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Structural Characterization of Thermal Prebiotic Polypeptides

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Summary. Thermal polycondensation of amino-acids as a possible prebiotic path of chemical evolution of life has been critically examined.

The polymeric materials studied by nmr methods have scarce resemblance to natural peptidic material because β, γ and ε peptide bonds largely predominate over α̃-peptide bonds.

Key words: NMR - Prebiotic Polypeptides - Proteinoids - Polypeptide Thermal Synthesis

It has been claimed that the essential steps of chemical evolution can be simulated in the laboratory in a very short period by means of thermal syntheses (Fox, 1971). As outlined by S.W. Fox, who first formulated this hypothesis, the essential steps are: i) thermal synthesis of aminoacids from methane, ammonia and water at very high temperatures (900 ^{o} to 1060 ^{o}C), ii) condensation of aminoacids to polypeptides at temperatures of the order of 200 ^{o}C, iii) aggregation of these polypeptides (the so-called proteinoids) to form pseudo cellular systems (the so-called microspheres).

Step i) has been critically reexamined (Lawless & Boynton, 1973) and found of scarce relevance to the prebiotic synthesis of natural aminoacids. In fact, the yields of natural aminoacids, if any, are much lower than those of other prebiotic syntheses, e.g. those based on discharge experiments (Miller & Urey, 1959).

We have investigated step ii) with the aim of a structural characterization of the polypeptides obtained by pyrocondensation. In fact, these products, although studied from many points of view, have never been properly characterized and their similarity with naturally occurring peptides has never been demonstrated unambiguously. In our

view the knowledge of the structure of these polypeptides is crucial to assess the importance of thermal condensation in prebiotic syntheses. In fact, although geological condition were probably not very favourable to localized heating at temperature between 100 ^{o}C and 200 ^{o}C over limited periods of time (Miller et al., 1959) this method of condensation has two advantages over alternative prebiotic condensations, i.e. the high molecular weight of the products and the ease of water elimination. On the other hand, it is conceivable that a substantial portion of peptide bonds is not of the α-peptide type (as in natural peptides) since thermal condensation can be performed only with mixtures containing large amounts of trifunctional aminoacids to prevent extensive carbonization of the starting aminoacids (Fox, 1971). The presence of constitutional differences such as β, γ, or ε peptide bonds, is usually very difficult to detect by means of either chemical or physical methods. In particular, the spectroscopic methods traditionally employed in the field of synthetic or natural polypeptides (UV, IR, ORD, CD) can only give information on the overall structure of the chains but not on parts of the specific residues (Bradbury er al., 1973). It is possible however to resort to nmr spectroscopy since this technique can give useful information on the constitution and conformation of the single chemical groups in the polypeptide residues, as demonstrated by numerous studies on synthetic (Bradbury et al., 1973) and natural (Dwek, 1973) polypeptides. Besides, when ionizable groups are present on the chains, the chemical shifts of adjacent nuclei depend in a characteristic way on the degree of ionization (Sheinblatt, 1966; Andini et al., 1975). Thus, for instance, the chemical shift of the β-CH_2 of an aspartic acid residue is displaced of ca. 0.3 ppm downfield by the protonation of the adjacent carboxylate, whereas the chemical shift of the α-CH group is displaced of only 0.10 ppm (Sheinblatt, 1966; Andini et al., 1975).

We have studied, by means of ^{1}H nmr spectroscopy, a number of homo and co-polypeptides obtained by pyrocondensation of aminoacids. In particular polyaspartic acid, polylysine, copoly(aspartic $acid_1$, glutamic $acid_1$), copoly ($lysine_1$, aspartic $acid_1$), copoly($lysine_1$, $glycine_1$) and copoly($glycine_2$, glutamic $acid_1$). Two proteinoids were also obtained from mixtures of all 20 aminoacids (Fox & Harada, 1966). A lysine rich proteinoid from a mixture containing 10 moles of lysine per each mole of the remaining aminoacids, and an aspartic-rich proteinoid containing 25 moles of glutamic acid and of aspartic acid per mole of each other aminoacid.

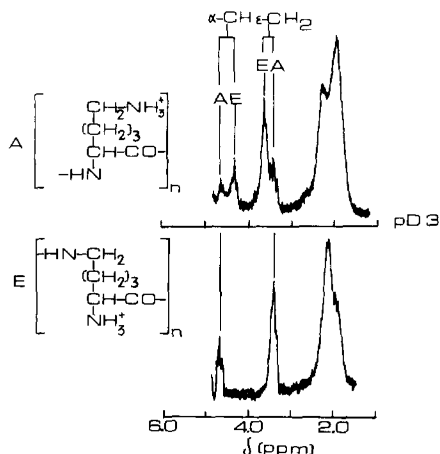

Fig.1
100 MHz spectra of poly-α-L-lysine and thermal poly-lysine. Only the region above the water peak is shown. Labels A and E refer to peaks of residues with α and ε peptide bonds respectively

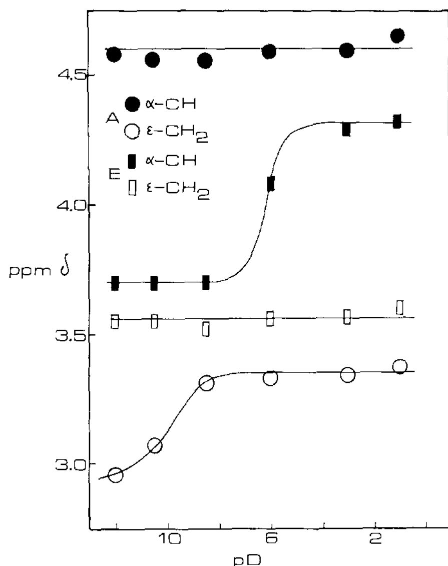

Fig.2
Chemical shift dependence (ppm from TMS) on pD of the α-CH and ε-CH_2 groups in poly-α-L-lysine (A: ● α-CH, o ε-CH_2) and poly-ε-lysine (E: ▮ α-CH, ▯ ε-CH_2)

All these polymers were prepared by heating the aminoacids in the solid state at 170 °C to 200 °C, according to the procedures described by Fox & Harada (1966). Purification procedures were essentially those described by Rohlfing (1967). Attempts to copolimerize trifunctional aminoacids with alanine or other apolar aminoacids led to intractable solids and/or to diketopiperazines.

