PEPTIDE FORMATION MEDIATED BY HYDROGEN CYANIDE TETRAMER: A POSSIBLE PREBIOTIC PROCESS

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Abstract.—Chemical evolution on the primitive earth must have involved condensation of α-amino acids to peptides. Under aqueous conditions consistent with current conceptions of primordial waters, heating glycerine with the hydrogen cyanide tetramer, diaminomaleonitrile, yields dipeptide. If nitrogen was cycled through primordial waters as cyanide, peptide synthesis by stepwise tetramer-mediated condensation of α-amino acids would have been a plausible process.

Many laboratories have studied the polymerization and condensation of molecules of biochemical interest under conditions believed to have been prevalent on the primitive Earth. Although, in many cases, special circumstances such as high temperatures and hypohydrous conditions were involved, several reports describe reactions in aqueous solutions in which condensing agents promoted the conversion of amino acids[1–4] to peptides, sugars[2, 5] and nucleosides[2, 6, 7] to phosphate esters, and inorganic orthophosphates[2, 7–10] to pyrophosphate. Because these agents are necessary to overcome the thermodynamic barrier to spontaneous dehydration-condensation reactions in aqueous media, they should be important elements in any concept of chemical evolution in which the oceans and other bodies of water served as primordial reaction vessels for the synthesis of compounds destined to be precursors of modern proteins and nucleic acids.

The relative merits of condensing agents should be judged on how likely their syntheses were in a primitive Earth environment and how consistent with such an environment were the conditions required for the condensation reactions. Since the probable state of the early Earth has been discussed, guidelines for experimental conditions are available;[11–13] and a reasonable criterion for the likelihood that a particular condensing agent had existed would be its detection in synthesis experiments performed under simulated primordial conditions. Of those compounds suggested as condensing agents, only cyanamide[14] has been detected in such experiments; however, recent work[4] has cast serious doubts about its effectiveness under relevant conditions. Clearly, the search for plausible primordial condensing agents is far from finished.

We have been guided in our investigations by observations that radioactive amino acids were incorporated in polymers during heating of ammonium cyanide solutions[15] and during spark discharges through methane, ammonia, and water atmospheres.[16] Acid hydrolysis[15, 16] and enzyme hydrolysis[16] of the polymers with leucine aminopeptidase[17] released intact labeled and unlabeled amino acids, thereby providing strong evidence for their polypeptide nature and indicating the occurrence of a dehydration-condensation mechanism in the aqueous phase of the reactions. In the spark discharge experiments, cyanide was detected in concentrations as high as 0.1 *M*.[16, 18] Apparently, conditions were conducive to cyanide polymerization[19] in both types of experiments, and possibly one or more of the cyanide polymers may have acted as a condensing agent. Such a finding would provide further support for the belief[19–23] that hydrogen cyanide and its polymers played unique and central roles in prebiotic syntheses.

The formation, hydrolysis, polymerization, and photochemistry of the hydrogen cyanide tetramer, diaminomaleonitrile, has been studied;[19–24] however, its role as a possible condensing agent has not been elucidated. This report describes the conversion of glycine to diglycine in the presence of tetramer under plausible primitive earth conditions.

Experimental.—Diaminomaleonitrile was prepared as described in ref. 25. Gas chromatography was performed on a Varian 1520 chromatograph, which was equipped with flame ionization detectors using a 6-ft by $^1/_8$-in. aluminum column packed with 5% Carbowax 20M on 80/100 mesh Chromosorb W-AWDMCS. Mass spectra were obtained with a Consolidated Electrodynamics Model 21-110 mass spectrograph. A modified Phoenix automatic analyzer was used for amino acid and dipeptide analyses. Ultraviolet irradiations were carried out in a Hanovia photochemical reactor.

A 2-ml solution 0.025 *M* in glycine and diaminomaleonitrile at pH 6.1 was heated at 94°C for 24 hr; a 200-μl aliquot of the amber solution was banded on Whatman 3M paper and chromatographed for 1 hr at 1000 v in pH 9.2 borate buffer. The band, which coincided in R_F value with that of diglycine, was eluted with water. Half the eluate was hydrolyzed in HCl. Chromatography of the unhydrolyzed half on the amino acid analyzer revealed two minor products and a major product (13 nmole) having the same retention time as diglycine. The only peak observed in the hydrolyzed sample corresponded to glycine. The presence of diglycine was confirmed by micropreparative isolation from the amino acid analyzer and conversion to the *N*-trifluoroacetyl methyl ester, which was purified by gas chromatography. The mass spectrum of the product was identical with that of an authentic diglycine derivative (70 ev): 242(6.0), 211(11), 183(17), 163(3.8), 154(6.4), 141(5.8), 127(82), 116(100), 97(10), 88(77), 69(60), 56(65). (We list the most abundant ion in each 14 mass unit interval ($2 + 14\text{ n} <$ nominal mass $\leq 16 + 14\text{ n}$) followed in parentheses by its abundance relative to the base peak set at 100.)

