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<https://doi.org/10.15760/etd.2372>

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AN ABSTRACT OF THE THESIS OF Z. Badri Adnani-Gleason for the Master of Science in Chemistry presented February 20, 1976.

Title: Polymerization of Amino Acids on Kaolinite

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Dennis Barnum, Chairman

Gary Gard /

David Roe

Joann Loehr

John Myers

In the origin of life on the primitive earth a major step must have involved the condensation of amino acids to form the first polypeptides. Several suggestions as to how this might have occurred have been made by other workers. One of the more appealing proposals is that the polymerization was catalyzed by clay minerals. It has been reported, for example, that L-aspartic acid polymerizes significantly faster than D-aspartic acid in the presence of kaolinite in aqueous suspension at

90°. In this work an attempt was made to repeat this report and extend the pH range to include values presumably present on the prebiotic earth. No evidence for polymerization of L-aspartic acid was found.

Polymerization of glycine under dry conditions in the presence of kaolinite and sodium borate was also investigated. Although small amounts of glycylglycine and glycylglycylglycine were detected there was no evidence that the reaction is enhanced by the presence of kaolinite.

POLYMERIZATION OF AMINO ACIDS ON KAOLINITE

by

Z. BADRI ADNANI GLEASON

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
CHEMISTRY

Portland State University
1976

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of
Z. Badri Adnani-Gleason presented February 20, 1976.

Dennis Barnum, Chairman

Gary Gard

David Roe

Joann Loehr

John Myers

APPROVED:

Gary Gard, Head, Department of Chemistry

Richard Halley, Dean of Graduate Studies and Research

February 20, 1976

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INTRODUCTION

In 1952 Urey and Miller (22) reported the synthesis of several amino acids under conditions that may have existed on the prebiotic earth. It suddenly became apparent that questions regarding the source of organic molecules necessary for the origin of life could be attacked by laboratory experiments that simulate primordial conditions; especially, that positive results could be obtained in the laboratory without the passage of eons of time as was previously assumed to be necessary.

The Urey - Miller experiment assumed that the primitive atmosphere consisted of methane, ammonia, hydrogen and water. It was also assumed that frequent electrical storms occurred. In a laboratory simulation they passed an electrical discharge through such a mixture for several days and found that 6 different amino acids and 14 other organic compounds accumulated in the condensed aqueous phase. Since that time this basic experiment has been repeated by many other workers using different atmospheres and different sources of energy. For example, amino acids were synthesized from HCN using shock waves (4), from CH_4 , NH_3 , H_2 , and H_2O using electrical discharge (3), UV light (19), or heat (2), and a variety of other conditions (5, pp. 128-9) including β , γ , and x-rays (23). It is now clear that amino acids form very easily under a variety of conditions. In fact, it would seem very difficult to imagine primordial conditions under which they would not form.

Assuming that the amino acids would have spontaneously formed on the prebiotic earth, the next question is how they might have been concen-

trated and polymerized, and this is the problem investigated in this thesis.

If the amino acids accumulated in primitive oceans about the volume of the current oceans of the earth, experiments suggest that the concentration may have reached about 10^{-5} M. (49). This is probably much too low for polymerization to occur. A number of mechanisms for the accumulation of higher concentrations might be visualized. For example:

- 1) By evaporation of ocean water in lakes, lagoons or tidal pools.
- 2) By adsorption on mineral surfaces.
- 3) Organic matter tends to accumulate in sea foam which may have been blown onto beaches where it would accumulate in the sand.
- 4) Concentration by freezing.

A spontaneous polymerization is thermodynamically impossible, unless coupled with some other reaction that will provide the necessary energy or unless one of the products of polymerization, e.g. water, is removed as soon as the peptide bond is formed. Several mechanisms have been suggested. One of the more appealing is dry thermal polymerization in which a mixture of dry amino acids containing some acidic or basic amino acid are heated to 120 - 200°C. Reaction is driven to completion because the water that is formed escapes (5). Some other methods are described below in Review of the Literature.

