Experimental study of electro-optical rotation of helical polypeptides

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The optical rotation of anisotropic macromolecules depends on the direction of propagation of linearly polarized light through the molecule. The molecular optical activity is thus a tensor property, the components of which characterize the molecular structure. A study is reported in which these components have been measured for three polypeptides in helix-promoting solvents. These were poly(γ-benzyl-L-glutamate), poly(γ-benzyl-D-glutamate) and poly(β-benzyl-L-aspartate). The measurement technique involved imposing orientational alignment upon the molecules. This alignment was achieved using pulsed electric fields and the optical rotation recorded over a range of visible light wavelengths. From the rates of response to the field changes, helical lengths were obtained. The implications for molecular conformations are discussed.

(Keywords: optical activity; polypeptide; helical polypeptides)

INTRODUCTION

With dilute macromolecular solutions, optical rotation has two origins. One is the inherent structural asymmetry associated with the composite chemical residues of the polymer whilst the other is the asymmetry of the overall conformation. The observed rotation (θ) of the linear polarization state after passing through a length L (dm) of solution at concentration c (g ml^{-1}) defines the specific rotation, where

$$[\theta] = \theta/Lc \qquad (1)$$

Measurements of $[\theta]$ and its wavelength (λ) dependence have been the basis for many years of determining helical conformations. Of special historical note was the use of the Moffit–Yang[1] concept to ascertain the helical state of polypeptides and by considering these as model compounds for proteins, estimating the protein content of complex macromolecules[2].

For a macromolecule, the optical rotation is anisotropic due to the anisotropy of the molecular geometry. Hence, the specific rotation is tensorial in form. For rigid molecules in random array

$$[\theta] = [\theta_{33}] + [\theta_{22}] + [\theta_{11}]/3 \qquad (2)$$

where the components $[\theta_{ii}]$ are associated with the major molecular axes. For cylindrically symmetric molecules, equation (2) becomes

$$[\theta] = \{[\theta_{33}] + 2[\theta_{11}]\}/3 \qquad (3)$$

with 3 indicating the unique axis. It is these principal tensor components which relate to the molecular structure and which have been the basis of theoretical predictions for helices of various form[3].

Equation (3) is based upon a dilute, non-interacting solution of randomly oriented cylinders. If subjected to an external force field so that the molecules align, at least partially, with their long axes towards the direction of optical propagation, then the change in the specific rotation from that of equation (3) is given by[4]

$$[\Delta\theta] = 2\{[\theta_{33}] - [\theta_{11}]\}\Phi/3 \qquad (4)$$

Here Φ is the orientation function defining the degree of alignment in the field.

Heliocoidal polypeptides are known to have a permanent electric dipole moment (μ) directed along the long axis, and in non-polar solvents, to exhibit no significant induced dipole moments from the anisotropy of electrical polarizabilities. For such, in an applied field of amplitude E at a temperature T, with k the Boltzmann constant, then[5]

$$\Phi = \left[1 - \frac{3(\coth\beta - 1/\beta)}{\beta}\right] \qquad (5)$$

with $\beta = \mu E/kT$.

For high fields, where the torque between the field and the molecular dipole exceeds the influence of thermal disorienting energies (kT), complete molecular alignment is achieved and

$$\Phi_{E\to\infty} = 1 \qquad (6)$$

At low fields, the orientation function demonstrates a

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quadratic dependence on field strength and

$$\Phi_{E\to 0} = \frac{1}{15}\left(\frac{\mu E}{kT}\right)^2 \tag{7}$$

This condition is the most easily achieved experimentally but gives smaller measurable responses. Hence, in terms of the pre-field specific rotation $[\theta]$ and the observed change $[\Delta\theta]$ in this parameter

$$\left.\begin{aligned}[\theta_{33}] &= [\theta] + [\Delta\theta]/\Phi \\ [\theta_{11}] &= [\theta] - [\Delta\theta]/2\Phi\end{aligned}\right\} \tag{8}$$

Knowledge or measurement of the dipole moment then allows a determination to be made of the specific axial parameters $[\theta_{ii}]$ at various experimental wavelengths (λ).

