THE OPTICAL ROTATORY DISPERSION OF TWO BETA STRUCTURES[1]

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(Publication #489)

Received March 16, 1967

The importance of the conformational evaluation of proteins increases as the topology of enzymes, antibodies, etc. becomes evident through X-ray diffraction studies, active site investigations and antigen-antibody interactions. Optical rotatory dispersion (ORD) has played a significant role in the estimation of the conformation of proteins and synthetic polypeptides (Blout, 1960; Urnes and Doty, 1961; Fasman, 1963). The recent discovery of the ORD of the β structure in solution (Davidson *et al.*, 1966; Sarkar and Doty, 1966) has now made available the ORD patterns of the three basic structures, α, β and random coil, thought to exist in proteins. Three β conformations have been postulated for proteins (Pauling and Corey, 1951; Rudall, 1952), the intermolecular parallel and antiparallel hydrogen-bonded sheets, and the intramolecular hydrogen bonded cross β structures. Their differentiation has been based mainly on infrared dichroism studies (Miyazawa and Blout, 1961; Krimm, 1962) and has not yet been possible by ORD. We wish to report the ORD spectra of films of

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[1] Supported in part by grants from the National Science Foundation (GB 5576), The National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U.S. Public Health Service (AM-0582) and the U. S. Army Medical Research and Development Command, Department of the Army, under Research Contract #DA49193-MD-2933.

several poly-α-amino acids, all of which have been found to exist in the β conformation by previous infrared dichroic studies (see Table I). These ORD spectra can be divided into two classes. Form I-β has a trough at 230 mμ and a peak at 205 mμ; Form II-β has troughs at 238 mμ and 194 mμ with a peak in the 211-215 mμ region. It is suggested that these spectra represent two different β forms.

The poly-α-amino acids listed in Table I were prepared by the polymerization of their respective N-carboxyanhydrides. Films were cast onto quartz discs by evaporation of solutions of the polymers in trifluoroacetic acid. Samples were prepared in a glove bag, containing potassium hydroxide, through which flowed a mild stream of nitrogen. Oriented samples were obtained by

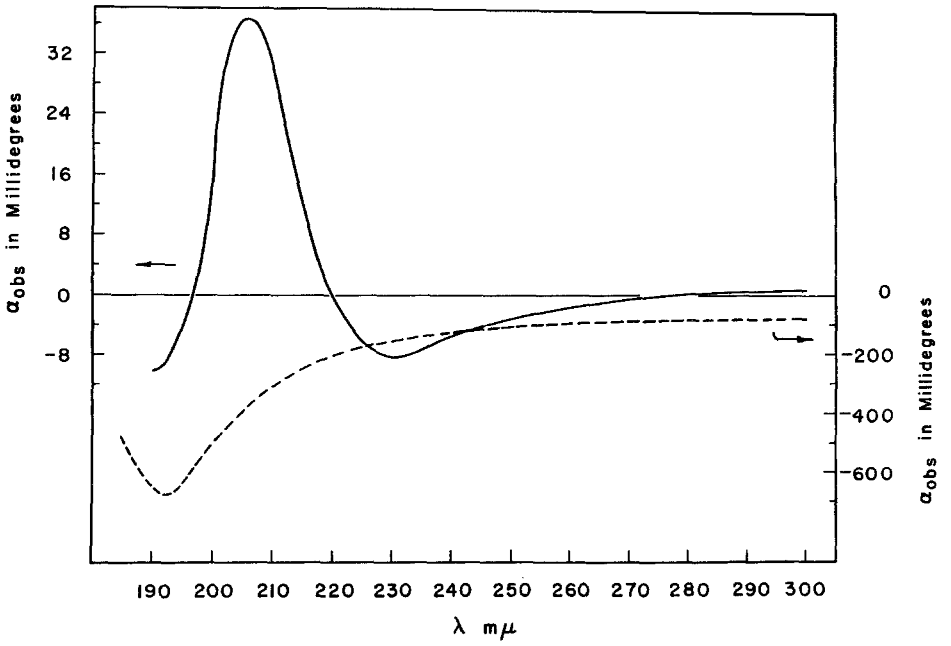

Figure 1. The ORD spectra of films of poly-O-acetyl-L-serine: ———, unoriented, $O.D._{195} = 1.2$; -----, oriented, $O.D._{195} = 0.3$.

stroking the film during evaporation with a teflon clad finger. The films were placed under vacuum (10^{-2} mm Hg) in a dessicator for final removal of solvent. The ORD was run on samples having A_{195} = 0.2 to 1.3 using a Cary 60 spectropolarimeter.

