INTRODUCTION

The formation of primitive amino acids has been discussed with respect to both terrestrial [1,2] and extra terrestrial [2,3] origins. The results of simulation experiments involving Miller-type spark-discharge [4,5] in an assumed reducing atmosphere to yield hydrogen cyanide, aldehyde and ammonia; in a second step, a solution-phase reaction proceeds via aminoacetonitrile to give amino acids. However, when the same experiment is carried out in a non-reducing atmosphere, the yield of amino acids is very low. Contact glow discharge electrolysis onto the aqueous phase, which simulates an energy source for chemical evolution, converted aminoacetonitrile via glycinamide to glycine. The mechanism of glycinamide formation was explained by considering the addition of hydrogen and hydroxyl radicals to the C-N triple bond and subsequent transformation into the amide, which was then oxidized to the amino acid. This research suggests that amino acid amides and amino acids can be obtained through oxidation-reduction with H and OH radicals in the primitive hydrosphere whether under reducing or non-reducing conditions.

Keywords: Aminoacetonitrile, Chemical evolution, Contact glow discharge electrolysis.
Contact glow discharge electrolysis (CGDE): A glass reaction vessel (114 × 35 mm i.d.) was used for CGDE as reported previously [49,50]. The reaction mixture (20 mL) containing aminoacetonitrile (20 mM) and inorganic salt buffer (200 mM, pH 1-10) in the reaction vessel was cooled in an ice-water bath to 10-30 °C. After bubbling argon gas through the reaction mixture for 15 min, discharge electrolysis (440-520 V, 20-30 mA) was carried out between the platinum anode above the solution and the platinum cathode in the solution over bubbling argon gas. Electric power was supplied by a Model PS-1515 (Toyo Solid State, Tokyo) power supply. An aliquot of the reaction solution was taken at regular time intervals and analyzed with an amino acid analyzer.

Hydrolysis of aminoacetonitrile at room temperature without CGDE: Aqueous solutions containing 20 mM aminoacetonitrile in 200 mM buffer were stirred with bubbling argon. An aliquot (about 0.200 mL) of the reaction solution was taken at regular time intervals and analyzed with an amino acid analyzer.

Amino acid analysis: The composition of amino compounds in the reaction mixtures were analyzed with a JLC-300 amino acid analyzer (JEOL, Tokyo, Japan). Typical chromatograms of standard compounds and of a reaction mixture are shown in Fig. 3. Glycine, aminoacetonitrile, glycinamide and ammonia were almost baseline separated; their typical retention times were as follows: glycine (39.6 min), aminoacetonitrile (73.9 min), glycinamide (77.0 min) and ammonia (80.1 min). Eluted compounds were reacted with ninhydrin to enable detection by measuring absorption at 570 nm. Concentration of ammonia in the reaction mixtures were determined by drawing the initial concentration out of the actual concentration at each reaction time.

RESULTS AND DISCUSSION

Contact glow discharge electrolysis onto aminoacetonitrile solution: The changes in concentration of aminoacetonitrile and products during the CGDE at pH 10 were plotted against the reaction time (Fig. 4(d)). After 60 min...
reaction, the amount of aminoacetonitrile decreased to 45% (9 mM) compared with the initial concentration; at this point, amino acid analysis revealed the presence of glycine (0.14 mM, 0.7%) and glycinamide (2.9 mM, 14.5%). Contact glow discharge electrolysis using aminoacetonitrile was also performed under acidic and neutral pH conditions. However, glycinamide was either not detected or was found in very low levels under these conditions.

**Contact glow discharge electrolysis onto glycinamide solution:** Glycinamide solution (20 mM) was irradiated by CGDE. The reactions at basic pH showed a gradual decrease in the amount of glycinamide and formation of ammonia and glycine. Fig. 5 shows the time course of the CGDE with glycinamide (20 mM). Of the three pH conditions examined, degradation of glycinamide induced by CGDE was most rapid at pH 10. Glycinamide was more stable under acidic pH conditions. However, the formation of glycine was very slow compared with the rate of decrease in the amount of glycinamide. For instance, the concentration of glycinamide decreased to approximately 70% after 40 min reaction under CGDE, but the concentration of glycine was only 5% at that time point. This result suggests that the remaining 25% was converted into other compounds. The identities of these compounds are not known; however, the negative response to analysis with ninhydrin suggests that the compounds do not contain an amino group.

**Hydrolysis of aminoacetonitrile:** Hydrolysis of aminoacetonitrile at pH 1, 6 and 11 without CGDE is shown in Fig. 6. The rate of degradation of aminoacetonitrile was much lower than those observed under CGDE conditions. Indeed, no hydrolysis occurred at either pH 1 or pH 6 and no glycinamide or glycine was observed even after reaction for 150 h. However, hydrolysis at pH 11 gave glycinamide (65%) and glycine (10%) after reaction for 300 h.

