these changes in the overall rate of protein synthesis. This implies that a common mechanism is involved. Moreover, Pipeleers *et al.* (*5*) found two treatments (cycloheximide and 2,4-dinitrophenol) which reduce the rate of protein synthesis without affecting the preferential synthesis of proinsulin. These two treatments primarily influence the rate of elongation, and thus support the hypothesis that the rate of polypeptide chain initiation determines how efficiently the proinsulin mRNA is translated.

Lodish's kinetic model for translational regulation (*7*) predicts that any increase in the overall rate of polypeptide chain initiation will allow mRNA's with lower rate constants (that is, affinities) to be translated more efficiently. Hence, under low glucose conditions, proinsulin mRNA may compete (relative to the other mRNA's in the $\beta$ cell) less effectively as a result of its lower intrinsic binding affinity for rate controlling translation elements. This competition may be so severe that, at any one time, only a fraction of the proinsulin mRNA will be found engaged on an active ribosome. The high concentration of glucose increases the overall rate of polypeptide chain initiation (for example, by increasing the concentrations of guanosine triphosphate) (*18*). This permits the less competitive proinsulin mRNA to be initiated more rapidly, and mobilization of the proinsulin mRNA pool. This combination will account for the relative increase in proinsulin synthesis. Hence, if the rate of glucose oxidation within the $\beta$ cell regulates the overall rate of polypeptide chain initiation, which in turn regulates the rate of proinsulin synthesis, then the $\beta$ cell possesses a simple mechanism to replenish insulin levels following glucose-stimulated insulin secretion.

Our results are clearly compatible with Lodish's kinetic model for translational regulation. However, they in no way prove the hypothesis, nor do they exclude the possibility that glucose modulates either the concentrations of specific initiation factors for proinsulin mRNA or the amount of proinsulin mRNA available for translation. Experiments to test these possibilities await production of a labeled complementary DNA probe to measure the amount of proinsulin mRNA.

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## Deuterolysis of Amino Acid Precursors: Evidence for Hydrogen Cyanide Polymers as Protein Ancestors

Abstract. *Deuterolysis experiments suggest that hydrogen cyanide polymers rather than aminoacetonitriles are major precursors of α-amino acids obtained from spark reactions and other studies on chemical evolution. These results are consistent with the hypothesis that the original heteropolypeptides on the earth were synthesized spontaneously from hydrogen cyanide and water without the intervening formation of α-amino acids.*

After the pioneering demonstration by Miller and Urey (*1*) that α-amino acids are readily obtained from methane, ammonia, and water subjected to electric discharges, it has become widely accepted that the prebiotic formation of primitive proteins occurred in two stages: α-amino acid synthesis initiated by the action of natural high-energy sources on the components of a reducing atmosphere, followed somehow by polycondensation of the accumulated monomers in the oceans or on land (*1, 2*). Limited progress in research on possible condensation reactions (*2*), however, suggests that the inherent thermodynamic barrier to spontaneous polymerization of α-amino acids is not easily overcome. An alternative model for the origin of proteins (*3–5*) bypasses this problem by

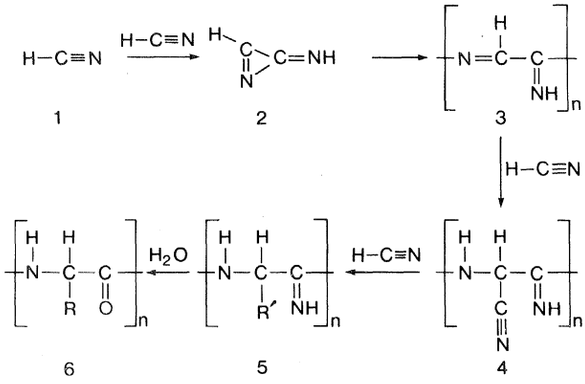



Fig. 1. Proposed route for heteropolypeptide synthesis from hydrogen cyanide and water (**1** → **6**). R′ represents precursors of protein side chains R.

