THE CIRCULAR DICHROISM OF THE β STRUCTURE OF POLY-L-LYSINE

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Optical rotatory dispersion (ORD) studies carried out over the past few years in the far ultra-violet region have resulted in indentification of the Cotton effects associated with the α-helical and disordered conformations of polypeptides and proteins (Blout _et al._, 1962; Yang and McCabe, 1965). The third general type of structure, the β, or pleated sheet conformation, has not yet been characterized in detail by ORD, since polypeptides are usually insoluble when in this form. The recent demonstration that poly-L-lysine will assume the β conformation in dilute aqueous solution when heated gently (Rosenheck and Doty, 1961; Applequist and Doty, 1962; Davidson _et al._, 1966) has permitted measurement of the far ultra-violet ORD parameters of this important hydrogen-bonded structure (Davidson _et al._, 1966).

ORD spectra consist of infinitely broad bands which frequently overlap

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Abbreviations used: ORD, optical rotatory dispersion; CD, circular dichroism.

and thus render impossible an unequivocal assignment of the responsible electronic transitions. These transitions, however, are also reflected in the circular dichroism (CD) spectrum. The bands observed here are relatively narrow, so that complications due to overlapping are greatly reduced and band assignment is usually more feasible. Furthermore, the proper resolutions of a CD spectrum into discrete bands can be checked by calculating from them the corresponding ORD spectrum and comparing it with experiment. Since each CD band is associated with a Cotton effect in ORD, the calculated ORD spectrum, if bands have been resolved correctly, will be identical with the experimentally determined one. Such an analysis of the CD spectra of some polypeptides in α-helical and disordered conformations have been reported recently (Holzwarth _et al._, 1962; Brahms and Spach, 1963; Grosjean and Tari, 1964; Beychok and Fasman, 1964; Holzwarth and Doty, 1965; Velluz and Legrand, 1965).

In this paper, we report a far ultra-violet CD study of poly-L-lysine in aqueous solution in both the β and α-helical conformations, and compare the ORD parameters calculated from these results with those found experimentally on the same material.

The poly-L-lysine samples used in this study were the same as those used in the preceding article (Davidson _et al._, 1966). Experimental conditions were essentially identical to those described in that paper and are summarized in Fig. 1. The CD spectra were measured from 250 mμ to 184 mμ with a Durrum-Jasco ORD/UV 5 apparatus.[+] Polymer concentrations were determined by micro-Kjeldahl analysis. Theoretical ORD curves were calculated from the CD data by using the Krönig-Kramers transform (Moffitt and Moscowitz, 1959) as previously described (Beychok and Fasman, 1964; Moscowitz, 1960, 1961); this computation was carried out on a CDC-160A computer[*] using a Fortran program.

[+] It is not implied the U. S. Department of Agriculture recommends the above company or its product to the possible exclusion of others in the same business.

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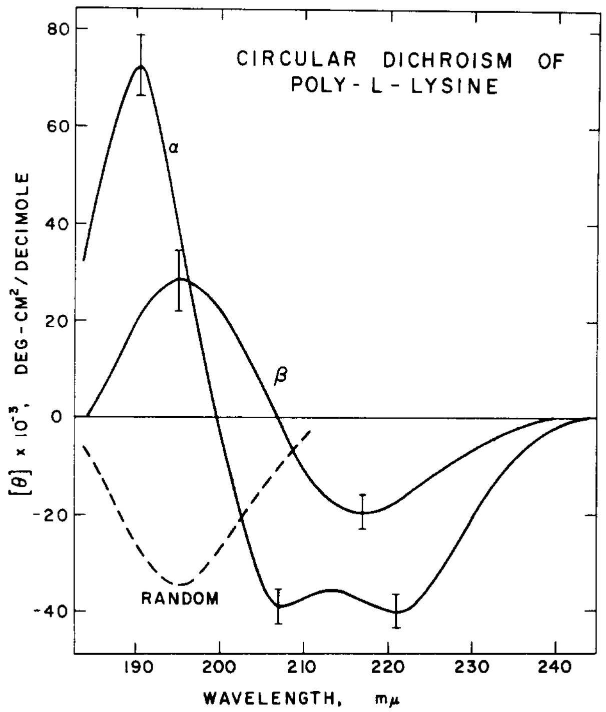

