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PEPTIDE FORMATION FROM AMINOMALONONITRILE (HCN TRIMER)

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We have recently shown (1, 2) that among the products of base-catalyzed polymerization of hydrogen cyanide are polymers that are readily converted by water to peptide-like solids which can be further hydrolyzed to a mixture of a dozen of the twenty common α-amino acids. We now report that similar peptidic products are formed from aminomalononitrile (I), a trimeric form of hydrogen cyanide, after decomposition and mild hydrolysis.

In a typical experiment, an aqueous solution of the p-toluenesulfonate of aminomalononitrile (3, 4) (5 g in 30 ml) was neutralized by a saturated sodium bicarbonate solution (30 ml). Extraction with ether (6 x 30 ml) followed by evaporation under vacuum gave aminomalononitrile (3) as a yellow oil. Cold water (50 ml, 25°C) was added to half of the oil with occasional shaking in a closed flask. After a week an orange solution had formed together with insoluble brown solids which were filtered off. The filtrate was freeze-dried and extracted with acetone and ether to remove diaminomaleonitrile (HCN tetramer) shown to be present by color tests (2). A yellow-brown solid (A) remained. The rest of the aminomalononitrile was allowed to stand under vacuum in a sealed tube. A tarry mass which soon formed with evolution of hydrogen cyanide was stirred with water (25°C, 24 h) after seven days and filtered to give a clear orange solution. Freeze-drying to a brown solid followed by extraction with acetone and ether left a light brown powder (B). Part of this was sealed in an ampoule with water and heated in an oven (100°C, 24 h). After insoluble black residues were separated by filtration, the filtrate was evaporated to give a dark brown solid (C).

Products A, B and C resembled the peptide-like solids (1, 2) arising from hydrogen cyanide polymerization reactions. Essentially similar infrared spectra were obtained showing nitrile (2200 cm^{-1}) and imino/keto (1650 cm^{-1}) peaks. Automatic peptide analysis (Technicon AutoAnalyzer) (5) produced a series of chromatograms (Figure 1) indicating that each solid contained a mixture of peptide-like polymers. TLC-ninhydrin tests showed that free α-amino acids were absent from A and B. After acid hydrolysis (6N HCl, 100 °C, 24 h), glycine and at least six other α-amino acids were shown to be present by automatic α-amino acid analysis (Beckman Amino Acid Analyzer, Model 120B).

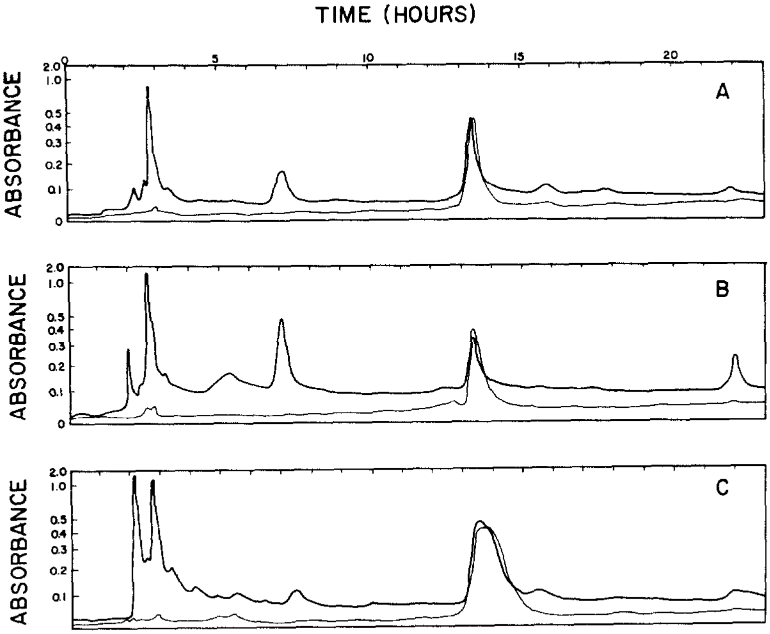

FIGURE I. CHROMATOGRAMS (TECHNICON AUTOANALYZER) OF PEPTIDIC PRODUCTS OBTAINED FROM AMINOMALONITRILE
A. AFTER TREATMENT WITH COLD WATER (25°)
B. AFTER TREATMENT OF DECOMPOSITION PRODUCT WITH COLD WATER (25°)
C. " " " " " BOILING WATER
RECORDED BEFORE (LOWER CURVES) AND AFTER (UPPER CURVES) ALKALINE HYDROLYSIS.

