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Synthesis of Polypeptides by Microwave Heating I. Formation of Polypeptides during Repeated Hydration–Dehydration Cycles and Their Characterization

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Summary. Amino acid amides effectively reacted to produce polypeptides in response to microwave heating during repeated hydration–dehydration cycles. The polypeptides, formed from a mixture of glycinamide, alaninamide, valinamide, and aspartic acid α-amide, had molecular weights ranging from 1000 to 4000 daltons. Amino acids were incorporated into the polypeptides in proportion to the starting concentrations, with the exception of glycine whose incorporation was 1.5 times higher than that of the other amino acids. The polypeptides had some definite secondary structure, such as α-helix and β-sheet, in aqueous solution. This reaction provides not only a convenient method for abiotic peptide formation but also a convenient method for the chemical synthesis of peptides.

Key Words: Origin of life — Chemical evolution — Prebiotic — Polypeptide — Amino acid amide — Secondary structure

Introduction

One of the major objectives in the study of life's origin is to understand significant events in the evolution of polypeptide synthesis from the assembly of nondirected amino acid sequences to that of extrinsically directed ones. The evolution of polypeptide synthesis is dependent on many factors: kinds of amino acids, optical form, reactivity and concentration of amino acids, energy availability, and intrinsic stereochemistry of resulting polypeptides. We have been trying experimental approaches to study the evolution of polypeptide synthesis under conditions that simulate possible primitive sea environments (Egami 1974) and have found a variety of systems favorable for the formation of polypeptides and protocellular structures (Yanagawa et al. 1988): The first system involves heating at 50–105°C in a modified sea medium enriched with transition metal ions (Yanagawa 1980; Yanagawa and Egami 1980); this system simulates a warm and hot sea. The second system uses high temperatures (200–350°C) and high pressures (120–160 atm) in aqueous solution (Yanagawa and Kojima 1985; Yanagawa et al. 1988); this system simulates a submarine hydrothermal vent in the deep sea. The third system consists of heating of 80°C in alternating aqueous solution and solid phase conditions (Nishizawa et al. 1983; Yanagawa et al. 1984, 1988). This system simulates a hydration–dehydration cycle in a freshwater tide pool.

We synthesized polypeptides from amino acid amides (Oró and Guidry 1960) in a system involving repeated hydration and dehydration at 80°C (Yanagawa et al. 1984, 1988). Release of ammonia from the amide group is a rate-determining step in this peptide formation system.

$$NH_2CH(R_1)CONH_2 + NH_2CH(R_2)CONH_2 \rightarrow NH_2CH(R_1)CONHCH(R_2)CONH_2 + NH_3$$

Recently, we have found a novel procedure that can synthesize polypeptides more efficiently than heating at 80°C. In this paper we report the remarkably

Abbreviations. CD, circular dichroism; IR, infrared spectroscopy; UV, ultraviolet spectroscopy; MW, molecular weight; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; MeOH, methanol; D_2O, deuterium oxide; poly(GAVD), polypeptides containing glycine, alanine, valine, and aspartic acid

rapid synthesis of polypeptides from amino acid amides by using microwave heating, and the characterization of the resulting polypeptides.

Materials and Methods

Materials. Glycinamide hydrochloride, L-alaninamide hydrobromide, L-valinamide hydrochloride, L-leucinamide hydrochloride, L-methioninamide hydrochloride, L-phenylalaninamide hydrochloride, L-serinamide hydrochloride, triglycine, hexaglycine, dansyl chloride, and Tris were from Sigma Chemical Co.; glycine, phosphoric acid, urea, TFA, SDS, hydrochloric acid for hydrolysis, and guanidine hydrochloride were from Wako Pure Chemicals Ind. Ltd.; TFE and MeOH for spectroscopy were from Merck; L-histidinamide hydrochloride was from Kokusan Chemical Works Ltd.; a standard solution of amino acids was from Takara Kosan Co. Ltd.; kaolin was from Kukita Yakuhin Kogyo Co. Ltd.; insulin β-chain was from Boehringer Mannheim GmbH; Bio-Gel P-4 (200–400 mesh) was from Bio-Rad Laboratories. L-aspartic acid α-amide hydrochloride was prepared from *N*-carbobenzoxy-L-aspartic acid-β-benzylester (Anderson et al. 1964). A synthetic polypeptide (YRKLRKRLLRD) was kindly provided by Dr. K. Sato of this institute. All chemicals were of analytical grade.