Degens, Matheja and Jackson (6,21) have reported that an aqueous suspension of kaolinite (a clay mineral that might reasonably be expected to have existed on the primitive earth) catalyzes the polymerization of aspartic acid at 80-95°C and furthermore that L-aspartic acid is polymerized more rapidly than the D- isomer. This is an exceptionally attractive proposal because it solves three problems at once; the problem

of concentration of amino acids from dilute aqueous solution, the formation of peptide bonds, and the origin of the overwhelming preference of living organisms for L-amino acids over D-amino acids.

In the present work an attempt was made to duplicate these experiments because the results are, for the most part, erratic and the statistical treatment of the data is not convincing. Furthermore, poly-aspartic acid was never actually isolated and identified, and the work was done only at pH 3.2 to 3.8, whereas the pH of the primordial ocean was probably close to the present value of 8.1 (1). In the course of the present work two papers were published by other laboratories, reporting that they could not reproduce the work of Degens et al. (21) and Jackson (6). However, they did not take pH into account and the experiments were not designed to isolate small amounts of polymer that might be present. In this work experiments were performed with the pH of the aqueous phase adjusted to 6, 7, 8, 9, and 10 and an ion exchange method was devised to isolate small amounts of poly-aspartic acid.

Thermal polymerization of amino acids, under dry conditions was also investigated in this work. Here again, the object is to determine if kaolinite is capable of catalyzing the polymerization. Dr. Jose Flores (20) suggested that sodium borate might act as a condensing agent, in the same way as poly-phosphates, so additional experiments were done in the presence of kaolinite and sodium borate. Kaolinite, glycine and sodium borate were heated together at 105°C and 140°C for 15, 30 and 45 days.

REVIEW OF THE LITERATURE

The formation of amino acids under simulated primitive conditions is reviewed by Miller and Urey (12), Lemmon (13) and several books on the origin of life (14,15,16,17,18). This aspect of the problem will not be reviewed here as it has already been amply demonstrated that amino acids form easily under prebiotic conditions.

One of the steps in molecular evolution must have been the formation of polypeptides from amino acids and this review is concerned with possible polymerization reactions in a primitive earth environment.

Very limited progress has been made in the study of prebiotic polymerization reactions. Many interesting scraps of information are available, but there is no indication that the correct pathway has yet been discovered. This is one of the most important problems in prebiotic chemistry and one in which considerable progress can be anticipated in the near future.

Thermodynamic Considerations

A basic problem for the spontaneous generation of life is that the polymerization of amino acids requires an increase in free energy. For the formation of a single peptide bond we have

If polymerization is to be made to occur, some method must be provided to remove the thermodynamic barrier. Examples of possible methods are outlined below.

1) Removal of water as peptide bonds are formed (5). This can be accomplished simply by heating the dry amino acids above 100°C. Another method might be the strong binding of water by the poly-amino acid molecules themselves (38).

2) Removal of water by condensing agents such as polyphosphates, diaminomaleonitrile, cyanamide, dicyandiamide, etc. (5). Polyphosphate for example, helps to remove water by reacting with it as follows:

3) Reaction is coupled with another energy-yielding reaction such that the coupled reactions are thermodynamically favored (5).

4) Removal of polymer by adsorption on mineral surfaces (25). If polymer is strongly adsorbed and stabilized by the mineral surface this may be sufficient to eliminate the thermodynamic barrier. Bernal (25) was the first to suggest that prebiotic synthesis may have taken place on clay particles in aqueous media, where they simultaneously protected the prebiotic monomers from photolytic degradation and provided a catalytic region for the promotion of subsequent polymerization and chemical development. Polymerization is essentially occurring outside the aqueous phase, i.e., on the surface.

Formation of a "Fore-Protein".

Akabori (39) has suggested that protein could have originated from the polymerization of aminoacetonitrile according to the following

sequence of reactions:

It was suggested that once the polyglycine was formed subsequent reactions altered the side groups and some possible mechanisms for this were investigated. For example, when an aqueous suspension of polyglycine adsorbed on kaolinite was reacted with formaldehyde for 7 days at 80°C, 3% of the glycine groups were converted to serine groups. The main difficulty with this hypothesis is that the conditions required for polymerization of the aminoacetonitrile are not very likely to have occurred on the primitive earth. Thus, Hanafusa and Akabori (39) obtained only digly and trigly by heating aminoacetonitrile neat with kaolinite at 120° to 140°C for 5 hours.