postulating the direct synthesis of heteropolypeptides from hydrogen cyanide and water without the intervening formation of $\alpha$-amino acids. The proposed low-energy pathway (Fig. 1) would allow atmospheric hydrogen cyanide to polymerize to polyaminomalononitrile (**4**) via azacyclopropenylidenimine (**2**) (*5*) and its polymer (**3**). Subsequent reactions of hydrogen cyanide with the activiated nitrile groups of **4** then yield heteropolyamidines (**5**) which would be converted to heteropolypeptides (**6**) after contact with water. According to this hypothesis then, the amino acids isolated from spark reactions, such as Miller's, could actually be secondary products resulting from hydrolytic breakdown of heteropolypeptides (mechanism A) rather than primary products formed directly by the hydrolysis of aminoacetonitrile intermediates (mechanism B), as is generally assumed (*1, 2*). We report here results of deuterolysis experiments which support mechanism A, and point out some implications for studies of chemical evolution.

To distinguish between the two mechanisms (Fig. 2) deuterolysis was carried out on the products obtained by subjecting a mixture of methane and ammonia to an electric discharge (*6*), water being excluded during sparking in order to avoid any ambiguities caused by the introduction of oxygenated intermediates. Mechanism A predicts that the hydrogen cyanide formed in high yield from methane and ammonia would rapidly polymerize to polyaminomalononitrile (**4**) and to heteropolyamidines (**5**). Acid hydrolysis of **5** would yield many $\alpha$-amino acids, while treatment of **4** with DCl-$D_2O$ would give **7** and then perdeuterated glycine **8**, after hydrolysis of its nitrile groups to carboxylic acid groups and subsequent decarboxylation. By contrast, glycine (**11**) obtained after DCl-$D_2O$ treatment of aminoacetonitrile (**10**)—the addition product of HCN and formaldimine (**9**)—should possess essentially no carbon-bound deuterium (mechanism B). To establish the validity of these assumptions, deuterolysis (*6*) was also carried out on the reference compounds (a) glycine and polyglycine, (b) aminoacetonitrile, (c) hydrogen cyanide polymers (*7*), and (d) poly-$\alpha$-cyanoglycine (**12**), the polyamide analog of **4** (*8*). In each case, the glycine obtained was analyzed for carbon-bound deuterium by combined gas chromatography–mass spectrometry (GC-MS), with selective monitoring of the ion fragments 172, 173, and 174 (Fig. 3) derived from *N*-trifluoroacetyl-*n*-butyl derivatives (*9*). As

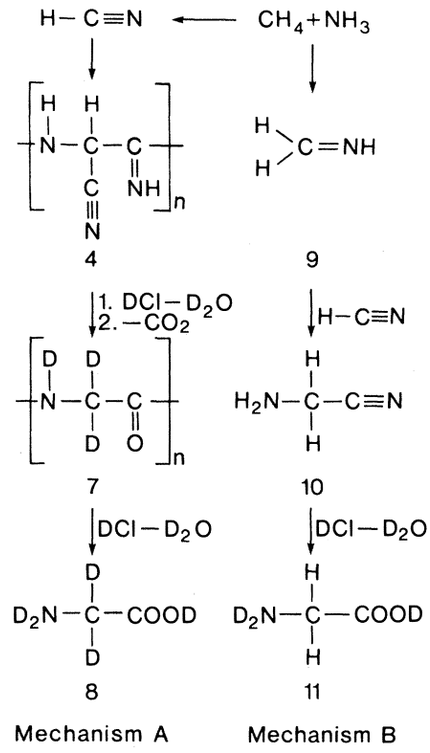



Fig. 2. Two proposed pathways—via polymeric HCN (mechanism A) and aminoacetononitrile (mechanism B)—leading to glycine from spark experiments on methane and ammonia followed by deuterolysis with DCl-$D_2O$.

was expected, most of the glycine from the model polymer **12** and from the HCN polymer (*7*) was found to be perdeuterated, possessing two atoms of deuterium per molecule more than glycine obtained from aminoacetonitrile or polyglycine (Table 1, items a to d), whereas glycine from the spark experiment (Table 1, item e) was perdeuterated to the extent of 38 percent (45 percent in a second experiment). It appears, therefore, that both pathways might have been involved in the formation of amino acid precursors, particularly since, in previous experiments with methane and ammonia subjected to electric discharges, Ponnamperuma and Woeller (*10*) were able to isolate aminoacetonitrile—a colorless liquid—from their reaction products, while Matthews and Moser (*3*) extracted yellow-brown solids hydrolyzable to $\alpha$-amino acids from theirs.