It is experimentally expedient to apply the electric field as a short rectangular pulse of duration sufficient just to allow statistical alignment to be achieved. This restricts the influence of heating and electrophoretic phenomena, and results in fast experimentation. Furthermore, the field-free disorienting relaxation, characterized by a time τ, allows a measurement to be made of the molecular size. The decay follows a typical first-order rate equation with[6]

$$[\Delta\theta] = [\Delta\theta]' \exp - t/\tau \tag{9}$$

where the prime indicates the value at the cessation of the field for which $t = 0$. For all rigid spheroids, the major dimension l relates to τ via the equation[7]

$$l = \left(\frac{18kT}{\pi\eta}\tau\nu\right)^{1/3} \tag{10}$$

Here ν is a shape factor, which for cylinders of axial ratio r has the form[8]

$$\nu = \ln(2r) - 0.8 \tag{11}$$

whilst η is the medium viscosity.

For monodisperse media, it is possible in principle to estimate the major molecular dimension simultaneously with the data for $[\theta_{ii}]$.

EXPERIMENTAL

Three polypeptides have been studied as follows. A sample of poly(γ-benzyl-L-glutamate) (PBLG) with a molecular weight of 260000 was dissolved in 1,2-dichlorethane to 0.01 g ml^{-1} concentration. In this solvent a right-handed helical conformation is adopted. Care has to be taken as at greater concentrations this medium readily forms lyotropic liquid crystalline phases[9]. As the electro-optical rotation effect is small and concentration-dependent, the highest practicable concentration was sought. In these studies, aggregation was avoided as monitored from measured τ data.

A sample of the left-handed helix forming poly(γ-benzyl-D-glutamate) (PBDG) with a relative molecular mass of 150000 was also studied in the same solvent and at the same concentration. Both polymers were obtained from the Sigma Chemical Company.

The third system was a sample of poly(β-benzyl-L-aspartate) (PBLA) with a molecular weight of 230000 (Pilot Chemical Co.). This was chosen because it has a L configuration but adopts a left-handed helical conformation in helix-promoting solvents, of which chloroform was chosen. A 5×10^{-3} g ml^{-1} solution was principally studied. Aggregation was apparent for higher concentrations.

The apparatus has been described in detail elsewhere[10]. Briefly, light from a helium–neon laser or an argon-pumped dye laser passed through a noise-reducing unit and a linear initial polarizer. The polarized output traversed a pair of Faraday rotators before meeting the sample cell. The transmitted beam, with its rotated polarization state, passed through an analysing polarizer which was 'crossed' with the initial polarizer, and fell onto a high sensitivity photodetector. One Faraday rotator was energized with a direct current which was adjusted to compensate for, and hence measure, the rotation θ introduced by the sample. The other rotator was supplied with an alternating current which provided a polarization modulation on the transmitted light beam onto which a phase-sensitive detector could frequency-lock for accurate setting of the compensator. For field-induced transient changes $\Delta\theta$, the alternating current compensator was switched off and the linear compensator slightly activated to give a low intensity transmitted light beam at the detector. The pulsed electric field was applied to the sample, colinear with the light propagation direction through the cell. The transient change in the photodetector response accompanying the field-induced rotation change, was recorded directly. The enhanced sensitivity obtained with this arrangement and details of the special cell design have been given previously[10,11].

For the studies described here, fields of up to 4 kV cm^{-1} were applied across the solutions for durations of the order of a few milliseconds. Optical rotation data were recorded over the wavelength range from 488 to 633 nm. It is highlighted that the field direction must be along the light path to avoid spurious contributions from the larger magnitude electric birefringence contributing to the observed signals[10].