Polymerization in Dilute Aqueous Solution.

Calvin (48) has discussed the possible role of the carbodiimide structure ($-\text{N}=\text{C}=\text{N}-$) as a condensing agent. This structure is a tautomeric form of various nitriles that are likely to have been present on the prebiotic earth. Thus, reaction of HCN with ammonia could produce cyanamide, dicyanamide, and dicyandiamide:

A peptide bond is formed by the reaction:

For example, reaction of 0.005 F glycine in 0.1 M HCl while slowly adding 0.1 M sodium dicyanamide over a period of 60 minutes produced digly, trigly, and tetragly (40). The yield of tetraglycine was about 8% of the original monomer. The reaction is accelerated by montmorillonite (41).

Chang et al. (42) heated a solution that was 0.025 F in both glycine and diaminomaleonitrile at pH 6.1 for 24 hours at 94°C and obtained about a 1% yield of diglycine. Irradiation of the reacting solution with UV light enhanced the yield of diglycine to 2.0% at pH 8.0 and 6.4% at pH 10.5. Sanchez et al. (43) and Miller (44) have shown that diaminomaleonitrile is indeed formed under various possible primordial conditions.

Flores and Ponnamperna (45) found that diglycine and triglycine are formed if a solution of glycine and ammonium cyanide is allowed to stand at room temperature for three months.

The significance of these results for the prebiotic formation of polypeptides is questionable. Generally, only dimers and trimers have been detected and the concentrations of reactants are much larger than one would expect to have been present in a primordial ocean. Of course,

one can always argue that concentration occurred, or that millions of years were required to form sufficiently long polymers.

A greater degree of polymerization was obtained by Horowitz et al. (46). They reacted 0.001 F alanyl adenylate in an aqueous suspension of montmorillonite or illite at pH 7.8 to 8.5. Reaction required in a few hours at room temperature and the polymer contained 9 to 56 monomer units. This reaction has also been investigated with several pairs of amino acid adenylates (47). Certain bonds are more likely to be formed than others. For example, ala-ala bonds are more likely than gly-gly which in turn are more likely than ala-gly or gly-ala. No catalytic activity was observed with kaolinite, apatites, or permutites.

Also, of particular interest is the reported polymerization of aspartic acid on the surface of kaolinite. This reaction has already been described in the introduction and is the topic of this thesis.

Dry Thermal Polymerization.

Dry thermal polymerization has been extensively studied by Fox and others (5,26,27,28,31,32,33,34).

Thermodynamic theory indicates that amino acids would be condensed by heating to a temperature above the boiling point of water (5). Fox obtained 10 - 40% conversion to polymer by heating a mixture of 20 amino acids containing at least a small proportion of non-neutral amino acids at 170°C for 6 hours (5). The molecular weight of the product can be as high as 20,000 (35). These organic macromolecules have been called "proteinoids" or "thermal polypeptides".

Burton and Neuman heated glycine with hydroxyapatite and pyrophosphate for 4 days at 120° and 140°C. The presence of the hydroxyapatite

retards the thermal decomposition of glycine. Up to 46% of the glycine was converted to peptide bonds but significant amounts of diketopiperazine were formed (36).

Flores and Leckie prepared a mixture of glycine, potassium cyanate and hydroxyapatite and heated it in a sealed tube at 95°C for 2 to 20 days. They obtained up to 1.2% yield of diglycine in the presence of potassium cyanate and smaller yields in its absence (37). Solid sodium dihydrogen phosphate plus potassium cyanate is an even better catalyst giving up to 12% yield of diglycine. However, with other solid orthophosphates the presence of cyanate decreased the yield of peptide.