Synthesis of Polypeptides during Repeated Hydration–Dehydration Cycles. A reaction mixture (10 ml) containing 0.1 M (each) of glycinamide hydrochloride, L-alaninamide hydrobromide, L-valinamide hydrochloride, and L-aspartic acid α-amide hydrochloride and 2 g of kaolin was adjusted to pH 7.2 with 4 N NaOH, placed in a beaker (100 ml, 60 × 70 mm), and heated in an electronic oven (Toshiba model ER512: 258 mm width, 300 mm depth, 226 mm height; 100 V, 500 W; oscillation frequency, 2450 MHz) equipped with a turntable. The solution was completely evaporated to dryness by microwave heating for 5 min. The same reaction mixture (10 ml) as described above, except that kaolin was absent, was then added to the resulting powder, adjusted to pH 7.2 with 4 N NaOH, and heated to complete dryness for 5 min. This series of operations was repeated 10 times. The resulting powder was dissolved with 20 ml of distilled water and centrifuged at 3000 rpm for 10 min. The precipitate was washed with distilled water and centrifuged at 3000 rpm for 10 min. The washing and centrifugation were repeated two more times. The supernatants were pooled and passed through a Nuclepore membrane filter (pore size 0.2 μm). The filtrate was concentrated on an Amicon YM-2 membrane filter. Lyophilization of the fraction remaining on the membrane filter gave a pale brown powder.

Gel Filtration. The molecular weights of resulting polypeptides were estimated by gel filtration on Bio-Gel P-4. A sample of resulting polypeptides (1.05 mg) remaining on an Amicon YM-2 membrane filter was dissolved in 200 μl of elution buffer, 2.5 mM H_3PO_4–Tris–0.25% SDS–2 M urea (pH 6.8), and heated at 40°C for 1.5 h. The sample (95 μl) was then applied to a Bio-Gel P-4 column (0.95 × 54 cm) equilibrated with the same elution buffer. Fractions (0.5 ml) were collected at a flow rate of 3 ml/h, and their absorbance was measured with a Gilford 2400-2 spectrophotometer at 230 nm. The column was calibrated by chromatographing the standard peptides: triglycine (MW 189), hexaglycine (360), synthetic peptide (YRKLRKRLLRD, 1500), insulin β-chain (3500). Blue dextran was used to determine the void volume.

Sedimentation Analysis. The molecular weights of resulting polypeptides were also estimated by sedimentation equilibrium in a Hitachi model 282 analytical ultracentrifuge equipped with an ultraviolet absorption system. For the equilibrium run, the RA-72 Tc rotor was used with a multichannel cell. The initial polypeptide concentrations were 40 μg, 80 μg, and 120 μg/ml, respectively. The polypeptide solution was centrifuged at 30,000 rpm for 16 h at 25°C. Partial specific volumes were calculated from the amino acid composition (McMeekin et al. 1949). Polypeptide molecular weights were calculated and corrected as described by Richards and Schachman (1959).

Amino Acid Analysis. Polypeptides (1 mg) were hydrolyzed with 6 N HCl in an evacuated and sealed glass tube at 105°C for 72 h. After hydrolysis, hydrochloric acid was removed by rotary evaporation and the residues were dissolved at 0.2 M sodium citrate buffer (pH 2.2). Polypeptides containing valine residues were also hydrolyzed with TFA–12 N HCl (1:1) at 175°C for 24 h or TFA–12 N HCl (1:2) at 167°C for 50 min. Amino acid analysis was performed with a Hitachi KLA-5 or an Irica model-5500 amino acid analyzer.

