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Chemical Evolution of Aminoacetonitrile to Glycine under Discharge onto Primitive Hydrosphere: Simulation Experiments Using Glow Discharge

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Aminoacetonitrile is an important precursor of abiotic amino acids, as shown in the mechanism that was developed to explain the results of the Miller-type spark-discharge experiment. In present experimental setup, a spark discharge is generated in a simulated 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.

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 (CH_4, NH_3, H_2O, H_2), glow discharge onto aqueous solutions [6] and other energy sources [2,7,8] have supported the conclusion of a terrestrial origin of amino acids. Although the same Miller-type spark-discharge experiment gave very low yield of amino acids [9] in an assumed non-reducing [10] atmosphere (CO_2, N_2, H_2O), the value of Miller-type spark-discharge has been supported by the research results [11] that the early earth atmosphere would have been more hydrogen-rich and reducing rather than non-reducing. On the contrary, a recent report [12] on the oxidation state of Hadean magmas suggests that the early earth atmosphere would have been oxidizing. Discussion about whether the early atmosphere had been reducing or non-reducing is still controversial. Amino acid formation processes under Miller-type spark-discharge conditions has been explained by a two-step mechanism (Fig. 1): the first step is the formation of acetaldehyde and hydrogen cyanide in the gas phase and the second step is a Strecker-type synthesis in the solution phase [13,14] to give aminoacetonitrile, which hydrolyzes to glycine. Amino acid formation would have actually proceeded in a primitive hydrosphere, if the primitive atmosphere would have supplied the majority of materials for amino acid formation on the primitive earth.

On the other hand, the presence of amino acids and their precursors in interstellar solid medium [15,16], interstellar gas phase [17-20], comets [21,22] and meteorites [23-25] has been reported. Experiments involving use of high-energy particles [26,27] and ultraviolet light [28-30] to irradiate simulated interstellar media have provided results that support formation of amino acids and their precursors. The survival of such compounds within extra terrestrial bodies during atmospheric entry to earth has been discussed [31,32]. Several experiments that have been designed to simulate atmospheric entry have supported the conclusion that amino acids remain intact, although the amount of degradation might depend on the size of the extra terrestrial bodies and on the nature of the amino acids [33-42].

Aminoacetonitrile is known to be an important precursor for abiotic amino acid formation [3,43,44] and subsequent peptide formation (Fig. 2) [45] in the primitive earth and interstellar medium [46]. Herein, the formation of glycine from aminoacetonitrile *via* glycinamide in aqueous solutions induced by contact glow discharge electrolysis (CGDE; also termed Harada discharge [47-50]) as a simulation energy source in a chemical evolutionary process is reported. The author uses

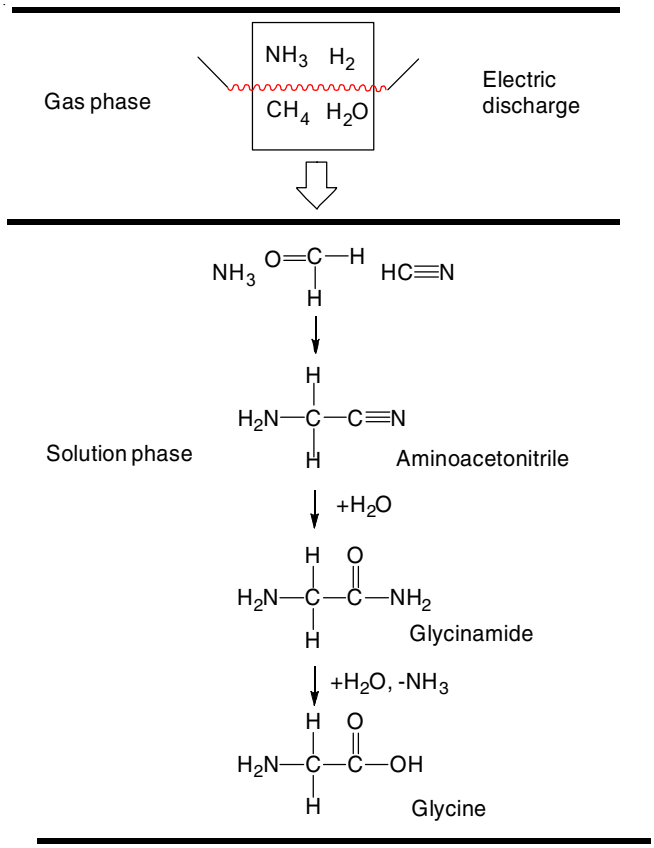

Fig. 1. Plausible reaction pathway of amino acid formation in solution under Miller type discharge

