrocarbons, the singlet NH will insert into the C–H bonds of carboxylic acid to form amino acids. The formation of amino acids on the primitive earth is an interesting problem in the study of prebiotic molecular evolution. The formation of singlet NH has been found in the discharge and vacuum ultraviolet photolysis of ammonia.[4] Since ammonia is known to have been an important component of the primordial atmosphere, the insertion reaction of the singlet NH into the C–H bonds of carboxylic acid, if it occurred, might have been important for the formation of amino acids on the primitive earth.

For these reasons, the photolysis of hydrogen azide was studied in liquid propionic acid, as was shown in a previous letter, and the formation of α- and β-alanine was observed.[5] The present paper will report the details of the previous letter, together with new results obtained with acetic acid and isobutyric acid.

Experimental

The methods of the preparation and purification of hydrogen azide have already been described.[2,3] Acetic acid, propionic acid, and isobutyric acid (Tokyo Kasei Co.) were used after distillation *in vacuo*.

The reaction cell was a Pyrex tube, 10 mm o.d., in which about 2 cm³ of carboxylic acid and a small amount of hydrogen azide were introduced *in vacuo*. A medium pressure mercury lamp (Toshiba HP 400) was used to illuminate the reaction cell through a transparent quartz Dewar flask filled with distilled water. Since a Pyrex tube was used as a reaction cell, the effective wavelengths absorbed by hydrogen azide were limited within about 280—320 nm.

After the irradiation, non-condensable products at −196 °C were collected with a Toepler pump and their amount was measured with a gas burette. Unreacted hydrogen azide and carboxylic acid were evacuated *in vacuo* at about 50—90 °C. The residue in the cell was dissolved in water and subjected to the analysis by color reactions.[6] A D_2O solution of the residue was analyzed with an NMR spectrometer (JEOL FX 100). The NMR spectra were compared with those obtained with D_2O solutions of glycine, α- and β-alanine, and α- and β-aminoisobutyric acid (Tokyo Kasei Co.). To measure the amount of the products, a known amount of maleic acid was added to the NMR sample, and the integrated intensities were compared.

Results

The non-condensable product at −196 °C was nitrogen. The mass spectrometric analysis showed neither methane nor hydrogen. The yield of nitrogen increased with the increase in the concentration of hydrogen azide and in the irradiation time.

When the solvent was evacuated together with unreacted hydrogen azide, a white solid was left on the

TABLE 1. THE RATIOS OF THE YIELDS OF AMINO ACIDS TO THAT OF NITROGEN IN THE PHOTOLYSIS OF HYDROGEN AZIDE IN CARBOXYLIC ACIDS

$\frac{[HN_3]}{\text{mol dm}^{-3}}$	Irradiaton time/h	α/N_2	β/N_2	β/α
CH_3COOH				
0.10	4	0.21	—	—
0.10	3	0.19	—	—
0.10	2	0.21	—	—
0.05	4	0.22	—	—
0.05	2	0.22	—	—
	Mean	0.21±0.02		
CH_3CH_2COOH				
0.10	3	0.19	0.25	1.3
0.10	2	0.13	0.21	1.6
0.10	1	0.20	0.32	1.6
0.05	3	0.11	0.18	1.6
0.05	2	0.16	0.24	1.5
	Mean	0.16±0.04	0.24±0.05	1.5±0.1
$(CH_3)_2CHCOOH$				
0.10	4	0.082	0.47	5.7
0.10	3	0.091	0.44	4.8
0.10	2	0.083	0.41	4.9
0.05	5	0.079	0.40	5.1
0.05	3	0.083	0.38	4.6
	Mean	0.084±0.005	0.42±0.04	5.0±0.4

wall of the reaction cell in the cases of acetic acid and propionic acid. In the case of isobutyric acid, the residue was a yellowish liquid. When methanol was added to this liquid, the white solid remained undissolved. The white solid was soluble in water.

In the case of acetic acid solution, the formation of glycine was confirmed by the color reactions with ninhydrin and with chromotropic acid. The color observed for the products agreed well with that observed with an authentic glycine.

The D_2O solution of the residue was subjected to the NMR analysis. The NMR spectra of the products coincided with those obtained with a mixture of amino acids and the reactant acid: glycine with acetic acid, α- and β-alanine with propionic acid, and α- and β-aminoisobutyric acid with isobutyric acid. An attempt to eliminate the reactant acid from the products was not successful. The ratios of the yield of amino acid to that of nitrogen are summarized in Table 1, as functions of the concentration of hydrogen azide and of the irradiation time.

