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The Synthesis of Poly[N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine] and Its Secondary Structure

Hiroyuki YAMAMOTO and Tadao HAYAKAWA

Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Ueda

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In a previous paper,[1,2] poly-γ-(*l*)-menthyl glutamate and poly-β-(*l*)-menthyl aspartate were synthesized and the secondary structures of these polymers were studied in order to investigate the effect of the side-chain with optically-active protect-group contributions to the polypeptide structure.

The syntheses of poly-L-lysine derivatives have been achieved in the preparation of the starting N^{ε}-benzyloxycarbonyl[3–5] and N^{ε}-trifluoroacetyl-L-lysine[6] as a monomer, and their chemical, physical, and biological

1) H. Yamamoto, Y. Kondo, and T. Hayakawa, *Biopolymers*, **9**, 41 (1970).

2) H. Yamamoto and T. Hayakawa, *ibid.*, **10**, 309 (1971).

3) E. Katchalski, J. Crossfeld, and M. Frankel, *J. Amer. Chem. Soc.*, **70**, 2094 (1948).

4) R. R. Becker and M. A. Stahmann, *ibid.*, **74**, 38 (1954).

5) T. Hayakawa, Y. Onchi, and J. Noguchi, *Nippon Kagaku Zasshi*, **80**, 81 (1959).

6) M. Sela, R. Arnon, and I. Jacobson, *Biopolymers*, **1**, 517 (1963).

properties have been studied extensively.[7-10] In the present study, poly-N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine was synthesized, and the secondary structure of the polymer was studied by means of the optical rotatory dispersion (ORD), the circular dichroism (CD), and infrared spectroscopy (IR) techniques. The route of synthesis is as follows:

$$\text{lys} \xrightarrow{CuCl_2} \text{lys}\cdot 1/2\text{Cu} \xrightarrow[(2)\ \text{EDTA}\cdot 2\text{Na}]{(1)\ (l)\text{-Menthyl chloroformate}} N^{\varepsilon}\text{-}(l)\text{-Menthyloxycarbonyl lys} \xrightarrow{COCl_2} N^{\varepsilon}\text{-}(l)\text{-Menthyloxycarbonyl lys NCA} \xrightarrow{TEA} \text{Poly-}N^{\varepsilon}\text{-}(l)\text{-menthyloxycarbonyl lys}$$

Since the menthyl chloroformate decomposes almost instantly on contact with water, the N^{ε}-menthyloxycarbonylation of lysine is unsuccessful when the Schotten-Baumann method is used in an aqueous solution. The copper complex was prepared by the reaction of lysine with cupric chloride in methanol. The complex was reacted with menthyl chloroformate to give N^{ε}-(*l*)-menthyloxycarbonyl lysine. Poly-N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine was prepared *via* N^{ε}-(*l*)-menthyloxycarbonyl-N^{α}-carboxy-L-lysine anhydride (NCA). The resulting polymer was obtained in a high yield and had a high molecular weight, as Table 1 shows. It was soluble in ethyl ether, tetrahydrofuran (THF), chloroform, dimethylformamide (DMF), trifluoroacetic acid (TFA), and dichloroacetic acid (DCA), and was insoluble in methanol.

Table 1. Molecular weight of poly[N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine]

Solvent	Monomer NCA (mg)	Yield mg (%)	Molecular weight (DP)[a]	$[\eta]$[b] dl/g
Dioxane	460	354(88)	12300(40)	0.158
DMF	460	331(82)	10100(33)	0.156

a) By titration of the amino endgroup with 0.02N perchloric acid.
b) In DCA at 25°C.

Experimental

(l)-Menthyl Chloroformate. *(l)*-Menthyl chloroformate was prepared from (*l*)-menthol, quinoline, and phosgene as has been described by Westley and Halpern.[11]