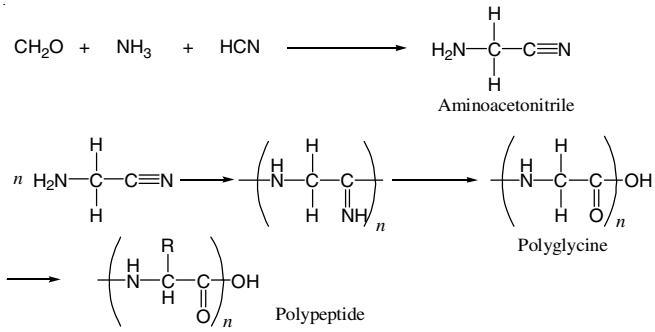

Fig. 2. Aminoacetonitrile as an important intermediate in the formation of polypeptides

an inert gas, argon atmosphere as a reaction condition to monitor the solution phase reactions less susceptible to whether the atmosphere is reducing or non-reducing. The results demonstrate that amino acid amides and amino acids are formed dominantly by oxidation-reduction rather than hydrolysis of aminoacetonitrile.

EXPERIMENTAL

Inorganic buffer solutions (200 mM) were prepared from sulfuric acid (pH 1), sodium hydrogen phosphate (pH 4-7) and sodium carbonate (pH 10-11). The inorganic salts and aminoacetonitrile sulfate were purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Glycinamide hydrochloride was purchased from Kokusan Chemical Co., Ltd. (Tokyo, Japan). Glycine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

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.

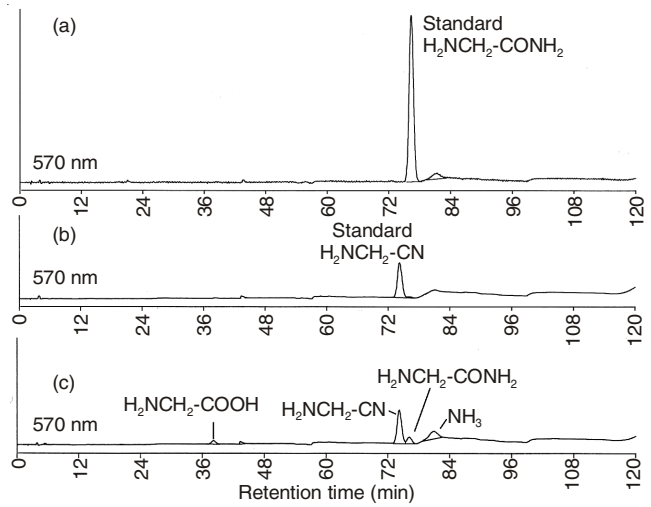

Fig. 3. Typical chromatograms (a, b) of standard compounds and (c) of a reaction mixture after exposure to CGDE. A JLC-300 amino acid analyzer was used for analysis. Eluted compounds were reacted with ninhydrin to enable detection by measuring absorption at 570 nm

