Formation of Aspartic Acid from Aqueous Mixture of Maleic Acid and Ammonia by γ-Irradiation

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Received February 10, 1969

The formation of amino acid from maleic acid and ammonia aqueous solution by 60Co γ-ray irradiation has been studied.

The irradiated solutions were analyzed by paper electrophoresis and amino acid analyzer. Aspartic acid was identified and estimated by spectrophotometry. The yield of aspartic acid formed was found to be dependent on the radiation dose and the concentrations of maleic acid and ammonia. The reaction mechanism was proposed as the combination of radicals induced by radiation.

1. Introduction

Synthesis of amino acids by irradiation has been studied by several investigators1~4). Hasselstrom1), et al. reported the formation of glycine by irradiation with high-energy electron in solutions of ammonium acetate. Palm and Calvin4) observed the formation of glycine and alanine after irradiating methane in aqueous ammonia solution. The authors previously reported the formation of aspartic acid from fumaric acid and ammonia solution by γ-irradiation. The present study deals with the formation of aspartic acid from aqueous solutions of maleic acid and ammonia by γ-irradiation. Since fumaric acid and maleic acid are geometrical isomers, one can expect similar reaction mechanisms and experimental results as have been elucidated by the present paper.

2. Experimental

2-1 Sample solution

All reagents used in this study were of analytical reagent grade from Kishida Kagaku Co. Solutions were prepared with maleic acid and ammonia giving several kinds of concentrations shown in Table 1.

2-2 Gamma irradiation

The glass tubes each containing 10 ml of sample solution were degassed on the vacuum line, sealed off and irradiated with 60Co γ-rays at a dose rate of 4×10^4 R/hr or 5.6×10^5 R/hr. Total doses ranged from 0.7 MR to 4.8 MR.

2-3 Analysis

The amino acid formed in the irradiated solution was analyzed by paper electrophoresis and amino acid analyzer. The quantities of the amino acid were determined by spectrophotometry which was described in the previous paper5).

3. Results and Discussion

In general the yield of aspartic acid is dependent on the dose and concentrations of maleic acid and ammonia.

Table 1 Concentrations of maleic acid and ammonia in sample solution

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solute</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>maleic acid, ammonia</td>
<td>0.05 0.05 0.05 0.05 0.05</td>
</tr>
<tr>
<td></td>
<td>ammonia</td>
<td>0.5 1 2 3 4</td>
</tr>
<tr>
<td>B</td>
<td>maleic acid, ammonia</td>
<td>0.02 0.05 0.1 0.2 0.3</td>
</tr>
<tr>
<td></td>
<td>ammonia</td>
<td>2 2 2 2 2</td>
</tr>
<tr>
<td>C</td>
<td>maleic acid, ammonia</td>
<td>0.0063 0.0125 0.025 0.05 0.1</td>
</tr>
<tr>
<td></td>
<td>ammonia</td>
<td>0.25 0.5 1 2 4</td>
</tr>
</tbody>
</table>

Solution of 0.01M aspartic acid was used as the standard solution.

In Fig. 1, the concentrations of aspartic acid formed in the irradiated solutions are plotted against doses. It increases with rising of doses but tends to reach a maximum value at 3 MR.
This can be explained by the reason that the aspartic acid already formed after having reached a certain limiting concentration is attacked by the products of water radiolysis. The yield of aspartic acid also depends on the concentration of solutes. Relation between yield of aspartic acid and concentration of ammonia is shown in Fig. 2. G values calculated at 3 MR are plotted in Fig. 3, which increases with increase of ammonia concentration but reach a limiting value at 2 M and with further increase in concentration of ammonia the yields remain almost constant. This fact suggests that the sufficient concentration of ammonia for the formation of aspartic acid seems to be about 2 M at 3 MR and the higher ammonia concentration should not contribute to the reaction. The yield of aspartic acid is also strongly depends on the concentration of maleic acid, as shown in Fig. 4.

The yield of aspartic acid reaches a maximum value at 0.05 M of maleic acid but decreases rapidly with increasing concentration of maleic acid. This decrease may be due to the competition between maleic acid and NH2 radical for the reaction with the intermediate radical, HOOCCH=CHCOOH, resulting from adding H to the double bond of maleic acid. This competitive reaction is verified by the detection of the dimer succinic acid (1,2,3,4-butane-tetracarboxylic acid) by paper-chromatography, which appears proably...
by the dimerization of this intermediate radical (cf. eq. 6) or by the combination of this radical with maleic acid followed by the addition of hydrogen (cf. eq. 7).