N$^{\varepsilon}$-*(l)-Menthyloxycarbonyl-L-lysine.* To a suspension of L-lysine hydrochloride (3.66 g, 0.02 mol) in 3 m*l* of methanol, 0.46 g of sodium in 10 m*l* of methanol and 1.70 g (0.01 mol) of cupric chloride in 10 m*l* of methanol were added.[12] After 30 min, the mixture was cooled to 0°C and 2.80 m*l* of triethylamine (TEA) were added. Then 0.024 mol of *(l)*-menthyl chloroformate in 30 m*l* of toluene and 3.34 m*l* of TEA were added. This solution was allowed to stand at 0°C for 2 hr and then at room temperature for 2 hr. The reaction mixture was filtered and dried; yield, 6.0 g. A suspension of the copper complex of N^{ε}-menthyloxycarbonyl-L-lysine in an aqueous ethylenediamine-tetraacetic acid 2Na (EDTA 2Na) was treated by the method described by Kuwata and Watanabe;[13] yield, 1.20 g (30%). The product was recrystallized from 20% acetic acid. 1.05 g (26%), mp 228°C. $[\alpha]_D^{22} = -40.9$ (*c* 1.0, acetic acid).

Found: C, 62.25; H, 9.97; N, 8.34%. Calcd for $C_{17}H_{32}N_2O_4$: C, 62.16; H, 9.82; N, 8.53%.

N$^{\varepsilon}$-*(l)-Menthyloxycarbonyl-L-lysine NCA.* Dry phosgene was passed through a suspension of N^{ε}-*(l)*-menthyloxycarbonyl-L-lysine (1.1 g) in 25 m*l* of dry dioxane for 1 hr at 50°C. Nitrogen was then passed through the reaction mixture for 30 min. The solvent was removed at 40°C under reduced pressure. The residual oily product was crystallized by treating it with *n*-hexane; yield, 1.0 g. This was recrystallized from ethyl acetate and *n*-hexane; yield, 0.97 g (82%). Mp 106—107°C.

Found: C, 61.04; H, 8.32; N, 7.81%. Calcd for $C_{18}H_{30}N_2O_5$: C, 60.99; H, 8.53; N, 7.90%.

*Poly-*N$^{\varepsilon}$*-(l)-menthyloxycarbonyl-L-lysine.* N^{ε}-*(l)*-Menthyloxycarbonyl-L-lysine NCA was dissolved in dioxane and DMF at the concentration of 10%. Triethylamine was added to each solution at NCA/initiator ratios of 100/1. The mixture was polymerized in a sealed tube at 15°C for 3 days, at 40°C for a day, and at 100°C for 3 hr. The results are summarized in Table 1.

Found: C, 65.14; H, 9.66; N, 8.86%. Calcd for $C_{17}H_{30}N_2O_3$: C, 65.78; H, 9.73; N, 9.03%.

Methods. The ORD, CD, and IR measurements were made on the respective ORD/UV 5 instruments, both made by the Japan Spectroscopic Co., Ltd. The optical rotations are expressed as a reduced molar residue rotation. As for CD, the measured values of $\varepsilon_L - \varepsilon_R$ were converted to the molar ellipticity.

Results and Discussion

The IR spectrum of the polymer showed absorptions at 1663 cm^{-1} for amide I and at 1530 cm^{-1} for amide II, suggesting the existence of the α-helical conformation.

The ORD and CD curves of the polymer in ethyl ether are shown in Fig. 1. The polymer exhibits a trough at 233 mμ with $[m']_{233} = -12100$ deg-cm²/dm. Moreover, two negative dichroism bands near 222 mμ and 206 mμ, with $[\theta]_{222} = -20000$ and $[\theta]_{206} = -26600$, are observed. This behavior is essentially identical with that of the right-handed α-helical form of the other polyamino acids. The polymer gave similar α-helical curves in THF ($[m']_{233} = -10000$) and in chloroform ($[m']_{233} = -9500$), and exhibited the ORD behavior of a random coil in TFA. The lower $[m']_{233}$ and $[\theta]_{222}$ values can be considered to result from the fact that this phenomenon arose from the interaction of the solvent and the bulky menthyloxycarbonyl chromophore.[1]

The helix→random coil transition for poly-N^{ε}-benzyloxycarbonyl-L-lysine (PCLL) occurs at 40% DCA in a DCA-$CHCl_3$ mixed solvent.[14] Plots of the $[\alpha]_{546}$ values *versus* the solvent composition of poly-N^{ε}-*(l)*-menthyl-