To appreciate the true significance of these studies, however, it is necessary to explore the effects of *p*H and time brought about by the addition of water (acid or alkaline) to the anhydrous intermediates, aminoacetonitrile (**10**) in particular. To this end, aminoacetonitrile (0.04*M*) was allowed to stand in a buffer at *p*H 8.7 (0.05*M* $NH_4Cl$ and $NH_3$) approximating the *p*H of the primitive oceans (*1*). Portions were removed at 4-day intervals, lyophilized, treated with DCl-$D_2O$, and analyzed for glycine (by GC-MS) as before. The uptake of carbon-bound deuterium increased with time, reaching a maximum of 32 percent after 16 days. When a higher concentration of aminoacetonitrile (0.2*M*) was used, the maximum deuterium uptake was 48 percent after 10 days (*11*). Evidently a mildly alkaline environment favors both the elimination of hydrogen cyanide from aminoacetonitrile (**10**) and its subsequent polymerization, thus gradually bringing about the irreversible conversion of **10** to formaldimine (**9**) and to HCN polymers that, on deuterolysis, yield perdeuterated glycine (**8**) and other $\alpha$-amino acids. On further standing, as was expected, a gradual decline in deuterium uptake was observed, presumably as those polymers possessing nitrile groups became converted through hydrolysis and decarboxylation to polymers such as polyglycine or **13**, which on deuterolysis yield glycine (**11**) essentially free of carbon-bound deuterium. Other evidence for the ready release of HCN from aminoacetonitrile in an alkaline medium was obtained from a series of experiments in which aminoacetonitrile (**10**) was allowed to stand for 6 days in

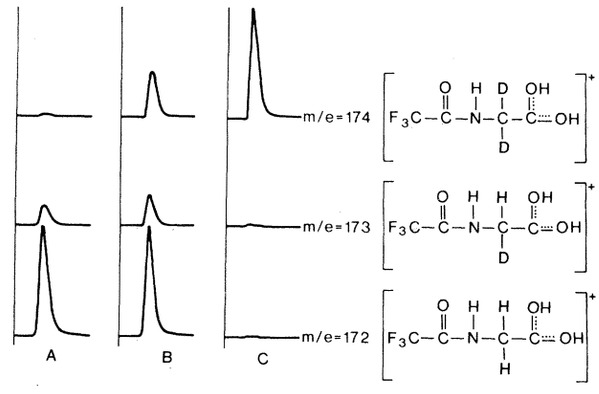
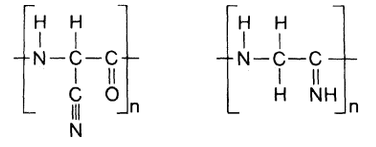



Fig. 3. Typical mass fragmentograms for glycine ions [*m/e* (mass to charge) = 172, 173, and 174] obtained upon deuterolysis (DCl-$D_2O$) of (A) glycine, (B) a methane-ammonia spark experiment, and (C) hydrogen cyanide polymer.

buffer solutions from *p*H 2 to 12. When the lyophilized products were treated with DCl-$D_2O$ and analyzed for glycine (GC-MS) it was found that maximum deuterium uptake occurred within the range of *p*H 4 to 9. These results are in accord with the original demonstration (*12*) that alkaline hydrolysis of aminoacetonitrile yields mixtures of α-amino acids rather than pure glycine, which is obtained only under strongly acidic conditions that inhibit the polymerization of hydrogen cyanide. Similar behavior was shown (*13*) by HCN oligomers such as aminomalononitrile (**14**), a trimer of HCN, and diaminomaleonitrile (**15**), a



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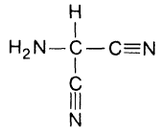

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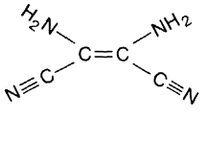

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tetramer, other possible intermediates (*14*) in reactions involving hydrogen cyanide. We conclude, then, that aminoacetonitriles and HCN oligomers in alkali act mainly as sources of hydrogen cyanide and its polymers, rather than of free α-amino acids.