Fig. 1. The CD of poly-L-lysine in the α-helical and β conformations. The poly-L-lysine was dissolved in H_2O and the pH was adjusted to 11.2. The resulting 0.24 g/l solution was filtered through an ultra-fine sintered glass filter and the α-helical CD curve was determined at 25° C. The sample was heated then to 51° C until the shape of the CD band between 200 and 240 mμ did not change any longer (after cooling to 25° C for the measurements). All measurements were carried out between 184 and 250 mμ in 0.11 and 1.0 mm cells with fused silica windows. The signal to noise ratio was 4:1 at 190 mμ and 7:1 at 220 mμ in the case of the β structure experiments and 8:1 at 190 mμ and 16:1 at 220 mμ in the α-helix experiments. The dashed line represents similar data on poly-L-lysine in the random conformation (pH 8.0).

Results obtained with poly-L-lysine in the α-helical and β conformations are shown in Fig. 1 and summarized in Table I. The CD spectrum of the β conformation is characterized by two bands, a negative one centered at 217 mμ with an ellipticity, [θ], of -19,300 degree-cm^2/decimole, and a positive band at 195 mμ with an ellipticity of +27,900. The crossover point is at 207 mμ, and the CD spectrum rejoings the baseline at 184 and 240 mμ. The ORD curve calculated from this CD spectrum is shown in Fig. 2. Each CD band gives rise to a Cotton effect, a negative one centered at 217 mμ with a trough at 230 mμ

Table I

Circular Dichroism Parameters of Poly-L-Lysine

Peak Position	[θ]	Crossover Position
A. β Structure		
195 mμ	27,900 $\pm$ 4,400	207 mμ
217	-19,300 $\pm$ 3,000	
B. α-Helix		
190.5 mμ	71,500 $\pm$ 6,100	200 mμ
207	-38,900 $\pm$ 3,900	(199)[a]
(207)[a]	(-33,000)[a]	
221	-39,800 $\pm$ 3,900	
(222)[a]	(-32,000)[a]	

[a] Data of Holzwarth and Doty, 1965.

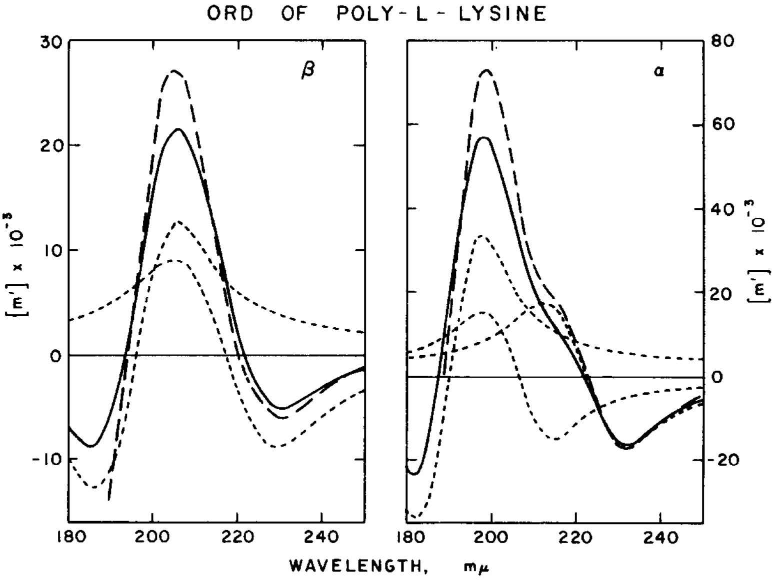

Fig. 2. ORD curves of poly-L-lysine calculated from CD data. Dotted lines: Cotton effects resulting from each individual CD band; solid lines: theoretical ORD spectrum (sum of dotted lines); dashed lines: experimental data of Davidson et al. (1966).

