Molecular Crystals and Liquid Crystals

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Circular Dichroism of the Liquid Crystals of Poly(γ-benzyl-L-glutamate) Under Static Electric Fields†

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Liquid crystals of poly(γ-benzyl-L-glutamate) show circular dichroism (CD) from the benzyl chromophores. In an electric field the shape, magnitude and sign of the spectrum depend on the angle between the applied field and the direction of polarization. The inherent CD is restored after subtraction of the linear dichroism component.

Solution of poly(γ-benzyl-L-glutamate) (PBLG) in certain organic solvents such as dioxane, methylene chloride, chloroform and *m*-cresol above a certain concentration becomes liquid-crystalline, is birefringent, and exhibits strong "form"-optical rotation.[1,2] The cholesteric structure of the polypeptide has visible equidistant parallel lines, associated with an oscillating value of retardation, that can be observed with light parallel to the layers of molecular alignment. However, this cholesteric structure has not been detected by means of polarization micrograph or optical rotation if we use a dilute polypeptide solution, a quick-drying film prepared from a dilute solution, or even a concentrated solution enclosed in a cell having a thickness less than 0.25 mm.[1]

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We wish to report that concentrated chloroform solution of liquid-crystalline PBLG and film prepared from such a solution also show significant circular dichroism (CD), $\varepsilon_L - \varepsilon_R$, in wavelength range of the aromatic absorption bands. (Here ε is the molar absorptivity. The subscripts L and R refer to the left and right circularly polarized components of a linearly polarized light. By convention the CD of a polymer is expressed on a monomer basis, that is, the concentration used in the calculation is the number of moles of monomer residues per liter.) It is well known that PBLG in a poor solvent such as chloroform is in an α-helical conformation, which has its characteristic CD spectrum below 240 nm.[3] But in a dilute solution the benzyl groups of this helical polypeptide do not induce optical activity. What we observe arises from the formation of liquid-crystal structures of the particular polypeptide, but it is not a "form"-CD that is directly related to the pitch of the helicoidal structure of the cholesteric structure.[4] The "form"-CD of helical polypeptides has been reported to appear in the region of 450 to 600 nm for films of poly(γ-methyl-L-glutamate) or its D-isomer.[5]

Figure 1 shows the multiple CD bands between 245 and 280 nm of liquid-crystalline PBLG and its optical isomer, poly(γ-benzyl-D-glutamate) (PBDG). $\varepsilon_L - \varepsilon_R$ does not obey the Beer's law; its magnitude (per unit

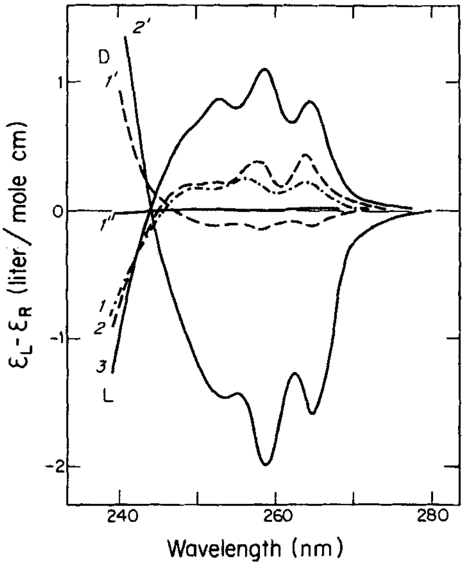

FIGURE 1 Circular dichroism (CD) spectra of liquid-crystalline poly(γ-benzylglutamate) in chloroform. L-glutamate: 1, 14.5% (v/v), 2, 19.6% (v/v); 3, film prepared from a liquid-crystalline solution. D-isomer: 1', quick-dried film from a isotropic solution; 2', film prepared from a liquid-crystalline solution. Racemic mixture (equimolar D- and L-isomer): 1'', 13.5% (v/v).

length per unit residue concentration) increases with the concentration of the polypeptides (curves 1 and 2) and the films have the strongest CD (curves 3 and 2′). Clearly, the polypeptide molecules are more ordered at higher concentration and their side chains are more restricted to rotate and more oriented. There is an additional possibility that the benzene rings in the side chains of the polypeptide might associate with each other, thus enhancing the intermolecular interactions and induced optical activity. In this respect we note the work of Caveney on the structure of the liquid-crystallike-polypeptide-structures in the integument of some beetles.[6] Therein the extraordinary $n_L - n_R$ for circularly polarized light has been attributed to the birefringence contributed by what seems to be epitaxial uric acids associated with the oriented protein molecules. The CD of PBLG and PBDG is opposite in sign as expected. The racemic mixture of the L- and D-isomers shows no CD at all (curve 1″). The fact that the films of the two isomers (curves 3 and 2′) differ in magnitude is probably due to the uncertainty in the determination of the film thickness.

