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Possible Role of Aminoacetonitrile in Chemical Evolution

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Aminoacetonitrile can condense with an amino acid such as alanine, in a basic aqueous solution, to give low yields of products that appear to be dipeptides, on the basis of elution times and enzymatic hydrolysis. The predominant reactions, however, are hydrolysis and condensation to give amino and imino acids.

INTRODUCTION

In simulated primitive earth and contemporary Jovian atmosphere experiments, the formation of some α-aminonitriles has been demonstrated (*1*). In such studies, the product resulting from an electric discharge through methane and ammonia has been shown to give some amino and imino acids on acid hydrolysis (*2*). The role of aminonitriles, as precursors to amino acids and peptides in studies dealing with chemical evolution, is of primary importance. Akabori (*3*) proposed that peptides could result by the polymerization of α-aminoacetonitrile on a solid surface, followed by the hydrolysis of the polymer to polyglycine and ammonia. Side chains could then be introduced by reaction of polyglycine with aldehydes or with unsaturated hydrocarbons. In partial support of this hypothesis, Hanafusa and Akabori (*4*) reported the formation of diglycine and triglycine when aminoacetonitrile sulfate was heated on clay to 120–140°C for 3–5 hrs.

Moser and Mathews (*5*) have examined the products resulting from acidic, neutral, and basic hydrolysis of α-aminoacetonitrile. Upon neutral and basic hydrolysis, in addition to the expected major product glycine, they claimed that significant amounts of aspartic acid as well as traces of alanine, serine, threonine, and lysine were formed. They proposed that α-aminoacetonitrile decomposed partially to hydrogen cyanide, which rapidly underwent base-catalyzed polymerization via HCN dimer to give HCN tetramer, peptide-like polymers, and intractable black solids. They postulated that the various amino acids were obtained from the breakdown of HCN tetramer and the peptidic compounds under the drastic hydrolytic conditions.

Morimoto et al. (*6*) have studied the polymerization of α-aminopropionitrile under anhydrous conditions and have reported the formation of polymeric material. This material, after mild hydrolysis and separation by Sephadex gel filtration, has been shown to give fractions with infrared absorption at 1650 and 1550 cm^{-1} characteristic of peptide bonds. On hydrolysis (6 *N* HCl), the α-aminopropionitrile polymer has been shown to give alanine and ammonia.

The present study explores the chemistry of aminoacetonitrile at pH 9.0 in an aqueous medium. The pH was selected because the products of electric discharge through

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methane and ammonia on dissolution in water result in solutions with pH ~9–10. It was expected that coupling of the aminonitriles might occur by the intermolecular reaction of the amino group and nitrile group to give amidine bonds which could then hydrolyze to peptide bonds. The effect of inorganic phosphate in this coupling reaction was also explored because the phosphate ion might exert a catalytic effect through the formation of a reaction intermediate, an iminophosphate:

$$NH_2CH_2CN + HOPO_3^{2-} \rightleftarrows NH_2CH_2C(=NH)OPO_3^{2-}$$

It was also of interest to see if the nitrile group of the aminoacetonitrile would interact with the amino group of an amino acid. Consequently, experiments were set up in which (a) alanine and aminoacetonitrile were the reactants and (b) inorganic phosphate was added to the reaction mixture.

METHODS

The following reaction mixtures were prepared and adjusted to pH 9.0 by the addition of 6 *N* NH_4OH. A. Aminoacetonitrile (0.1 *M*), by dissolving aminoacetonitrile acid sulfate in distilled water. B. Aminoacetonitrile (0.1 *M*), disodium hydrogen phosphate (0.1 *M*). C. Aminoacetonitrile (0.1 *M*), alanine (0.5 *M*). D. Aminoacetonitrile (0.1 *M*), alanine (0.5 *M*), and disodium hydrogen phosphate (0.1 *M*).

The solutions were maintained in sealed vials at room temperature (22°C) and at 75°C for 1, 2, 4, and 8 days. Aliquots were examined for dipeptide formation by the use of an automatic amino acid analyzer and by thin-layer chromatography.