RESULTS

The dispersion of the optical rotation was measured for dilute solutions of all three polypeptides. The data are displayed in *Figure 1*. Various studies have been reported for PBLG[12,13], whilst there are only a few data for PBDG and PBLA[14]. The results presented here are in general agreement with accepted literature values when allowance is made for the effect of the polymer degree of polymerization. The sign of $[\theta]$ and the trends of the dispersion curves are consistent with the predictions of the PBLG helix being of right-handed form whilst PBDG and PBLA have a left-handed sense. Whilst the backbone conformation is the dominant factor in each case, values of $[\theta]$ also contain a contribution from optically active chromophores within the polypeptide residues.

Transient changes in the observed rotation were recorded over a complete range of field strengths at each available wavelength for each polymer system. Typical responses are shown in *Figure 2* for PBDG and PBLA. Figure 5 of reference 10, which contained details of the apparatus and its operation, provides a representative trace for PBLG in the same solvent as used here. The field strength dependence of the amplitudes of the traces always followed the predictions of the Langevin function as shown in *Figure 3*. At the lowest field strengths, the linear dependence of $[\Delta\theta]$ on E^2 is apparent, in accord with equations (4) and (7). With increasing field

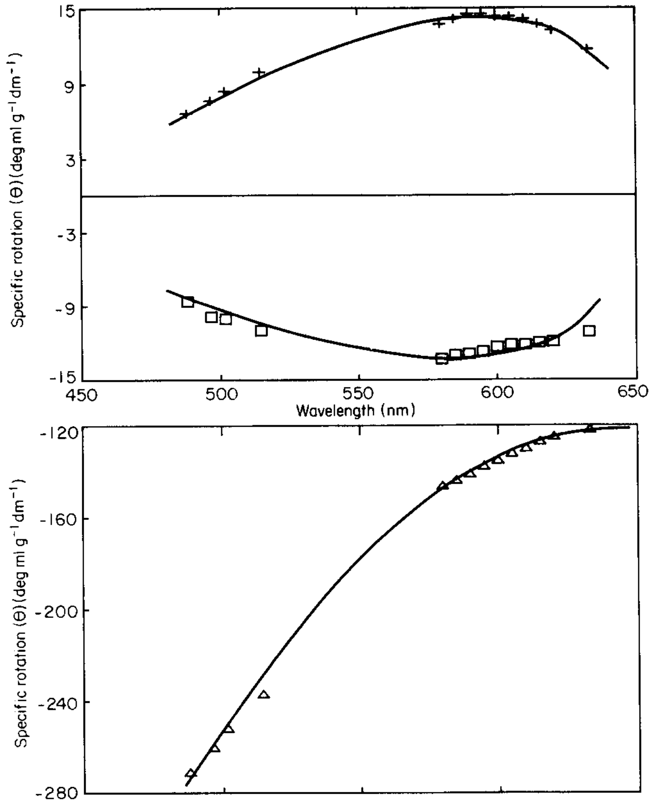

Figure 1 Optical rotatory dispersion for the three polypeptides: (+) 1% PBLG; (□) 1% PBDG; (△) 0.5% PBLA

