Optical rotatory dispersion of polypeptides and proteins in the near infrared region

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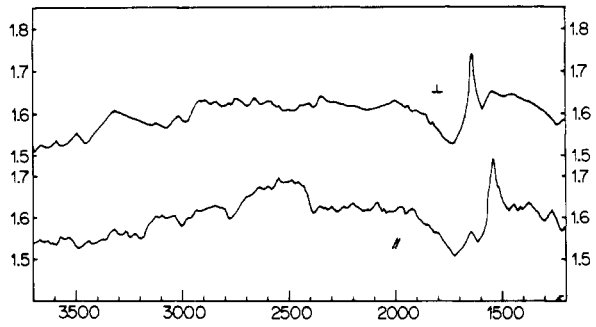

Figure 1.

peak. Both these observations refer to the (stronger) perpendicular transition moment. The parallel moment would give rise to less variation.

Poly(vinyl alcohol). The film examined was the Cipoviol, made by the Rhône Poulenc Co. This material is thought to be plasticized. It was drawn at 20 and 100°, at 500%/min, to a ratio of 3:1. It was clamped in a specially designed sample holder before release from the jaws. The most reliable index measurements, made at 2400–1800 cm^{-1}, show little difference between the parallel and perpendicular directions. An average value of 1.70 was calculated. The reflection spectra show that the index varied between 1.63 and 1.77 within the zone 4000–1200 cm^{-1}. At lower frequencies numerous absorptions made measurements unreliable. The reflection spectra show that the index varies little until it rises sharply between 500 and 440 cm^{-1}.

Polyamide 6. This material, showing several markedly dichroic absorptions, was chosen for its excellent drawing behavior. Films were drawn at 20° to a ratio of 7:1. The average value of the index, by the $\Delta K/\Delta\nu$ method, in the 2500–1800-cm^{-1} zone, was 1.62 both for the parallel and perpendicular directions.

Figure 1 shows the perpendicular and parallel refractive indices in this frequency region, together with perturbations accompanying the absorption peaks at 1660 and 1580 cm^{-1}. As might be expected from the presence of dichroic peaks in the 1700–1100-cm^{-1} zone, most absorbing strongly in the parallel direction, the indices in the 1000–800-cm^{-1} zone were not equal; parallel 1.79, perpendicular 1.72.

These values are in fair agreement with the value of 1.82 found for an amorphous peak at 1140 cm^{-1} [5] in polyamide 6-6, but higher than the reported value of 1.44 for a crystalline peak at 936 cm^{-1} in this polymer. The latter value is surprising in view of the rather flat reflection spectrum for polyamide 6-6 in the 1200–800-cm^{-1} zone,[4] which would signify a rather constant index over this range.

Conclusions

Even for a highly dichroic polymer such as polyacrylonitrile, the use of a single value of refractive index determined at a wavelength of low opacity would be a useful approximation. We believe our average value of 1.51 to be reliable for such use within ±5%, the reported value of 1.8[2] being too high by about 20%. For finer work it is necessary to make index measurements at the frequency of the absorption maximum, in at least two of the ordinate directions, making all associated absorption measurements at carefully calibrated wavelengths. No reliance can be placed on index measurements in the visible spectrum, the pleochroic infrared absorptions having a cumulative effect progressing toward lower frequencies.

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Optical Rotatory Dispersion of Polypeptides and Proteins in the near-Infrared Region†

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ABSTRACT: It has been shown for polypeptides that the value of the ir term of optical activity which depends on the conformation of the polypeptide chain can be determined by ORD in the near-infrared region. This term corresponds to the sum of rotatory powers of optically active vibrational transitions. For the random state of this value is equal to zero, for the α-helical state, though small, it exceeds the error of measurement and for the β form it is higher by almost an order than for the α-helical state. Measurements carried out for a number of globular proteins with a known structure show a high sensitivity of the method. It is possible to detect the β conformation of three to five residues in a molecule consisting of 100–200 amino acid residues. The possibility of using the method for studies of protein structures is discussed.

Optical rotatory dispersion (ORD) of polypeptides and proteins in the ultraviolet and visible regions greatly depends on their conformations. ORD in these regions is accounted for by the interaction of light with the electronic transitions of the peptide group. In principle, the existence of optical activity of the vibrational transitions is not excluded, although at present the possibility of this is shown only theoretically.[1] Indirectly the optical activity of the vibrational transitions must be reflected in the rotatory dispersion in the near-infrared region and this was shown in measurements for crystal quartz.[2,3] On this basis an attempt was made to investigate ORD in the near-infrared region for polypeptides in different conformations.[4,5]

† This paper is one of the group presented at the 10th Prague IUPAC Microsymposium on Macromolecules, August 28–31, 1972.