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

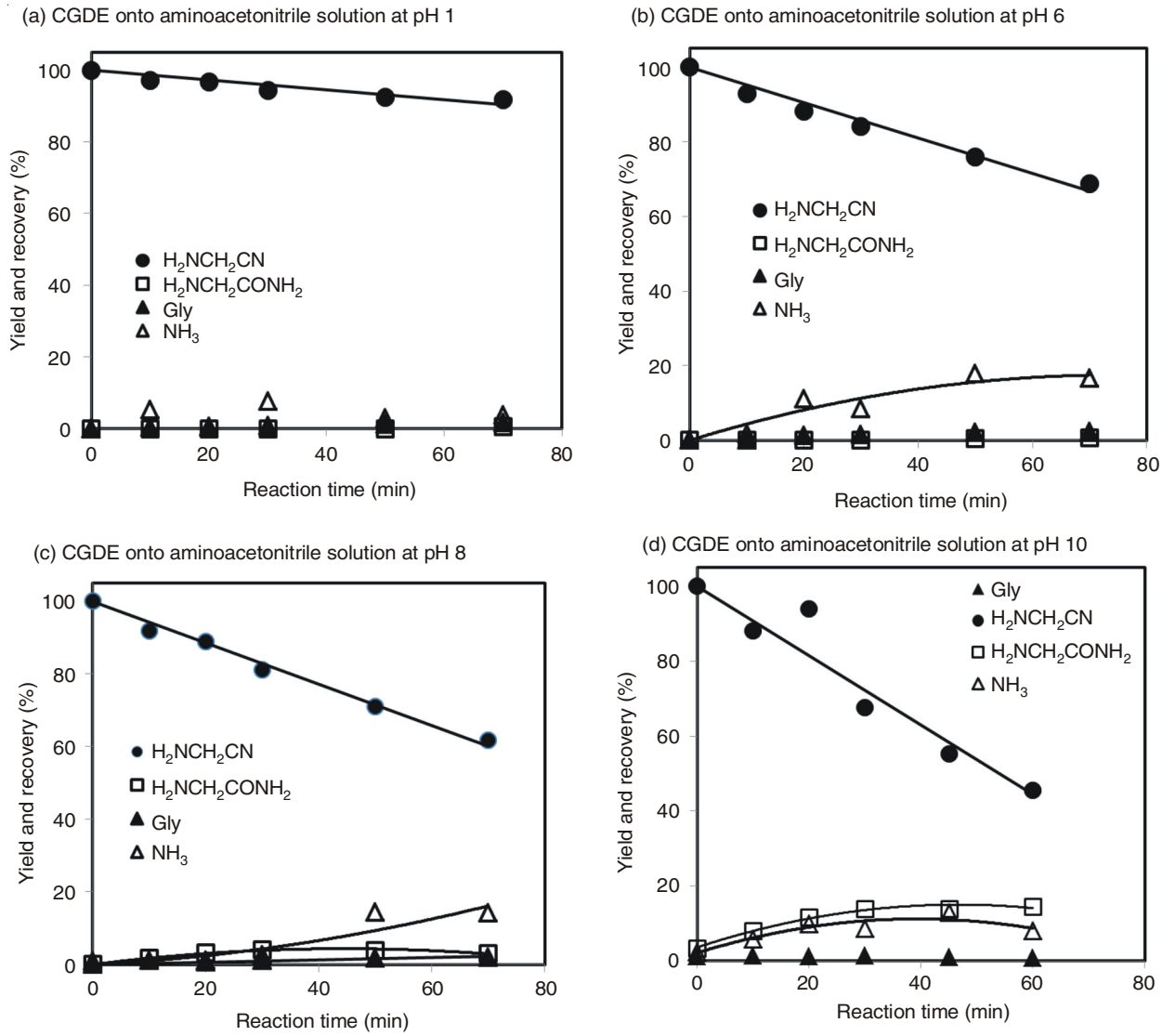

Fig. 4. CGDE onto aqueous solutions containing aminoacetonitrile (20 mM) at pH 1, 6, 8 and 10. Conditions of CGDE: 400-440 V, 20 A

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 graduation 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:

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

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-

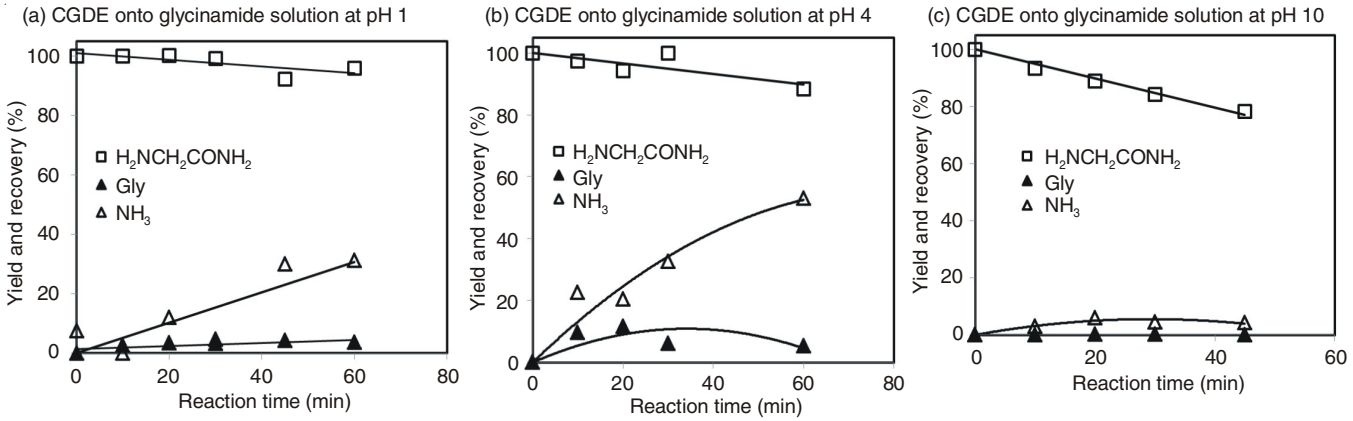

Fig. 5. CGDE onto aqueous solutions containing glycinamide (20 mM) at pH 1, 4 and 10. Contact glow discharge electrolysis conditions: 400-440 V, 20 A

