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Deuterium N.M.R. studies of polypeptides

I. Sidechain orientation in poly(γ-benzyl L-glutamate) and the mechanism of the cholesteric sense inversion

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The mechanism of thermally- and solvent-induced cholesteric sense inversions in lyotropic polypeptide liquid crystals has been discussed based on deuterium N.M.R. observations for poly(γ-benzyl L-glutamate) with perdeuteriated sidechain benzyl ester groups. Comparison of the deuterium quadrupolar splitting pattern with the macroscopic helical twisting power indicates that the sense inversion does not necessarily require sidechain conformational transitions (or changes in the helix surface chirality). The new data support a less specific mechanism for sense determination in polypeptide liquid crystals: anisotropic intermolecular interactions between helices are influenced by the solvent dielectric medium.

1. Introduction

Lyotropic cholesteric liquid crystals formed in concentrated solutions of the synthetic polypeptide, poly(γ-benzyl L-glutamate) (PBLG), exhibit thermally- and solvent-induced cholesteric sense inversions. Typical examples of the former kind of inversion are seen in *m*-cresol and 1,2,3-trichloropropane solutions [1–3]. The cholesteric sense in these systems, indicated by the sign of form optical rotation or induced circular dichroism, reverses from right to left upon heating. The reciprocal value of the cholesteric pitch, a measure of the helical twisting power, changes linearly with temperature passing through zero at a certain critical temperature at which the compensation of right- and left-handed cholesteric twists takes place. The cholesteric sense of the PBLG liquid crystal depends also on the supporting solvent, thus it undergoes an inversion in appropriate binary solvent mixtures. In most cases the solvent-induced sense inversion is single-fold [4–6], but even a double-fold inversion (e.g. right- to left- then back to right-handed sense) has been reported [3]. The sense inversion and the corresponding twist compensation exhibited by PBLG liquid crystals are quite anomalous in comparison with the general trend seen for low molecular weight, thermotropic liquid crystals, i.e. the cholesteric sense in the latter class of liquid crystals is simply dictated by the chirality of the constituent molecules and remains unchanged throughout the mesomorphic temperature range.

Since the PBLG mainchain preserves its right-handed α-helical conformation in liquid-crystalline solutions (the elongated, rod-like shape of the helix is essential for generating liquid-crystalline phases) [6], the backbone chirality alone seems not to be sufficient for interpreting the sense inversion phenomena. For the sidechain, on the other hand, a number of experimental observations have been reported which suggest that it possess a high degree of orientational freedom. In particular, deuterium N.M.R. studies on a labelled PBLG sample, PBLG-d_7, carrying perdeuteriated benzyl ester groups at the termini of sidechains, have demonstrated that the average orientation of the labelled benzyl fragment changes substantially with temperature and solvent [8, 9]. A new concept introduced as a result of these studies was a helix surface chirality, i.e. a chirality associated with the average sidechain secondary structure on the exterior of the α-helical mainchain. It was suggested that this local chirality reverses itself and this leads to an inversion of the macroscopic cholestric sense, even if the mainchain persists as a right-handed α-helix.

In this study, we re-examine the deuterium N.M.R. characteristics of PBLG-d_7 and discuss the proposed mechanism of the sense inversion in comparison with experimental observations. For a critical test, measurements of the deuterium quadrupolar splitting patterns are carried out in systems which undergo well-defined thermally- and solvent-induced cholesteric sense inversions. The results indicate that the sense inversions in PBLG liquid crystals do not necessarily require sidechain conformational transitions. This implies, in turn, that the sense determination may not be a direct consequence of the backbone or sidechain chirality, but a result of anisotropic intermolecular interactions between PBLG helices via the solvent dielectric medium.

2. Experimental

2.1. *Synthesis of PBLG-d_7*

2.1.1. *Benzyl alcohol-d_7*

Perdeuteriated benzyl alcohol-d_7 was prepared from toluene-d_8 (> 99 per cent isotopic purity, Merck, Sharp and Dohme) via benzylic bromination and subsequent hydrolysation reactions. The former reaction was carried out using the prescription of Ouertani *et al.* [10] and the resultant benzyl bromide-d_7 was hydrolysed with 4N aqueous sodium hydroxide. The product was extracted with ether and dried *in vacuo* over anhydrous sodium sulphate. The hydrolysation reaction was repeated three times until a single spot of benzyl alcohol-d_7 was obtained in the thin layer chromatography in benzene-ether (2 : 1).

