# LINKAGES IN THERMAL COPOLYMERS OF LYSINE



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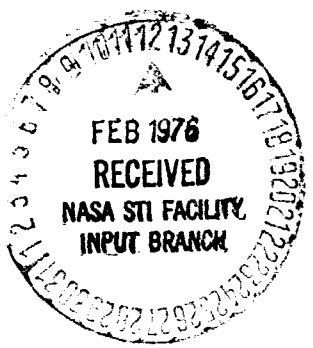


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## Abstract

The thermal copolymerization of lysine with other α-amino acids has been studied further. The identity of the second amino acid influences various properties of the polymer obtained, including the proportion of α and ε linkages of lysine. A review of linkages in proteinoids indicates α and β linkages for aspartic acid, α and γ linkages for glutamic acid, α and ε linkages for lysine, and α linkages for other amino acids. Thermal proteinoids are thus more complex in types of linkage than are proteins.

The propensity of lysine to copolymerize by heating with other amino acids has been reported (Harada, 1959; Fox et al., 1962; Harada and Fox, 1965; Heinrich et al., 1969). Some data have been presented for the proportion of bonding of lysine through the α- and ε-lysine groups. In this paper more data are reported; these permit comparing the influence of some other amino acids on the type of linkage produced.

## METHODS

### Copolycondensation of lysine

Free DL-lysine (1.02 g) was heated at 170° to form the clear lactam. The second amino acid was added after 45 min of total heating. The mixture was then heated with stirring at 195° under nitrogen gas for 2½ h. Gas evolved vigorously. Water, 20 ml, was added to the brownish solid, and the mixture was dialyzed for 5 days with 3 changes of water each 24 h. The water-soluble and water-insoluble fractions of the contents of the sac were separated; the water-soluble fraction was lyophilized.

In other experiments, lysine monohydrochloride was used instead of free lysine. Since these latter reaction mixtures were not fluid, 1.0 ml of phosphoric acid (Baker, 85% orthophosphoric acid) was added per each 0.02 mol (0.64 g) of lysine HCl. The liquid was heated at 195°C under nitrogen for 10 h. The product was worked up in the same way as the polymer from free lysine.

### Amino acid composition of polymers

Copolymers were dried in the Abderhalden pistol, using tetralin of b.p. 208°, or water, until the sample was at constant weight. Aliquots were dissolved in 6 N HCl, sealed in tubes, and heated for hydrolysis at 100° for 72 h. The hydrolyzate was dried in a vacuum desiccator, water was added, and drying was repeated. The solution was analyzed on a Phoenix K-5000 analyzer.

### Infrared absorption spectra

Copolymers were pressed into KBr pellets and examined on a Baird Atomic Inc. Model KM-1 infrared spectrophotometer.

### Assay of ratio of α- and ε-linkages (Folk, 1956)

Polymer, 0.1 g, was dissolved in 1.0 ml of water containing 0.1 g of sodium bicarbonate, and 2.0 ml of 2,4-dinitrofluorobenzene solution (5% in ethanol) was added. The mixture was shaken mechanically for 2-3 h at room temp.

A yellow ppt formed. Water, 2.0 ml, was added to dilute the ethanol for extraction, and the unreacted dinitro compound was extracted with 3-4 ml of ether several times. The ether layer was separated by centrifugation.

The water layer was acidified with several drops of conc. HCl to form additional precipitate which was washed with ether and acetone and dried. A sample of the DNP-polymer, 30 mg. was then hydrolyzed in a sealed tube for 24 h with 2.0 ml of 6 N HCl at 100°C. This solution was extracted with several 5 ml portions of ethyl acetate to remove any N-terminal di-DNP-lysine.

The ethyl acetate solution was washed with dilute HCl to extract ε-DNP-lysine into the water layer completely. The combined aqueous solution was then evaporated in a vacuum desiccator over sodium hydroxide. The residue was dissolved in a small amount of water and chromatographed on paper with 1 M citrate buffer of pH 6.2. Each derivative was eluted with 1.5% sodium bicarbonate and assayed at 360 nm in a Bausch and Lomb Spectronic 20 colorimeter. The amounts of α- and ε-DNP-lysine were estimated from standard curves.

