THE OPTICAL ROTATORY DISPERSION OF THE β STRUCTURE OF POLY-L-LYSINE AND POLY-L-SERINE[1]

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Optical rotatory dispersion (ORD) has become an important technique among those used to study the conformation of proteins and synthetic polypeptides in solution (Blout, 1960; Urnes and Doty, 1961; Fasman, 1963). Although the α-helical and random conformations have been characterized in aqueous solution by far-ultraviolet ORD (Blout et al., 1962; Yang and McCabe, 1965; Iizuka and Yang, 1965), the β-conformation has been studied only in the visible and near ultraviolet spectral regions (Fasman and Blout, 1960; Wada et al., 1961; Bradbury et al., 1962; Harrap and Stapleton, 1963; Ikeda et al., 1964; Imahori and Yahara, 1964). These measurements are far removed from the electronic transitions responsible for the Cotton effects. The solid state far ultraviolet ORD spectrum has been reported for one β-structure (Blout and Shechter, 1963).

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[1] For the previous paper in this series see Sage, H. J. and Fasman, G. D., Biochemistry 5, 286 (1966).

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We wish to describe the far ultraviolet (280 to 185 mμ) ORD parameters of the β conformation of a poly-L-serine film and of poly-L-lysine in aqueous solution.

X-ray diffraction studies and infrared data (Imahori and Yahara, 1964; Bohak and Katchalski, 1963) provide evidence that in the solid state poly-L-serine possesses the β conformation as does its O-blocked derivatives (Fasman and Blout, 1960; Bradbury et al., 1962; Imahori and Yahara, 1964; Yahara and Imahori, 1963; Yahara et al., 1963). The ORD curve, in the 200-290 mμ region, of a film of poly-L-serine is seen in Fig. 1. This curve is similar to that reported by Blout and Shechter (1963) for the ORD of an oriented film of the β-structure of poly-L-isoleucine.

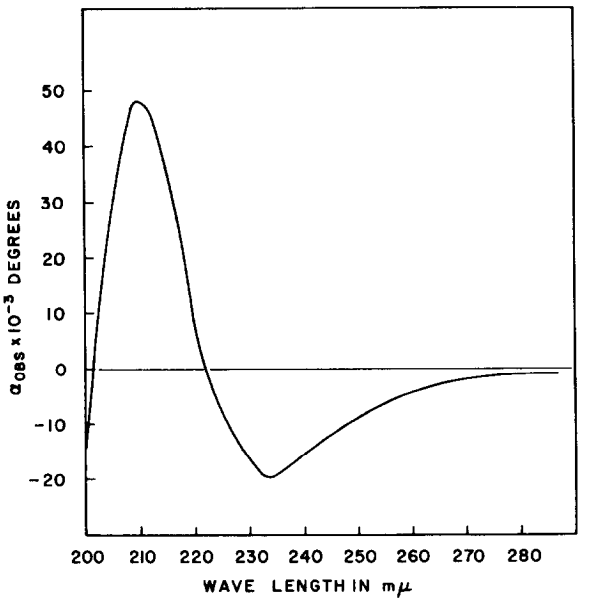

Fig. 1. Poly-L-serine (#NT-2-374-27) cast on a quartz disc from trifluoroacetic acid. The polymer was prepared by unblocking poly-O-tertiary-butyl-L-serine, with anhydrous HCl and HBr in benzene. Specific viscosity of blocked polymer in dichloroacetic acid (0.2%) is 0.52.

Previous studies, utilizing the techniques of ultraviolet and infrared spectroscopy (Blout and Lenormant, 1957; Applequist and Doty, 1958, 1962; Rosenheck and Doty, 1961) and X-ray diffraction (Shmueli and Traub, 1965), have shown that under certain conditions poly-L-lysine undergoes a transition from an α-helical to a β conformation. In the work reported here, the α → β transi-

