

OPTICAL ROTATION AND HELICAL POLYPEPTIDE
CHAIN CONFIGURATION IN α -PROTEINS

Sir:

A theory of optical rotatory dispersion for helical macromolecules has recently been developed by Moffitt.¹ This has been applied successfully to the rotatory behavior of synthetic polypeptides in the α -configuration,^{2,3,4,5} and of "globular" proteins.⁶ Here we present the result of an examination of a series of "fibrous" proteins which yield in the condensed state the α -type wide-angle X-ray diffraction diagram.

In the native state the proteins examined have a rotatory behavior in aqueous solution similar to that of synthetic polypeptides in helix-inducing solvents. With the exception of fibrinogen, none of these proteins shows "simple" dispersion, *i.e.*, follows a one-term Drude equation. The data fit Moffitt's equation for helical systems

$$[\alpha]_{\lambda} = \left(\frac{100}{M}\right) \left(\frac{n^2 + 2}{3}\right) \left[\frac{a_0 \lambda_0^2}{(\lambda^2 - \lambda_0^2)} + \frac{b_0 \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \right]$$

where M is the molecular weight per residue and n is the refractive index of the solvent. Within the experimental error of about $\pm 100\text{\AA}$., λ_0 equals 2100\AA ., in agreement with the λ_0 reported for poly- γ -benzyl-L-glutamate and poly-L-glutamic acid.³ A value of near -600° for the constant b_0 characterizes a fully-coiled, right-handed α -helix of synthetic polypeptides.³ Assuming that λ_0 for the helical and for the non-helical configurations are close, one can use b_0 as a measure of helical content. Table I lists the b_0 's obtained on this assumption, using a value of λ_0 equal to 2100\AA .. The

TABLE I

	Native ^a		Denatured ^b	
	$[\alpha]_{5780}^c$	b_0^d	$[\alpha]_{5780}$	λ_0 (\AA) ^e
Light meromyosin fraction I'	-13.0°	-660°	-118°	2120
Tropomyosin	-16.0°	-620°	-118°	2130
Paramyosin	-11.1°	-600°	-63°	...
Light meromyosin	-20.4°	-490°	-107°	2150
Myosin	-28.7°	-370°	-108°	2180
Heavy meromyosin	-34.5°	-300°	-103°	2150
Fibrinogen	-58.2°	-210°	-110°	2130

^a In $0.6M$ KCl, pH 7.0; fibrinogen in $0.3M$ NaCl, pH 6.2. ^b In $9.5M$ urea. ^c Measurements were made with a Rudolph High Precision Polarimeter with photoelectric attachment at four wave lengths: 3650, 4360, 5460, 5780\AA ., isolated by glass and interference filters from a mercury arc; room temperature, $20 \pm 3^\circ$. ^d Obtained from plots of $[\alpha]_{\lambda}(M/100) (3/(n^2 + 2)) (\lambda^2 - \lambda_0^2)$ vs. $1/(\lambda^2 - \lambda_0^2)$, $\lambda_0 = 2100\text{\AA}$.. ^e Obtained from plots of $\lambda^2[\alpha]_{\lambda}$ vs. $[\alpha]_{\lambda}$. ^f Part of light meromyosin resistant to ethanol treatment representing about half of light meromyosin.

negative sign indicates that the helices in the "fibrous" proteins have the same sense of twist as those in synthetic polypeptides and other proteins. Since the macromolecules of these α -proteins prob-

ably consist of cables of α -helices in a supercoiled configuration^{7,8,9} it would appear that this association in aqueous solution, as well as superimposed backbone dissymmetry, do not markedly affect rotatory behavior.

In the denatured state one expects absence of helical backbone contribution to the rotation.¹⁰ Indeed, we have found that b_0 is approximately zero for all the proteins except paramyosin whose b_0 is of the order of -190° . Moffitt's equation then reduces to a one-term Drude equation, and the λ_0 's found from the latter are close to 2100\AA .. (see Table I). This fact supports our use of b_0 as a measure of helical content. There is independent evidence that paramyosin is not completely denatured by the urea treatment.¹¹ The values of the specific rotations listed in Table I are in agreement with those found for other denatured proteins¹⁰ and synthetic polypeptides in the random coil configuration.²

The rotation of myosin is close to the proportional sum of the rotations of light and heavy meromyosin; hence it would appear that there is no large-scale unfolding of helical domains in the myosin molecule when split by trypsin.

We note that although there is a variation of helical content in "fibrous" proteins, the values are generally higher than those characterizing "globular" proteins. Asymmetry is thus an expression of high helical content.

The relationship of proline content to helical configuration in these α -proteins will be described in another communication.

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THE STRUCTURE OF THE TRANSITION STATE FOR
ELECTROPHILIC AROMATIC SUBSTITUTION

Sirs:

Present theories of aromatic electrophilic substitution allow only an indefinite formulation of

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