NOTE

Nonrandom Thermal Polymerization of Amino Acids

Peptide polymers have been produced by thermal reaction,[1] condensations using dicyanamide,[2] and condensations using *N*-carboxy-α-amino acid anhydrides.[3] In contradistinction to suggestions that these polymerizations are random processes, Steinem[4] and Fox[5] have provided experimental evidence that the polymerizations are not random. Whereas Steinem and Fox's work involved di- and tripeptides, the work presented here involves hexa- and heptapeptide fragments of thermal polyamino acids with molecular weights in the range of 10,000. These findings are consistent with Fox and Steinem's results in that these polymers have been found to be very limited in the actual amino acid sequences that are formed and with the complete absence of Met–Phe sequence.

The copolymers prepared by the *N*-carboxy-α-amino acid condensation procedure as described by Sela, Fuchs, and Arnon[10] and Sela and Berger[11] have been described as being random polymers, although no sequence work has been carried out. More recently these copolymers have been studied as synthetic antigens,[10] immunosuppresants,[12] substrates for the purification of protein kinases,[13] and in biodegradable sustained release systems.[14]

Thermal polyamino acids were synthesized by heating mixed combinations of amino acids by weight (five parts glutamic acid and one part of an equimolar mixture of alanine, methionine, and phenylalanine) in a filtering flask.[6,7] The flask was heated in an oil bath at 194°C for 17 h under a stream of nitrogen. The resulting product, a dark brown glass, was dissolved in water and was fractionated by using paper chromatography in butanol/water/acetic acid, 5 : 4 : 1, v/v. The polymers were detected by spraying the dried paper with the hypochlorite–starch–iodide method of Rydon and Smith.[8] There were at least three thermal polymer fractions formed, as detected by the Ryden and Smith reagent.

Polymer separations were done by streaking the samples at the origin on 3MM Whatman chromatography paper and chromatographed in the same way as the polymer fractionation procedure. Polymers were eluted with 10% acetic acid. Acid hydrolysis of each peptide fraction was performed with 6*N* HCl at 110°C for 24 h in evacuated, sealed tubes, and amino acid analysis was done by paper chromatography. The amino acids were detected by spraying with ninhydrin reagent. Quantitative analyses of the polymer were done by high-performance liquid chromatography (HPLC). The results indicated that glutamic acid, methionine, alanine, and phenylalanine were incorporated in the high R_f (S_4–F_4) value (hydrophobic) polymer, and glutamic acid and alanine were incorporated into the low R_f value polymer.

Cyanogen bromide was used for fragmentation of the fraction that contained methionine (S_4–F_4). The sequences of these peptides were studied by using the Edman procedure.[9] In the last step of this procedure, phenylthiohydantoin amino acid (PTH–AA) was extracted with benzene. The benzene was dried under nitrogen gas, and the small amount of solvent B (0.05 *M* ammonium acetate and 50% CH_3CN, pH 6.8) for HPLC was added to the dried sample and redried to remove benzene. Solvent B was added and the solution was filtered through a 0.3-μm Millipore filter paper. Sample (4 μL) was injected into HPLC. A Spectra Physics HPLC system was used, which included a SP8700 solvent delivery unit, SP8750 organizer module (injector and column bypass valve mounted), a SP8400 variable wavelength detector (fixed wavelength at 254 nm), and a SP4100 computing integrator. An Eldex 7251010 constant-temperature oven was used for the column. The sensitivity of the detector was routinely set at 0.32 AUFS (absorption unit, full scale). PTH–amino acids were analyzed on an Alltech C_{18} column (10 μm: 25 cm $\times$ 4.6 mm).

The six rounds of Edman degradation of thermal polyamino acids (S_4–F_4) after cyanogen bromide treatment are shown in Table I. There are glutamic acid and alanine at the N-terminal positions of the low R_f fraction of polyamino acids (S_4–F_4CN_1). The fraction must therefore contain a mixture of polyamino acids. The same results were found for every fraction.