Analysis of N-Terminal Amino Acids. N-terminal amino acids of polypeptides were analyzed using dansyl chloride (Gray 1972). Polypeptides (100 μg) were dissolved in 0.2 M aqueous sodium bicarbonate solution, evaporated and dried in vacuo, and then dissolved in 100 μl of water. After the addition of 100 μl of 0.25% dansyl chloride in acetone, the solution was heated at 37°C for 1 h and evaporated to dryness under reduced pressure. The residues were hydrolyzed with 6 N HCl at 105°C for 18 h. After hydrolysis, hydrochloric acid was removed by rotary evaporation, and the residues were dissolved in 100 μl of MeOH. Dansyl amino acids were analyzed by HPLC on a C_8 reversed-phase column (Senshu-Pak P-5C8, Senshu Science, 4.6 mm × 15 cm) with solvent systems A (0.01 M Tris-HCl, pH 7.75) and B (MeOH) (Kaneda et al. 1982). The elution was carried out by a linear gradient from 100% A to 60% A : 40% B in 15 min at 50°C. The flow rate was 1 ml/min, and the column effluent was monitored at 254 nm.

Other Analytical Methods. Infrared spectra were measured on a Hitachi 260-50 infrared spectrophotometer. CD spectra were measured on a Jasco J-40A automatic recording spectropolarimeter. Usually, a quartz cell of 1-mm light path was used. ^1H and ^{13}C NMR spectra were obtained with a Brucker WM400 MHz spectrometer at ambient temperature. HPLC was carried out on a Jasco system equipped with a Tri Rotar-VI solvent pump, a Uvidec-100VI variable-wavelength UV monitor, and an AS-L350 intelligent processor.

Results

Glycine, alanine, valine, and aspartic acid were more abundantly formed in the simulated prebiotic synthesis of amino acids (Miller and Orgel 1973) and were also found in the Murchison meteorite (Kvenvolden et al. 1970). Thus, glycine, alanine, valine, and aspartic acid could have been formed in abundance at an early stage of chemical evolution (Eigen and Schuster 1978; Shimizu 1980; Egami 1981). Amino acid amides are formed from aldehyde, hydrogen cyanide, and ammonia by the Strecker synthesis, and this is a possible route to the prebiotic formation of amino acids (Oró et al. 1959; Kamaluddin et al. 1979; Kobayashi et al. 1987). We chose

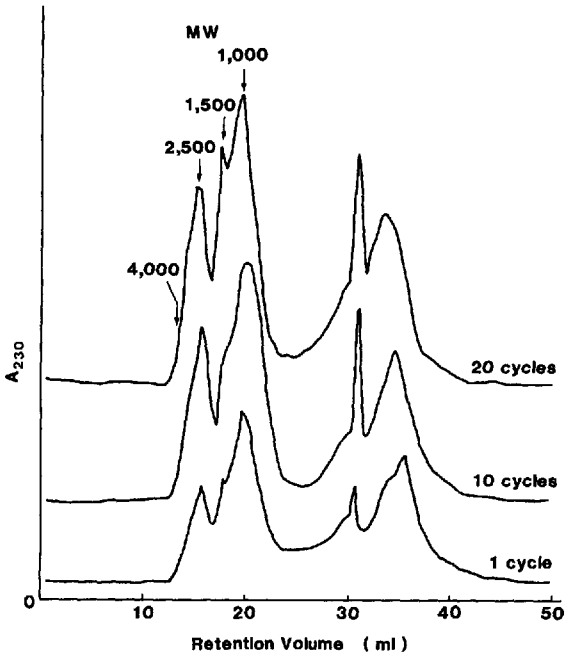

Fig. 1. Gel filtration of reaction mixtures obtained during repeated hydration–dehydration cycles on Bio-Gel P-4. A reaction mixture contained 0.1 M (each) glycinamide, L-alaninamide, L-valinamide, and L-aspartic acid α-amide. Further experimental details on the synthesis and gel filtration of polypeptides were described in the Materials and Methods. Three peaks with retention volumes of 13–23 ml were attributed to polypeptide fractions and two peaks with retention volumes of 25–40 ml to starting amino acid amide fractions.

four amino acid amides as starting material for our studies.

