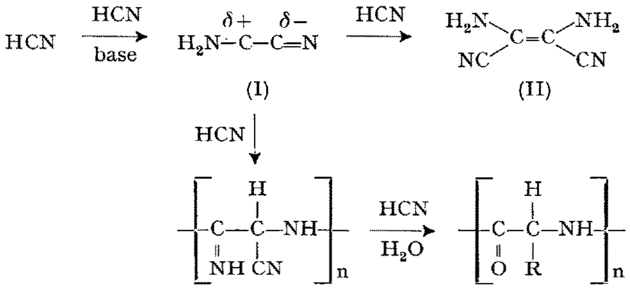
Hydrolysis of Aminoacetonitrile: Peptide Formation

Free α-amino nitriles as a class are among the most unstable nitriles known[1], reverting readily to hydrogen cyanide and ammonia in the presence of alkali. Conversion to α-amino acids, as in the final step of the Strecker synthesis, is therefore usually carried out under acidic conditions. Glycine, for example, can be obtained in 92% yield from aminoacetonitrile after hydrolysis with 40% sulfuric acid[1]. We now report that neutral or alkaline decomposition of aminoacetonitrile gives a mixture of several other α-amino acids in addition to glycine.

When aminoacetonitrile (b.p. 65 °C at 8 mm)[2] was dissolved in cold water (25 °C), the colorless solution gradually darkened and became converted to a black suspension. After 30 days a black residue was filtered off and the resulting filtrate freeze-dried to a dark brown solid resembling the peptidic products obtained by base-catalyzed polymerization of hydrogen cyanide[3,4]. Following acid hydrolysis (6N HCl, 100 °C, 24 h) of the solid, automatic α-amino acid analysis showed that in addition to glycine, the main product, at least 6 other α-amino acids were present – lysine, aspartic acid, threonine, serine, glutamic acid and alanine.

To investigate the effect of pH on the hydrolysis of aminoacetonitrile, 7 samples (0.5 ml) were sealed in ampoules containing HCl or NH_4OH solutions (10 ml) of known pH ranging from lower than 1 to higher than 12 (Table I). After heating at 100 °C for 3 days, the solutions (ranging from colorless to black) were transferred to larger ampoules and diluted with enough 12N HCl to lower the pH of each of these to less than 1. After the sealed ampoules had been reheated to 100 °C for 24 h, the contents of each were decolorized with acid-washed charcoal and freeze-dried. The residue was then analyzed automatically for α-amino acid composition (Beckman Amino Acid Analyzer, Model 120 B) (Table II). As expected, under strongly acidic conditions glycine was the only α-amino acid product. At neutral or slightly alkaline pH, however, the yields of glycine were lower and appreciable amounts of at least 6 other α-amino acids were detected. In strong alkali higher yields of glycine were obtained together with the other α-amino acids. Results of parallel experiments on the hydrolysis of α-aminobutyronitrile (m.p. 138–139 °C as the hydrochloride)[5] showed a similar trend, with α-aminobutyric acid being the major product of each reaction. Small amounts of other α-amino acids, including glycine, were again found to be present under neutral or alkaline conditions.

We interpret these hydrolysis reactions of α-amino nitriles as occurring in 2 ways, both ultimately leading to α-amino acids. Hydrolysis under strongly acidic conditions leads directly to the expected α-amino acid. While a similar conversion of nitrile to carboxylic acid groups occurs in alkali, partial decomposition to hydrogen cyanide also takes place and is followed rapidly by base-catalyzed polymerization via HCN dimer [(I) aminocyanocarbene][6] to give a mixture of products[7] including a tetramer [(II) diaminomaleonitrile], peptide-like polymers[3,4] and black intractable solids.



Under drastic hydrolytic conditions, as in our experiments, mixtures of α-amino acids are then formed by breakdown of the tetramer[8,9] and the peptidic compounds. We have previously shown[4] that at least 12 of the 20 common α-amino acids can be obtained by hydrolysis of such polymers obtained directly from hydrogen cyanide.

Table I. Aminoacetonitrile hydrolysis conditions (pH)

Sample No.	Hydrolysis medium	Initial pH	Final pH	Color after hydrolysis
1	HCl, 6.0N	< 1	< 1	Colorless
2	HCl, 1.0N	1.10	2.09	Orange
3	HCl, 0.1N	6.40	9.50	Black
4	H_2O	8.10	9.48	Dark Brown
5	NH_4OH, 0.1N	10.85	9.70	Dark Brown
6	NH_4OH, 1.0N	11.50	9.80	Dark Brown
7	NH_4OH, 15.0N	12.61	–	Light Brown

In the context of chemical evolution studies, the above results suggest that the important role usually assigned[10–17]

Table II. α-Amino acid composition[a] of hydrolysis products of aminoacetonitrile

Amino acid	1	2	3	4	5	6	7
Lysine	–	–	3	7	5	5	7
Ammonia	4310	4560	5917	4270	4364	4255	3309
Aspartic acid	–	–	113	113	141	83	114
Threonine	–	–	3	4	4	3	4
Serine	–	–	4	7	6	4	6
Glutamic acid	–	–	6	9	7	4	9
Glycine	4920	2400	883	958	1094	753	1283
Alanine	–	–	10	9	11	9	11

[a] Adjusted to μ moles α-amino acid/g hydrolyzate.