Nmr spectra of D_2O solutions were recorded on a Varian HA-100-15 spectrometer at probe temperature (ca. 29 °C). The pH of the samples was measured immediately before each run with a radiometer PH6 Phmeter. pH adjustments were made with either DCl or NaOD solutions in D_2O.

Spectra of authentic samples of poly-α-L-aspartic acid, poly-α-L-glutamic acid and of poly-α-L-lysine were run for comparison in the same pH ranges employed for thermal poly-

peptides. The structural use of nmr is well illustrated by the case of the homo-polylysine. Fig.1 shows a comparison of the proton spectra of thermal polylysine and poly-α-L-lysine at pD3. The spectrum of the thermal polymer, besides all the peaks characteristic of lysine residues linked by α-peptide bonds (A peaks in the figure), contains extra CH and CH_2 peaks, arising from lysine units linked by ε-peptide bonds (E peaks in the figure). This assignment is clearly substantiated by the pH dependence of the α-CH and ε-CH_2 chemical shifts shown in Fig.2. As expected, the peaks of α-CH and ε-CH_2 groups directly linked to an NH_2 group shift upon protonation of 0.60 ppm (E α-CH) and 0.40 ppm (A ε-CH_2) respectively. The backbone CH and CH_2 do not show appreciable shifts. Although the areas of the peaks can not be measured very accurately it is easily seen that the resonances of the "unnatural" ε-peptide units account for the larger part of the peak area. By an approximate evaluation of the intensities, thermal polylysine was thus found to consist of about 70% ε-peptide units and only 30% α-peptide units.

Even more striking is the case of thermal polyaspartic acid. In this case the proton spectrum of the thermal polymer shows only one β-CH_2 peak (while the α-CH region is almost completely obscured by the HDO peak). The pH dependence of this peak indicates that the β-CH_2 is not adjacent to a carboxyl group, i.e. that all the residues are linked as β-peptide units. Fig.3 shows a comparison of the titration curves of the β-CH_2's of authentic poly-α-L-aspartic and of thermal polyaspartic acid. The two types of peptide units can be easily distinguished both by the pH induced shift and by the slightly different inflection points, reflecting a small difference in the pk's of the carboxylic groups (Sheinblatt, 1966; Andini et al., 1975).

All copolypeptides could be studied by this method using the simple criteria of pH induced shifts and of a comparison with authentic samples of corresponding homopolymers to evaluate the type of peptide units present along the chain. The results are summarized in Table 1 which shows the relative amounts of α, β, γ, or ε peptide units of each residue in the polymers studied. It is apparent that the presence of α-peptide units is the exception rather than the rule.

The spectra of the two proteinoids were very similar to those of thermal polylysine and of thermal poly-aspartic acid respectively. Owing to the much larger area of peaks arising from Lys and Asp residues it was not possible to assign the other peaks to specific residues with any degree

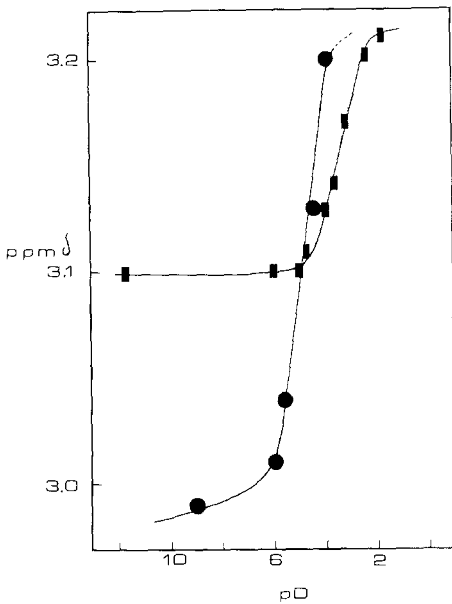

Fig.3
Chemical shift dependence (ppm from TMS) on pD of the β-CH_2 groups in poly-α-L-aspartic acid (●) and thermal poly-aspartic acid (▮)

Table 1
Relative amounts of different types of peptide bonds in thermal polypeptides

Residues \ Polymers	Homo	Copoly $(Glu\text{-}Asp)_n$	Copoly $(Lys\text{-}Asp)_n$	Copoly $(Gly\text{-}Glu)_n$	Copoly $(Lys\text{-}Gly)_n$
Asp	100%β	100%β	100%β		
Glu		100%α		100%γ	
Lys	70%ε		60%ε		50%

of confidence. It is significant however that even when mixed with many different aminoacids lysine and aspartic acid give rise to polymers containing a preponderance of ε and β units respectively, as in the corresponding homopolymers.

The general pattern emerging from our investigation is that trifunctional aminoacids, when subjected to thermal condensation tend to form β, γ, or ε peptide units more easily than α-peptide units. This finding, if added to the low probability of the proper geological conditions (Miller et al., 1959), shows that the relevance of thermal condensation in the synthesis of prebiotic polypeptides ought to be critically reexamined.

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