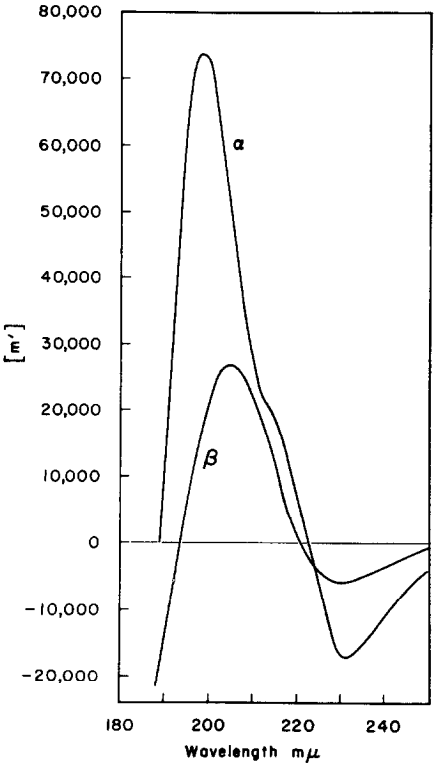

Figure 2. The ORD of α and β poly-L-lysine

α: Poly-L-lysine·HCl (Sample BD-1-10-28, intrinsic viscosity in 1M NaCl at pH 4.0 = 0.67) was dissolved in water and the pH adjusted to 11.06 with NaOH. The 0.0123% solution was filtered through an alkali resistent millipore filter into a water jacketed cell of 1 mm path length. The ORD was determined at 22.%°C.
β: After obtaining curve α, the sample was heated, 51°C, in situ for about 15 minutes. When no further decrease in $[m']_{199\ m\mu}$ was noted, the cell and contents were cooled to 22.5° and curve β was obtained.
A Cary 60 spectropolarimeter was used for the measurements.
The solvent reference solution was water adjusted to pH 11.08.
Polymer concentration was determined by a modified micro-biuret procedure (Zamenhof and Chargaff, 1963).

tion was accomplished by heating (Applequist and Doty, 1958, 1962; Rosenheck and Doty, 1961) an aqueous solution of the helical polymer at around 50° until no further change in $[m']_{199}$ mμ was noted. At this point the transition was judged complete. ORD curves of the initial α and final β form of poly-L-lysine are shown in Fig. 2. The experimental conditions are described in the legend. The rate of the α → β transition was increased by increasing either the temperature or concentration of the polymer solution. The latter observation might indicate an intermolecular β rather than an intramolecular cross-β structure. Table I summarizes the ORD parameters of aqueous solutions of α and β poly-L-lysine and a film of β poly-L-serine. The positions of the ORD peak and trough for the α and β forms of poly-L-lysine agree well with the circular dichroism data (Townend et al., 1966) for the same preparation. The wavelength of the ORD trough of the random form of poly-L-lysine is similar in position, but perhaps different in magnitude than that reported for random poly-L-glutamic acid (Blout et al., 1962; Iizuka and Yang, 1965) (Table I). The cross over points at wavelengths in the 190 mμ region correspond to the reported positions of the ultraviolet maxima for the α-helical and β forms of the polymer (Rosenheck and Doty, 1961; Tinoco et al., 1962)(Table I). It should be noted that the ORD film parameters are slightly displaced from those in solution.

The a_o and b_o values obtained from the Moffitt equation (Moffitt, 1956; Moffitt and Yang, 1956) have frequently been used to evaluate the various polypeptide conformations. Estimates of the b_o and a_o values of the β-structure, using λ_o = 212 mμ, have varied from b_o = 0 to +420, and a_o = 350 to 840 (Fasman and Blout, 1960; Wada et al., 1961; Bradbury et al., 1962; Harrap and Stapleton, 1963; Ikeda et al., 1964). The values found in this study using the wavelength region 460-278 mμ are b_o = -241±17 and a_o = -62±5.

Table I

	ORD Trough		ORD Peak		Cross-over wavelengths	Reported U.V. Maximum
	position	[m']a	position	[m']a		
Poly-L-serine, film	≈233 mμ		210 mμ		222 mμ,	
Poly-L-lysine, α	233 mμ	-15,700±870b	198.6 mμ	+74,900±2470b	223 mμ, 190 mμ	190 mμ[1,2]
Poly-L-lysine, β	230 mμ	-6,220±590b	205 mμ	+29,200±1380b	220 mμ, 194 mμ	194 mμ[1]
Poly-L-lysine·HCl random conformation	204.5 mμ	-25,000±300			196-197 mμ	192 mμ[1]
Poly-L-glutamate random conformation						
(Blout et al., 1962)	204 mμ	≈-16,000			≈198 mμ	
(Iizuka and Yang, 1965)	204 mμ	≈-17,500			≈197.5 mμ	
(Holzwarth and Doty, 1965) ORD, calculated from circular dichroism	205 mμ	≈-22,000			≈202 mμ	

[a] Reduced mean residue rotation; Fasman, 1963

[b] Average of eight determinations

[1] Rosenheck and Doty, 1961

[2] Tinoco et al., 1962

From the wide range of values reported it becomes evident that the various β structures, parallel, anti-parallel and cross-β, may yield different b_o and a_o values. Moreover, the extent of β formation must also be dependent on chain length, degree of overlap of chains, etc.

These experiments illustrate that a single polypeptide chain is capable of assuming more than one conformation. As the final structure is the β conformation, it must have a greater thermodynamic stability than the α. We propose the following mechanism of formation: α → random chain → β. The added stability of the β structure may result from side chain hydrophobic interactions not present in the α-form. The enthalpy necessary to break the hydrogen bonds of the helical structure, and to associate the hydrophobic side chains is derived from the heating process.

It should be noted that the β-conformation has significant rotation at 233 mμ. Thus, estimates of helical content of proteins based on the measurement of $[m']_{233}$ will be in error to the extent that β-structures are also part of the protein secondary structure.

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