Taken together with other research (*3, 4, 8*), this body of evidence invites the reexamination and possible reinterpretation of several kinds of studies concerned with the origin of α-amino acids and proteins. It seems likely to us that where α-amino acids or peptides have been detected in spark experiments simulating planetary atmospheres—whatever the nature of the starting materials or energy sources used—hydrogen cyanide polymers were major precursors derived from HCN formed either as a spark product or by elimination from intermediates such as aminoacetonitriles and HCN oligomers. Similar reasoning applies to the amino acids and peptides found in studies of aqueous cyanide reactions based on the original work of Oró (*15*). In none of the above investigations yielding peptides (*16*) do we believe that "the amino acids appear to have been linked together as soon as they were synthesized" (*10*). Nor does it seem probable that the original proteins on the earth were formed by polymerization of aminoacetonitrile to a polyamidine fore-protein (**13**) which was then hydrolyzed to polyglycine and modified by aldehydes to heteropolypeptides, as suggested by Akabori (*17*). Our deuterolysis experiments demonstrate both the instability of aminoacetonitrile in an alkaline medium and the inertness of polyglycine compared to polyaminomalononitrile (**4**), the true ancestor, we believe, of all proteins.

Turning to extraterrestrial chemistry, we think that this ubiquitous series of reactions (Fig. 1, **1** to **5**) provides a better explanation (*4*) than has yet been offered for the presence in carbonaceous chondrites of water-soluble, yellow-brown solids that can be hydrolyzed to α-amino acids (*18*), particularly since deuterolysis brings about the incorporation of carbon-bound deuterium in glycine obtained from the Murchison meteorite (*19*). Comparable extracts obtained from lunar fines (*18*) might also have similar structures (*4*). Hydrogen cyanide polymerization along these lines could account, too, for much of the yellow-brown-orange coloration of Jupiter (*4*) with its reducing environment, a continuing reminder that optimum conditions for heteropolyamidine synthesis might well have existed in the upper atmosphere of the primitive earth. Instead of the relatively crude material found in condensed phase reactions, aqueous solutions, or spark chambers—or in meteorites and moondust—true heteropolyamidines of high molecular weight would have been generated spontaneously in the stratosphere from clouds of hydrogen-bonded HCN molecules formed by the photolysis of methane and ammonia. As these helical macromolecules settled on the earth—together with other stable products of atmospheric photochemistry derived from acetylene, hydrogen cyanide, and formaldehyde (*4, 20*)—a proteinaceous matrix developed capable of participating in and promoting the chemical interplay that eventually led to the emergence of life.

Table 1. Distribution of deuterium label in glycine derived from the following sources upon deuterolysis (DCl-$D_2O$).

| Item | Source | Mole percent of glycine with carbon-bound deuterium atoms: | | |
|---|---|---|---|---|
| | | Zero | One | Two |
| a | Glycine | 87 | 13 | 0 |
| a | Polyglycine | 86 | 14 | 0 |
| b | Aminoacetonitrile (**4**) | 88 | 12 | 0 |
| c | HCN polymer | 1 | 1 | 98 |
| d | Poly-α-cyanoglycine (**10**) | 2 | 3 | 95 |
| e | Spark experiments | 47 | 15 | 38 |
| | | 39 | 16 | 45 |