Polypeptides were rapidly synthesized from a mixture of glycinamide, alaninamide, valinamide, and aspartic acid α-amide by microwave heating during multiple hydration–dehydration cycles. The effect of repetition of a hydration–dehydration cycle on the yield and molecular weight of the polypeptides was examined in the range of 1–20 cycles (Fig. 1). Even a single hydration–dehydration cycle yielded considerable amounts of polypeptides with molecular weights of up to 4000 daltons. Furthermore, 10 hydration–dehydration cycles resulted in only a 1.5-fold increase in yield and no increase in molecular weight compared to the first cycle. Twenty hydration–dehydration cycles did not further increase the yield of polypeptides beyond that obtained after 10 cycles. Therefore, a saturating yield (10%) of the polypeptides with molecular weights of 1000–4000 daltons was obtained during 10 hydration–dehydration cycles.

Elemental analysis of the resulting polypeptides showed a percentage composition of C, 48.49; H, 5.26; and N, 17.95. The composition was similar to that predicted for polypeptides containing glycine, alanine, valine, and aspartic acid residues in equimolar mixture. The IR spectrum (KBr disk) of the resulting polypeptides showed strong absorption at 3375, 3080, 2980, 1708, 1665, 1540, 1400, 1335, 1175, and 620 cm^{-1}. The band at 3375 cm^{-1} could be attributed to the NH stretching of peptides. Similarly, the two frequencies at 1665 and 1540 cm^{-1} corresponded to the amide I and amide II absorptions of peptide bonds, respectively, and a frequency at 1708 cm^{-1} to the absorption of the carboxyl groups of aspartic acid residues.

Gel filtration of the resulting polypeptides on Bio-Gel P-4 gave two peaks at 2500 and 1500 daltons, respectively (Fig. 2). The profile of the gel filtrate indicated that the polypeptides had molecular weights ranging from 1000 to 4000 daltons. The molecular weights of the polypeptides were estimated to be 1900 daltons by ultracentrifugal analysis. The peaks on the gel filtration chromatogram completely disappeared after hydrolysis of the sample with 6 N HCl at 105°C for 72 h. The amino acid composition of the polypeptides, determined after acid hydrolysis, was glycine, 34.2; alanine, 20.6; valine, 22.1; and aspartic acid, 23.1%. The incorporation ratio of glycine into the polypeptides was about 1.5-fold that of alanine, valine, or aspartic acid, which were incorporated into the polypeptides almost in proportion to their starting concentration. The N-terminal amino acid composition of the polypeptides was determined using dansyl chloride: glycine, 16.0; alanine, 29.7; valine, 34.9; and aspartic acid, 19.4%. This suggests that the reactivity of the N-terminal amino acids decreases in the order glycine, aspartic acid, alanine, and valine.

^1H NMR analysis of the resulting polypeptides in D_2O showed relatively broad signals at 0.98 (valine γ-CH_3), 1.42 (alanine β-CH_3), 2.12 (valine β-CH), 2.90, 3.22 (aspartic acid β-CH_2), 4.03 (glycine α-CH), 4.15 (valine α-CH), and 4.34 ppm (aspartic acid α-CH). The amino acid composition of the resulting polypeptides was calculated from each peak area in the ^1H NMR spectrum: glycine, 31.1; alanine, 23.9; valine, 18.7; aspartic acid, 26.3% moles. The amino acid composition was very similar to that determined by acid hydrolysis of the polypeptides. The ^1H NMR in d_6-DMSO showed signals at 7.05–7.25 ppm and 8.18 ppm, suggesting the presence of primary amide bonds and peptide bonds. The ^{13}C NMR analysis of the resulting polypeptides in D_2O showed signals at 17.29–18.94 (alanine β-CH_3), 19.23–20.29 (valine γ-CH_3), 30.43–31.35 (valine β-CH), 35.46–35.64 (aspartic acid β-CH_2), 41.52–43.11 (glycine α-CH_2), 49.66–50.77 (alanine α-CH), 51.00–52.80 (aspartic acid α-CH), 59.20–60.52 (valine α-CH), 171.55–175.59 (carbon of amide bonds), and 176.31–178.72 ppm (carboxyl group of aspartic acid residues).