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Table I
Infrared Term of Optical Activity C_0 for Polypeptides[a]

No.	Compound	Solution	C_0 ((deg cm^2) dmol^{-1})
		α-Helix High Content (80–100%)	
1	Poly(γ-benzyl L-glutamate)	Chloroform	−1.0 ± 0.2
2	Poly(L-glutamic acid)	0.2 *M* NaCl + dioxane, 2:1, pH 5.4	−0.6 ± 0.2
3	Poly(L-lysine)·HCl	0.2 *M* NaCl, pH 11	−1.0 ± 0.5
		β-Form Content about 30–50%	
4	Poly(*S*-carbobenzoxymethyl-L-cysteine)	1,2-Dichloroethane + dichloroacetic acid, 99:1	−3.0 ± 0.2
5	Poly(*S*-carboxymethyl-L-cysteine)	0.1 *M* NaCl, pH 4.6	−2.8 ± 0.2
6	Silk fibroin *Bombyx mori*	Water + methanol, 1:1, pH 7.3	−4.7 ± 0.1
7	Poly(L-lysine), b_0 = +240	Water, pH 10.95, 10 min at 52°	−30.0 ± 2.0

[a] Random form has $C_0 = 0$ for all samples.

Table II
Infrared Term of Optical Activity C_0 for Several Globular Proteins and Calculation C_0 (100% β)[a]

No.	Protein	Structure (%) α	Structure (%) β	Ir Optical Act. Term ((deg cm^2) dmol^{-1}) C_0^{exp}	c_0 (β)	C_0 (100% β)
1	Lysozyme	30	10	−1.1 ± 0.2	−0.8 ± 0.2	−8.0 ± 2.0
2	Lactate dehydrogenase	24	20	−1.8 ± 0.7	−1.56 ± 0.1	−7.8 ± 0.5
3	Ribonuclease A	14	32	−2.7 ± 0.15	−2.55 ± 0.15	−8.0 ± 0.5

[a] $C_0(\beta) = C_0^{exp} - C_0(\alpha)$, here C_0 (100% α) = −1.0.

Molecular rotation $[m']$ far from the absorption bonds is expressed by[6]

$$[m'] = \frac{0.96\pi N}{hc}\sum_k \frac{R_k\lambda_k^2}{\lambda^2 - \lambda_k^2}$$

In the near-infrared region we have

$$(\lambda_k^{ir})^2 \gg \lambda^2 \gg (\lambda_k^{uv})^2$$

and thus

$$[m'] = \frac{0.96\pi N}{hc}\left\{\sum_k \frac{R_k^{uv}(\lambda_k^{uv})^2}{\lambda^2} - \sum_k R_k^{ir}\right\}$$

The second term is summarized only by vibrational transitions. Designating it as C_0 we obtain the infrared term of optical activity as

$$C_0 = -\frac{0.96\pi N}{hc}\sum_k R_k^{ir}$$

where R_k is the rotational strength of kth transition. It should be noted that in a more detailed theoretical treatment the different arrangement of polymer molecules in relation to the vector of the light wave intensity must be taken into account.[7] Thus, the ORD of polypeptides can be described by the following modified Moffitt-Yang equation

$$[m'] = a_0\frac{\lambda_0^2}{\lambda^2 - \lambda_0^2} + b_0\frac{\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} + C_0$$

where a_0, b_0, and λ_0 are parameters determined from measurements in the visible region.

A simple method of determining C_0 is

$$C_0 = \lim[m'] \text{ at } 1/\lambda^2 \longrightarrow 0$$

For higher precision we must use special coordinates. One of the few possible transformations is

$$[m']\frac{\lambda^2 - \lambda_0^2}{\lambda^2} - b_0\frac{\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} = (a_0 - C_0)\frac{\lambda_0^2}{\lambda^2} + C_0$$

Building the dependence of the left part of the equation from $1/\lambda^2$ and extrapolating the obtained straight line to a value at which $1/\lambda^2 = 0$ we get the C_0 value (Figure 1).

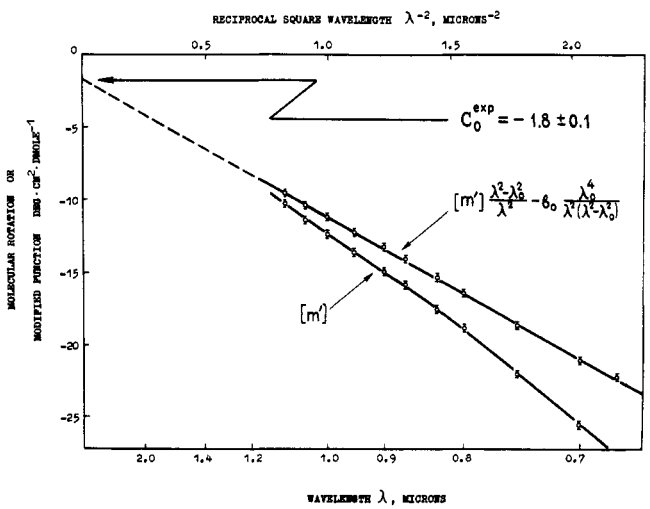

Figure 1. A representation of the rotatory dispersion data in the near-infrared region for lactate dehydrogenase. The procedure of determining infrared optical activity term C_0 is shown. Protein concentration 0.25%, 0.1 *M* phosphate buffer, pH 7, 22°, cell length 1 dm, $b_0 = -240$ (deg cm^2) dmol^{-1}.

