Studies of Copolymers Containing L-Proline Residue as a Main Constituent. I. The Synthesis of Copolymers of L-Proline with the γ-Ester of L-Glutamic Acid

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Polyamino acids are very useful as a model for elucidating the physico-chemical properties of proteins.[1] Many investigators have studied collagen, the major protein component of the connective tissues, which is very specific because of its amino acid composition and structure. The vertebrate collagen contains about 30% glycine residues and more than 20% L-proline residues, inclusive of L-hydroxyproline residues, and its molecular structure is similar to that of poly-L-proline II.[2] Therefore, it is interesting to investigate how the conformation of copolypeptide containing L-proline changes with the L-proline content. We have studied polyamino acids which contain L-proline residue as the main constituent.

In the present communication, the synthesis of copolypeptides with proline will be descirbed. The other constituent is γ-methyl-L-glutamate (γMLG) or γ-benzyl-L-glutamate (γBLG). Some properties of the copolypeptides obtained will also be described briefly. The reason why we have chosen γMLG or γBLG as the other constituent in the copolypeptides is that poly-γ-methyl-L-glutamate (PMLG) and poly-γ-benzyl-L-glutamate (PBLG) have already been thouroghly studied by many investigators.[3] Moreover, the copolypeptides which contain these constituents can be readily converted to water-soluble copolypeptides by the cleavage of γ-ester groups in side chains.

We have carried out the copolymerization in various solvents, including dioxane, pyridine, acetonitrile and nitrobenzene, and have found that nitrobenzene is a solvent suitable for the preparation of copolymers of L-proline with γMLG or γBLG at various molar ratios.

To prepare the copolymers, about 4 g. of *N*-carboxy anhydrides (NCA's) of L-proline and L-glutamate in a desired molar ratio were dissolved or suspended in 100 ml. of a polymerization solvent. Sodium methoxide was used as the initiator for the polymerization. The solution was left standing for about a month. The solution, excepting that in nitrobenzene, became turbid with time, and a precipitate separated out in the course of the reaction. The precipitate thus separated is designated as a precipitate a. After the precipitate had been removed, ethyl ether was added to the solution. Then further precipita-

1) E. Katchalski and M. Sela, "Advances in Protein Chemistry," XIII, Academic Press, New York (1958), p. 243.

2) W. F. Harrington and P. H. von Hippel, ibid., XVI (1961), p. 1.

3) E. R. Blout, R. H. Karlson, P. Doty and B. Hargitay, *J. Am. Chem. Soc.*, **76**, 4492 (1954); P. Doty, J. H. Bradbury and A. M. Holtzer, ibid., **78**, 947 (1956).

TABLE I. PROPERTIES OF POLYPEPTIDES

Polypeptide	Content of L-proline			$[m']^*_{546}$	$[\eta]^{***}$ dl./g.
	at polymerization	from amino acid analysis	from absorption of benzyl residue		
Poly-L-proline	1.0	—	—	−41°**	0.436
Copoly-3:1-(L-proline, γ-benzyl-L-glutamate)	0.75	0.767	0.756	−74°	0.251
Copoly-1:1-(L-proline, γ-benzyl-L-glutamate)	0.50	0.535	0.528	−89°	0.165
Copoly-1:3-(L-proline, γ-benzyl-L-glutamate)	0.24	0.274	0.26	−50°	0.150
Poly-γ-benzyl-L-glutamate	0	—	0	+16°	0.405

* Measured in 1,2-dichloroethane.
** Measured in 1,2-dichloroethane - acetic acid (99.5—0.5%).
*** Measured in dichloroacetic acid at 25°C.

tion occurred. This precipitate is designated as the precipitate b. On the other hand, the solution in nitrobenzene remained clear throughout the reaction. When ether was added to the solution, the precipitate separated out as well. The precipitate thus obtained was different in composition from the precipitates a and b. In fact, the specific rotation at 546 mμ in dichloroacetic acid was −376° for the precipitate a and −266° for the precipitate b, when the NCA's of L-proline and γMLG were copolymerized in dioxane at a molar ratio of 4:1. As the specific rotation of PMLG was found to be −37°, and that of poly-L-proline, −510°, in dichloroacetic acid, the precipitate a would contain more proline residues than b. Copoly-1:1-(L-proline, γMLG) which was prepared in nitrobenzene was found to be readily soluble in chloroform, although both poly-L-proline and PMLG were insoluble in it.

Copolymerizations at different L-proline-to-γBLG ratios were performed in nitrobenzene. The amino acid compositions of these copolypeptides were determined by amino acid analysis and by means of studying the ultraviolet absorption at 258 mμ. It was found that the composition is as is shown in Table I.

Poly-L-proline, PBLG and the copolymers obtained above were all soluble in *m*-cresol, dichloroacetic acid and nitrobenzene. In chloroform, PBLG, copoly-1:3-(L-proline, γBLG) and copoly-1:1-(L-proline, γBLG) were soluble, whereas copoly-3:1-(L-proline, γBLG) was soluble in this solvent if the temperature of solution was raised. However, poly-L-proline was not soluble in chloroform. On the other hand, poly-L-proline is the only water-soluble polypeptide among these substances. When copoly-1:1-(L-proline, γBLG) was shaken with a water-chloroform system, no significant amount of the copolymer was found in the water layer. All of these polypeptides excepting poly-L-proline were soluble in 1, 2-dichloroethane. Poly-L-proline was soluble in 1, 2-dichloroethane containing only 0.5% acetic acid. This solution showed little mutarotation, just as the solutions of other copolymers in 1, 2-dichloroethane. The final values of the effective mean residue rotation at 546 mμ are also shown in Table I. As is well known, poly-L-proline can exist in two different forms in solution according to the nature of the solvent.[4] One of these is designated as poly-L-proline I, the specific rotation of which at 546 mμ is about +40°. The other, namely, poly-L-proline II, has its specific rotation of about −500°. Acetic acid is a solvent converting poly-L-proline I to II. Consequently, 1, 2-dichloroethane may be a solvent which stabilizes Form I of poly-L-proline. It has already been reported by Katchalski et al.[5] that copolymers can be formed in dioxane with great difficulty. They assumed that the marked tendency of the terminal prolyl residue of a growing chain to react with the NCA of L-proline in preference to the NCA of γBLG was the cause.

However, all the findings reported here support the conception that the true copolypeptides of L-proline with γ-L-glutamate can be formed when nitrobenzene is used as the polymerization solvent.

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4) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela and E. Katchalski, ibid., **82**, 5263 (1960).
5) E. Katchalski, A. Berger and J. Kurtz, "Aspects of Protein Structure," Ed. by G. N. Ramachandran, Academic Press, New York (1963), p. 205.