**Analysis of rate constants:** The pseudo-first-order reaction rate constant \( k_{AN} \) for aminoacetonitrile was calculated from the graph of the straight line representing the linear correlation between the reaction time (min) and \( \ln([AN]/[AN]_0) \) in Fig. 7. The linear correlation is as follows:

\[
\ln([AN]/[AN]_0) = -k_{AN}t \quad (1)
\]

where, \([AN]\): concentration of aminoacetonitrile; \([AN]_0\): initial concentration of aminoacetonitrile; \( k_{AN} \): pseudo-first-order reaction rate constant; \( t \): reaction time (min). The pseudo-first-
order reaction rate constant $k_{GA}$ for glycinamide was calculated from the straight line representing the linear correlation between the reaction time (min) and $\ln (1-\frac{[GA]}{[AN]_0})$ in Fig. 7. The linear correlation is as follows:

$$\ln (1-\frac{[GA]}{[AN]_0}) = -k_{GA} \cdot t$$  \hspace{1cm} (2)

where, $[GA]$: concentration of glycinamide; $[AN]_0$: initial concentration of aminoacetonitrile; $k_{GA}$: pseudo first-order reaction rate constant; $t$: reaction time (min).

The pseudo-first-order reaction rate constants for the consumption of aminoacetonitrile, for the formation of glycine and for either consumption or formation of glycinamide are compared in Fig. 8. The consumption rate of substrates and the formation rate of products are proportional to the concentration of substrates, since CGDE constantly delivers OH and H radicals onto a limited area of the surface of the reaction solutions [51]. In order to distinguish between the consumption and the formation, minus signs are put in front of the rate constants for the consumption of substrates.

The absolute value of the pseudo-first-order reaction rate constant for aminoacetonitrile consumption under CGDE was larger in solutions with higher pH values. The rate constant was $-1.4 \times 10^{-3}$ (pH 1), $-5.5 \times 10^{-3}$ (pH 6), $-6.9 \times 10^{-3}$ (pH 8).
without CGDE was 0.00 (pH 1), $-3.2 \times 10^{-6}$ (pH 6) and $-8.7 \times 10^{-5}$ (pH 11). Thus, the amount of aminoacetonitrile decreased with higher pH values, as described above. Glycinamide and glycine were obtained with a maximum yield of glycinamide and glycine under CGDE. The rate of consumption of aminoacetonitrile and the maximum yield of glycinamide and glycine under CGDE was greater in solutions with higher pH.

Maximum yield of glycinamide and glycine with the recovery of aminoacetonitrile: Table 1 shows the recovery of aminoacetonitrile and glycine with and without CGDE (minus signs are put in front of the rate constants for the consumption of substrates) and $-1.3 \times 10^{-2}$ (pH 10), whereas the rate constant for hydrolysis without CGDE was 0.00 (pH 1), $-3.2 \times 10^{-6}$ (pH 6) and $-8.7 \times 10^{-5}$ (pH 11). Thus, the amount of aminoacetonitrile decreased 1700 (pH 8) times faster under CGDE than the corresponding hydrolysates without CGDE. The rate constant for glycinamide formation from aminoacetonitrile was also greater in solutions with higher pH values. Contact glow discharge electrolysis onto aminoacetonitrile solutions at pH 6 gave the highest rate constant for glycinamide formation (3.8 $\times 10^{-4}$); the reactions at pH 8 and pH 1 gave rate constants of 1.5 $\times 10^{-4}$ and 2.5 $\times 10^{-4}$, respectively. The reaction rate constant for glycinamide consumption under CGDE was greater in solutions with higher pH.

Table 1 shows the recovery of aminoacetonitrile and the maximum yield of glycinamide and glycine under CGDE. The rate of consumption of aminoacetonitrile increased with higher pH values, as described above. Glycinamide and glycine were obtained with a maximum yield of 12.7 % at pH 10 and 2.0 % at pH 6. A balance between the rates of formation and consumption of products governed the maximum yields of glycinamide and glycine.

### Table 1

| pH | Recovery (%) | Maximum yield (%) |
|----|--------------|-------------------|
|    | H$_2$NCH$_2$CN | H$_2$NCH$_2$CONH$_2$ | H$_2$NCH$_2$COOH |
| 1  | 18.4 (90)     |          | 0.36 (1.8)        |
| 6  | 14.8 (74)     | 0.12 (0.6)   | 0.44 (2.2)         |
| 8  | 13.7 (69)     | 0.80 (4.0)   | 0.38 (1.9)         |
| 10 | 12.4 (62)     | 2.9 (14.5)   | 0.12 (0.6)         |

*The value at 50 min reaction time.
*Calculated from initial concentration of aminoacetonitrile.

**Reaction pathway for the formation of amino acid from aminoacetonitrile:** Although the transformation (Radziszewski reaction) [52-58] of nitriles into the corresponding carboxamides by hydrogen peroxide under basic conditions is known, the ionic reaction of hydrogen peroxide with nitrile cannot be used to explain the mechanism under neutral and acidic conditions. On the other hand, radical addition to the C-N triple bond may explain the reactions. The dissociation of water to hydrogen (H) and hydroxyl (OH) radicals has been proposed [49,50,59] to be a trigger that initiates oxidation-reduction of organic compounds under CGDE in aqueous solutions. Published results tend to support the conclusion that the mechanism involves H and OH radicals. These radicals can give different adducts (Intermediates 1 and 2) [60,61] upon addition to the carbon atom of the nitrile group, as shown in Fig. 10.