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In the first place we investigated some well-known polypeptides. Measurements were carried out on an automatic recording spectropolarimeter which was constructed on the basis of a Jasco ORD-UV-5 spectropolarimeter. The spectral range was 0.5–2.3 μm, the maximum accuracy, 2×10^{-4} deg. The results for polypeptides are shown in Table I. It should be noted that in all cases the random form (coil) gave a value of $C_0 = 0$. In comparison with the α helix, the β form had an essentially greater value.

At present there are no methods permitting to evaluate the β-form content in polypeptides and therefore we had no possibility of obtaining the $C_0(\beta)$ value for the pure structural form. We measured C_0 for several globular proteins with a known structure. The C_0 value proved to correlate with the β-form content. Recalculating $C_0(\beta)$ to $C_0(100\%\ \beta)$ we obtained the same value $C_0 = -8.0$ (deg cm^2) dmol^{-1} for all the three investigated proteins (Table II). Thus the approximated values of C_0 for the different forms will be

Form	Random	α Helix	β Form
C_0 ((deg cm^2) dmol^{-1})	0	−1.0	−8.0

The selectivity of C_0 for the β form allows to identify it in proteins with very high precision. Thus, in lysozyme only twelve amino acid residues are in the β form and, as seen in Table II, C_0 is determined with an error of 25%, *i.e.*, the β conformation of three to five residues in a molecule consisting of 100–200 amino acid residues can be detected.

The practical application of the revealed effect is evident, and the theoretical explanation should be expected in the near future.

Optical Properties of Poly(L-azetidinecarboxylic acid) in Solution†

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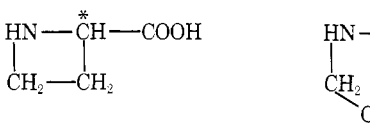
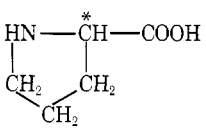
ABSTRACT: The effect of the rigidity of the four-membered ring of L-azetidinecarboxylic acid residue on the conformation of a new polymer structurally related to poly(L-proline) has been studied. As in the case of poly(L-proline), uv and CD results point to the existence of two different conformations in solution.

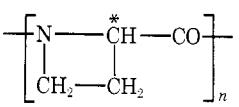
The replacement of L-proline with L-azetidine-2-carboxylic acid (L-Aze-COOH) in polypeptide chains of plant proteins and collagens results in drastic alterations of structures and biological functions, in spite of the close chemical similarity between the imino acids.[1-4] The "toxicity" of L-Aze-COOH has been attributed to the structural difference between its rigid four-membered ring and the more flexible pyrrolidine ring of proline, which would significantly affect the secondary and tertiary structure of polypeptide chains.[5] To elucidate these effects, the synthesis of a homopolymer of the following structure



azetidine-2-carboxylic acid



proline



has been recently accomplished.[6]

The usual *N*-carboxyanhydride procedure was not followed because the *N*-chloroformyl-L-azetidine-2-carboxylic acid intermediate could not be cyclized.[7,8] Poly(L-azetidinecarboxylic acid) (PLAze) was conveniently prepared by the polymeric self-condensation of an active ester (*i.e.*, the pentachlorophenyl ester of the imino acid), through the sequence shown in Scheme I.

The polymer was purified and fractionated by elution on Sephadex (G-50 fine). All spectroscopic measurements were performed using a fraction having a weight-average molecular weight, $\bar{M}_w$, of 7600 (~90 residues), determined by the Yphantis midpoint method.[6]

We report in this communication the uv adsorption and circular dichroism results of a preliminary study of PLAze conformation in solution.

The uv and CD spectra (Figures 1–3) of PLAze in water were recorded immediately after sample dissolution and registered repeatedly at regular time intervals. No time dependence was observed, excluding further conformational change. In the ethanol–water mixture (the polymer is insoluble in pure ethanol and, generally, in simple aliphatic alcohols), the spectra were recorded at the equilibrium conditions, *i.e.*, the changes of the circular dichroism at 219 nm were less than the uncertainties in the measurement. The similarity with the corresponding poly(L-proline) spectra, particularly in the curves recorded in the ethanol–water mixture, suggests that the conformation of PLAze in this medium resembles that of poly(L-proline I).[9-11]

† This paper is one of a group which was presented at the 10th Prague IUPAC Microsymposium on Macromolecules, August 28–31, 1972.

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