2.1.2. *L-Glutamic acid γ-benzyl ester-d_7*

L-Glutamic acid (7 g) was dissolved in a mixture of benzyl alcohol-d_7 (3 ml) and sulphuric acid (60 per cent, 5 ml) at 70°C with stirring. After a reaction period of 2 h, the excess water was removed by an azeotropic distillation with 9 ml of benzene. The solution was then neutralized with aqueous sodium bicarbonate, and the precipitate was recrystallized from a hot aqueous solution to give the desired ester.

2.1.3. *N-Carboxyanhydride of L-glutamic acid γ-benzyl ester-d_7 (NCA)*

Purified ester (1·5 g) was suspended in 60 ml of dry tetrahydrofuran at 40°C, to which trichloromethyl chloroformate (0·54 ml) was added. After complete dissolution of the ester, the reaction product was solidified *in vacuo* and purified by repeated recrystallization from the *n*-hexane/ethyl actate system to furnish NCA as needles.

2.1.4. *PBLG-d$_7$*

Polymerization of NCA (1·2 g) was carried out in dry dioxane (25 ml) using triethylamine as an initiator. The molar ratio of NCA to initiator was about 50. The reaction was continued at room temperature for 5 days, then the polymer was precipitated with a large excess of methanol and dried *in vacuo*.

2.2. *N.M.R. measurements*

All deuterium N.M.R. spectra were recorded at 41·3 MHz on a JEOL JNM–GX–270 Fourier transform spectrometer equipped with a variable temperature controller. Liquid-crystalline solutions prepared at 0·20 polymer volume fraction (318 mg PBLG-d$_7$ + 1 ml solvent) were equilibrated in the spectrometer magnetic field before measurements to achieve homogeneous alignment. Spectra were recorded using complete proton decoupling without sample spinning; typically 500 FID signals were accumulated with a spectral width of 100 kHz.

3. Results and discussion

3.1. *Thermally-induced sense inversion*

Deuterium N.M.R. spectra in uniaxially oriented liquid-crystalline phases appear as a superposition of discrete quadrupolar doublets. The separation of each doublet $\Delta\nu$ is related to the orientational order of the C–D bond with respect to the field direction. Liquid-crystalline PBLG helices have a positive diamagnetic anisotropy and consequently align parallel to the applied magnetic field. For deuterons located within the flexible PBLG sidechains, the quadrupolar interactions are averaged by the following two mechanisms: namely the *extra*molecular orientational motions of the helix as a whole (librations of the rod-like molecule in the oriented liquid-crytalline phase) and the *intra*molecular segmental motions in the pendent sidechains. The former averaging process is decoupled from the latter and thus merely scales all sidechain $\Delta\nu$ values by the same factor. The relative magnitudes of sidechain $\Delta\nu$'s should, therefore, provide information solely concerned with the average sidechain secondary structure.

Typical examples of deuterium N.M.R. spectra are shown in figure 1 for PBLG-d$_7$ in 1,2,3-trichloropropane. The innermost, intense doublet is due to the four equivalent *ortho*- and *meta*-phenyl deuterons. Three relatively weak doublets are assigned based on their isotropic chemical shifts (centre frequencies of the corresponding doublets): the outermost doublet is assigned to the *para*-phenyl deuteron and the remaining two doublets to the benzyl-methylene deuterons. Inequivalent $\Delta\nu$ values for the methylene deuterons indicate that the two C–D bond vectors adopt, on average, different orientations relative to the helix axis. It can be seen in figure 1 that these two doublets invert their relative magnitudes when the temperature is increased. Figure 2 illustrates this change more clearly. The doublet (A) having a larger splitting at room temperature undergoes a rapid reduction upon heating; on the other hand, the doublet (B) with a smaller splitting exhibits a slight expansion. As a consequence, the two doublets coalesce and the orientational anisotropy at the benzyl-methylene group vanishes at about 50°C. The difference between the two quadrupolar splittings $\Delta\nu_A - \Delta\nu_B$ may be used to delineate the degree of the orientational anisotropy (or the local chirality) at the helix surface [9]. In figure 3, we compare this quantity with the strength of the macroscopic cholesteric twist. Figure 3 (*a*) summarizes the results of pitch measurements [3] where we can see that the helical twisting power S^{-1} (the reciprocal of the