Hydrogen (H) and OH radicals are proposed to attack the C-N triple bond of aminoacetonitrile to give adduct intermediates 1 and 2, respectively. Similar radical adducts formed by pulse radiolysis [60,61] and irradiation [60,62] of cyanate solutions have been observed. Intermediate 1 would react with a water molecule and decompose via amino ethanol [62] to glycine. Intermediate 2 would decompose via glycinamide to glycine. However, amino acid analysis of the reaction mixtures did not give peaks corresponding to aminooanethanol on the chromatograms. The latter result is not unexpected considering that the yield of the hydrogen adduct was much lower than that of the hydroxyl adduct with cyanate and because the high susceptibility of aminooanethanol towards attack by OH radicals means that it decomposes very rapidly even if formed.

Another plausible reaction starts with hydrogen abstraction [60,63-67] from aminoacetonitrile to give Intermediate 3, which would react with OH radicals and lead to a deaminated product that would be undetectable by ninhydrin-based analysis (Fig. 11). Hydrogen abstraction also explains the decomposition of glycinamide to glyoxylic acid amide, which is not detectable by ninhydrin-based analysis (Fig. 12). However, glyoxylic acid (HC(=O)-C(=O)-OH) produced from glycine by plasma-jet blowing [51], which is an OH radical supplying process, supports glyoxylic structure (HC(=O)-C(=O)-) produced from glycinamide.

**Dependence of reaction pathway on pH:** The rates of degradation of both aminoacetonitrile and glycinamide were higher under higher pH conditions. The pH dependence may be explained by the stability of the OH radical under basic conditions. Addition of OH radical to the nitrile group is faster under basic conditions, whereas H radicals react with each other to form a hydrogen molecule under acidic conditions. The second-order reaction rate constants for the reaction of H radical with aminoacetonitrile have been reported [68] to be

![Fig. 10. Plausible reaction pathway for the formation of glycine from aminoacetonitrile via adducts under CGDE](image-url)
5.2 \times 10^7 \text{ mol L}^{-1} \text{ s}^{-1} \text{ at pH 7 and } 6.1 \times 10^6 \text{ mol L}^{-1} \text{ s}^{-1} \text{ at pH 1. The former conditions mainly lead to the nitrile group adduct (intermediate 1), whereas the latter conditions give the hydrogen-abstracted compound (intermediate 2). The second-order reaction rate constant for the reaction of OH radical with glycinamide at pH 10 is reported [67] to be 2.8 \times 10^9 \text{ mol L}^{-1} \text{ s}^{-1}, which is approximately 34 times greater than that at pH 5 (8.3 \times 10^7 \text{ mol L}^{-1} \text{ s}^{-1}). Similarly, the second-order reaction rate constants for the reaction of OH radical with glycine at higher pH (about 10^9 \text{ mol L}^{-1} \text{ s}^{-1}) are greater than those recorded at lower pH (about 10^7 \text{ mol L}^{-1} \text{ s}^{-1}). On the other hand, glycine forms from aminoacetonitrile slower under basic and acidic pH conditions than under neutral pH conditions, where deamination of glycine is depressed because the amino group remains protonated. The second-order reaction rate constants described above are proportional to the first-order reaction rate constants of the corresponding reactions if the concentration of active species like H and OH radicals is constant [51]. The plasma-jet blowing into aqueous solutions resulted in the linearity between the second-order reaction rate constant and the first-order reaction rate constant [51]. Both the plasma-jet blowing and CGDE have each small reaction zone, which is located in each fixed position and constantly renewed by the generation of active species [51].

**Conclusion**

Terrestrial and extra terrestrial formation of amino acids in the prebiotic era has been discussed many times and many points of view exist. Common issues have included a consideration of the materials, energy sources and the condition of the reaction. The primary materials that have been considered for the formation of amino acids are amino nitriles, amino acid amides and similar compounds.

Strecker synthesis can be invoked to explain the mechanism of amino acid formation by discharge into simulated early earth atmosphere, although the reaction includes two steps: the first reaction is in the gas phase and the second step is in the solution phase. However, this explanation using the Strecker mechanism is restricted to the hydrolysis of amino nitrile via amino acid amides to amino acids. Whereas the energy source for hydrolysis is heat, amino acid formation from aminoacetonitrile can also occur by the discharge onto the hydrosphere as well as photolysis and radiation [66].

These energy sources would have supplied radical species in the primitive hydrosphere. Hydrolytic degradation of aminoacetonitrile is controlled by the nucleophilicity and the concentration of reagents like hydroxyl anion, but the rate is much smaller than that of radical-catalyzed degradation in the solution phase. The results reported herein suggest that the
nature discharge onto the hydrosphere containing amino nitrile to amino acids should be considered as a different process for the primitive amino acid formation.

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