In subsequent experiments the yields of diglycine were measured by direct examination of 100- to 350-μl aliquots of reaction mixtures on the amino acid analyzer. The use of calibration curves permitted quantitative analysis in the nanomole range with ±5% precision. In some instances, the decrease in ultraviolet absorption of the tetramer was monitored at 298 nm. In this report yields were calculated on the basis of the limiting reagent and, when appropriate, involved corrections for unreacted tetramer. It was assumed that one molecule of tetramer was consumed for every molecule of diglycine formed. The pH of reactant solutions were initially adjusted when desired by the addition of small amounts of 4 *N* NaOH or 4 *N* HCl, which did not alter significantly the reactant concentrations.

Results and Discussion.—When dilute aqueous solutions of diaminomaleonitrile and glycine were heated at 94°C a number of reactions took place, one of which yielded diglycine as product. When ^{14}C-labeled glycine was used, radioactivity was incorporated in several unidentified products in addition to diglycine. Other reactions presumably entailed hydrolysis[19] and polymerization[24] of the tetramer. Thin-layer electrophoresis on cellulose-F plates also revealed several unidentified ultraviolet absorbing compounds. The course of tetramer destruction and diglycine formation is shown in Figure 1. The increase in diglycine is paralleled by the decrease of ultraviolet absorption arising from the tetramer, indicating participation of the tetramer in the condensation reaction.

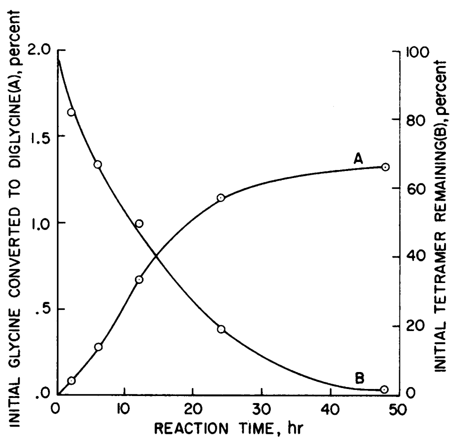
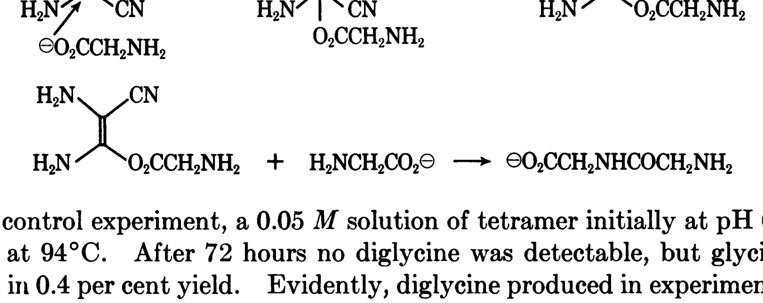


FIG. 1.—Rates of diglycine formation and tetramer destruction in solutions 0.025 *M* in glycine and tetramer and initially at pH 6.1.

Diglycine yields obtained with different concentrations of starting materials are shown in Table 1. Dipeptide yields responded positively to increases in initial glycine concentrations. However, decreases in tetramer concentration below 0.025 *M* produced little diminution in yields. Interestingly, at 0.001 *M* tetramer, diglycine formation competed significantly with tetramer hydrolysis, suggesting that, at higher tetramer concentrations where yields were comparable, polymerization diverted tetramer that might otherwise have served to condense amino acids. The effect of varying the initial pH of solutions is also shown. At low pH no diglycine is detectable. However, in slightly basic solutions (conditions compatible with current conceptions of primordial waters[13]) diglycine yields increased markedly. The influence of pH on the extent of condensation suggested the importance of carboxylate and uncharged amino groups on glycine in the reaction. A possible mechanism under investigation involves formation of an intermediate enol ester by addition of the carboxylate end of glycine to the tetramer followed by elimination of cyanide. Reaction at the carbonyl carbon of the intermediate with the amino group of a second molecule of glycine would yield diglycine. Analogous addition-elimination reactions of cyanoethylene derivatives have been reported,[26, 27] and enol esters have been involved as intermediates in peptide synthesis.[28]