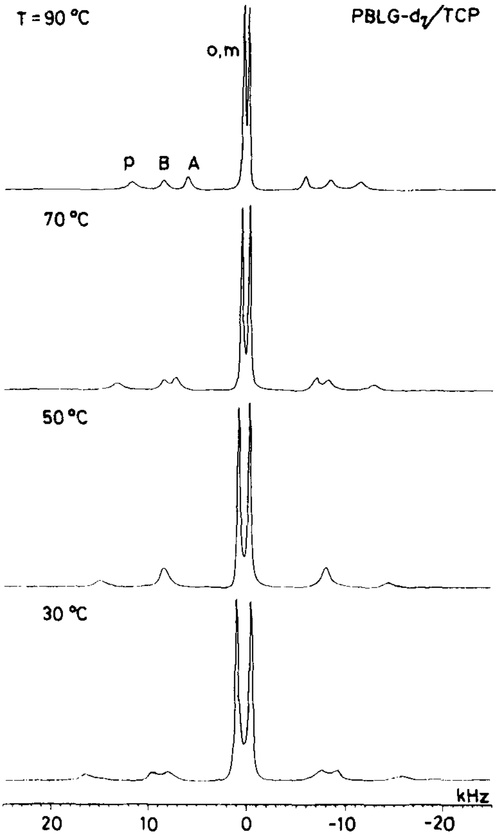

Figure 1. Deuterium N.M.R. spectra of PBLG-d_7 in 1,2,3-trichloropropane (TCP) at various temperatures.

cholesteric half-pitch) changes linearly with temperature and the cholesteric sense reverses from right ($S^{-1} > 0$) to left ($S^{-1} < 0$) upon heating. The corresponding $\Delta\nu_A - \Delta\nu_B$ values in figure 3 (*b*) also give a linear plot accompanied by a sign inversion. Although there are strong resemblances between these two plots, as will be seen later, such a coincidence does not hold generally.

m-Cresol is another solvent which supports thermally-induced sense inversion [1, 2]. The temperature dependence of the quadrupolar splitting pattern in this solvent (figure 4) shows, however, some substantial differences from that in 1,2,3-trichloropropane (figure 2). In particular, the two benzyl-methylene doublets are widely separated from each other and their differences ($\Delta\nu_B > \Delta\nu_A$) persist across the entire range of temperature (see also figure 8 for inductive assignments of doublets A and B.) In other words, the sense inversion of *m*-cresol does not necessarily require an inversion of the enantiotropic orientation of the two benzyl C–D bond vectors. (Although a coincidence of the benzyl doublet B with the *para* doublet is seen at about 80°C, this bears no relation to the sense inversion in figures 2 and 6.) The absence of any straightforward correlations between the cholesteric sense and the $\Delta\nu$ pattern either precludes the concept that the helix surface chirality is responsible for determining the cholesteric sense in this solvent, or questions the reliability of N.M.R. for measuring local chirality.

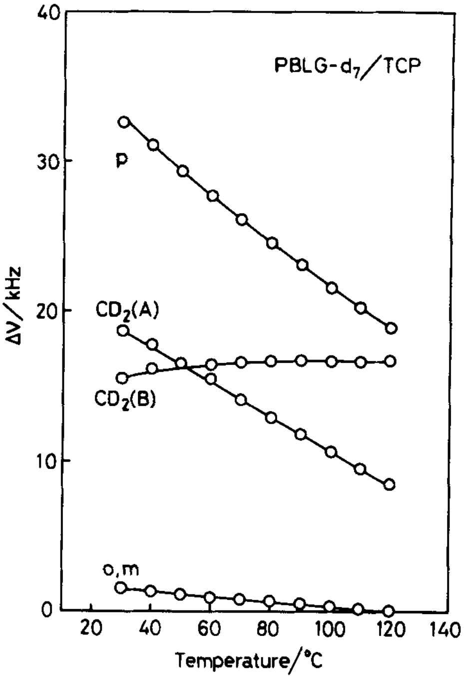

Figure 2. Temperature dependence of the quadrupolar splitting pattern of PBLG-d_7 in 1,2,3-trichloropropane (TCP).