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to α-amino nitriles needs to be reconsidered. It is widely believed, for example, that α-amino acids obtained from several types of simulation experiments were formed by the direct hydrolysis of corresponding α-amino nitriles[10–13]. In these alkaline reactions, any formation of α-amino nitriles would soon be followed by elimination of hydrogen cyanide. It therefore seems more likely to us that in such experiments most of the α-amino acids were products of alkaline hydrolysis of the peptides formed by the polymerization of hydrogen cyanide present either as a starting material or a reaction intermediate[3,4]. It has also been proposed[14–17] that the formation of triglycine from aminoacetonitrile (by heating with kaolinite and extracting with water)[14] occurred through a prior condensation between amine and nitrile groups yielding polyamidines. Since black solids were also a major product, it again seems more probable to us that the peptides were formed by base-catalyzed polymerization of hydrogen cyanide following decomposition of the starting material. We conclude, therefore, that in the reducing and basic environment of primitive Earth, α-amino nitriles played little or no direct part in the sequence of events leading to the prebiological synthesis of polypeptides and proteins. The main role, instead, was taken by hydrogen cyanide[3,4,18].

Zusammenfassung. Neutrale oder alkalische Hydrolyse von Aminoacetonitril gibt mindestens 6 α-Aminosäuren neben Glycin, welches das einzige Produkt bei saurer Hydrolyse ist. Die anderen α-Aminosäuren entstehen durch die Hydrolyse der peptidähnlichen Polymere, welche durch die Polymerisierung des aus dem Aminoacetonitril gebildeten Cyanwasserstoffes mit basischen Katalysatoren gebildet werden. Im Zusammenhang mit der chemischen Evolutionstheorie weisen diese Resultate darauf hin, dass α-Aminonitrile nur eine kleine oder gar keine direkte Rolle in der Aufeinanderfolge der Reaktionen gespielt haben, welche zur vorbiologischen Synthese von Polypeptiden und Proteinen führten.

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Solid Phase Synthesis and Some Pharmacoogical Properties of 4-Ser-8-Gln-Oxytocin (Glumitocin)[1]

Glumitocin (4-Ser-8-Gln-Oxytocin) was first isolated from *Raia clavata*[2] and later identified in 3 additional elasmobranchs, *R. batis*, *R. fullonica* and *R. naevus*[3]. Until very recently the proposed structure had not been confirmed by laboratory synthesis[4]. As part of a continuing investigation on the phylogeny of the neurohypophyseal hormones the synthesis of 4-Ser-8-Gln-Oxytocin was undertaken using the adaptation of the MERRIFIELD solid phase method[5] which has recently been applied toward the synthesis of oxytocin[6]. The synthetic product has been pharmacologically evaluated by methods previously described[7] and the results obtained are presented in the Table.

The required protected nonapeptide amide intermediate was synthesized in a stepwise manner beginning with 5.0 g of *t*-butyloxycarbonylglycyl resin containing 0.985 mmole of glycine according to the general procedure of MERRIFIELD[8], using the modifications previously described[6] with one additional precaution, i.e. the trifluoroacetic acid, dimethylformamide and triethylamine used in the synthesis were all fractionally redistilled before use. 8 cycles of deprotection, neutralization and coupling were carried out with appropriate Boc-amino acids[9] producing the protected nonapeptide esterified to the resin. Boc-amino acids with protected side chains were S-Bzl-Cys, O-Bzl-Ser and O-Bzl-Tyr. The final cysteine residue was added as the *N*-Carbobenzoxy-S-Benzyl (*N*-Z-S-Bzl) derivative. All coupling reactions to form peptide bonds were mediated by dicyclohexylcarbodiimide[10] in methylene chloride except those involving the carboxyl groups of Asn and Gln, which were allowed to react in dimethylformamide (DMF) as their nitrophenyl esters[11].

Following the coupling of the final residue, the dried resin weighed 6.057 g. The weight increase of 1.057 g represents the incorporation of 0.81 mmole of protected nonapeptide in the resin. This is 82.2% of the amount expected, based on the original glycine content of 0.985 mmole of the esterified resin. Ammonolytic cleavage of the protected nonapeptide resin (2.9 g) was carried out as previously described[6] to give the protected nonapeptide amide Z-Cys(Bzl)-Tyr(Bzl)-Ile-Ser(Bzl)-Asn-Cys(Bzl)-Pro-Gln-Gly(NH_2) as a white amorphous powder, weight 464 mg; m.p. 243.5–245°, $[\alpha]_D^{22.5}$ $-29.0°$ (c, 1, dimethylformamide). Anal. calcd. for $C_{76}H_{92}N_{12}O_{15}S_2$: C, 61.77; H, 6.27; N, 11.37. Found: C, 61.97; H, 6.20; N, 11.28.

The yield of the protected nonapeptide amide from the cleavage was 82% of the amount expected based on the increase in weight of the resin. The overall yield based on the amount of glycine originally esterified to the resin

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