7) E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 243 (1958).
8) M. Sela and E. Katchalski. *ibid.*, **14**, 391 (1959).
9) J. Applequist and P. Doty, "Polyamino acids, Polypeptides and Proteins," ed by M. Stahmann, Univ. Wisconsin Press, Madison, Wisc. (1962), p. 161.
10) E. Daniel and E. Katchalski, *ibid.*, p. 183.
11) J. W. Westley and B. Halpern, *J. Org. Chem.*, **33**, 3978 (1968).
12) R. W. Hay and L. J. Porter, *Aust. J. Chem.*, **20**, 675 (1967).
13) S. Kuwata and H. Watanabe, This Bulletin, **38**, 676 (1965).
14) G. D. Fasman, "Polyamino acids, Polypeptides and Proteins," ed. by M. Stahmann, Univ. Wisconsin Press, Madison, Wisc. (1962), p. 221.

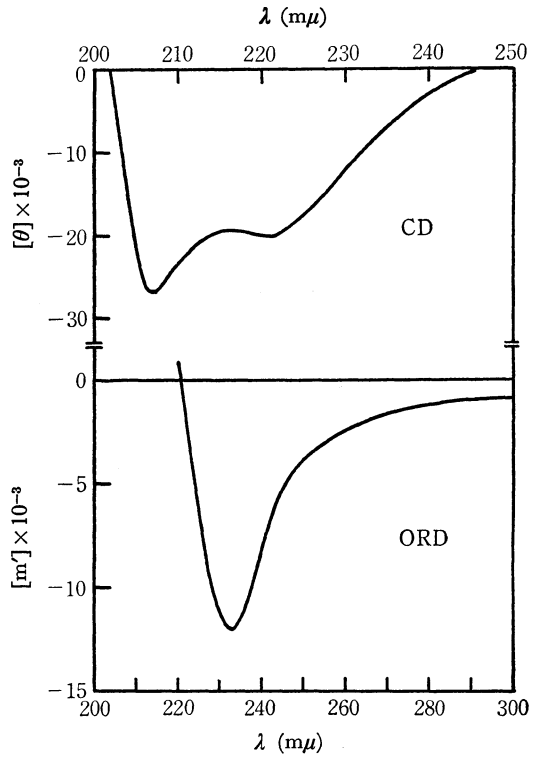

Fig. 1. ORD and CD of poly[N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine] at 22°C in ethyl ether.

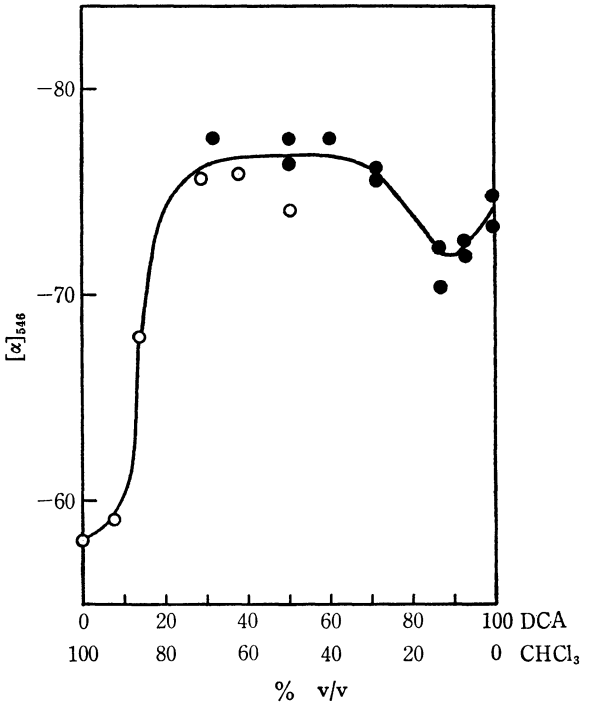

Fig. 2. Specific rotation $[\alpha]_{546}$ values of poly[N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine] of varying solvent composition: (○), polymer dissolved in chloroform (solution diluted with DCA); (●), polymer dissolved in DCA (solution diluted with chloroform); at 22°C.

oxycarbonyl-L-lysine are shown in Fig. 2. This polymer causes a sharp transition at about 15% DCA. In comparison with PCLL, the helical structure of poly-N^{ε}-(*l*)-menthyloxycarbonyl-L-lysine is made rather unstable by the introduction of the N^{ε}-(*l*)-menthyloxycarbonyl group.