The nonpeptidic products were characterized by gas chromatography and combined gas chromatography/mass spectrometry using the procedures discussed earlier (*2*).

For qualitative glc and mass spectral determinations, aliquots of solutions were evaporated to dryness, methylated with 3 *N* methanolic HCl, and then trifluoroacetylated (trifluoroacetic anhydride in methylene chloride).

The derivatized products were first examined on an OV-17 column then analyzed by combined gas chromatography/mass spectrometry using a Loenco 160× gas chromatograph and a CEC 491 mass spectrometer. The gas chromatograph was connected to the mass spectrometer by means of a single-stage Llewellyn separator (Varian V-5620).

Confirmation of structure was obtained by comparing gas chromatographic retention times and mass spectral fragmentation patterns for the reaction products and standard compounds.

For quantitative estimation, the products were converted to their *N*-trifluoroacetyl, *n*-butyl ester derivatives. [The methyl-*N*-trifluoroacyl derivatives of some amino acids, e.g., alanine and glycine, are known to give nonquantitative results (*7*).]

RESULTS AND DISCUSSION

Preliminary observations using tlc and ion exchange showed that at room temperature little or no transformation took place. However, there was evidence for the formation of significant amounts of products, including some possible dipeptides from those

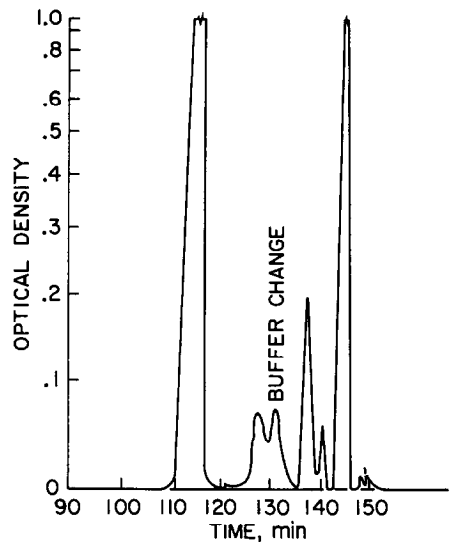

FIG. 1. Ion-exchange chromatogram of dipeptides from 0.1 *M* AAN, 0.5 *M* alanine after 8 days at 75°C, pH 9.

experiments run at 75°C for 8 days. Therefore these reaction mixtures were examined in detail.

Evidence for peptide linkage formation. The ion exchange chromatogram of the solution containing aminoacetonitrile (AAN) alone (75°C, 8 days) gave no evidence for the formation of any dipeptide. However, from AAN and inorganic phosphate (P_i), a peak with the approximate elution time of gly-gly was observed. The solution containing AAN + alanine showed a number of peaks in the dipeptide region (Fig. 1).

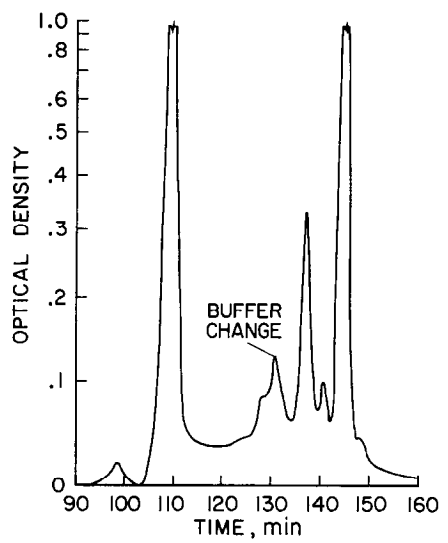

FIG. 2. Ion-exchange chromatogram of dipeptides from 0.1 *M* AAN, 0.1 *M* Na_2HPO_4, 0.5 *M* alanine after 8 days at 75°C, pH 9.

The ion-exchange chromatographic pattern (Fig. 2) for AAN + alanine + P_i reaction mixture was quite similar to the previous solution.