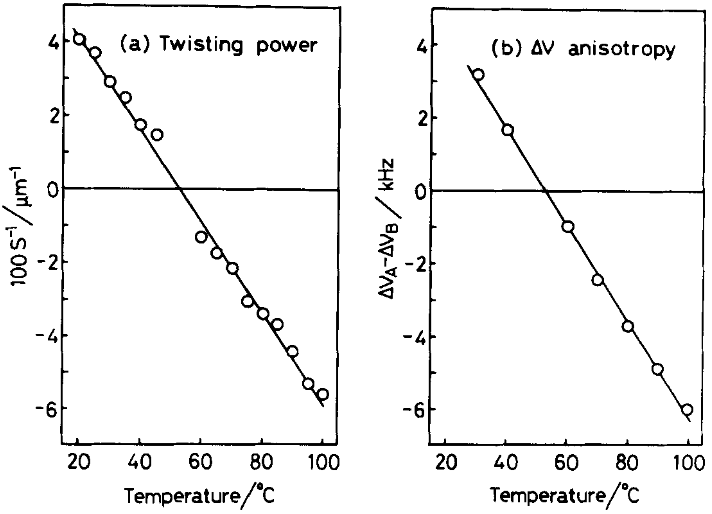

Figure 3. Comparison of (*a*) the macroscopic helical twisting power S^{-1} and (*b*) the sidechain orientational anisotropy measured by $\Delta\nu_A - \Delta\nu_B$ for the PBLG liquid crystal in 1,2,3-trichloropropane.

3.2. *Solvent-induced sense inversion*

Figure 5 summarizes the S^{-1} values measured in a mixed solvent system of 1,4-dioxane and 1,2-dichloroethane [6]. In dioxane, PBLG forms a right-handed cholesteric phase whose positive twisting power linearly decreases with temperature,

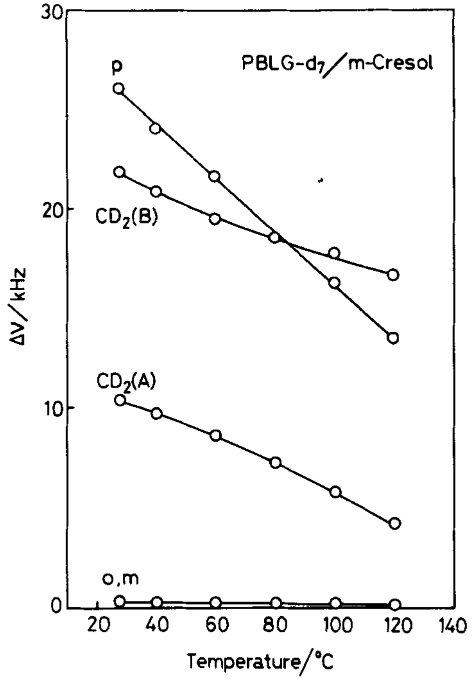

Figure 4. Temperature dependence of the quadrupolar splitting pattern for PBLG-d_7 in *m*-cresol. The cholesteric sense inversion in this solvent takes place at about 60°C [2].

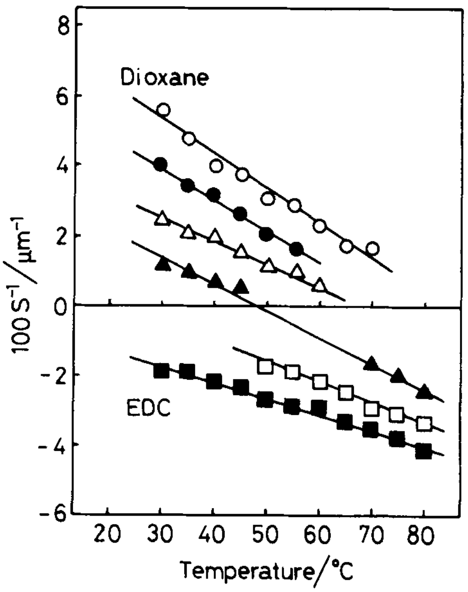

Figure 5. Temperature dependence of the helical twisting power S^{-1} in dioxane-1,2-dichloroethane (EDC) [6]: the PBLG concentration is 15 vol per cent and the 1,2-dichloroethane content in the solvent mixtures are (○) 0, (●), 20, (△) 40, (▲) 60, (□) 80, and (■) 100 vol per cent.