Another possible explanation for this decrease is that the direct effect of radiation becomes significant at the higher concentrations of maleic acid and the radiolytic products of maleic acid are produced capturing NH₂ radicals and lowering the formation of aspartic acid. This is supported by the observation of ninhydrin positive substances, which are not identified, tailing to a broad zone on the electromigration paper.

Fig. 5 Relation between yield of aspartic acid and concentrations of solutes, keeping mole ratio maleic acid vs. ammonia to be 1:40.

As is shown in Fig. 5, the yield of aspartic acid increases linearly with increasing concentrations of solutes, in which mole ratio between maleic acid and ammonia is kept to be 1:40, and reach 2×10⁻³ M at 0.05 M maleic acid and 2 M ammonia.

The experiment carried out by the dose rate of 4×10⁴ R/hr and 5.6×10⁵ R/hr shows that changes in dose rate do not affect the yield of aspartic acid at 2 M and higher concentration of ammonia (cf. Fig. 6).

The result is similar to that obtained from the experiment concerning fumaric acid and ammonia solutions. The isomeric effect on the yield of aspartic acid is not discriminable and this fact suggests the similar reaction sequence to that of fumaric acid. The reaction mechanism is assumed to be as follows:

\[
\begin{align*}
\text{H}_2\text{O} & \rightarrow \text{H}, \text{OH}, e_{aq}, \text{H}_2\text{O}_2, \text{H}_2 \\
\text{NH}_3 + \text{OH} & \rightarrow \text{NH}_2 + \text{H}_2\text{O} \\
\text{HOOC-CH=CH-COOH} + \text{H} & \rightarrow \text{HOOC-CH}_2-\text{CH-COOH} \\
\text{HOOC-CH=CH-COOH} + e_{aq} & \rightarrow \text{HOOC-CH}_2-\text{CH-CHOH} + \text{OH}^- \\
\text{HOOC-CH}_2-\text{CH-COOH} + \text{NH}_2 & \rightarrow \text{HOOC-CH}_2-\text{CHNH}_2-\text{COOH} \\
\text{HOOC-CH}_2-\text{CH-COOH} + \text{HOOC-CH=CH-COOH} + \text{H} & \rightarrow \text{CH}_2\text{COOH(CHOCH)}_2\text{CH}_2\text{COOH} \\
\text{HOOC-CH}_2-\text{CH-COOH} + \text{HOOC-CH=CH-COOH} + \text{H} & \rightarrow \text{CH}_2\text{COOH(CHOCH)}_2\text{CH}_2\text{COOH}
\end{align*}
\]

Fig. 6 Effect of dose rate on the yield of aspartic acid.
by reaction (5) decreases. This would support that the reaction mechanism is a process involving free radicals, especially OH plays an important role.

Fig. 7 Effect of potassium iodide concentration on the yield of aspartic acid.

4. Conclusion
Formation of aspartic acid induced by irradiation has been shown to occur in the mixture of maleic acid and ammonia. The yield of aspartic acid depends on the radiation doses and concentrations of maleic acid and ammonia. The sufficient dose is about 3MR which is independent of dose rate in the range of $4 \times 10^4$ to $5.6 \times 10^5$ R/hr. The optimum concentration is found to be $0.05 \text{ M}$ of maleic acid and $2 \text{ M}$ of ammonia (mole ratio; 1:40). The isomeric effect of fumaric acid and maleic acid on the yield of aspartic acid is negligible under the condition of the experiment in the present paper.

References
1) Hasselstrom, T., Henry, M. C., Murr, B.: Science, 125, 350 (1957)

要 旨
マレイン酸・アンモニア水溶液のγ線照射によるアスパラギン酸の生成

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マレイン酸とアンモニアの混合水溶液に $^{60}\text{Co}$ γ線を照射し、照射試料を電気泳動法およびアミノ酸自動分析計で分析したところ、アスパラギン酸が生成していることが判明したので比色法により定量を行なった。アスパラギン酸の収量は照射線量とマレイン酸およびアンモニアの濃度により異なり、照射線量 3 MR でマレイン酸 0.05 M、アンモニア 2 M の試料中に生成したアスパラギン酸の濃度は $2 \times 10^{-4} \text{ M}$ である。生成機構としては水の放射線分解により生じた OH がアンモニアと反応して NH$_3$ を生じ、一方 Hがマレイン酸に付加して二重結合が開き、それに NH$_3$ が結合してアスパラギン酸が生成したと考えられる。