Only the dry thermal method under simulated geological conditions has given polymers of amino acids that 1) contain all the amino acids common to contemporary protein, 2) have molecular weights of many thousands, 3) possess an array of catalytic or rate enhancing activities of the kinds from which enzymes and metabolism could evolve, 4) exhibit limited heterogeneity comparable to that found in contemporary populations of organismic protein, and 5) give, on contact with water, organized units with many of the properties of contemporary cells.

While this list of properties is impressive it is not evidence that the polypeptides were actually formed under dry thermal conditions. That is, these are properties inherent in the polymer and should be observed regardless of how the polymer is synthesized.

The importance of these thermal syntheses in prebiotic chemistry is controversial. Some workers do not believe that suitable combinations of heat source and accumulations of amino acids could have existed in prebiotic time. Another way of examining this problem is by asking whether there are places on the earth today with appropriate temperatures where

one could drop, say, 10 grams of a mixture of amino acids and obtain a significant yield of polypeptides.

EXPERIMENTAL

Materials and Apparatus

Ninhydrin. In order to obtain a low blank in the photometric procedure it is necessary, in some cases, to recrystallize the ninhydrin from 2 N hydrochloric acid according to the method of Hamilton and Ortiz (7). Ninhydrin obtained from Matheson, Coleman & Bell must be recrystallized but the product from Sigma Chemical Company (St. Louis, Mo. 63178) could be used as received.

Poly-aspartic Acid: Poly-aspartic acid was obtained from Miles Laboratories, Inc. (Kankakee, Ill. 60901). The number average molecular weight is 8000.

Kaolinite: Kaolinite was obtained from Fisher Scientific Company (3382 Edward Ave., Santa Clara, Calif. 95050). Particle sizes of less than 2 microns effective spherical diameter (esd) were obtained by sedimentation (8). The time required for particles 2 microns (esd) to settle from a suspension of kaolinite in water was calculated from Stokes Law:

$$v = \frac{2(D-D')g r^2}{9\eta}$$

where v is the rate of settling, D is the density of water, D' is the density of kaolinite (2.65 g/ml), η is the viscosity of water at room temperature (0.9548×10^{-2} poise) $g=981 \text{ cm sec}^{-2}$ and r is the radius of a spherical particle. Kaolinite (100 grams) was suspended in 1500 ml of water, 30 drops of 2 N NaOH was added to the suspension and the mixture thoroughly stirred. After the calculated period of time the supernatant

liquid was poured off and the suspended particles less than 2 microns (esd) separated by centrifugation. The product was washed four times with distilled water and dried 3 hours at 60°C.

3 N HCl in Butanol. To 150 ml of n-butanol was added 2-3 g of CaH_2 to remove water. This was allowed to stand overnight and then distilled. The first 50 ml of distillate was discarded and about 90 ml dry butanol was collected. Gaseous hydrogen chloride was generated in an all-glass apparatus by adding 98% H_2SO_4 dropwise to 12 N HCl. The liberated HCl gas was passed into dry butanol. At intervals, 1.00 ml aliquots of butanol were withdrawn and titrated with 0.100 N NaOH. When the concentration of HCl reached 3 N the generation of HCl was stopped and the 3 N solution stored in a glass-stoppered flask.

All other chemicals were reagent grade and were used without further purification.

Absorbance Measurements: Absorbance measurements were made with a Bausch and Lomb SPECTRONIC 20 spectrometer using "test tube" absorption cells.

Vapor Phase Chromatography: The analysis for amino acids by vapor phase chromatography was done with a Hewlett-Packard model 5750 Chromatograph, using a flame ionization detector. An all-glass column was used, 1 meter x 4mm ID, packed with 3% SE-30 on 80-100 mesh Chromosorb W AB. The column was conditioned before use with dimethyldichlorosilane. Samples were injected directly onto the column.

Analysis for Amino Acids

Amino acids were analyzed colorimetrically by the ninhydrin method and later in this work by vapor phase chromatography.