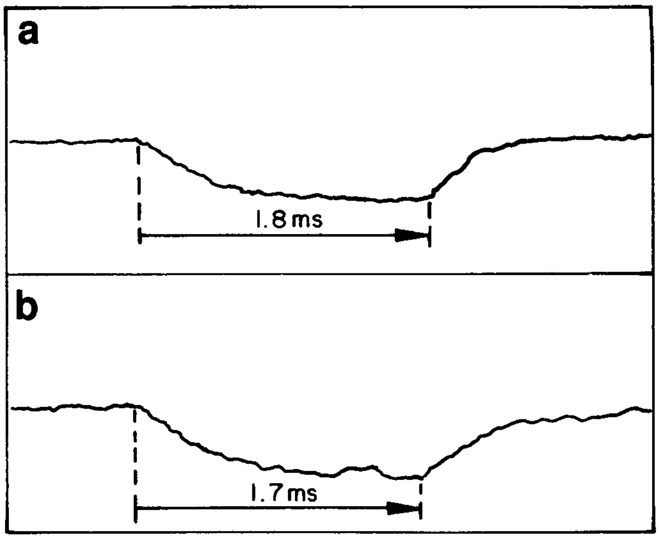

Figure 2 Typical electrically induced transient responses: (a) 1% sample of PBDG, $E = 7.6$ kV cm^{-1}, $\Delta\theta = -0.059°$, $[\Delta\theta] = -19.7$ deg ml g^{-1} dm^{-1}, $\lambda = 620$ nm; (b) 0.5% sample of PBLA, $E = 7.2$ kV cm^{-1}, $\Delta\theta = -0.029°$, $[\Delta\theta] = -19.2$ deg ml g^{-1} dm^{-1}, $\lambda = 497$nm. Time runs from left to right

strength, $[\Delta\theta]$ tends towards the asymptotic limit of the full molecular orientation condition of equation (6).

Data recorded for each λ and polymer were analysed in the following manner, based on equations (1), (3), (4) and (7) for the low-field data. To evaluate the various $[\theta_{ii}]$ with $i = 1$ and 3, from $[\theta]$ and $[\Delta\theta]$, knowledge is required of Φ or, at low fields, of μ. It is possible to assume a value for μ applicable to the expected α-helical structure for the sample molecular weight. A better, more rigorous approach, is to measure the parameter Φ under given experimental conditions. This has been the approach adopted here. In principle, this should accommodate the influence of sample polydispersity on Φ. To achieve this, transient electric birefringence experiments have been carried out on the same samples using the same field conditions as the optical rotation experiments. Details of the electric birefringence methodology can be found elsewhere[15]. The birefringence (Δn) induced in a solution of concentration c of macromolecules which exhibit an anisotropy ($\Delta g = g_1 - g_2$) of the optical polarizability (g), is given by

$$\Delta n = \left(\frac{2\pi c}{n\rho}\right)\Delta g\Phi \tag{12}$$

with n the refractive index of the solvent and ρ the density of the solute. Relevant data for the variation of Δn with E^2 for each system is given in *Figure 4* from which the behaviour of Φ could be interpolated and used in conjunction with *Figure 3* and equation (4). Values for $[\theta]$ and $[\Delta\theta]$ were thus analysed to enumerate $[\theta_{33}]$ and $[\theta_{11}]$ assuming the helices were rigid. The variations of these parameters are given in *Figures 5–7*.

It is noteworthy that the electric birefringence experiments are of greater sensitivity than the electro-optical activity data. This is a result of the magnitudes of the optical effects for these systems and of the

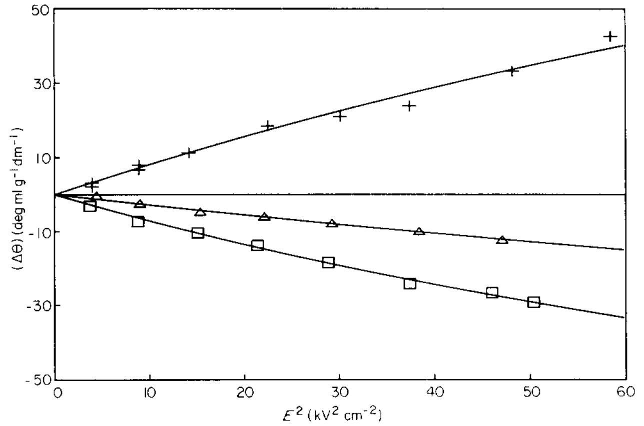

Figure 3 Plots of the specific electro-optical rotation $[\Delta\theta]$ as a function of the field strength for each sample. Data for wavelength 580 nm. The solid lines are the corresponding fits of the orientation function. Symbols as in *Figure 1*