In Fig. 1 is seen the ORD spectra of poly-O-acetyl-L-serine of both unoriented and oriented specimens. The unoriented film displays a trough at 230 mμ, a peak at 206 mμ with cross over points at 220 mμ and 197 mμ. This ORD spectrum is very similar to that reported for the β form of aqueous solutions of poly-L-lysine (Davidson _et al._, 1966) and for a film of poly-L-isoleucine (Blout and Shechter, 1963). In Fig. 2 is seen the ORD spectrum of poly-S-benzyl-L-cysteine, unoriented and oriented. In the unoriented specimen, the trough lies in the 240 mμ region, peak at 211-215 mμ with a second trough at 194 mμ. Fig. 3 displays the

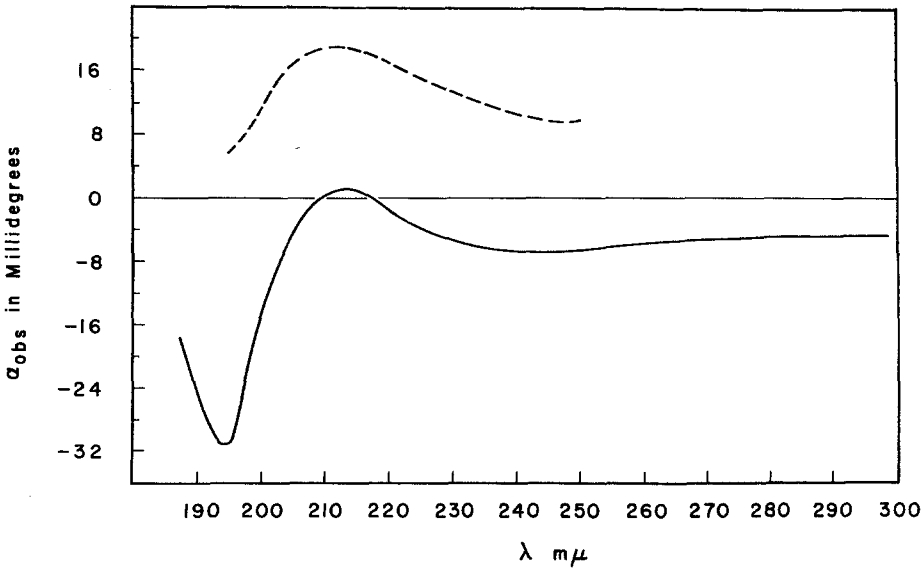

Figure 2. The ORD spectra of films of poly-S-benzyl-L-cysteine: ———, unoriented, $O.D._{195}$ = 1.21; -----, oriented, $O.D._{195}$ = 0.48.

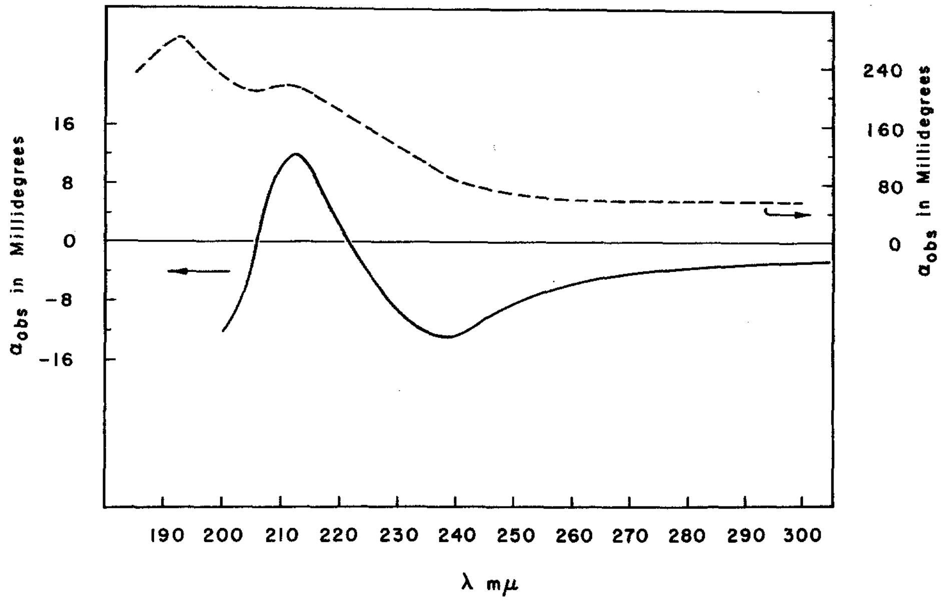

Figure 3. The ORD spectra of films of poly-S-methyl-L-cysteine: ———, unoriented, O.D.$_{195}$ = 0.87; -----, oriented, O.D.$_{195}$ = 0.51.

ORD spectra of poly-S-methyl-L-cysteine (unoriented and oriented); the unoriented spectrum is similar to that found in Fig. 2. These ORD spectra are similar to those found in solution for poly-L-serine (Tooney and Fasman, 1967) and poly-S-carboxymethyl-L-cysteine (Ikeda and Fasman, 1967).

Thus, two different ORD spectra can be identified, to be designated form I-β and form II-β. Form I-β is seen in Fig. 1 and form II-β in Figs. 2 and 3. In Table I is listed several poly-α-amino acids classified into forms I and II.