The physical data described above clearly indicate that the resulting polymers are polypeptides

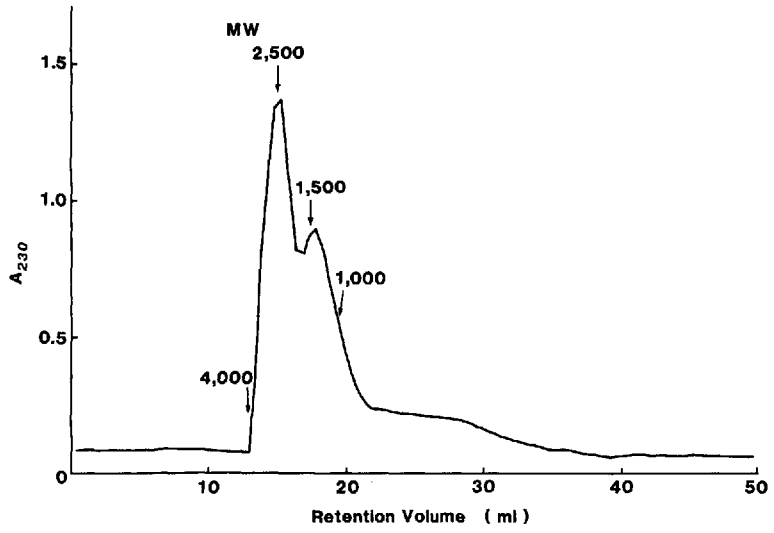

Fig. 2. Gel filtration of resulting poly(GAVD) on Bio-Gel P-4. Poly(GAVD) was synthesized from a mixture of 0.1 M (each) glycinamide, L-alaninamide, L-valinamide, and L-aspartic acid α-amide during 10 hydration–dehydration cycles. The resulting polypeptides were filtered through an Amicon YM-2 membrane filter to remove starting materials, and the fraction remaining on the membrane filter was applied to a Bio-Gel P-4 column. Further details were described in the Materials and Methods.

Table 1. Physical and chemical properties of the resulting polypeptides

Polypeptides[a]	Molecular weights[b]	Amino acid composition (%)						IR (peptide bond, cm^{-1})	
		G	A	V	D	M	L		
Poly(GAVD)	1000–4000	39.5	18.3	24.3	17.4			1670	1540
Poly(GAMD)	1000–4000	34.0	21.2		23.6	21.2		1660	1530
Poly(GALD)	1000–4000	33.9	23.8		20.6		21.7	1670	1540
Poly(GAV)	1000–4000	43.2	30.9	25.9				1665	1530
Poly(GAD)	1000–4000	43.4	26.6		30.0			1660	1535
Poly(GVD)	1000–4000	43.0		21.2	35.8			1660	1530
Poly(AVD)	1000–4000		32.1	29.6	38.3			1660	1530
Poly(D)	1000–4000				100			1670	1540

[a] Polypeptides were synthesized from a mixture of 0.1 M (each) amino acid amides during 10 hydration–dehydration cycles. Experimental details were described in the Materials and Methods.
[b] Molecular weights of polypeptides were estimated by gel filtration on Bio-Gel P-4

with molecular weights ranging from 1000 to 4000 daltons consisting of glycine, alanine, valine, and aspartic acid residues. Table 1 shows the physical and chemical properties of polypeptides synthesized from mixtures of different amino acid amides by the method described above. These resulting polypeptides had molecular weights ranging from 1000 to 4000 daltons. The amino acids were incorporated into polypeptides essentially in proportion to their starting concentration, except that glycine was incorporated 1.5 times as frequently as the other amino acids. Characteristic amide I and II bands of peptide bonds at 1530–1670 cm^{-1} were also present in the IR spectra of the polypeptides.