Colorimetric Method for Amino Acids. The following method is essen-

tially that of Yemm and Cocking (9). In each experiment 1.00 ml of amino acid solution containing 3.7×10^{-7} to 2.1×10^{-5} mole amino nitrogen was mixed with 0.5 ml pH = 5.0 citrate buffer (0.20 M citric acid). Ninhydrin in methyl cellosolve (0.2 ml 5% w/v) and 1.0 ml of KCN (0.01 M) were added to this solution. The homogeneous solution was heated for 15 minutes at 100°C and then cooled for 5 minutes in running tap water. The solution was made to a convenient volume (5.7 ml) with 60% v/v ethanol/water and the absorbance determined with a SPECTRONIC 20 at 570 nm. Ammonia reacts with ninhydrin, so it is necessary to guard against accidental contamination by even traces of ammonia.

Analysis for Amino Acids by Vapor Phase Chromatography. Amino acids were also analyzed quantitatively by vapor phase chromatography. In this technique amino acids were converted to volatile derivatives (10). The derivatization consists of conversion of the carboxyl group to a n-butyl ester and the amino group to an N-trifluoroacetate group according to the following reactions:

n-Butyl Esterification:

Acylation:

For samples containing 10 mg or less of amino acid, $50 \mu\text{l}$ of standard leucine solution containing $1 \mu\text{g}/\mu\text{l}$ was added as internal standard. The esterification was done by adding $150 \mu\text{l}$ of 3 N HCl in n-butyl alcohol

per 100 μ g of amino acid and heating at 100°C for 15 minutes in a culture tube sealed with a Teflon-lined screw cap. The excess n-butanol was removed with a rotary evaporator and then 60 μ l of CH_2Cl_2 and 20 μ l of trifluoroacetic anhydride (TFAA) for each 100 μ g of amino acid was added. Acetylation was carried out at 150°C for 5 min., as before, in a sealed culture tube. Generally a 1.0 μ l sample was injected into the chromatograph. Chromatography was done in an all glass column of 3% SE-30 at 150°C.

Separation of Poly-Aspartic Acid from Monomer by Ion Exchange

Initially an anion exchange resin (Amberlite CG-400, $\text{R}(\text{CH}_3)_3\text{N}^+\text{Cl}^-$, 100-200 mesh) was used but the eluate gave high non-reproducible blanks by the ninhydrin method. Low blanks were obtained using a cation exchange resin (Amberlite CG-120, $\text{RSO}_3^-\text{Na}^+$, 100-200 mesh). The size of the resin bed was 1.07 cm in diameter and 8.5 cm in length. After conditioning the column with 50 ml of 2 N sodium acetate, 50 ml of 2 N HCl and 25 ml of 0.10 N HCl, 10 ml of 0.10 N HCl effluent was collected and reacted with ninhydrin so that a base line could be determined. Next 1.0 to 2.0 ml of sample was introduced and eluted with 0.10 N HCl. For analysis for polyaspartic acid in fractions 1 to 12, 2.00 ml of sample was added to 2.00 ml of 12 N HCl and hydrolyzed in a sealed tube for 20 hours at 110 ± 1 °C. A blank, consisting of 2.00 ml of eluent (0.10 N HCl) and 2.00 ml 12 N HCl was also run. After hydrolysis the samples were analyzed by the ninhydrin method. For fractions 13 to 24, 1.00 ml samples were analyzed by the ninhydrin reaction directly. A chromatogram showing the separation of polyaspartic acid from its monomer is shown in Figure 1. It will be seen that complete separation is achieved.

To test the separation and recovery of monomer and polymer by this

Figure 1. Separation of aspartic acid from poly-aspartic acid by cation exchange chromatography.

method a series of known mixtures was prepared and analyzed. The results are tabulated in Table I. It will be seen that very good recoveries were obtained.

Hydrolysis of Poly-Aspartic Acid. Equal volumes (2.0 ml) of those fractions containing poly-aspartic acid and 12 N HCl were mixed and sealed under vacuum in pyrex tubes. Each tube was heated for 20 hours at $110 \pm 1^\circ\text{C}$ (11). At the completion of the heating time the hydrolyzate was transferred to a 10-ml round bottom flask and evaporated to dryness using a rotary evaporator. Amino acid analysis using the ninhydrin method was then performed.