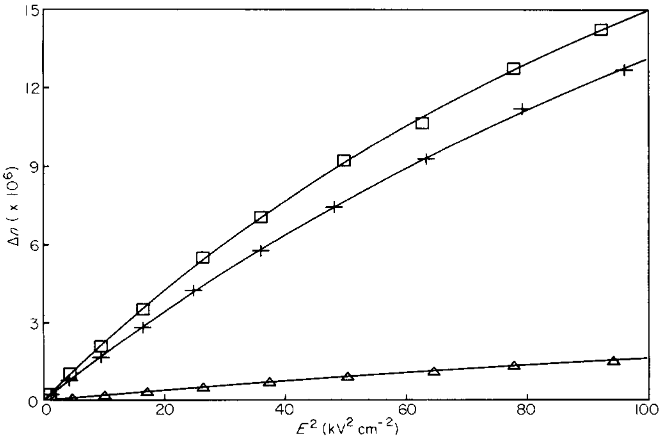

Figure 4 Plots of the measured electric birefringence (Δn) as a function of the field strength for each sample. The solid lines are the corresponding fits of the orientation function. Symbols as in *Figure 1*

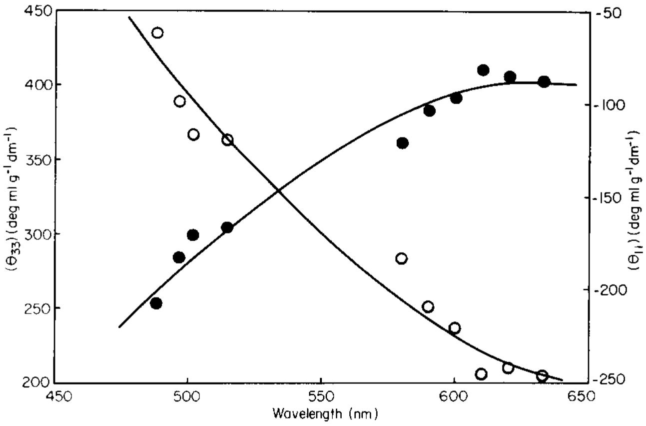

Figure 5 Dispersion of the optical rotation tensor components for 1% PBLG: (○) $[\theta_{33}]$; (●) $[\theta_{11}]$

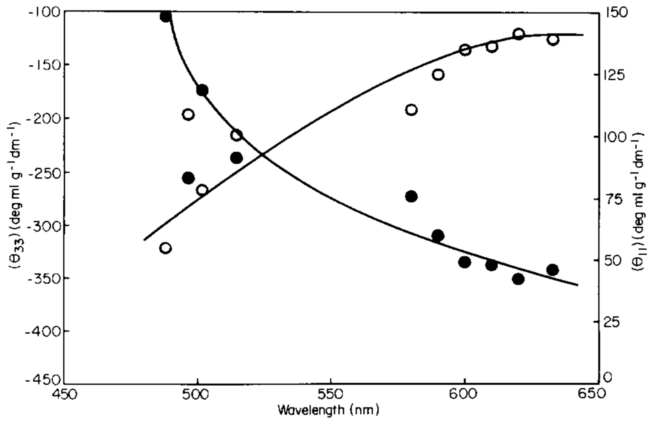

Figure 6 Dispersion of the optical rotation tensor components for 1% PBDG. Symbols as in *Figure 5*

larger signal-to-noise ratio for these measurement procedures[10,16]. Although not the primary aim of these measurements, the electric birefringence data give supplementary information on the measured systems. Under low-field conditions, the initial quadratic response of Δn on field amplitude affords a means of evaluating the product $(\Delta g)\mu^2$ from a combination of equations (7) and (12). Alternatively, in the high field asymptotic limit, the birefringence data enable (Δg) alone to be evaluated. Hence, both the permanent dipole moment and the optical polarizability anisotropy can be determined for each system. The data so obtained are given in *Table 1*.