The CD spectra of three resulting polypeptides are shown in Fig. 3. Poly(GAMD) and poly(GALD) had one negative maximum at ~190 nm. Poly(GAVD), however, had a negative maximum at 192 nm and a broad negative band between 200 and 230 nm. Estimation of the secondary structure parameters based on the method of Provencher and Glöckner (1981) yielded 6% α-helix, 42% β-sheet, and 52% random conformations. These results suggest that parts of poly(GAVD) have definite secondary structures, such as α-helix and β-sheet, in aqueous solution, and that valine was necessary for the formation of the secondary structure. The negative value of molecular ellipticity of poly(GAVD) between 200 and 230 nm greatly decreased in the presence of 8 M guanidine-HCl and completely disappeared after 6 N HCl hydrolysis.

The CD spectra of poly(GAVD) in water at 3°C, MeOH, and TFE are shown in Fig. 4. Two definite negative bands with minima at 213 and ~225 nm were observed in both MeOH and TFE, while a single negative band at ~212 nm was observed in water at 3°C. These results indicate that poly(GAVD) can form more definite secondary structures in organic solvents or in water at low temperature than in water at 25°C.

Discussion

We have efficiently synthesized polypeptides with molecular weights of 1000–4000 daltons by microwave heating during repeated hydration–dehydration cycles. The yield of the polypeptides was 100-

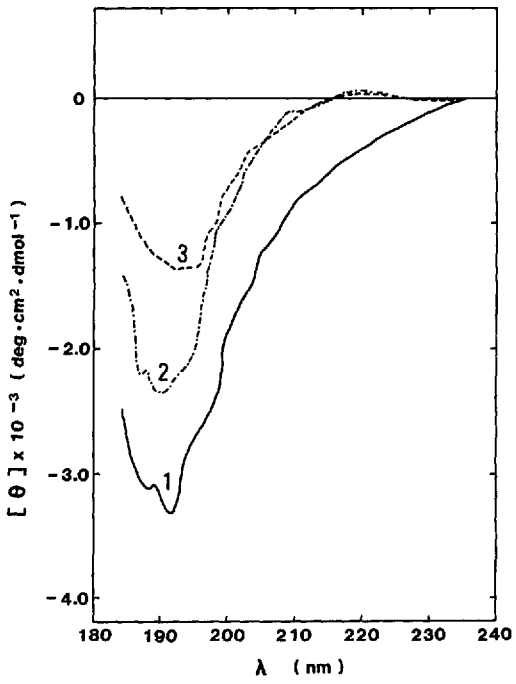

Fig. 3. CD spectra of different polypeptides in water at 25°C. Curves: 1, poly(GAVD); 2, poly(GAMD); 3, poly(GALD). The concentration of polypeptides was 90 μg/ml in each case. Methods for the synthesis of the polypeptides were given in Table 1.

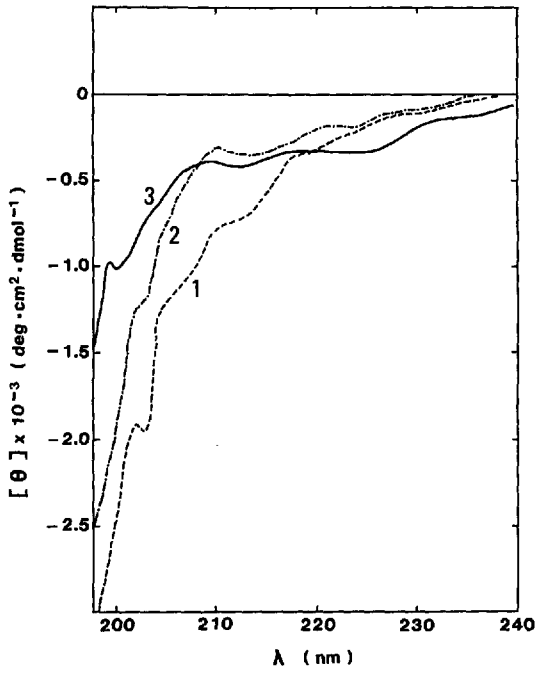

Fig. 4. Effect of MeOH, TFE, and low temperature on the CD spectra of poly(GAVD). Curves: 1, water at 3°C; 2, TFE at 25°C; 3, MeOH at 25°C. The concentration of poly(GAVD) was 90 μg/ml.