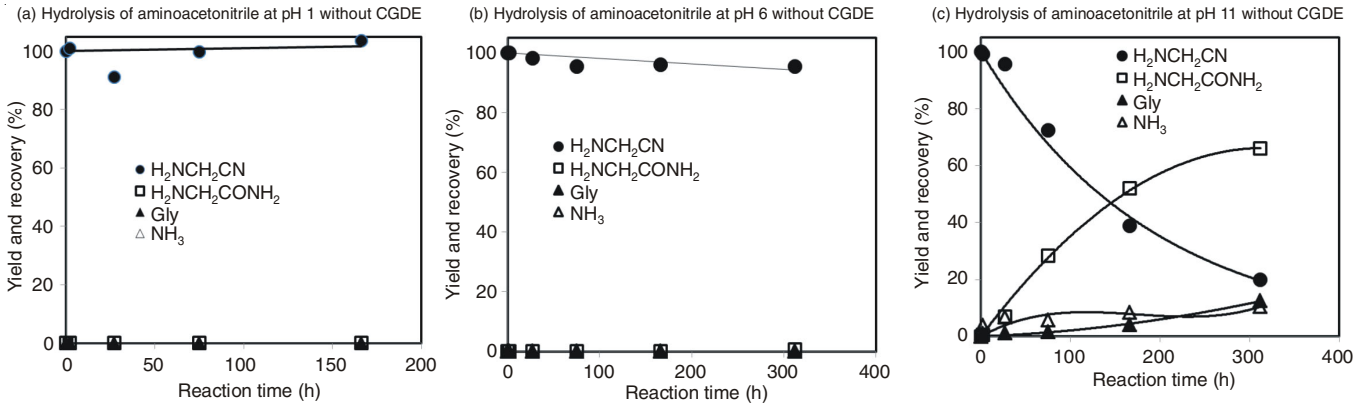

Fig. 6. Hydrolysis of aminoacetonitrile at pH 1, 6 and 11 without CGDE

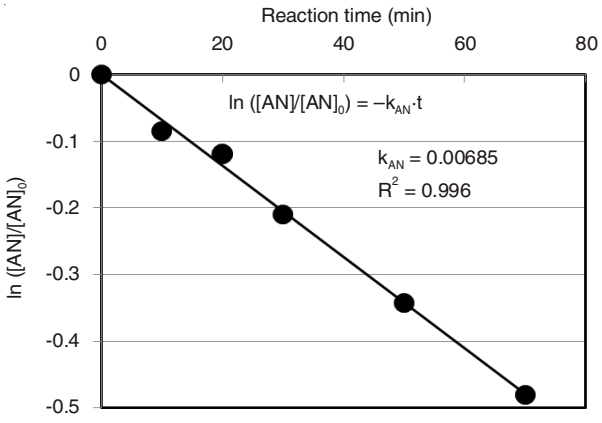

Fig. 7. First-order reaction rate constant for aminoacetonitrile assumption by CGDE onto 20 mM aminoacetonitrile at pH 8

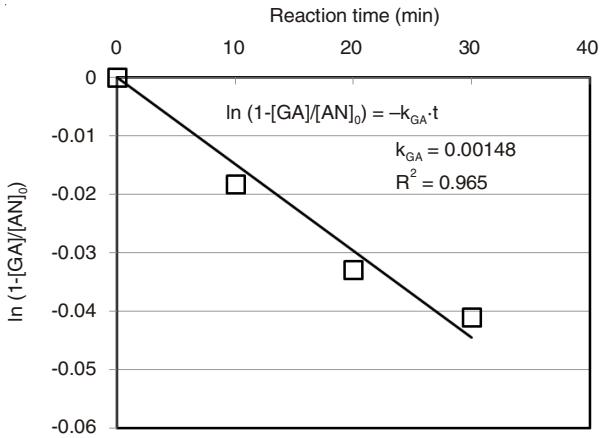

Fig. 8. First-order reaction rate constant for glycinamide formation by CGDE onto 20 mM aminoacetonitrile at pH 8