In a control experiment, a 0.05 *M* solution of tetramer initially at pH 6.2 was heated at 94°C. After 72 hours no diglycine was detectable, but glycine was formed in 0.4 per cent yield. Evidently, diglycine produced in experiments with

TABLE 1. *Yields of diglycine obtained at different reactant concentrations and initial pH after heating 24 hr at 94°C.*

pH	Initial Concentration (*M*) Tetramer	Glycine	Per cent yield
6.1	0.025	0.500	3.1
6.1	0.025	0.100	1.49
6.1	0.025	0.025	0.91
6.1	0.010	0.025	0.86
6.1	0.005	0.025	1.09
6.1	0.001	0.010	1.20
2.0	0.025	0.025	<0.02
9.0	0.025	0.025	1.41
10.5	0.025	0.025	3.8

added glycine could not have arisen through any combination of hydrolysis or polymerization of the tetramer. Furthermore, the minute amount of glycine resulting from tetramer hydrolysis could not have contributed significantly to diglycine formation.

Irradiation of tetramer solutions with 254- or 350-nm light was reported to cause photorearrangement to an imidazole derivative, the reaction proceeding fastest at the latter wavelength.[19] To investigate the possible competition between condensation and photorearrangement, solutions of tetramer and glycine were simultaneously irradiated with 350 nm light and heated at 85° to 90°C for 24 hours. The results presented in Table 2 also include experiments where only heating or irradiation were involved.

After irradiation alone, no diglycine could be detected and more than 40 per cent of the tetramer remained. Yields resulting from heating alone were comparable to those obtained at 94°C in Table 1. Simultaneous irradiation and heating, however, more than tripled the yield at pH 8.0 and 9.0 and nearly doubled it at pH 10.5. In another experiment the effect of stepwise irradiation and heating on a solution of 0.025 *M* in tetramer and glycine initially at pH 8.0 was explored. After 24 hours of irradiation at 25°C the pH dropped to 6.8, the tetramer was reduced to 0.012 *M*, and no diglycine was detectable. Subsequent heating at 94°C for 24 hours without irradiation afforded diglycine in 1.20 per cent yield, more than doubling the amount obtained at pH 8.0 by heating alone. When heated at 75°C for 72 hours, the irradiated solution yielded 0.92 per cent diglycine. These findings indicated that irradiation actually enhanced dipeptide formation, possibly by promoting formation of an intermediate that subsequently underwent thermal reaction with glycine to afford dipeptide. In all

TABLE 2. *Yields of diglycine obtained from solutions 0.025 M in tetramer and glycine by irradiation with 350 nm light, heating, and simultaneous irradiation and heating.*

	T(°C)	Initial pH	Per cent yield
Irradiation only	25	8.0	<0.02
	25	10.0	<0.02
Heating only	85–90	8.0	0.45
	85–90	9.0	1.50
	85–90	10.5	3.3
Simultaneous irradiation and heating	85–90	8.0	2.0
	85–90	9.0	4.8
	85–90	10.5	6.4

irradiation experiments, soluble colored material appeared in the solutions after an hour or two and probably served to absorb some of the light, thereby diminishing photorearrangement of tetramer.

We have shown that the tetramer is an effective condensing agent for amino acids under plausible primitive Earth conditions. At high concentrations where polymerization was possible,[24] at low concentrations where hydrolysis was facile,[19] and even after ultraviolet irradiation, significant amounts of dipeptide formation occurred in the temperature range 75°–94°C.

The formation of tetramer from dilute aqueous solutions of cyanide under various possible primordial conditions has been amply demonstrated[19, 29] so that its presence on the primitive Earth was highly probable. A continual source of tetramer would have been available if, as has been suggested,[19] nitrogen could have been recycled through primitive bodies of water as cyanide. In light of our findings, tetramer-mediated stepwise condensation of amino acids appears to be a plausible pathway for peptide formation under dilute aqueous conditions on the primitive Earth. Furthermore, it provides a possible explanation for the polypeptide formation observed in some prebiotic synthesis experiments.[15, 16] Our results add a new dimension to the importance of hydrogen cyanide and its polymers as primordial chemical reagents. Possibly they served as mediating agents in other condensation reactions relevant to chemical evolution.

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