The transient decay of the electro-optic response following the termination of the applied field pulse, has the same exponential form for electric birefringence as for optical rotation [Equation (9)]. Either can be used to evaluate an average molecular relaxation time. For the reasons mentioned above, the birefringence data were the more accurate. For a polydisperse sample, all components contribute, so that the observed birefringence has the form

$$\Delta n = \Sigma[(\Delta n_0)_i \exp - 6t/\tau_i] \qquad (13)$$

where $(\Delta n_0)_i$ is the steady-state birefringence amplitude and τ_i the relaxation time for the component i. A semi-log plot of $\ln(\Delta n/\Delta n_0)$ *versus* t expresses curvature with t. It was convenient to analyse the initial slope and obtain an average value $\bar{\tau}$. This was then interpreted for each sample to obtain a helical length l using equations (10) and (11). From the degrees of polymerization of each sample, values of the dipole moment (μ_0) and rod length (l_0) per monomer unit were obtained. These are also listed in *Table 1*.

The signs of the pre-field optical rotation are in agreement with the literature data and the right-handed conformations of PBLG and left-handed geometry for both PBDG and PBLA. The forms of the dispersion curves over the restricted wavelength range relate to the chromophores activated in each system. For electrically induced optical activity, PBLG has been the only chiral polypeptide previously studied. The data presented here are in accord with former results[17,18]. For all systems therefore, the values presented herein are regarded as the best to date. In that values of the specific tensor components are given for both left- and right-handed material, methodology is revealed by which theoretical model predictions can be experimentally assessed. A cursory thought might lead to the expectation that the data for PBLG and PBDG would differ only in sign due to the different helical senses. That this is not so has its origins in the existence of a finite contribution from the chemical residues themselves apart from the helical conformation factor. From such experiments as these, it should be possible to assess the residue contributions.

A further difficulty arises from the influence of sample polydispersity on specific parameters. Definitive parameters can only be evaluated for homogeneous samples. Even with this limitation, the data are useful. In the present study, it is instructive to consider the parameters l_0 and μ_0. For the well-established α-helix, $l_0 = 0.15$ nm and $\mu_0 = 3.4$ D (ref. 19). Data in *Table 1* indicate approximate conformity to this model. A sensitive differentiation between the α-helix and the 3.0_{10} helix, for which $l_0 = 0.20$ nm cannot be made on these samples. Equivalent measurements on highly

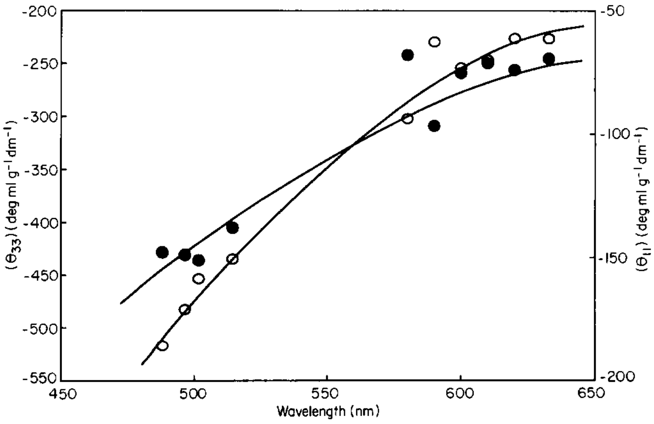

Figure 7 Dispersion of the optical rotation tensor components for 0.5% PBLA. Symbols as in *Figure 5*

Table 1 Supplementary physical parameters evaluated for the samples

	$(g_3 - g_1)_{\lambda=633}$ ($\times 10^3$)	μ_0 (D)	τ (μs)	l_0 (nm)
PBLG	1.85	2.1	41	0.15
PBDG	1.63	4.4	21	0.21
PBLA	0.63	1.5	50	0.19
α-Helix	–[a]	3.4	–[a]	0.15
3_{10}-Helix	–[a]	–[b]	–[a]	0.20

[a] Dependent on particular sample
[b] Value not available

fractionated polymers dissolved in pure solvents should enable such differentiation to be achieved.

Apart from the value of the method for molecular analysis, the phenomenon might also be the basis for electrically activated polarization modulators or switches in opto-electronics and molecular electronics. Suitable materials might be used in conjunction with an analysing polarizer to form an electrically addressable amplitude modulator, or optical shutter for binary logic. This facet is being further explored.

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