Of the several peaks (Fig. 1) from AAN and alanine, two were found to coincide with the elution times for gly-gly and gly-ala. The presumed gly-ala peak (146 min) represents 0.3% incorporation of the AAN moiety. The peak coinciding with gly-gly (138-min peak) represents about 0.05% incorporation. A preparative ion-exchange run was made on 1 ml of the AAN + alanine solution and a fraction collected (10-min period) which contained the components in the dipeptide region. This fraction was desalted by charging on a 50-ml Dowex 50 column (H^+ form) and eluting successively with water and 2 *N* NH_4OH. The NH_4OH eluent containing the components in the dipeptide region was evaporated to dryness and the residue dissolved in water. An

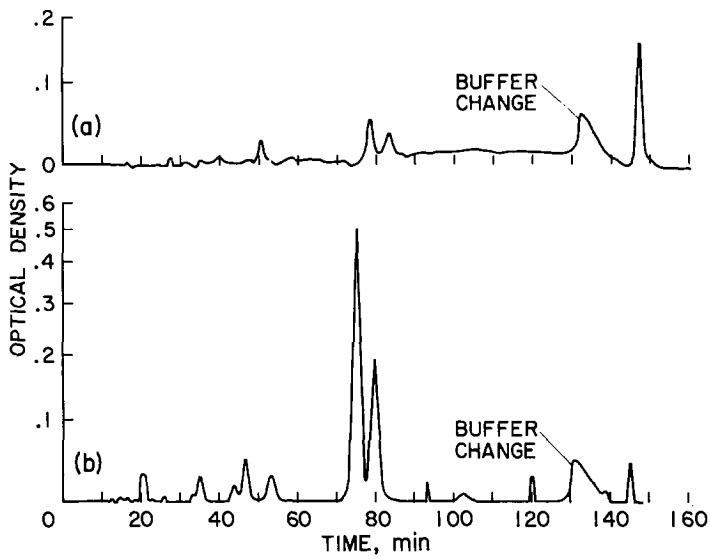

FIG. 3. Ion-exchange chromatograms of 10-min dipeptide fraction from 0.1 *M* AAN, 0.5 *M* alanine 8 days, 75°C, pH 9; before (a) and after (b) LAP hydrolysis.

aliquot of this fraction was found to give a single peak (146 min) on analytical ion-exchange chromatography (Fig. 3).[2] Another portion of this fraction was incubated with leucine aminopeptidase (LAP) at 35°C for 48 hr. Ion-exchange analysis showed a 50% diminution in the 146-min peak and a corresponding increase in the peaks characteristic of glycine and alanine (Fig. 3). The above data suggest the possibility of the 146-min peak being gly-ala.

Characterization of products resulting from hydrolysis and condensation reactions. In addition to the peptide material, a number of other products were detected and characterized by gas chromatography and mass spectrometry.

Only three significant peaks were observed in the chromatogram (Fig. 4) of the sample from the reaction mixture which initially contained only AAN. The major products were identified as glycine and iminodiacetic acid (IDAA), and the minor product as nitrilotriacetic acid (NTA). Results from the reaction mixture containing AAN and

[2] Lack of material prevented chromatographing a larger portion of the desalted fraction to reveal the 132-min peak.

