ESTIMATION OF HELICITY OF PROTEINS FROM OPTICAL ROTATION DISPERSION MEASUREMENTS

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The phenomenological equation proposed by Moffitt and Yang (1956) has been widely used for the estimation of helicity of proteins from optical rotation dispersion (ORD) data. Implicit in the use of this equation is the assumption that the ORD in the visible and near ultraviolet regions is a reflection solely of Cotton effects associated with the peptide linkage in helix and coil forms. Recently, in studying Cotton effects in tyrosine and tyrosine polymers, Hooker and Tanford (1964) and Fasman et al. (1964) have pointed out that uncritical interpretation of ORD data for proteins containing large amounts of amino acids with chromophoric side chains may lead to serious errors.

The measurements to be reported here for α lactalbumin are a striking illustration of the large errors that may be incurred with such a protein. α Lactalbumin contains 5 tyrosine and 5 tryptophan residues out of a total of 126 (Gordon and Ziegler, 1955). The comparative ORD properties of the native protein (pH 6) and the acid denatured form (pH 4 and below) (Kronman et al. 1964, Kronman et al. 1965) are the subject of this communication.

ORD Measurements: Measurements of ORD were made with a Rudolph MSP-4 double monochromator manual spectropolarimeter. Data for the Yang-Moffitt analysis was obtained with lines (313, 334, 365, 405, 436, 547, and 578 mμ) from a low pressure mercury lamp, while ORD spectra in the ultraviolet region were measured with an Osram XBO-450 xenon source. Band widths were 1.5 mμ or less over the entire wavelength range measured. Protein concentrations were chosen to insure an absorbance of 2 or less over the range of ORD measurement.

Results: Plots of ORD data (578 to 313 mμ) in the form of the Yang-Moffitt equation revealed small, but quite reproducible deviations from linearity at pH 6 but good fits below pH 3. The results shown in Table I

TABLE I

Helix Content and b_0 for Native and Acid Denatured α Lactalbumin

Protein[b]/range	$-b_0$		% helix[a]	
	313-578 mμ	365-578 mμ	313-578 mμ	365-578 mμ
	(1)	(2)	(3)	(4)
Native (pH 6)	247 ± 10.4	214 ± 2.4	39	34
Denatured (pH 2)	151 ± 1.5	151 ± 3.3	24	24

[a] % helix = $-100 \frac{b_0}{630°}$ [b] Solvent 0.15 M KCl, temperature 25°

for each pH were obtained with a single protein solution of concentration of about 0.7 g/100 ml. Regression lines were computed using a value for λ_0 of 212. Values of b_0 for two wavelength ranges were computed from the same set of data. At pH 6, b_0 values obtained from the full wavelength range were invariably higher than those from the truncated range and the average deviation was much larger. Values of b_0 obtained for acid denatured protein, on the other hand, were insensitive to the wavelength range used. This type of behavior was noted in all measurements made at these pH values (about 12 sets of experiments at each pH).

The conventional interpretation of the data would lead to the conclusions that: (a) About 1/3 of the backbone of the α lactalbumin molecule is in helical form; and (b) denaturation is accompanied by melting out of about 1/3 of this structure. Extension of the ORD measurements to wavelengths below 313 mμ demonstrates that neither of these conclusions is correct.

Shown in Figure 1 are ORD spectra for the wavelength range 250 to 360 mμ plotted in Yang-Moffitt form. The pH 6 curve shows distinct peaks at about 300 and 290 mμ, a trough at 292 mμ and a relatively flat region at about 275 mμ. The pH 2 curve, although devoid of well defined