Attempted Polymerization of Aspartic Acid on Kaolinite in Aqueous Suspension

Aliquots (5.00 ml) of aqueous (0.001000 M or 0.01000 M) solutions of L-aspartic acid, D-aspartic acid, or DL-aspartic acid were added to 500 mg of kaolinite. The solutions were adjusted to the desired pH using 12 N ammonia solution. The volume of 12 N ammonia required is so small that no significant increase in total volume occurs. The tubes were then sealed under nitrogen and heated at 90°C for several days in a continuously shaking bath. Aspartic acid solutions without clay were adjusted to the same pH values to serve as blanks. After heating for the desired time the tubes were opened and treated according to the flow diagram in Figure 2. The kaolinite was separated by centrifugation. A portion of supernatant liquid (A) was passed through a cation exchange column. Fractions 13 to 24 were analyzed for free aspartic acid and fractions 1 to 12 were hydrolyzed and then analyzed for free aspartic acid. A second portion of supernatant liquid (A) was hydrolyzed in 6 N HCl before analysis for amino acid.

TABLE I

RECOVERY OF ASPARTIC ACID AND POLY-ASPARTIC ACID
FROM KNOWN SAMPLES USING CATION
EXCHANGE CHROMATOGRAPHY

μg Aspartic Acid added	μg Asp recovered	% Asp recovered	μg Poly-Asp added	μg Poly-Asp recovered	% Poly-Asp recovered
1000	995.2	99.5	1000	956.3	95.6
500	492.8	98.6	500	477.3	95.5
250	246.8	98.7	250	240.7	96.3
100	98.3	98.3	100	97.5	97.5

Figure 2. Flow diagram showing work up of the reaction products.

The kaolinite remaining after reaction was washed three times with 4.0 ml portions of 2 N ammonia, separating the kaolinite between each washing by centrifugation. The washings were combined. Since ammonia interferes with the ninhydrin analysis it was removed by allowing the wash solution to stand overnight in a desiccator over 98% sulfuric acid. The resulting ammonia free solution was analyzed in the same manner as supernatant liquid (A) above. The data are presented in Table II.

Thermal Polymerization under Dry Conditions

Each sample was composed of 75 mg DL-glycine, 100 mg $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$, 1.00 g kaolinite and was mixed by grinding in an agate mortar. The mixtures were heated for 15, 30 and 45 days at 105° and 140°C. They were then extracted with 3.0 ml of H_2O , centrifuged, and reextracted with 2.0 ml of 2 M ammonia. The samples were then centrifuged again. Both the water and ammonia washings were combined and analyzed for oligomers by Dr. Jose Flores using an amino acid analyzer (20). Blanks were also prepared containing glycine plus kaolinite, glycine plus sodium borate, kaolinite plus sodium borate, or glycine only. A flow diagram showing work-up after reaction is shown in Figure 3.

RESULTS AND DISCUSSION

Attempted Polymerization of Aspartic Acid in Aqueous Suspensions of Kaolinite

In the polymerization experiments described by Degens et al. (21) and Jackson (6) two methods were used for the detection of polymerization. In the first method the kaolinite was separated by centrifugation and the supernatant liquid analyzed first for aspartic acid, then hydrolyzed and analyzed again. Any increase in aspartic acid concentration was assumed to be due to polymer. The kaolinite was washed with 2 N ammonia and the washings also analyzed before and after hydrolysis. The second method used was analysis for polymer by the biuret method.

As a criterion for polymerization the analysis for aspartic acid before and after hydrolysis can be criticised on several accounts. First, small amounts of polymerization cannot be detected because it depends on taking the difference between two numbers that are nearly equal. Second, any diketopiperazide (a cyclic dimer) present would be mistaken for linear poly-peptide because it does not react with ninhydrin but is converted back to aspartic acid on hydrolysis. Third, the method does not actually isolate and identify the product, and fourth, it has not been established that poly-aspartic acid is desorbed from the kaolinite surface by washing with 2 N ammonia.