## RESULTS

Free L-lysine was successfully copyrocondensed with L-glutamic acid, DL-aspartic acid (with phosphoric acid as a solvent), DL-glutamine, L-leucine, L-isoleucine, or L-valine; the products gave positive biuret tests. Amino acids that did not give positive biuret tests for the products of polymerization with lysine were L-histidine, L-arginine, L-tyrosine, and L-tryptophan. The thermal cocondensation of free lysine with aspartic acid was examined at three different ratios; the results are presented in Table 1.

Table 2 indicates the composition of thermal copolymers of lysine. Included for comparison is the polymer of aspartic acid with lysine hydrochloride as well as with free lysine. Total recovery of amino acids on analysis ranged between 40-80 percent, incomplete recoveries being known for the analysis of thermal lysine copolymers. This could not be overcome by prolonged hydrolysis. The copoly (lys HCl, asp) yielded a recovery of 35% after 24 h of hydrolysis, a value that progressed to 45% at 92 h of hydrolysis.

Infrared maxima of copoly (lys HCl, asp), copoly (lys, glu), and copoly (lys, gluNH$_2$) were 3300, 3080, 1650, and 1550 cm$^{-1}$, which indicate peptide bonds (Fox and Harada, 1960; Fox et al., 1962). In copoly (lys, asp) and copoly

Table 1

Thermal Copolymerization of Aspartic Acid and Free Lysine in Varying Molar Ratios

| Aspartic Acid | Lysine | Molar Ratio | Yield | | |
|---|---|---|---|---|---|
| | | | Water-insoluble | Water-soluble | Total |
| g | g | | g | g | % |
| 1.24 | 0.66 | 2:1 | 0.9 | tr. | 49 |
| 0.93 | 1.02 | 1:1 | 1.2 | tr. | 61 |
| 0.66 | 1.36 | 1:2 | 1.2 | tr. | 59 |

tr = trace.

Table 2

Amino Acid Composition of Thermal Lysine Copolymers

| Monomers | Composition of Polymer | |
|---|---|---|
| | Lysine % | Other Amino Acid % |
| Lys HCl, Asp | 71 | 26 |
| Lys, Asp[a/] | 82 | 13 |
| Lys, Glu | 44 | 50 |
| Lys, $GluNH_2$ | 40 | 51 |
| Lys, Ala | 61 | 29 |

[a/] The water-insoluble fraction of polymer was analyzed. For all other polymers, the water-soluble fraction was analyzed.

(lys HCl, asp) additional absorption was observed at 1700 and 1790 $cm^{-1}$. These correspond to imide bonds (Fox and Harada, 1960).

All polymers of lys with asp or glu or glu($NH_2$) yielded, in 1% aqueous solution, pH of 6.0 or less. The copoly (lys, ala) gave a pH of 8.3, consistent with an excess of basic groups in this polymer.

Table 3 presents the proportions of α- and ε-linkages in three copolylysines. The proportion of α-linkage is equivalent to the proportion of ε-DNP-lysine found, while the proportion of ε-linkage is proportional to the α-DNP-lysine recovered. The α,ε-di-DNP-lysine, having been removed before analysis, is ignored in these calculations. The results with aspartic acid copolymer are in close agreement with the findings of others (Harada, 1959; Fox, 1966).

The proportion of α-linkage is major in the case of the glutamic acid copolymer and minor for the aspartic acid copolymer. This indicates that the second amino acid significantly influences the mode of linkage of the one under study, in this case lysine.

## DISCUSSION

The behavior of lysine in these studies of thermal polycondensation with other amino acids is consistent with earlier observations (Harada, 1959; Fox et al., 1962; Harada and Fox, 1965; Heinrich et al., 1969).

The catalog of the tendency of lysine to copolymerize thermally with other amino acids to yield peptidic products is further extended by the data presented here. Although lysine undergoes thermal condensation with several α-amino acids it does not do so with some others. This reveals a tendency that is less than that observed for aspartic acid with comonomeric amino acids, or that of glutamic acid with additional amino acids, excepting proline (Harada and Fox, 1958). The ability of all common proteinous amino acids to condense thermally with lysine (Fox et al., 1962) is thus to be explained by the accessory action of aspartic acid, glutamic acid, and perhaps other amino acids during the same polymerization.