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-[GA]/[AN]$_0$) in Fig. 8. The linear correlation is as follows:

$$\ln(1-[GA]/[AN]_0) = -k_{GA} \cdot t \quad (2)$$

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. 9. 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 -1.4×10^{-3} (pH 1), -5.5×10^{-3} (pH 6), -6.9×10^{-3} (pH 8)

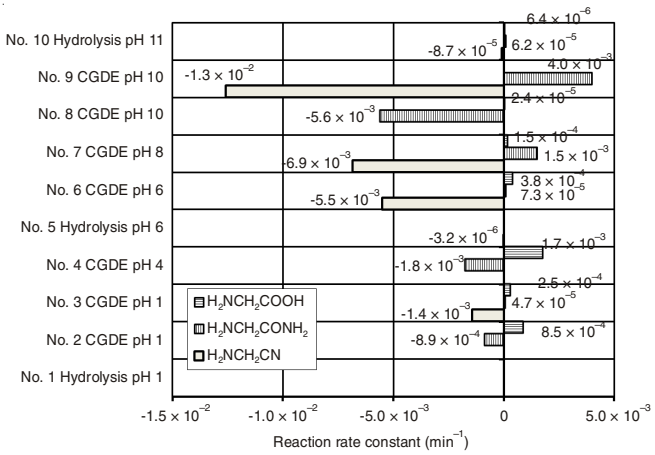

Fig. 9. Comparison of the reaction rate constants of aminoacetonitrile, glycinamide and glycine with and without CGDE (minus signs are put in front of the rate constants for the consumption of substrates)

and -1.3×10^{-2} (pH 10), whereas the rate constant for hydrolysis without CGDE was 0.00 (pH 1), -3.2×10^{-6} (pH 6) and -8.7×10^{-5} (pH 11). Thus, the amount of aminoacetonitrile decreased 1700 (pH 8) times faster under CGDE than the corresponding hydrolyses 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 glycine formation (3.8×10^{-4}); the reactions at pH 8 and pH 1 gave rate constants of 1.5×10^{-4} and 2.5×10^{-4}, respectively. The reaction rate constant for glycinamide consumption 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 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
MAXIMUM YIELD OF GLYCINAMIDE AND GLYCINE BY CGDE ONTO AMINOACETONITRILE SOLUTIONS

pH	Recovery [a]/mM (%)[b]	Maximum yield/mM (%)[b]	
	H_2NCH_2CN	$H_2NCH_2CONH_2$	H_2NCH_2COOH
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)

[a]The value at 50 min reaction time.
[b]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 ethanal [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 aminoethanal 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 aminoethanal 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 a 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

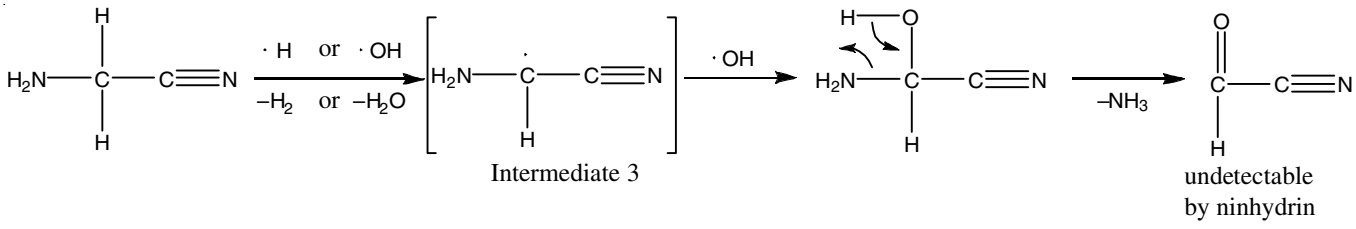

Fig. 10. Plausible reaction pathway for the formation of glycine from aminoacetonitrile *via* adducts under CGDE