Upon orientation of poly-O-acetyl-L-serine (Fig. 1) and poly-O-t-butoxy-L-serine, the spectra change to yield a trough[2] at 192 mμ, possibly changing from form I-β to form II-β, or giving yet

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[2] The sign of oriented films cannot be assigned. By exposing the opposite face of the quartz plate to the incident beam, the sign of the Cotton effect can be changed from plus to minus.

another β spectrum. Orientation of form II-β causes a splitting of the major peak, e.g. the poly-S-methyl-L-cysteine peak at 213 mμ splits into two peaks at 212 mμ and 195 mμ (Fig. 3). However, interpretation of oriented spectra will not be attempted due to the difficulties of anisotropy of absorption, refraction or scattering.

The assignment of two β forms is entirely based upon the ORD of the unoriented films and has been corroborated by recent solution studies (to be published). The rotation observed for unoriented films showed little or no dependence on rotating the quartz disc, and the magnitude of the observed rotation was proportional to the film thickness. This would indicate negligible birefringence. The absorption curves for these films do not coincide with the ORD curves and thus linear dichroism cannot be contributing greatly to the rotation.

The form I-β ORD curve of films has been found for the polypeptide O-derivatives of serine, poly-L-valine and poly-isoleucine. Previously, poly-O-acetyl-L-serine has been shown to be capable of forming both the cross-β structure and the parallel β structure (Johnson, 1959; Fasman and Blout, 1960; Yahara and Imahori, 1963; Yahara _et al._, 1963) while poly-L-valine has been reported as having the cross-β structure (Blout _et al._, 1960; Bloom _et al._, 1962; Fraser _et al._, 1965a). Solution studies on poly-L-lysine (Davidson _et al._, 1966; Sarkar and Doty, 1966) have yielded ORD curves of similar form. The theoretical calculations of Pysh (1966) and the infrared studies of Blout and Lenormant (1957), Rosenheck and Doty (1961) and Townend _et al._ (1966) have assigned the poly-L-lysine spectra to that of the antiparallel-β, while Rosenheck and Sommer (1967) suggest the parallel-β mixed with the antiparallel structure. The form II-β ORD curve of films has been found for the

Table I

Polypeptide	Form I-β			Form II-β			Conformation
unoriented film	Trough mμ	Peak mμ	Cross over mμ	Trough mμ	Peak mμ	Cross over mμ	as determined by infrared dichroism
Poly-L-lysine[a]	230	205	220, 194				antiparallel-β^{1-3}
Poly-O-acetyl-L-serine	230	206	220, 197				cross β^{4-7} antiparallel-β
Poly-O-t-butoxy-L-serine	230	205	222, 198				β^{8}
Poly-O-benzyl-L-serine	230	205	224, 222				cross $\beta^{9,10}$
Poly-L-valine	230	205	215, 201				antiparallel-β^{11-13} cross β
Poly-L-serine				233	210	222	$\beta^{14,10}$
Poly-S-benzyl-L-cysteine				238-240 194	211-215		cross $\beta^{11,15}$
Poly-S-methyl-L-cysteine				238	213	222, 205	β^{11}
Poly-S-carbobenzoxy-L-cysteine					215	204	cross β^{16}
Poly-S-carbobenzoxy-methyl-L-cysteine				238-242 194	215	232, 205	cross $\beta^{17,18}$

[a] Solution

[1] Blout and Lenormant, 1957

[2] Rosenheck and Doty, 1961

[3] Townend *et al.*, 1966

[4] Fasman and Blout, 1960

[5] Yahara and Imahori, 1963

[6] Johnson, 1959

[7] Yahara *et al.*, 1963

[8] Fasman and Tooney, 1967

[9] Bradbury *et al.*, 1962

[10] Bohak and Katchalski, 1963

[11] Blout *et al.*, 1960

[12] Bloom *et al.*, 1962

[13] Fraser *et al.*, 1965a

[14] Davidson *et al.*, 1966

[15] Fraser *et al.*, 1965b

[16] Elliott *et al.*, 1964

[17] Ikeda *et al.*, 1964

[18] Anufrieva *et al.*, 1965

polypeptide derivative of cysteine and for poly-L-serine. Similar curves in solution have been found for poly-L-serine (Tooney and Fasman, 1967) and poly-S-carboxymethyl-L-cysteine (Ikeda and Fasman, 1967). The polymers of S-benzyl, (Blout et al., 1960; Fraser et al., 1965b), S-carbobenzoxy (Elliott et al., 1964), and S-carbobenzoxymethyl (Ikeda et al., 1964; Anufrieva et al., 1965) derivatives of cysteine have been assigned the cross β structure.

Thus, both in films and in solution two distinct β ORD spectra have been identified. An assignment of these forms, based upon correlation with infrared dichroism spectra, is not possible at present. This is due to the fact that a single polypeptide is capable of existing in more than one β form.

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