Discussion

As shown in Table 1, the ratios of the yield of amino acid to that of nitrogen were independent of the changes in the concentration of hydrogen azide and in the irradiation time for every case examined. The trend observed is similar to that observed in the photolysis of hydrogen azide in liquid ethylene or propene at the temperature of Dry Ice–methanol.[7] The following mechanism can be considered to explain the results:

$$NH_3 + h\nu \rightarrow NH(a^1\Delta) + N_2 \qquad (1)$$

$$NH(a^1\Delta) + CA \rightarrow \text{Amino acids} \qquad (2)$$

$$\rightarrow NH(X^3\Sigma^-) + CA \qquad (3)$$

$$NH(X^3\Sigma^-) + NH_3 \rightarrow NH_2 + N_3 \qquad (4)$$

$$NH_2 + HN_3 \rightarrow NH_3 + N_3 \qquad (5)$$

$$2\,N_3 \rightarrow 3\,N_2, \qquad (6)$$

where CA is carboxylic acid. Reaction 2 is the insertion reaction of the singlet NH into the C–H bond of carboxylic acid. In the cases of propionic acid and isobutyric acid, α- and β-amino acids are formed, which correspond to the two types of the C–H bonds involved in the carboxylic acid. As shown in Table 1, the β/α ratios in alanine and in aminoisobutyric acid produced were 1.5 and 5.0 respectively; these are very close to the ratios of the numbers of the C–H bonds in the two types. These results agree well with the assumption that the amino acids are formed by the insertion reaction.

Reaction 3 is the quenching of the singlet NH by carboxylic acid. In the above mechanism, the triplet NH is assumed to abstract a hydrogen atom from hydrogen azide. The triplet NH might abstract a hydrogen atom from carboxylic acid:

$$NH(X^3\Sigma^-) + CA \rightarrow NH_2 + R. \qquad (7)$$

When the resultant radical, R, was assumed to abstract a hydrogen atom from hydrogen azide:

$$R + HN_3 \rightarrow CA + N_3. \qquad (8)$$

Reaction 7 followed by Reaction 8 is indistinguishable from Reaction 4.

In the present experiment, ammonia could not be analyzed, since it was difficult to isolate it from the reactant. If ammonia is formed, an ammonium salt will be formed by the reactions with acid. The ammonium salt may be evacuated together with the reactant and/or will give $RCOO^-$ in the D_2O solution. Thus the NMR spectra of the ammonium salt in D_2O are the same as those of the reactant acid.

According to the above mechanism, (1)—(6), the quantum yields of amino acid (ϕ_a) and of nitrogen (ϕ_n) should have the following relation:

$$\phi_n = 4 - 3\phi_a. \qquad (9)$$

Using the values of relative yields (ϕ_a/ϕ_n) shown in Table 1, the quantum yields of amino acid formation can be calculated. The results are shown in Table 2. As are shown in Table 2, the quantum yield of amino acid increased with an increase in the number of C–H bonds in the reactant acid.

TABLE 2. THE QUANTUM YIELDS OF AMINO ACID FORMATION

Reactant	α-Amino acid	β-Amino acid	Total
CH_3COOH	0.52	—	0.52
CH_3CH_2COOH	0.29	0.43	0.72
$(CH_3)_2CHCOOH$	0.13	0.67	0.80

Miller found the formation of amino acids in the electric discharge of a mixture of methane, ammonia, hydrogen, and water.[8] This mixture is considered to be the primordial atmosphere of earth. He considered that α-amino acids are formed by the hydrolysis of cyanohydrin:

$$RCHO + NH_3 + HCN \rightarrow RCH(NH_2)CN + H_2O \qquad (10)$$

$$RCH(NH_2)CN + 2H_2O \rightarrow RCH(NH_2)COOH + NH_3. \qquad (11)$$

For the formation of β-alanine, he assumed acrylonitrile as the intermediate:

$$CH_2{=}CHCN + NH_3 \rightarrow NH_2CH_2CH_2CN \qquad (12)$$

$$NH_2CH_2CH_2CN + 2H_2O \rightarrow NH_2CH_2CH_2COOH + NH_3. \qquad (13)$$

Miller also found the formation of carboxylic acids such as formic acid, acetic acid, and propionic acid.[8] Since it is known that the singlet NH radicals are formed in the electric discharge of ammonia,[9] the insertion reaction of the singlet NH into the C–H bond of carboxylic acid might explain the formation of amino acids in Miller's study.

The present study suggests a new possibility of the prebiotic synthesis of amino acids on the primordial earth.

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