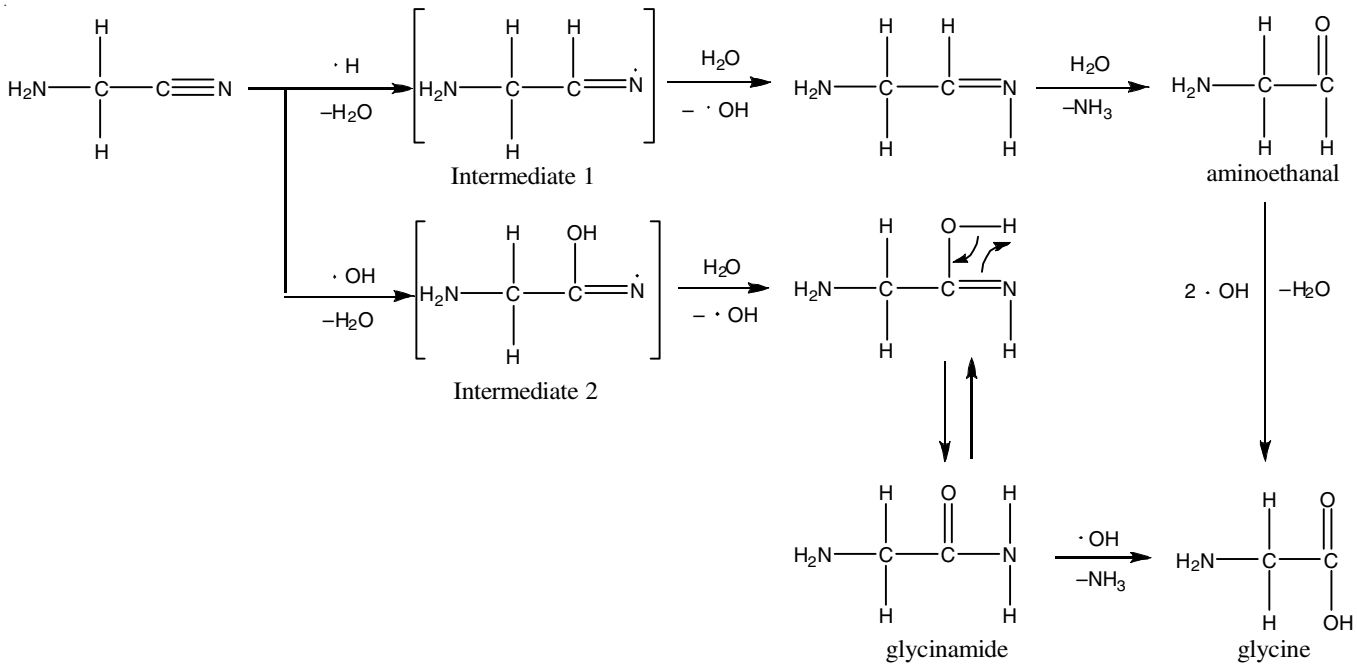

Fig. 11. Plausible reaction pathway from aminoacetonitrile through hydrogen abstraction to give non-amino compounds under CGDE

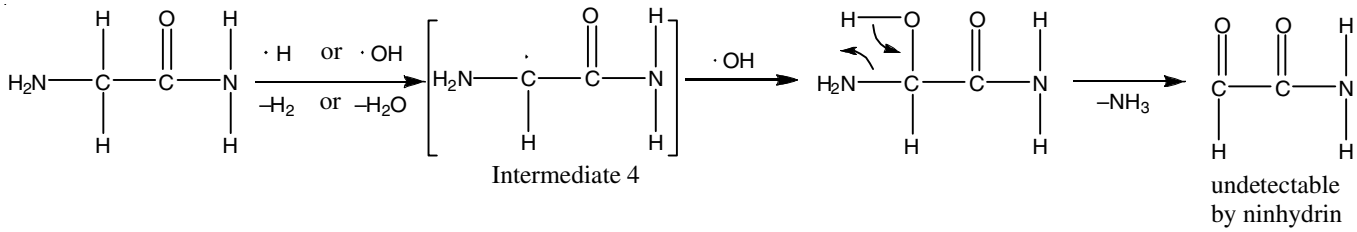

Fig. 12. Plausible decomposition mechanism of glycinamide by CGDE

5.2×10^7 mol L^{-1} s^{-1} at pH 7 and 6.1×10^6 mol L^{-1} s^{-1} 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 2.8×10^9 mol L^{-1} s^{-1}, which is approximately 34 times greater than that at pH 5 (8.3×10^7 mol L^{-1} s^{-1}). Similarly, the second-order reaction rate constants for the reaction of OH radical with glycine at higher pH (about 10^9 mol L^{-1} s^{-1}) are greater than those recorded at lower pH (about 10^7 mol L^{-1} 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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