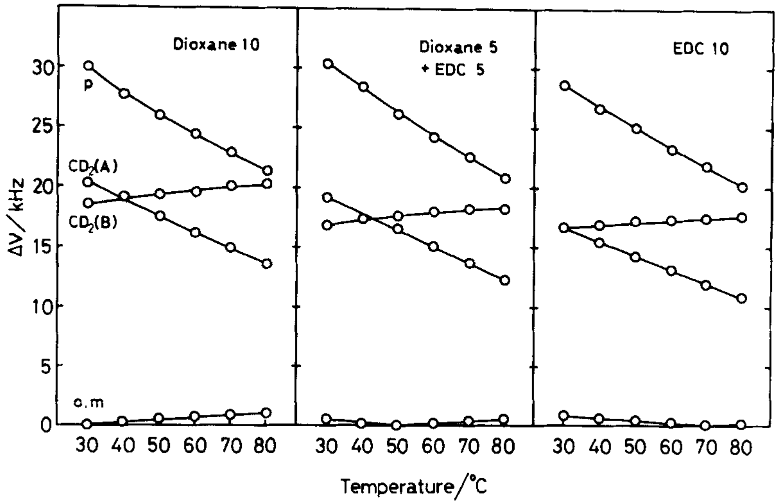

Figure 6. Quadrupolar splitting patterns for PBLG-d_7 in (left) dioxane, (centre) 1 : 1 dioxane : 1,2-dichloroethane (EDC) mixture, and (right) 1,2-dichloroethane.

T. The introduction of 1,2-dichloroethane to this system causes a downward shift of the linear plot of S^{-1} versus T along the ordinate, and results in a twist inversion in the 1,2-dichloroethane rich region. The deuterium quadrupolar splitting patterns in dioxane-1,2-dichloroethane are shown in figure 6. The predominant feature of these plots is their independence of the solvent composition. No major differences are seen between the quadrupolar splitting pattern in the right-handed phase in dioxane and that in the left-handed phase in 1,2-dichloroethane. The patterns in the intermediate region are also very similar to those in the pure solvents, thus they are represented by the one in 1 : 1 dioxane : 1,2-dichloroethane mixture. The comparison of figures 5 and 6 indicates clearly that the sense inversion in this system takes place without any major changes in the average sidechain conformation or its local chirality measured by N.M.R.

Another example of the solvent-induced sense inversion is shown in figure 7. The twisting power in *m*-cresol-1,2,3-trichloropropane exhibits a quadratic dependence on the solvent composition, accompanied by a twofold sense inversion (right → left → right) at low temperatures [3]. Although the sense inversion disappears at increased temperatures, the quadratic nature of the S^{-1} plot is still preserved. In contrast with this, the $\Delta\nu_A - \Delta\nu_B$ pattern in figure 8 shows a rather monotonic dependence on the solvent composition and undergoes sign inversion only once. A comparison of the two figures reveals again no direct correspondence between the sense inversion phenomena (especially those in a *m*-cresol rich region) and the sidechain conformational changes reflected in the quadrupolar splitting pattern.

Apart from such a comparison, it is still interesting to think about the curious solvent effect that *m*-cresol has on the PBLG sidechain orientation. In figure 8, an initial decrease in $\Delta\nu_A - \Delta\nu_B$ caused by a small addition of *m*-cresol should reflect the preferential solvation of the sidechain carbonyl ester groups due to hydrogen-bond formation with *m*-cresol [1]. The hydrogen-bonded ester groups may alter their average orientation relative to the helix axis [11, 12], so as to cause the reorientation of the terminal benzyl fragments. We have seen that the deuterium quadrupolar splitting pattern in *m*-cresol is substantially different from those in 1,2,3-trichloropropane and 1,2-dichloroethane. A rough estimate, assuming perfect

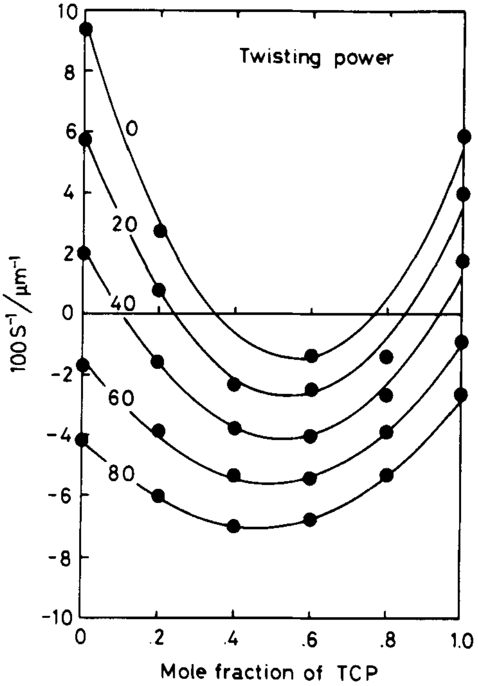

Figure 7. Dependence of the helical twisting power S^{-1} on the solvent composition in *m*-cresol-1,2,3-trichloropropane (TCP) mixtures [3]: the PBLG concentration is 15 vol per cent and the numbers indicate the temperature in °C.