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6. A mixture of methane and ammonia (equal volumes) introduced into a 5-liter flask (1 atmosphere) was subjected to an electric discharge via two tungsten electrodes with a 10-cm gap produced by a 15,000-volt neon lamp transformer with input voltage controlled by a variable transformer and output proceeding through a series of resistors. After 2 days, the dark brown products were rinsed out with 6 percent DCl in $D_2O$, lyophilized, and heated with 6*N* DCl-$D_2O$ for 20 hours at 100°C in a degassed sealed tube.
7. HCN (50 ml) was distilled into dry acetonitrile (100 ml). Gaseous ammonia (75 ml) was gradually added. In 6 weeks, the stirred solution turned from yellow to brown to almost black. The solvent was removed under vacuum, leaving a dark brown residue.
8. R. D. Minard, W. Yang, P. Varma, J. Nelson, and C. N. Matthews [*Science* **190**, 387 (1975)] report that hydrolysis of **12** yields glycine, whereas hydrolysis after treatment with HCN yields a mixture of α-amino acids including glycine, alanine, valine, aspartic acid, and glutamic acid. This shows that the nitrile groups of **12** and **4** are indeed reactive, as predicted.
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11. A comparable increase in uptake of carbon-bound deuterium (up to 79 percent after 21 days) was noted in parallel experiments where the products of a methane-ammonia spark experiment were allowed to stand in a *p*H 8.7 buffer.
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drawn from deuterolysis of the polymer mixtures remain unchanged, since separation of low-molecular-weight compounds including **15** from various HCN polymers (*3*) leaves polymers of higher molecular weight that can be further fractionated and hydrolyzed to mixtures of α-amino acids and other compounds.
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16. The water-soluble solids loosely described as peptides obtained from these and other experiments are probably partial hydrolysis products of cyanide polymers possessing segments of several kinds (*4*) including **3**, **4**, and **5**. More relevant to the proposed model are high-pressure liquid chromatography experiments now in progress with nonaqueous solvents to separate the various polymeric structures obtained from HCN polymers synthesized in solvents other than water.
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4 April 1977; revised 15 June 1977

## Mandelonitrile β-Glucuronide: Synthesis and Characterization

Abstract. *Mandelonitrile β-glucuronide, the compound patented as Laetrile®, has been synthesized from rabbit liver uridine diphosphate-glucuronosyl transferase immobilized on beaded sepharose, has been analyzed by thin-layer chromatography, nuclear magnetic resonance, and gas chromatography-mass spectrometry, and has been tested for cytotoxicity and mutagenic activity with* Salmonella typhimurium *strains TA 98 and TA 100. Several commercial laetrile preparations contained no glucuronide; they contained amygdalin and neoamygdalin instead. Mandelonitrile, mandelonitrile glucuronide, and a mixture of amygdalin and neoamygdalin were each found to be mutagenic.*

Significant structural differences exist between mandelonitrile glucuronide (**1**), originally patented (*1*) as Laetrile®, and amygdalin (**2**) (see Fig. 1), the mandelonitrile β-*d*-gentiobioside, reported (*2*) to be the major component of Mexican preparations marketed as laetrile.

Laetrile® (**1**) was originally reported (*1*) to be obtained from amygdalin by a multistep chemical synthesis. *R*-Amygdalin (**2**) is commercially derived from a variety of natural sources, including apricot pits (*2*), and it has also been synthesized chemically (*3*).

Considerable confusion exists (*2*) concerning the relation between the structure and nomenclature of these two compounds. We undertook to obtain physicochemical data on **1** and **2** which would permit the reliable identification of these compounds in products found on the market.

Although authentic amygdalin (**2**) was available commercially, we had to develop a synthesis of mandelonitrile glucuronide (**1**) in our own laboratories. The synthesis of **1** described in the original patents (*1*) has not been reproduced (as revealed by our search of the chemical literature); to us, a more feasible approach to this synthesis appeared to be through the Koenigs-Knorr reaction that has provided other glucuronides on a large scale (*4*). With this reaction we were successful in producing the glucuronide of benzyl alcohol, but not the glucuronide of mandelonitrile (**1**). However, the β-glucuronide of *dl*-mandelonitrile was easily prepared from UDP-glucuronosyl transferase (E.C. 2, 4, 1, 17) immobilized on beaded sepharose. The technique for this procedure has been described (*5*).