Fractions S_4–F_4–CN_3, S_4–F_4CN_4, and S_4–F_4–CN_5 did not contain Phe at the N-terminus. Thus there were no Met–Phe bonds formed in the thermal polyamino acids. Table I summarizes the results of the Edman analysis of the cyanogen bromide fragments of S_4–F_4. S_4–F_4–CN_2 with only Glu at the N-terminus indicates it is the N-terminal fragment from all the peptides in S_4–F_4. All the rest of the cyanogen bromide fragments of S_4–F_4 contain Glu and Ala at the N-terminus, which establishes that Glu and Ala occur at C-terminal of Met residues in the S_4–F_4. The fact that Phe does not occur at the N-terminus of S_4–F_4–CN_3, S_4–F_4–CN_4, or S_4–F_4–CN_5, the Phe containing fragments, demonstrates that Phe does not occur at the C-terminus of the Met residue. This means that in these thermal polypeptides no Met–Phe bond has been found.

If S_4–F_4–CN_1 had a single, unique amino acid sequence, there would only be one amino acid at each position in the polyamino acid. The Edman procedures indicate that more than one amino acid is at each position, which means the fraction contains more than one peptide. The total residues at each position for fragments CN_1 to CN_5 range

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Table I Edman Degradation of Thermal Polyamino Acids (S_4–F_4) after Cyanogen Bromide Treatment

		Concentration of PTH–Amino Acid (nmol/0.2 mL)					
Sample	PTH–AA	1	2	3	4	5	6
S_4–F_4–CN_1	PTH–Glu	1.0	1.0	1.0	1.0	1.0	1.5
	PTH–Ala	1.5	3.2	3.8	4.6	3.2	1.0
	Total	2.5	4.2	4.8	5.6	4.2	2.5
S_4–F_4–CN_2	PTH–Glu	1.0	2.1	1.0	1.0	1.0	1.0
	PTH–Ala	0.0	1.0	4.5	3.6	3.8	4.4
	Total	1.0	3.1	5.5	4.6	4.8	5.4
S_4–F_4–CN_3	PTH–Glu	2.9	1.8	1.0	1.0	1.6	1.0
	PTH–Ala	1.0	2.3	2.4	6.5	2.0	2.8
	PTH–Phe	0.0	1.0	2.0	2.6	1.0	0.0
	Total	3.9	5.1	5.4	10.1	4.6	3.8
S_4–F_4–CN_4	PTH–Glu	1.3	1.4	1.0	1.0	1.0	2.4
	PTH–Ala	1.0	2.2	1.5	2.6	1.2	2.7
	PTH–Phe	0.0	1.0	1.7	0.0	1.2	1.0
	Total	2.3	4.6	4.2	3.6	3.4	6.1
S_4–F_4–CN_5	PTH–Glu	2.3	1.5	1.0	1.4	1.9	3.0
	PTH–Ala	1.0	1.8	1.6	6.8	2.7	1.0
	PTH–Phe	0.0	1.0	1.8	1.0	1.0	2.3
	Total	3.3	4.3	4.4	9.2	5.6	6.3

from 2.5 to 10.1 with the majority falling in the range of 4 to 6 residues, which implies an average of five different sequences in each fraction or at least five different polyamino acids in each fraction. In the data presented in Table I, some data points such as S_4–F_4–CN_3 at position 4 (6.5), S_4–F_4–CN_5 at position 4 (6.8), S_4–F_4–CN_4 at position 4(0), and S_4–F_4–CN_3 at position 6(0) appear to be outside of the range of the rest of the data presented in the table. The average total residues at each position in these cyanogen bromide fragments ranges from 4 for CN_1 and CN_2 to 5 for CN_3 and CN_5. This is consistent with 4 to 5 polypeptides in each sample. Thus, conservatively there are 4 to 5 different sequences present rather than the possible 729 combinations of the 3 different amino acids in hexapeptide sequences.

The results obtained in these experiments thus indicate that only a small number of the possible polymers were formed with these conditions. Also the conditions used in these experiments did not allow any Met–Phe sequences in the polymer, which indicates some specificity in peptide bond formation.

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