Under the influence of a static electric field, molecular clusters of PBLG will be formed in liquid-crystalline solution regardless of the phase of liquid crystals and they tend to orient toward the direction of the applied field.[7,8] Under these conditions the liquid-crystalline polypeptides will show both linear and circular birefringence and linear and circular dichroism. Accordingly, the measured CD will show a dependence of the microscopic angle, α, that the fast optical axis of the oriented polypeptide clusters makes with respect to the plane of polarization of the polarizer in the CD instrument. Experimentally, we define a macroscopic rotation angle, θ, as the angle that the fast optical axis makes with respect to the vertical position, noting that the fast optical axis is perpendicular to the direction of the applied field and thereby the direction of orientation. The rotation angle is termed positive (or negative) when the cell containing the liquid crystals rotates clockwise (or counterclockwise) as seen by the observer facing the light source. This angle is related to α by[9]

$$\theta - \theta' = \alpha \tag{1}$$

where θ' is the value of θ when the fast optical axis coincides with the plane of polarization, i.e. $\alpha = 0$. Figure 2 shows the variations of the CD spectrum of a liquid-crystalline PBLG solution with the rotation angles. The shape, the magnitude, and even the sign of the CD can change markedly with the angle. Even a racemic mixture of liquid-crystalline PBLG and PBDG would show a θ-dependent CD spectrum (apparent) because of the appearance of linear dichroism. The curve "average" represents the real CD of liquid-crystalline PBLG; the method of its calculation will become evident in the following section.

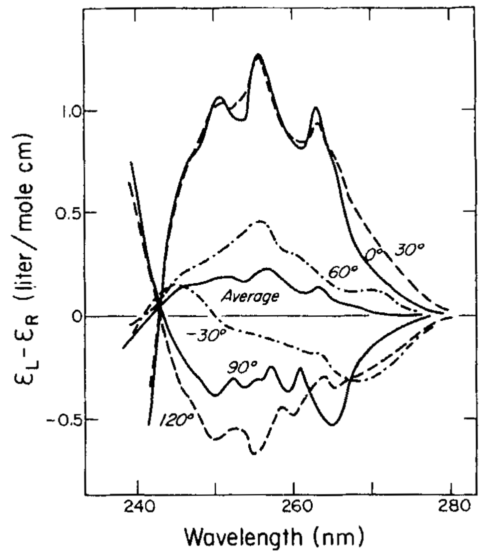

FIGURE 2 CD spectra of liquid-crystalline poly(γ-benzyl-L-glutamate) in an electric field at various rotation angles. PBLG concentration: 14.5% (v/v) in chloroform. Field strength: 169 V/cm. Infrared linear dichroic ration ($D_{\parallel}/D_{\perp}$): 1.6 at 3300 cm^{-1}. The numbers refer to the macroscopic rotation angle of the cell from the vertical position. The average is the mean of the six curves. See text for detail.

Tunis-Schneider and Maestre[9] have developed a general formula for the CD of oriented film of DNA, which can be reduced to a simple form for reasonably thin film (0.01–0.1 cm thick) or for partially oriented film (less than 5% orientation):

$$\delta_{\text{app}} = \delta_{\text{real}} - 0.298p \cos 2\alpha \tag{2}$$

Equation (2) for apparent CD consists of two terms, one for the true CD (δ_{real}) and the other for linear dichroism, p. The same equation should be applicable to our study, since our instrument Jasco J-10 has the same Grosjean–Legrand device as Cary 6001 which Tunis-Schneider and Maestre used. The light path of our solution was about 0.01 cm and its absorbance (optical density) was about one. Our polypeptide clusters were also partially oriented; for instance the infrared linear dichroic ratio at 3300 cm^{-1} (the NH-stretching frequency) ranged from 1.14 to 2.13 as compared with a ratio of 6.2 for a perfectly oriented helical polypeptide. Our conditions satisfy the requirements for Eq. (2). To eliminate the linear dichroism term in Eq. (2), we averaged two δ_{app} values at any chosen wavelength, whose rotation angles, θ, are 90° apart, noting that cos 2α in the two measurements will be equal in magnitude but opposite in sign (see Eq. 1). To further minimize experimental errors, we divided the six curves in Figure 2 into three sets and averaged them, that is,

$$\delta_{\text{real}} = (1/3)[\delta_{\text{av}}(-30^\circ, 60^\circ) + \delta_{\text{av}}(0^\circ, 90^\circ) + \delta_{\text{av}}(30^\circ, 120^\circ)] \tag{3}$$

The curve "average" so calculated is not unexpectedly close to curve 1 in Figure 1, which represents the CD of rodlike molecular clusters of PBLG that are randomly oriented in the absence of an electric field.

According to Eq. (2), the apparent CD of the liquid-crystalline PBLG at any chosen wavelength should show a periodic variation with respect to the rotation angle, θ, or the orientation angle, α (see Eq. 1). This is indeed the case in Figure 3, where the CD of a PBLG solution at 257 nm under a specific field strength is plotted against the rotation angle. Even for a racemic mixture for which δ_{real} is zero (Figure 1), the apparent CD is sinusoidal with a period of π. We also observed the same periodic behavior for CD at 252 nm. [According to Eq. (2), δ_{app} becomes maximum at $\cos 2\alpha = -1$ and minimum at $\cos 2\alpha = 1$. Thus, the average of the maximum and minimin in Figure 3 for PBLG at each wavelength will also give the real CD as does Eq. (3). On the other hand, the average for the racemic mixture in Figure 3 is essentially zero, as it should be.]