While the present work was in progress two papers appeared in the literature in which it was reported that Jackson's claim that L-aspartic acid polymerizes more rapidly than D-aspartic acid could not be repeated. In fact, no polymerization at all was observed (29,30). McCullough and

TABLE II

ATTEMPTED POLYMERIZATION OF ASPARTIC ACID ON KAOLINITE

Total Aspartic acid at start μg	pH	Reaction time, days	μg Asp recovered from supernate (A)		μg Asp recovered from Ammonia washed supernate (B)		% Recovery of Asp before hydrolysis	% Recovery of Asp after hydrolysis
			not hydrolyzed	hydrolyzed	not hydrolyzed	hydrolyzed		
665	6.0	12.5	—	—	—	—	97	—
665	7.0	12.5	4.4×10^2		2.4×10^2	2.2×10^2	102	99
665	8.0	12.5	4.5×10^2	4.5×10^2	2.15×10^2	2.1×10^2	100	99
665	10.1	12.5	3.8×10^2		3.1×10^2		104	—
6650	9.0	18	2.1×10^3	2.1×10^3	3.9×10^3	4.0×10^3	90	92

Figure 3. Work-up of glycine - kaolinite - sodium borate samples after dry thermal polymerization.

Lemmon (30) used radiotracer C^{14} to look for products of the reaction but only starting material was found; no polymer, no oligomers, and no thermal decomposition products. In both reports, however, the experiments were carried out only at pH 3.2 to 3.7 and in such a way that small amounts (say, 2 to 5%) of polymer adsorbed on the surface of the kaolinite would not have been detected.

In the present work the pH range was extended to include neutral and slightly basic solutions which were more likely the situation on the prebiotic earth. Also, a cation exchange technique was used to concentrate small amounts of poly-aspartic acid that would escape detection by looking only at the difference between hydrolyzed and unhydrolyzed analyses for aspartic acid.

Table II shows the results obtained in this work by heating solutions of aspartic acid with suspended kaolinite under different conditions. After reaction, samples were treated according to the flow diagram in Figure 2. Note that at pH 7, 8, and 10 essentially 100% of the aspartic acid was recovered unreacted. At pH 6 the recovery was 97%, which is probably within experimental error of 100%. It is clear that in those solutions that were initially 0.00100 F in aspartic acid the amount of polymerization is either very small or does not occur at all.

According to Jackson (6) the poly-aspartic acid is desorbed from the kaolinite surface by washing with 2 F ammonia and he observed the greatest amount of polymer in these solutions. In Table II, however, no increase in aspartic acid concentration occurred when the ammonia wash solutions were hydrolyzed, and there is therefore no evidence at all for polymerization.

Table III shows data for the polymer fraction from the cation ex-

TABLE III

ABSORBANCE OF POLY-ASPARTIC ACID FRACTION
FROM SUPERNATANT LIQUID (A) AND
AMMONIA WASH LIQUID (B)

pH	Supernatant Liquid (A)		Ammonia Wash Supernatant (B)	
	Blank	Polymer Fraction Absorbance After Hydrolysis	Blank	Polymer Fraction Absorbance After Hydrolysis
6.0	.062	.060	--	---
7.0	.057	.060	.057	.061
10.1	.061	.060	.061	.063

change column. In no case, after hydrolysis, was there an increase in aspartic acid concentration over the blank. Assuming that an increase in absorbance of 0.030 can be detected over the normal fluctuation in the blanks, the minimum detectable concentration of poly-aspartic acid in the supernatant liquids is $3\text{ }\mu\text{g/ml}$. This corresponds to 0.4% polymerization, based on 5 ml of 0.001 M aspartic acid at the start of the experiment or 0.04% based on 0.010 M aspartic acid at the start. Again, no evidence for polymer was found.

The only suggestion of polymer formation in Table II is the data obtained at pH 9.0. In this case, only 90 to 92% of the aspartic acid was recovered, so 8 to 10% of it might be present as polymer. If polymer were present it would have to be adsorbed on the kaolinite. According to Jackson (6) polymer is desorbed by 2 F ammonia, but since it is very doubtful that he ever produced any poly-peptide it is not really known whether the polymer is desorbed by this treatment or not.

