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PEPTIDE FORMATION FROM DIAMINOMALEONITRILE (HCN TETRAMER)

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Base-catalyzed polymerization of hydrogen cyanide (1-6) yields a mixture of products that includes a tetramer (I, diaminomaleonitrile), a pentamer (adenine), polymeric amino acid precursors (7,8) and black, intractable solids believed to have fused tetrahydropyridine structures (1). We have recently shown that after treatment with water, peptide-like solids (7,8) can be obtained from the reaction mixtures and can be hydrolyzed to yield a dozen of the twenty α-amino acids commonly found in proteins. We now report the formation of similar peptidic products from water and diaminomaleonitrile.

When diaminomaleonitrile (mp 182-184°C) in water was heated to 100°C for 24 h, a solid black product was formed. Removal of a black residue by filtration left a dark solution which was passed through filter paper pulp and a short Sephadex G-15 column and then freeze-dried to a yellow-brown solid. Fractionation of this material was carried out using a Sephadex column (G-15, 2.5 x 90 cm, ammonium acetate buffer, ionic strength 0.01). Portions of the effluent (3 ml) were collected automatically, the concentrations of material in each sample being followed spectrophotometrically at 230 mμ and recorded as an elution diagram (Figure 1). Collected fractions were combined into four solutions which were freeze-dried to give yellow-brown solids (2A-2D). Automatic peptide analysis (Technicon Auto Analyzer) (9) gave a series of chromatograms (Figure 2) indicating that each solid contained a mixture of peptide-like materials (see peaks at 1.9, 2.3, 2.4, 2.6 and 8.3 h on chromatogram 2A, for example). In addition, appreciable amounts of free glycine (10) were present in 2A and 2B (see peaks at 4.5 - 4.8 h) as had been shown also by prior TLC-ninhydrin tests. No other free α-amino acids were detected.

None of the chromatograms possessed the characteristic peaks of diaminomaleonitrile (Figure 3) and the absence of tetramer was further indicated by negative color tests (8).

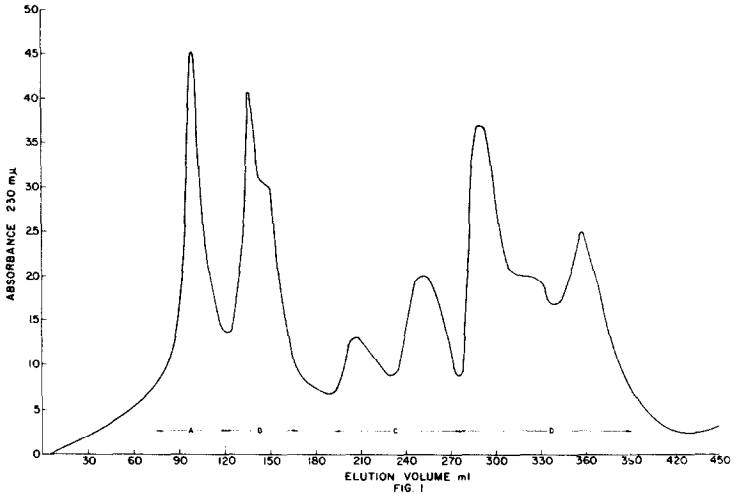

FIG. 1

ELUTION DIAGRAM (SEPHADEX G-15 COLUMN: AMMONIUM ACETATE BUFFER, IONIC STRENGTH 0.01) FOR FRACTION OF PEPTIDIC PRODUCTS (A-D) OBTAINED FROM HCN TETRAMER (DIAMINOMALEONITRILE) AFTER TREATMENT WITH BOILING WATER

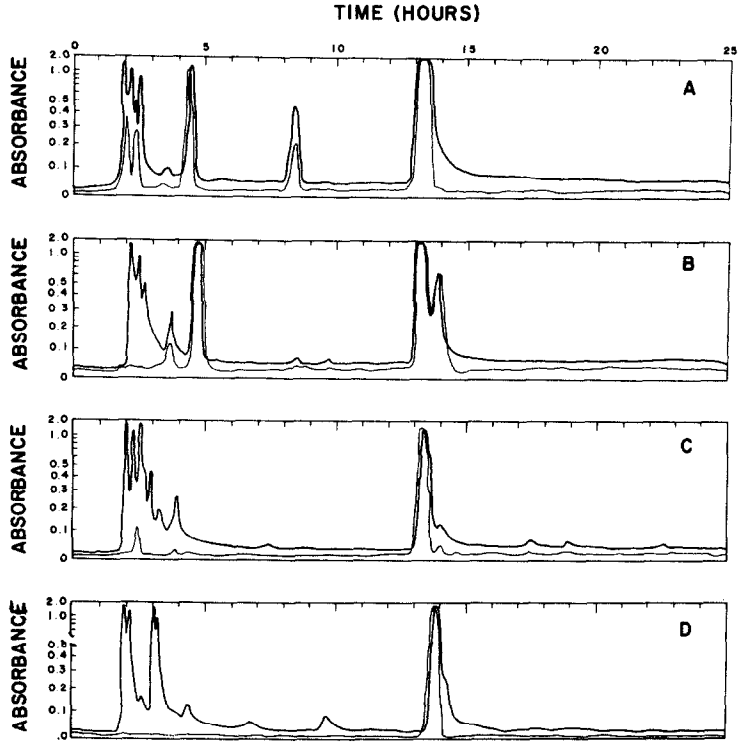

FIGURE 2. CHROMATOGRAMS (TECHNICON AUTO ANALYZER) OF SEPARATED PEPTIDIC PRODUCTS (A-D) OBTAINED FROM HCN TETRAMER (DIAMINOMALEONITRILE) TREATED WITH BOILING WATER, RECORDED BEFORE (LOWER CURVES) AND AFTER (UPPER CURVES) ALKALINE HYDROLYSIS.