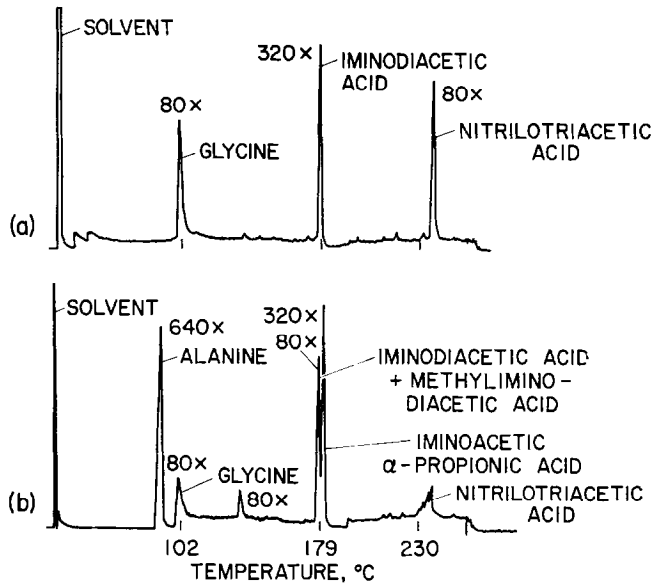

FIG. 4. Gas chromatogram of *n*-Bu-N-TFA derivatives of (a) 0.1 *M* AAN and (b) 0.1 *M* AAN, 0.5 *M* alanine, 0.1 *M* Na_2HPO_4. Program rate: 4 min, isothermal 80°C, 6°C/min to 230°C. Isothermal, 4 min at 230°C. Column: 1/4 in. o.d. × 4 ft glass column packed with 1.5% OV-17 on 80/100 mesh H.P.A.W. Chromosorb G. Flow rate: He 35 ml/min.

phosphate were similar to those from AAN only. Table 1 gives the yield of these products.

Analysis of the reaction mixture containing alanine and AAN showed iminoacetic-α-propionic acid (IAPA) to be the major product, with glycine, IDAA, and NTA being present in approximately the same amounts as before (Table 1). Examination of the derivatized sample from the reaction mixture containing alanine, AAN, and phosphate indicated the presence of another product, shown to be *N*-methyliminodiacetic acid (MIDAA). However, the peak from IDAA and MIDAA in the gas chromatogram of

TABLE 1

PRODUCT YIELDS[a]

Mixture	Product[b]			
	Gly	IDAA	NTA	IAPA
AAN	26	17	6	
AAN + P_i	18	13	6	
ala + AAN	29	14	2.5	59
ala + AAN + P_i	18	9.6[c]	2.5	57.5

[a] Percentage yield based on AAN.

[b] IDAA = iminodiacetic acid; NTA = nitrilotriacetic acid; IAPA = iminoacetic-α-propionic acid.

[c] Includes methyliminodiacetic acid (MIDAA); MIDAA + IDAA resolved as NTFA-methyl esters.

the NTFA-*n*-butyl esters overlapped so that individual yields could not be determined.

The formation of the imino acids, e.g., IAPA, may be explained by the following equilibria. The instability of α-aminonitriles to loss of HCN is well known (*8*). Alanine could condense with methyleneimine (I), formed by the loss of HCN from AAN, or its hydrolysis product, formaldehyde (*9*), to give II which could then add HCN, giving the iminonitrile acid III. Hydrolysis of III would yield IAPA.

$$NH_2CH_2CN \rightleftarrows HCN + \underset{\text{I}}{CH_2{=}NH} \overset{H_2O}{\rightleftarrows} H_2C{=}O + NH_3$$

$$\begin{array}{l} NH_2CHCO_2^- \\ \quad | \\ \quad CH_3 \end{array} + CH_2{=}NH \rightleftarrows \begin{array}{l} CH_2{=}NHCHCO_2^- \\ \qquad\quad | \\ \qquad\quad CH_3 \\ \qquad\quad \text{II} \end{array} + NH_3$$

$$\begin{array}{l} CH_2{=}NHCHCO_2^- \\ \qquad\quad | \\ \qquad\quad CH_3 \end{array} + HCN \rightleftarrows \begin{array}{l} NCCH_2NHCHCO_2^- \\ \qquad\quad\ | \\ \qquad\quad\ CH_3 \\ \qquad\quad\ \text{III} \end{array}$$

$$\begin{array}{l} NCCH_2NHCHCO_2 \\ \qquad\quad | \\ \qquad\quad CH_3 \end{array} + OH^- \rightleftarrows \begin{array}{l} {}^-O_2CCH_2NHCHCO_2^- \\ \qquad\quad\ | \\ \qquad\quad\ CH_3 \end{array} + NH_3$$

If AAN, instead of alanine, reacted with I, the ultimate product following hydrolysis would be IDAA. Repetition of the process yields NTA. The MIDAA probably results from a decarboxylation reaction, but the exact precursors are uncertain.

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