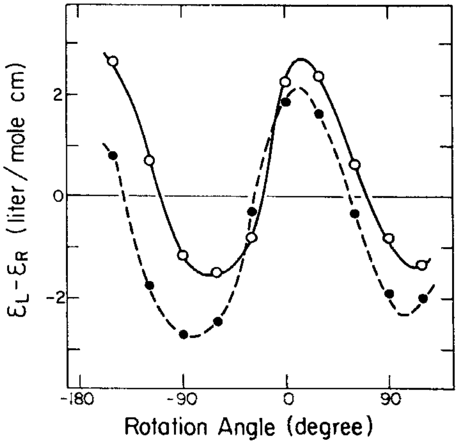

FIGURE 3 CD of electrically oriented liquid-crystalline poly(γ-benzylglutamate) at 257 nm as a periodic function of the rotation angle. Field strength: 253 V/cm. Symbols: ○, 13.7% PBLG in chloroform; ●, 13.5% racemic mixture of PBLG and PBDG. $D_{\parallel}/D_{\perp} = 1.45$ at 3300 cm^{-1}

Figure 4 shows the angular dependence of the apparent CD on the applied field strength. At any fixed rotation angle $\varepsilon_L - \varepsilon_R$ increases with increasing field strength. However, the real CD (the average) in the aromatic region seems to remain unchanged with the field strength, suggesting that the side-

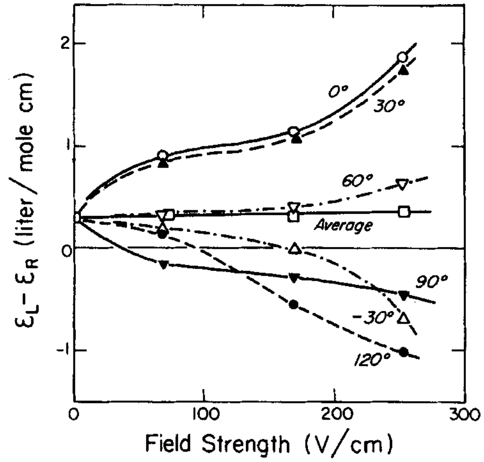

FIGURE 4 Effect of the electric field on the CD of poly(γ-benzyl-L-glutamate) at 257 nm. PBLG: 14.5% in chloroform. The $D_{\parallel}/D_{\perp}$ ratios were 1.14, 1.60 and 1.82 at 68, 169 and 253 V/cm, respectively. The numbers refer to the rotation angles.

chain orientation and molecular stackings in the molecular clusters are unaffected by the orientation process, although the linear dichroism, *p*, in Eq. (2) must increase with increasing orientation.

The CD of liquid crystals in a static electric field could provide additional information about the orientation of the chromophores such as the benzene rings and about the mechanism of the molecular aggregation in liquid-crystalline states. Recently, Tsuchihashi *et al.*[10] reported the CD of a concentrated solution of PBDG in methylene chloride under the influence of a magnetic field (but only at one fixed rotation angle). In their study the incident beam was parallel to the direction of the magnetic field, whereas in our case it was perpendicular to the direction of the electric field. Their CD spectrum was time dependent; furthermore, their aromatic CD of PBDG was positive, which contrasted with our finding in Figure 1. Possibly the aromatic ring has a different orientation when influenced by an electrical as contrasted with a magnetic field. Alternatively, the aromatic ring may have a diamagnetic birefringence in the opposite sense to the optical birefringence.

EXPERIMENTAL

PBLG (D.P. ca. 470) and PBDG (D. P. ca. 530) were gifts of Professor T. Hayakawa of Shinshu University. Polypeptides of higher molecular weights (PBLG, D. P. ca. 1460, and PBDG, D. P. ca. 1190, from the Pilot Chemicals, Inc.) were found to be unsuitable for our study probably because of noticeable wall effects that are inseparable from the optical cell. However, with utmost care we were able to obtain essentially the same results from high molecular-weight polypeptides as those reported in this work.

Concentrated solutions of polypeptides in reagent-grade chloroform were kept for 2 to 3 weeks at room temperature to insure full growth of the liquid crystals. They were then filled in a 1-mm quartz cell with a 0.90-mm spacer. Films were prepared on quartz windows from liquid-crystalline solutions by slowly evaporating off the solvent. The thickness of a film was determined from its weight and dimensions by assuming a density of 1.3 for the polypeptide.[11] The concentration of the film was taken as 5.93 moles per liter.

The cell was mounted on a cell holder and can be rotated in a plane perpendicular to the incident beam. A static electric field was applied to the solution by means of two platinum electrodes attached to the sides of the spacer.[12] The CD measurements of the film specimens were the average of at least duplicate runs, the cell being reversed in the second runs. All the data were expressed in terms of liters per mole (residue)-centimeter.

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