and a peak at 204 mμ, and a positive effect centered at 196 mμ with a peak at 206 mμ and a trough at 186 mμ. These are shown by the dotted lines of Fig. 2. Their sum is the theoretical ORD curve, shown by the solid line. This curve is characterized by a positive peak at 206 mμ, negative troughs at 186 and 230 mμ and crossover points at 193 and 222 mμ. Comparison of this calculated ORD curve with the experimental one (Davidson et al., 1966) (dashed line of Fig. 2) shows good agreement between the calculated and measured positions of the peak, the 230 mμ trough and the crossover points. The amplitudes of the calculated ORD peak and trough are less than the experimental ones, but since the error of measurement of the CD curves is of the order of 15% and the calibration of the instrument used results in an uncertainty of ca. 10%, this discrepancy is not surprising. Furthermore, under the conditions of the present experiments, it is uncertain whether the poly-L-lysine is totally converted into the β conformation; in fact, it is reasonable to expect that chain ends are disordered. Therefore, the reported amplitudes of the CD and ORD bands should be viewed with caution; the positions of these bands, on the other hand, can be considered as essentially established. While band assignments in terms of electronic transitions will not be attempted at this point, it should be noted that Rosenheck and Doty (1961) have reported that the UV absorption spectrum of poly-L-lysine in the β conformation is characterized by a band with a maximum at 194 mμ, skewed in the direction of longer wavelengths. The rotational strengths of the two β structure CD bands are calculated to be 19.0×10^{-40} and -14.5×10^{-40} erg-cm^3-rad. The Moffitt-Yang (1956) (Moffitt, 1956; Moffitt et al., 1957) a_o and b_o parameters, obtained from the calculated ORD curve, are 70 and -215, respectively. When λ_o is taken as 212 mμ, the data give a straight line from 350 to 570 mμ and the values of a_o and b_o are in reasonable agreement with experiments (Davidson et al., 1966). Examination of these a_o and b_o values suggests an explanation for certain reported "anomalous" combinations of these parameters, if the conformational analysis is limited to α-helical and disordered structures only, as has been a common practice when dealing with proteins (for a discussion of this, see, for example, Urnes and Doty, 1961).

The generic term "β conformation" is used to describe a number of related pleated sheet structures, some of which can be differentiated by the positions of their amide I absorption bands in the infrared spectral region (Miyazawa, 1960; Miyazawa and Blout, 1961; Krimm, 1962). With this in mind, infrared studies and CD experiments were done on poly-L-lysine solutions in D_2O, which is transparent in the region of amide I and amide II absorption. The resulting infrared spectra were characterized by an amide I band centered at 1637 cm^{-1} in the case of the α-helical structure and two sharp bands at 1612 and 1680 cm^{-1} for the β-structure. These positions have been reported previously for poly-L-lysine in the α and the β-conformations in D_2O (Rosenheck and Doty, 1961; Miyazawa, 1962; Elliott, 1962). The presence of the band at 1680 cm^{-1} suggests that the β structure formed by poly-L-lysine in solution is an antiparallel pleated sheet (Krimm, 1962; Miyazawa, 1962; Elliott, 1962). The conclusion of an intermolecular stacked structure was also reached from the concentration dependence of α → β conversion reaction (Davidson _et al._, 1966). The CD spectra in D_2O were found to be identical with those measured in H_2O.

Our CD data on the α-helical form of poly-L-lysine (Fig. 1) agree well with data reported by Holzwarth and Doty (1965) for wavelengths greater than 198 mμ (see Table I). Furthermore, the positive ellipticity maximum of 71,500 degree-cm^2/decimole at 190.5 mμ, reported in the present paper, compares well with the value reported by these authors for α-helical poly-L-glutamic acid (Fig. 2 of their paper). The calculated ORD curve is shown in Fig. 2. We have also determined the CD spectrum of poly-L-lysine below 210 mμ at pH 8.0, where it is in the random conformation. The resulting curve, shown by the dashed line of Fig. 1, exhibits a single negative band centered at 196 mμ. The ORD curve, calculated from this band, consists of a single negative Cotton effect, centered at 195.5 mμ with a trough at 206 mμ and a peak at 185 mμ.

The present determination of the circular dichroism parameters for at least one type of β structure should be of use in the study of the conformations of proteins in solution, particularly in view of the report by Blake *et al.*, (1965) that lysozyme contains a significant amount of pleated-sheet structure. Preliminary examination of the CD of native β-lactoglobulins (pH 5) has resulted in a spectrum with a positive maximum at 193 mμ and a negative one at 216 mμ. These data correlated well with a recent infrared examination of this protein (Timasheff and Susi, 1966) which shows that the predominant absorption band in the amide I region is at a position characteristic of the β structure.

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