To investigate the effect of pH on the hydrolysis of aminomalononitrile, seven samples of the yellow oil (0.5 g) were sealed in ampoules containing HCl and NH_4OH solutions (10 ml) of known pH ranging from lower than 1 to higher than 12 (Table I). After three days at 100 °C the acid samples were still a clear, pale yellow in color while the neutral and basic samples had become black and partially solid. Sufficient 12N HCl was added to each sample to lower the pH to less than 1. Reheating (100 °C, 24 h) followed by evaporation and freeze-drying gave residues which were analyzed automatically for α-amino acid composition (Table II).

TABLE I

Aminomalononitrile Hydrolysis Conditions

Sample Number	Hydrolysis Medium	Initial pH	Color after Hydrolysis
1	HCl, 6.0 N	<1	Yellow
2	HCl, 1.0 N	0.02	Yellow
3	HCl, 0.1 N	1.16	Yellow
4	H_2O	6.09	Black
5	NH_4OH, 0.1 N	10.30	Black
6	NH_4OH, 1.0 N	10.96	Black
7	NH_4OH, 15.0 N	11.87	Black

TABLE II

α-Amino Acid Composition of Hydrolysis Products†

Amino Acid	1	2	3	4	5	6	7
Lysine	-	-	-	5	34	7	-
Histidine	-	-	-	3	-	1	-
Ammonia	3690	6190	6285	5610	4170	5520	5195
Arginine	-	-	-	2	2	1	-
Aspartic Acid	-	-	-	15	19	8	8
Threonine	-	-	-	1	22	-	-
Serine	-	-	-	4	24	14	18
Glutamic Acid	-	-	-	8	181	49	130
Glycine	3505	2204	247	641	1091	302	462
Alanine	-	-	4	10	16	6	18
Valine	-	-	-	-	2	-	-
Leucine	-	-	-	-	2	5	6

†Adjusted to μmoles α-amino acid per g hydrolyzate.

As expected, in the acidic reactions glycine was the only α-amino acid product, whereas at neutral or alkaline pH yields of glycine were lower and appreciable amounts of up to ten other α-amino acids were detected including lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, alanine, valine and leucine.

We interpret these reactions of aminomalononitrile as occurring in two ways, both ultimately leading to α-amino acids. Under acid conditions, hydrolysis of nitrile groups to carboxyl groups followed by decarboxylation yields glycine as the sole α-amino acid product.

In alkali, however, or under anhydrous conditions, aminomalononitrile decomposes readily to hydrogen cyanide and its dimer, aminocyanocarbene (II) (6). Polymerization of these species yields a mixture of products (1, 2, 7) including diaminomaleonitrile (III) (8), black intractable solids and polymeric peptide precursors converted to peptidic solids after contact with water (Figure 2). Hydrolytic breakdown of diaminomaleonitrile (8) and the peptidic products then leads to the formation of mixtures of α-amino acids (1, 2).

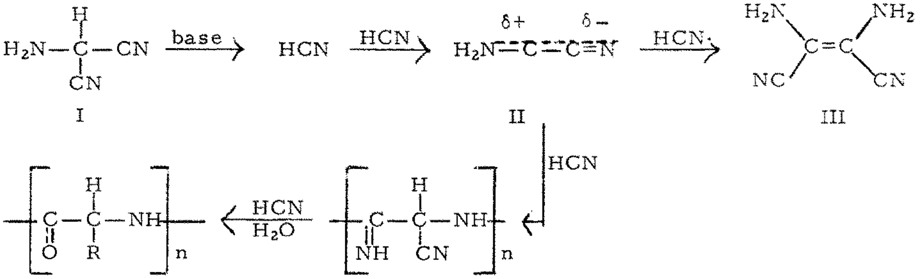

Figure 2

Polypeptide Formation from Aminomalononitrile (I).
R represents 15 possible α-amino acid side chains (2).

In the context of chemical evolution studies, this instability of aminomalononitrile suggests that reversion to hydrogen cyanide dimer and monomer would have been highly favored in the basic environment of primitive Earth. We therefore conclude that aminomalononitrile did not play an important part in the sequence of events leading to the prebiological synthesis of the prototypes of proteins. The main roles, instead, were taken by its precursors, aminocyanocarbene and hydrogen cyanide (1, 2).

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