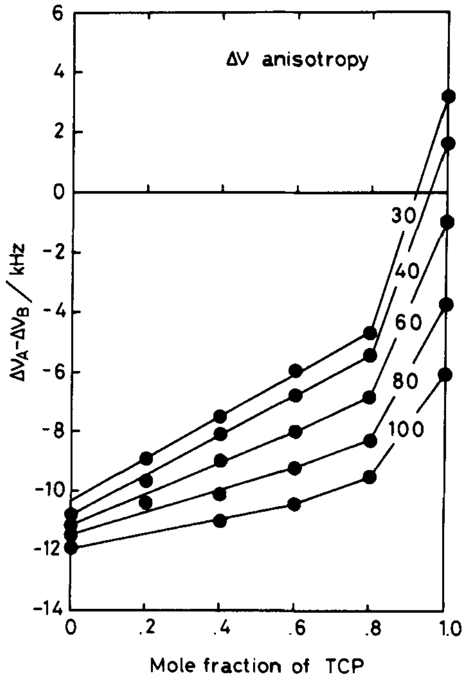

Figure 8. Plots of $\Delta\nu_A - \Delta\nu_B$ against the solvent composition in *m*-cresol-1,2,3-trichloropropane (TCP) mixtures. The numbers are the temperatures in °C.

solvation, suggests that approximately 20 per cent substitution of 1,2,3-trichloropropane by *m*-cresol would suffice to solvate all sidechain carbonyl groups. Subsequent addition of *m*-cresol should therefore cause only minor changes in the sidechain average conformation, as evidenced by a linear, slight decrease in the $\Delta\nu_A - \Delta\nu_B$ value in figure 8.

4. Concluding remarks

We have seen that the sense inversion phenomena in PBLG cholesteric liquid crystals cannot be ascribed simply to the sidechain conformational changes in so far as the latter are reflected in the sidechain quadrupolar splitting pattern. In figure 6, the $\Delta\nu$ pattern shows no appreciable differences in dioxane and 1,2-dichloroethane, although the cholesteric senses are mutually opposite in these two solvents. Moreover, an independent deuterium N.M.R. study on PBLG-γ-d_2 has demonstrated that the γ-methylene C–D bonds also adopt very similar orientations in both dioxane and 1,2-dichloroethane [13]. The PBLG rods with essentially the same average sidechain conformations can thus generate opposite cholesteric twists when embedded in different solvents. The sense inversion in *m*-cresol-1,2,3-trichloropropane (in a *m*-cresol rich region) also occurs without significant changes in the average sidechain conformation (compare the S^{-1} pattern in figure 7 with the $\Delta\nu_A - \Delta\nu_B$ pattern in figure 8). In view of these observations, it should be noted that Samulski and Samulski [14] have predicted by calculating the van der Waals interaction energies between two PBLG rods that the twist direction may reverse when the solvent dielectric constant (ε_m) is changed beyond a certain critical value. Toriumi *et al.* [3] have corroborated this prediction for a series of alkylchloride solvents, i.e. the room-temperature S^{-1} value decreases continuously from positive (right-handed twist) to negative (left-handed twist) with increasing ε_m. More recently, Osipov [15] has re-examined the Samulski–Samulski calculation and shown that even the twofold cholesteric sense inversion can be accounted for with changes in the dielectric properties of the solvent medium relative to those of the polypeptide α-helix.

The temperature dependence of S^{-1} observed in figures 3 (*a*) and 5 can be described by the simple equation $S^{-1} = A(T - T_c)$. The T_c value indicates how the compensation temperature shifts with the supporting solvent, while the coefficient $A \equiv dS^{-1}/dT$ always remains negative. In other words, an increase in temperature causes a linear enhancement of the left-handed twisting power, regardless of the cholesteric sense at room temperature (i.e. the solvent effect). A possible explanation for the linear dependence of S^{-1} on T may be given by assuming a hindered helix rotation within a plane normal to the axis of cholesteric torsion. This empirical equation has been reproduced from the Keating theory [16] by introducing an AT_c term which would represent the inherent twisting power [1]. Kimura *et al.* [17] and Osipov [15] have arrived at similar expressions by considering both attractive and repulsive interhelix interactions in their microscopic molecular theories.

Our present observations together with these theoretical considerations suggest strongly that the thermally- and solvent-induced cholesteric sense inversions in PBLG liquid crystals are the result of more general anisotropic intermolecular interactions between helices through the solvent dielectric medium. The specific nature of the backbone and/or sidechain chirality, although necessary to form the cholesteric supermolecular structure, probably plays a minor role in determining the cholesteric twist direction.

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