Conjugation of racemic mandelonitrile was expected to lead to two glucuronides, mandelonitrile glucuronide and a similar structure that is epimeric at the benzyl center. For characterization by gas chromatography–mass spectrometry (GC-MS), the product mixture was converted by standard methods to tetrakis (trimethylsilyl) derivatives. The gas chromatogram contained two peaks of equal height whose mass spectra were virtually identical. The GC retention times of the two tetrakis trimethylsilylated diastereomeric epimers (*6*) are given in Table 1. Each of the mass spectra contained molecular ions (M) at mass 597, accompanied by M − 15 ions of mass 582, peaks characteristic of persilylated glucuronides (*7*) at *m/e* 375, 333, 217, and 204, and peaks at *m/e* 116 and 133, corresponding to the ionized aglycon moiety. The product mixture was also converted to the tris(trimethylsilyl) methyl ester derivatives and analyzed by GC-MS. However, trifluoroacetylation did not provide satisfactory derivatives. The nuclear magnetic resonance spectrum of the product of enzymatic conjugation measured in [$^2H_6$]dimethyl sulfoxide exhibited absorptions at δ 7.49 (singlet, phenyl protons), 5.95, and 5.99 (chiral benzyl proton in the two diasteromers) and 2.72 to 4.93 (multiplet, glucosiduronic protons) (*8*). The two products were designated as the expected glucuronides of mandelonitrile (**1**) epimeric about the chiral benzyl center. The patent literature (*1*) appears to specify the epimer with the *R* configuration at the benzyl carbon as Laetrile®.

Commercial amygdalin (**2**) (*9*) was also converted to its per (trimethylsilyl) derivative for analysis by GC-MS. As indicated in Table 1, the retention time of the derivatized amygdalin (**2**) was quite

Table 1. Retention data for laetrile, amygdalin, and related samples. The TLC systems consisted of butanol, benzene, water, and methanol (2:1:1:1.25). Silica gel 60 F-254 plates (E. E. Merck) were used. The GC-MS system consisted of the DuPont Dimaspec 321 GC-MS; 3 percent OV-101, 1.3-m column temperature programming 200° to 300°C at 4° per minute; helium at 30 ml/min; and samples derivatized with BSTFA (Pierce Chemical Co.) in 1 percent trimethyl chlorosilane and pyridine. Other instruments were used with similar results.

| Sample | TLC ($R_F$) | GC-MS* (retention)* |
|---|---|---|
| Mandelonitrile | 0.91 | |
| Benzyl alcohol | 0.91 | |
| Benzyl alcohol glucuronide | 0.49 | 0.71 |
| Mandelonitrile glucuronides (**1**) | 0.50<br>0.50 | 0.78<br>0.81 |
| *R*-Amygdalin (**2**) | 0.55 | 1.90 |
| Commercial laetrile | | |
| R-amygdalin | 0.55 | 1.90 |
| Neoamygdalian | 0.55 | 1.83 |

*The ratio of retention time of the sample to the retention time of (tetrakis)trimethylsilated *p*-nitrophenol glucuronide. Mandelonitrile and benzyl alcohol were eluted in the solvent peak.

Table 2. Mutagenic activities on *S. typhimurium* (strains TA 100 and TA 98).

| $S_9$ treatment* | Compounds per plate (nmole) | Revertants per nanomole | |
|---|---|---|---|
| | | TA 100 | TA 98 |
| | *Mandelonitrile* | | |
| | 22.5 | 0.31 | |
| | 67.5 | 0.22 | 0.16 |
| | 225 | 0.24 | 0.14 |
| | 22.5 | 0.44 | 1.15 |
| | 67.5 | 0.40 | 1.08 |
| | 225 | 0.45 | 0.87 |
| | 22.5 | | 0.27 |
| | 67.5 | 0.32 | 0.33 |
| | 225 | 0.28 | 0.39 |
| | *Mandelonitrile glucuronide* | | |
| | 9.6 | 0 | 0 |
| | 28.8 | 0 | 0 |
| | 96 | 0 | 0 |
| Aroclor | 9.6 | 0.31 | |
| | 28.8 | 0.24 | 0.21 |
| | 96 | 0.19 | 0.24 |
| Phenobarbital | 9.6 | 0 | 0 |
| | 28.8 | 0 | 0 |
| | 96 | 0 | 0 |

*Livers were induced as indicated before preparation of microsomal fraction $S_9$ used in the mutagen test.