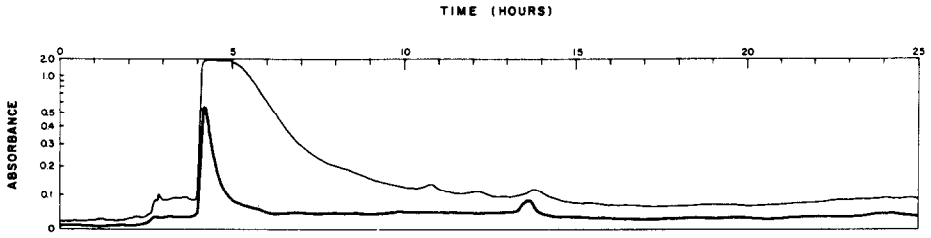

FIGURE 3. CHROMATOGRAM (TECHNICON AUTOANALYZER) OF DIAMINOMALEONITRILE (HCN TETRAMER), RECORDED BEFORE (LOWER CURVE) AND AFTER (UPPER CURVE) ALKALINE HYDROLYSIS

Acid hydrolysis (6N HCl, 100 °C, 24 h) of 2A-2D was followed by automatic α-amino acid analysis (Beckman Amino Acid Analyzer, Model 120B). Each hydrolyzate consisted of ammonia and glycine with lesser amounts of several other α-amino acids, at least 11 being detected in 2B (Table I, columns 2A-2D). As expected, fractions 2A and 2B contained much more glycine than 2C and 2D. Glycine was almost the sole α-amino acid product of acid hydrolysis (6N HCl, 100 °C, 24 h) of diaminomaleonitrile (Table I, column 3).

TABLE I

α-Amino Acid Composition of Hydrolyzates of Products from Diaminomaleonitrile Reactions†

Amino Acid	2A	2B	2C	2D	3
Lysine	2	4	4	24	-
Histidine	2	3	0.7	-	-
Ammonia	2463	2962	3043	4098	5410
Arginine	-	0.2	1	0.3	-
Aspartic Acid	10	32	11	8	-
Threonine	5	3	8	0.9	2
Serine	18	14	6	6	7
Glutamic Acid	1	1	1	0.5	-
Glycine	1220	2093	232	96	3156
Alanine	24	14	7	4	-
Valine	0.3	0.6	2	-	-
Isoleucine	0.5	0.2	0.7	0.5	-
Leucine	-	0.6	6	-	-

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

These results indicate that the primary products of hydrolytic breakdown of diaminomaleonitrile include glycine, hydrogen cyanide and ammonia. Under acid conditions, no polymerization of the hydrogen cyanide occurred (1). At neutral or alkaline pH, however, base-catalyzed reactions of hydrogen cyanide in water yielded the usual polymerization products formed via HCN dimer (II, aminocyanocarbene) (8,11) including peptidic solids and diaminomaleonitrile (Figure 4).

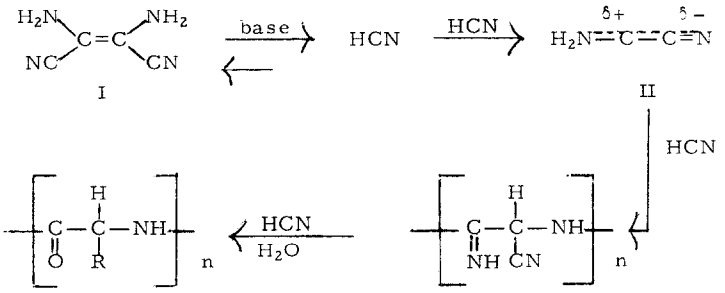

Figure 4

Polypeptide Formation from Diaminomaleonitrile (I).
R represents 15 possible α-amino acid side chains (8).

Breakdown of the newly-formed tetramer produced more hydrogen cyanide which again polymerized to a mixture of products including tetramer. Successive reactions of this type continued until no more tetramer was formed. The peptidic polymers—found together with glycine—were then further hydrolyzed to mixtures of α-amino acids. We believe that this explanation of diaminomaleonitrile hydrolysis accounts best for our results and for other work (1,6,12) identifying HCN tetramer as a source of α-amino acids.

In the context of chemical evolution studies concerning the origin of proteins in the basic environment of primitive Earth, we therefore suggest that reactions of diaminomaleonitrile giving rise to α-amino acids and peptides are only significant as part of the more general phenomenon (7,8) of peptide synthesis from hydrogen cyanide and water.

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