To detect adsorbed polymer, the infrared absorption spectrum of the recovered kaolinite (Figure 5, reaction at pH 9.0) was compared with that of poly-aspartic acid (Figure 4). The kaolinite spectrum is identical with that of the fresh kaolinite and there is no trace of adsorbed polymer, although additional aspartic acid monomer was extracted by washing a forth and fifth time with 2 N ammonia. The amount of aspartic acid was not measured quantitatively but it probably accounts for the missing 8 to 10%. It is not surprising that this aspartic acid was not detected by the infrared spectrum. This method is known to be very insensitive to small amounts of material in a major component.

It should be pointed out that Jackson used 0.0100 M aspartic acid whereas, in the results reported here, only the data at pH 9.0 are at

Figure 4. Infrared spectrum of poly-aspartic acid, M.W. 8000. KBr pellet, Miles Laboratories.

Figure 5. Infrared spectrum of kaolinite recovered after reaction with 0.0100 F aspartic acid at pH 9.0 for 18 days. KBr pellet. This spectrum is identical to fresh kaolinite. Poly-aspartic acid, if present, would show bands at about 5.88 and 6.60 μ .

this same concentration. The results at pH 6, 7, 8, and 10 used 0.00100 M aspartic acid.

As mentioned at the start, Degens et al. (21) and Jackson (6) also claimed to have detected polymerization by the biuret reaction. Dr. Barnum, in this laboratory, attempted to repeat these analyses. While Jackson calibrated the method against glycylglycylglycine, Barnum calibrated against poly-aspartic acid and found the gram absorptivity to be 0.11 liter gram⁻¹cm⁻¹. Assuming the smallest absorbance that can be measured with certainty is 0.005 leads to a minimum detectable concentration of polymer of 40 µg/ml. Since 2.00 ml samples are mixed with 8.00 ml of biuret reagent, the minimum detectable concentration in the supernatant liquid would be 200 µg/ml. The initial concentration of aspartic acid in Jackson's work was 1331 µg/ml so about 15% of the aspartic acid would have to polymerize before it could be detected by the biuret reaction.

Thermal Polymerization

Mixtures of glycine, kaolinite, and sodium borate were heated together, dry, for 15, 30, and 45 days at 105° and 140°C. Also, blanks were run in which glycine and sodium borate, glycine and kaolinite, or glycine alone were treated in the same manner. After heating, the mixtures were washed with water and 2 N ammonia and the combined washings analyzed for glycine oligomers by Dr. J.J. Flores with an amino acid analyzer. The following results were obtained:

1. No polymerization of glycine to diglycine or higher oligomers occurred at 105°C after 15 or 45 days.
2. After 15 days at 140°C only a small amount of diglycine was

found from glycine and sodium borate. The yield is about 0.25%; too small to be significant.

3. Heated at 140°C for 30 days, a good yield of diglycine from sodium borate and glycine was obtained. However, this combination also destroys most all of the glycine; a very serious disadvantage.

Therefore, there is no indication that kaolinite or sodium borate might have played a role in the prebiotic synthesis of polypeptides.

SUGGESTIONS FOR FURTHER WORK

There is a possibility that a small amount of polymer is so strongly adsorbed on the kaolinite surface that it is not removed by the usual washing operations. If this is the case it would not be detected either by the methods used in this work or by others. Jackson (6) and Degens et al. (21) claimed that polymer is removed by washing with 2 N NH_3 but there is no evidence to support this contention. Therefore, the next step in this research should be to investigate the adsorption isotherms of poly-aspartic acid on kaolinite at different pH values. It would then be possible to say for certain whether polymer is adsorbed on the surface or not.

Reactions should be carried out in the presence of ammonium cyanide, pyrophosphate, dicyandiamide, dicyanamide, and cyanamide.

Another possibility would be to try to activate the kaolinite surface by treating it with acid, base, transition metal salts, etc. That is, it is well known that kaolinite has a small cation exchange capacity and the catalytic activity may depend on the adsorbed ions as well as whether aluminum or silicon atoms are exposed at the active sites.

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