The results of Table 3, moreover, indicate that the linkage involved when a trifunctional amino acid like lysine is pyrocondensed is influenced by the other amino acids involved (Table 3) and by the temperature (Fox, 1966). Similar effects of neighboring amino acid residues in the hydrolysis of aspartoylimide to yield either α- or β-linkages have been predicted on the basis of results obtained

Table 3

Proportion of α- and ε-Linkages of Lysine Residues in Three Thermal Copolymers

| Copolymer | Linkage | |
|---|---|---|
| | α, % | ε, % |
| (Lys, asp) | 29 | 71 |
| (Lys, glu) | 55 | 45 |
| (Lys, gluNH$_2$) | 56 | 44 |

in the hydrolysis of various imides (Hoagland and Fox, 1973).

α-Linkages have been reported for thermal copolyaspartic acids, and the finding has been questioned. Andini et al. (1975) failed to find, by NMR, α-aspartyl linkages in homopolyaspartic acid, in contrast to the observations of Kovacs et al. (1961) who earlier reported α-aspartyl linkages in a number of preparations (Andini et al.do not cite this paper). Comparative examination of the results of each group of workers shows that each studied small fractions (< 5%) of the polyaspartic acid that each made; they obtained these fractions by very different techniques. Kovacs et al., for example,used copper salt to fractionate the crude polyaspartic acid. For formation of the polyaspartic acid, Andini et al. followed the procedure of Rohlfing (1967), who studied copolymers of aspartic acid, but not the homopolymer on which Andini et al. report. The heating conditions of Andini et al., patterned after Rohlfing, were thus 175° at 760 mm for 3½ h, whereas those for Kovacs were 200° at 0.1 mm for 120 h. Rohlfing's polymers have α-linkages as indicated by biuret tests on polymers of amino acids that are predominantly difunctional glycine, a structure that permits little linkage other than α (Saunders and Rohlfing, 1972). While Andini et al. acknowledge that methods of hydrolysis other than the kind they used might give α-peptide linkages they, as stated earlier, do not cite the most thorough study in which such report has already appeared (Kovacs et al., 1961).

Copolymers of glutamic acid having N-terminal pyroglutamyl residues must in their nature also have some α-linkage of the original trifunctional amino acid (Phillips and Melius, 1974).

Additional evidence for α-linkages in thermal copolyamino acids is found in the isolation of alanylalanine from copoly (ala, lys) as described by Harada and Fox (1965). The linkage between the two alanine residues of the dipeptide must of course be an α-peptide bond.

Andini et al. refer to linkages not commonly found in contemporary proteins as "errors". The antievolutionary views resultant from so labelling variant structures were pointed out long ago (Fox, 1953).

As a consequence of the above experimental reports, we must conclude that no evidence has been presented to indicate that α-linkages of aspartic acid, glucamic acid, or lysine cannot, or have not, been obtained in thermal copolyamino acids in addition to ω-linkages. It is of course true that proteinoids are not proteins; many of them are in large part peptides, and they are polyamino acids. In an evolutionary context, proteinoids should also not be regarded as prebiotic proteins, but rather as preproteins.

In overview, these interpretations are explained elsewhere (Fox, 1974), essentially as follows: Basic proteinoids functioned early in evolution to yield protoribosomes at the surface of which true contemporary proteins might have had their origin. The proteinoid (more properly referred to as preprotein than as prebiotic protein or protoprotein) did not bequeath its contained information directly to proteins. Rather, the synthesis of true protein would have begun in a protoribosome under influence of nucleic acids.* The fact that proteins and proteinoids exhibit compositional and other similarities (Mikelsaar, 1975) is understandable on the basis that they were produced under similar steric and other constraints. These constraints would have acted in a parallel manner at various stages of evolution, rather than in a serial manner in which proteinoid structure dictated later protein structure. The significant aspect of prebiotic proteinoids would thus have been their functional capabilities in yielding the first cells and the first ribosomes. A detailed identity of proteinoid to protein would be neither functionally nor structurally meaningful, nor even compatible with rudiments of such an evolutionary theory.

Acknowledgments

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*The relationship is illustrated by a synthesis of phenylalanine peptides from ATP and phenylalanine in the presence of particles composed of lysine-rich proteinoid and polyadenylic acid. Thus appear peptides having necessarily α-linkages, but requiring phase-separated particles formed from lysine-rich proteinoid which may not have required α-linkages (Fox et al., 1974).

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