fold higher than that obtained by heating at 80°C (Yanagawa et al. 1988). In the microwave heating system, incomplete evaporation to dryness of a reaction solution did give a poor yield of polypeptides. The production of white fumes of ammonia was observed at the last stage of drying, indicating a removal of ammonia from the reduction system. In addition, only a small amount of amino acids was detected in a final resulting mixture. These facts suggest that the higher yield of polypeptides is due to an effective removal of water and ammonia from the reaction system, and the prevention of hydrolysis of the terminal active amide groups of the starting amino acid amides and elongating peptides, because of the very rapid and short reaction period.

The thermochemistry shows that an amino acid amide bond stores more energy than a peptide bond according to a direct measurement of heat in aqueous solution. Heat of hydrolysis was estimated to be −5880 for benzoyl-L-tyrosinamide using chymotrypsin and −2550 cal/mol for carbobenzoxyglycyl-L-phenylalanine using carboxypeptidase (Dobry and Sturtevandt 1952). This shows that amino acid amides formed abiotically should have already overcome thermodynamically unfavorable situations to provide peptide bonds from thermochemical studies. The half-lives of glycine nitrile and glycinamide were estimated to be 1 year and 9 years at 25°C and pH 8, respectively (Miller and Van Trump 1981). Therefore, amino acid amides possibly could have accumulated in the primitive soup on the earth. In this study we used pure amino acid amides as starting materials. The primitive soup on the earth, however, would have contained various forms of amino acids, e.g., amino acids and their derivatives together with amino acid amides. Amino acids and their derivatives may have interfered with polymerization of amino acid amides in the primitive soup.

The microwave heating could not produce polypeptides with molecular weights higher than 4000 daltons. We have synthesized polypeptides under conditions simulating possible primitive earth environments, such as a warm and hot sea (Yanagawa et al. 1980), a submarine hydrothermal vent (Yanagawa and Kojima 1985), and a hydration–dehydration cycle of a freshwater tide pool (Yanagawa et al. 1984). To date, we have never obtained any polypeptides with molecular weights of more than 4000 daltons under different simulated primitive earth conditions. It is reported that Fox's proteinoid microspheres consisted of proteinaceous polymers with molecular weights of more than 10,000 daltons (Harada and Fox 1960). We have recently found, however, that the proteinoid microspheres consist of proteinaceous polymers with a mean molecular weight of about 2000 daltons using polyacrylamide gel electrophoresis after their complete denaturation in 1% SDS–8 M urea solution (Yanagawa et al. 1988). This evidence indicates that the proteinaceous polymers with a mean molecular weight of 2000 daltons aggregate with each other to form apparent high

molecular weight polymers. Therefore, these results suggest that it is too difficult to obtain polypeptides with molecular weights of at least more than 4000 daltons under simulated primitive earth conditions.

Why are polypeptides with molecular weight higher than 4000 daltons not synthesized in aqueous solution? In both chemical stepwise and segment synthesis of peptides in solution, the most common difficulty is poor solubility of some protected peptide intermediates. It is reported that the insolubility of protected intermediates are related at a molecular level to the tendency of some protected peptides to form intermolecular β-sheet aggregates, which both hinder couplings and tend to make the peptides precipitate (Kent 1985). The poor solubility and coupling are maximal at chain lengths of about 15 residues and rare at greater than 20 residues, consistent with the known maximal tendencies to form β-sheet aggregates or adopt α-helix and random coil conformations (Baron et al. 1978). High concentration, temperature, and ionic strength enhance the β-structure formation in homopolypeptides containing alanine or valine (Rajasekharan Pillai and Mutter 1981). The concentration effect emphasizes the importance of interpeptide hydrogen bonds in the stabilization of β-structure in these peptides. The enhancement of β-structure formation by heating or by the addition of salts suggests that hydrophobic interactions influence the stability of the aggregated species. In the present study, a dilute solution of the resulting poly(GAVD) showed slight β-sheet conformation (curve 1 in Fig. 3). However, higher concentrations of the poly(GAVD) provided the formation of more definite β-sheet conformation (data not shown). This indicates that a high concentration of the poly(GAVD) assumes a β-sheet conformation in solution. The polypeptides presumably formed β-sheet aggregates during synthesis. We assume that the coupling reaction occurs most efficiently as the solution evaporates and the reactants become more concentrated. Therefore, high polypeptide concentration and heating may enhance the formation of β-sheet aggregates during synthesis. Consequently, the formation of β-sheet aggregates and their poor solubility prevented further polypeptide chain elongation during the repeated hydration–dehydration cycles.

