ARCHITECTURE OF MODELS FOR PREBIOTIC SYNTHESIS OF PROTEINS: THE STRUCTURE AND FUNCTION OF POLYPEPTIDES SYNTHESIZED IN A FLUCTUATING SYSTEM

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We have recently found a novel procedure that can synthesize polypeptides more efficiently than heating at 80°C; that is, rapid synthesis of polypeptides from amino acid amides by microwave heating. In this paper we describe the structure and function of polypeptides synthesized in a fluctuating system repeating a hydration-dehydration cycle by microwave heating.

Glycine, alanine, valine and aspartic acid are more abundantly formed in prebiotic syntheses of amino acids and were found in the Murchison meteorite. Thus glycine, alanine, valine and aspartic acid were probably formed in abundance at an early stage of chemical evolution. Amino acid amides are formed from aldehyde, hydrogen cyanide and ammonia by the Strecker synthesis that is a prebiotically possible route to the formation of amino acid. We used the four amino acid amides as starting materials for rapid synthesis of polypeptides by microwave heating.

A reaction mixture (10 ml) containing 0.1 M (each) glycinamide, L-alaninamide, L-valinamide and L-aspartic acid amide adjusted to pH 7.2 was placed in a beaker and heated in an electronic oven equipped with a turn table. The solution was completely evaporated to dryness by microwave heating for 2 min, and then the same reaction mixture (10 ml) was added to the resulting powder, adjusted to pH 7.2, and heated to complete dryness for 2 min. This series of operations was repeated 10 times. The resulting powder was dissolved with 20 ml of distilled water and applied to ultrafiltration on an Amicon YM-2 membrane filter. Lyophilization of a fraction remaining on the membrane filter gave 400 mg of a pale brown powder in a 10% yield. The yield was 100-fold higher than that produced by heating at 80°C.

Elemental analysis of the resulting polypeptides showed a percentage composition of C, 48.49; H, 5.26; N, 17.95. The composition was similar to that of polypeptides containing glycine, alanine, valine

and aspartic acid residues. The IR spectrum of the resulting polypeptides showed strong absorptions at 3350, 3070, 2975, 2930, 2880, 1660, 1535, 1400 and 600 cm^{-1}. Two bands at 3350 and 3070 cm^{-1} could be attributed to NH stretching of peptides. Similarly, two frequencies at 1660 and 1535 cm^{-1} corresponded to the amide I and amide II absorptions of peptide bonds.

Gel filtration on Bio-Gel P-4 of the resulting polypeptides gave two peaks which appeared at 2500 and 1500 daltons. The profile of the gel filtration indicated that molecular weight of polypeptides was no more than 4000. These two peaks completely disappeared after hydrolysis with 6 N HCl at 110°C for 72 h. After such an acid hydrolysis, the amino acid composition of the polypeptides was determined: glycine, 34.2; alanine, 20.6; valine, 22.1; aspartic acid, 23.1%. This result suggests that amino acids were incorporated into polypeptides almost in proportion to the concentration of starting materials.

^1H NMR in D_2O of the resulting polypeptides showed relatively broad signals at 0.98 (γ-CH_3 of valine residues), 1.42 (β-CH_3 of alanine residues), 2.12 (β-CH of valine residues), 2.90, 3.22 (β-CH_2 of aspartic acid residues), 4.03 (α-CH_2 of glycine residues), 4.15 (α-CH of valine residues), and 4.34 ppm (α-CH of aspartic acid residues). 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%. 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 that suggested the presence of primary amide bonds and peptide bonds. ^{13}C NMR in D_2O of the resulting polypeptides showed signals at 17.29-18.94 (β-CH_3 of alanine residues), 19.23-20.29 (γ-CH_3 of valine residues), 30.43-31.35 (β-CH of valine residues), 35.46-35.64 (β-CH_2 of aspartic acid residues), 41.52-43.11 (α-CH_2 of glycine residues), 49.66-50.77 (α-CH of alanine residues), 51.00-52.80 (α-CH of aspartic acid residues), 59.20-60.52 (α-CH of valine residues), 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 indicates that the resulting polymers are polypeptides with molecular weights of at least 1000-4000 daltons consisting of glycine, alanine, valine and aspartic acid residues.

To confirm the secondary structures of the resulting polypeptides, we measured CD spectra of the polypeptides. Polypeptides composed of glycine, alanine, valine and aspartic acid had partly definite secondary structures such as α-helices and β-sheets. The content of the secondary structures was greater in methanol than that in water. Polypeptides composed of glycine-alanine-leucine-aspartic acid or glycine-alanine-methionine-aspartic acid had no definite α-helix and β-sheet structures. Valine was essential for the formation of the secondary structures. In addition, Polypeptides composed of glycine, alanine, valine, aspartic acid and histine had partly definite secondary structures and they had catalytic activities for hydrolysis of p-nitrophenyl phosphate and p-nitrophenyl acetate.