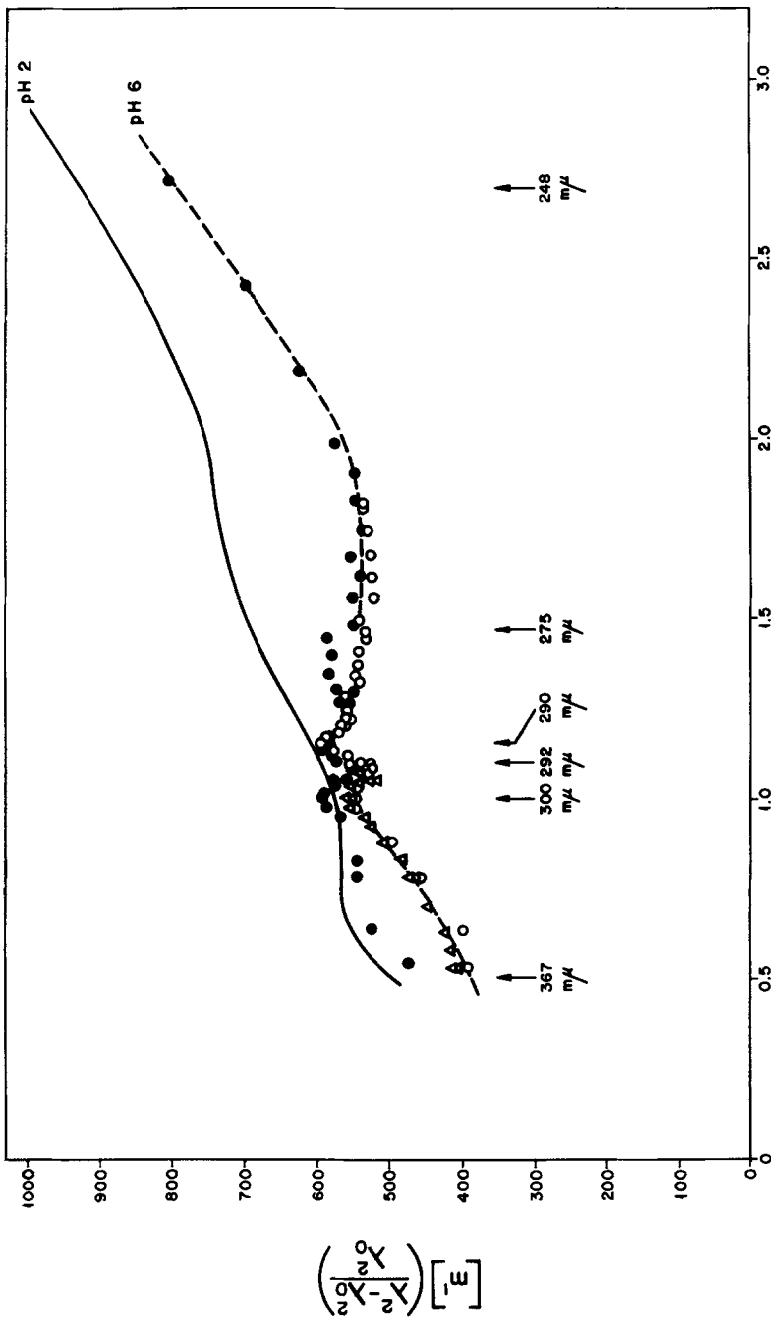

Figure 1. Yang-Moffitt plots of ORD data for α-lactalbumin. Native protein (pH 6); ●, conc. 1.045 g/100 ml., 0.1 cm. path length; ○, conc. 0.190 g/100 ml, 1 cm. path length; △, conc. 0.461 g/100 ml, 1 cm. path length. Denatured protein, (pH 2) solid curve. The experimental points have been omitted in the interest of clarity. Protein concentration comparable to those used at pH 6. Temperature 25° C. Solvent 0.15M KCl.

structure, shows indications of inflections near 300 mμ and below 275 mμ. These minima and maxima, occurring as they do in the 280 to 300 mμ absorption region are indicative of Cotton effects associated with tyrosine or tryptophan residues and appear to be the source of the systematic deviation in Yang-Moffitt plots at pH 6.

The pH dependence of the change in b_0, which will be described in greater detail in a future paper, roughly parallels that of the tryptophan difference spectrum (Kronman et al. 1965) and of tryptophan fluorescence (Kronman, manuscript in preparation) of α lactalbumin. It seems

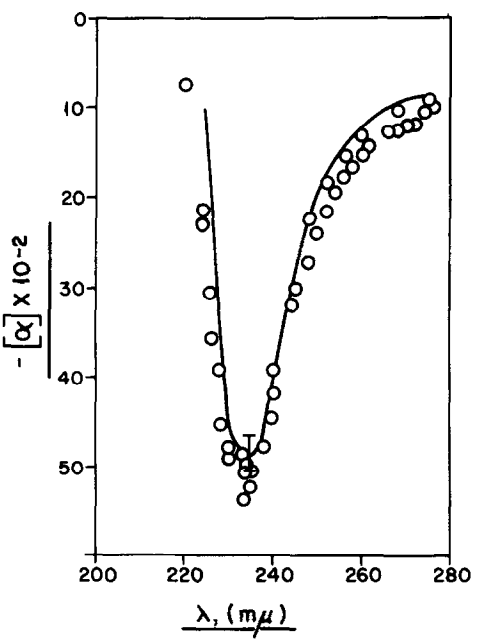

Figure 2. ORD of Native and Acid Denatured α Lactalbumin in the far ultraviolet region. Acid Denatured (pH 2) O . Native: _____. The experimental points have been omitted in the latter case in the interest of clarity. The error line at 233 mμ refers to data obtained with the native protein. Each set of data was obtained with a single protein concentration (ca. 0.01 g/100 ml) in a 1 cm path length cell. Temperature 25°C. Solvent 0.15M KCl.

reasonable, therefore, to identify the changes in the aromatic Cotton effects (Figure 1) with alterations in tryptophan environment. The observation of a Cotton effect trough at 275 mμ for free tryptophan (Iuzuka and Yang, 1964) is consistent with our association of this group with the aromatic Cotton effect (Figure 1). The above cited changes in tryptophan absorption and emission spectra for α lactalbumin appear to be the result of increased freedom of rotation of the three "buried" tryptophans in the denatured state (Kronman and Holmes, 1965). This increased freedom of motion appears in some way to eliminate certain symmetry properties of these tryptophan groups with concomitant reduction of their Cotton effect contributions.

Although the ORD curves at pH 2 and 6 show marked differences in the 250 to 360 mμ region (Figure 1) they appear to correspond at the trough of the 233 mμ Cotton effect (Figure 2) which has been associated with the helical configuration (Simmons et al. 1961). If we assume the absence of interference by chromophoric side chains in this wavelength region, we observe that the degree of helicity in native and denatured α lactalbumin are the same, in marked contrast with the conclusion reached from the b_0 data (Table I). This discrepancy can be explained on the basis of the Cotton effects seen in Figure 1. They appear to make sufficient contribution to the rotation over the range 578 to 365 mμ as to give anomalously high values of b_0 at pH 6. Depending upon the choice of $[m']_{233}$ for helix → coil for polyglutamic acid (-18,000 → 2,000, Yang and Semejima, 1964), (-15,000 → -2,000, Blout et al. 1962), $[m']_{233}$ calculated from the data of Figure 2 corresponds to 17 to 21% helix. This is in reasonable agreement with that obtained from the b_0 value at pH 2 (Table I), indicating that the slight inflection seen at this pH (Figure 1) has little influence on the ORD over the range 313 to 578 mμ. A comparison of the 17 to 21% value with the 39% estimated at pH 6 from the 313 to 578 mμ Yang-Moffitt treatment (Column 3, Table I) provides a striking example of the magnitude of the error that might be made in using ORD data to estimate helix content of proteins containing large amounts of aromatic amino acids.

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