The structure of small peptides in solution is influenced by a great number of factors such as chain length, the nature of side chains, solvent polarity, pH, concentration, temperature, and ionic interactions. Most homooligopeptides and small peptides without disulfide bonds can form definite secondary structures in aqueous solution. For example, homooligopeptides such as oligoalanine, oligovaline, and oligoleucine, with chain lengths greater than 5, could form definite β-sheet structures in aqueous solution (Ando et al. 1982). The neuroregulatory 11-peptide substance P self-associated to form a definite β-structure in aqueous solution (Toniolo et al. 1986). The minimal chain length for the formation of a β-structure was seven residues (Rueger et al. 1984). On the other hand, some short polypeptides appear to have a small amount of ordered structure in surfactant solution; for example, glucagon (29 residues), β-endorphin (31 residues), and β-lipotropin (91 residues) may have α-helix structures as judged from their CD spectra (Wu and Yang 1981). Short α-helices in water are unstable at room temperature in the absence of additional tertiary interactions such as those found in globular proteins. Brown and Klee (1971) found, however, that C-peptide (residues 1–13 of ribonuclease A) shows a partial α-helix in water at near 0°C. Baldwin and coworkers confirmed the α-helix formation and suggested that the salt bridge between Glu-9$^-$ and His-12$^+$ stabilized the helix structure (Bierzynski et al. 1982). What is the minimum number of amino acid residues for folding into a unique structure? In globular proteins, the average size of α-helices is about 10–11 amino acid residues. Such short helices are very unstable in water. It is suggested that a critical size of 8–9 residues of short polypeptides is required to form α-helices in surfactant solution (Wu and Yang 1981). β-sheets appear to be more easily formed than α-helices.

Can polypeptides with random sequences form secondary structures? We have recently tried to estimate the secondary structures of poly(GAVD) with random sequences (Yanagawa 1986): A random sequence consisting of 2000 amino acid residues was made using a table of random numbers and a computer. The amino acid composition in the sequence corresponded to the amino acid content of the resulting poly(GAVD). The prediction of the secondary structures based on the method of Chou and Fasman (1974) yielded 14.3% α-helix and 14.4% β-sheet structures. This result suggests that even polypeptides with random sequences may form definite secondary structures. It is experimentally estimated from the CD spectrum that the resulting poly(GAVD) yielded 6% α-helix, 42% β-sheet, and 52% random conformations in aqueous solution. In proteins, valine residues are strong β-sheet formers and leucine and methionine residues are strong α-helix formers (Chou and Fasman 1974). Therefore, we expected poly(GALD) and poly(GAMD) in which valine was replaced with leucine or methionine to form α-helix structures. Poly(GALD) and poly(GAMD), however, showed no definite secondary structure in aqueous solution. Kabsch and Sander (1984) recently showed that amino acid sequences of length five are in α-helical conformation in one protein and in another, quite different protein, in

β-sheet conformation. Thus, our results are most likely due to the difficulty of α-helix formation by short polypeptides in aqueous solution and/or differences in amino acid sequences of the resulting polypeptides. This result may also suggest that the combination of glycine, alanine, valine, and aspartic acid, which are considered good candidates for primitive amino acids (Eigen and Schuster 1978; Shimizu 1980; Egami 1981), is suitable for the construction of the main frame of enzymes.

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