Measurement and Analysis of the Molecular Ordering Tensors of Two Enantiomers Oriented in a Polypeptide Liquid Crystalline System

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High-resolution proton and natural abundance carbon-13 NMR spectra of a rigid chiral molecule, $(\pm)$-β-(trichloromethyl)-β-propiolactone, dissolved in the liquid-crystalline phase of poly(γ-benzyl-L-glutamate) (PBLG), in methylene chloride, were analyzed. From the NMR data and the X-ray structure, the full order matrix was determined and analyzed in detail for each optical isomer of the molecule. It is shown that the spectral discrimination of the enantiomers in this lyotropic medium arises mainly from the difference in their average molecular orientation and the principal axis systems of orientation are compared.

Introduction

The analysis of NMR spectra of molecules partially oriented in anisotropic media has been used as a powerful method for obtaining structural information and for the analysis of the order matrix.[1,2] The latter is an essential element for the understanding of intermolecular interactions responsible for the ordering. Nevertheless, the complete study of chiral molecules oriented in a chiral environment has never been performed before for two reasons: Firstly, considering the lack of symmetry elements in a chiral solute, five order parameters must be determined to describe the full order matrix, **S**. This requires a large number of dipolar coupling constants. For such case, the measured spectra are often despairingly complicated and the analysis difficult, even for a rigid molecule. Secondly, the liquid-crystal solvent must induce significant discrimination between the orientation of the enantiomers to enable the correct analysis of the spectra of each optical isomer. Experimentally, these conditions are not easy to fulfill.

Recently, it has been reported that a lyotropic cholesteric liquid crystal, poly(γ-benzyl-L-glutamate) (PBLG), dissolved in an organic solvent, can be used as a suitable medium for the measurement of enantiomer spectra and the determination of enantiomeric excess through deuterium,[3–6] proton or natural abundance carbon-13 NMR.[7] The discrimination is expressed in the NMR spectra in terms of different values for the quadrupolar or dipolar couplings and the chemical shift anisotropy for each enantiomer. This phenomenon arises because the two enantiomers exhibit different order parameters. The relationship between the elements of molecular ordering tensor, $S_{\alpha\beta}$, in the reference axis system and the dipolar couplings, D_{ij}, assuming no difference of geometry between the two optically active isomers, is[1,7]

$$\sum_{\alpha,\beta=x,y,z}(\cos(\varphi_{ij}^{\alpha})^{\text{R or S}}\cos(\varphi_{ij}^{\beta})^{\text{R or S}}S_{\alpha\beta}^{\text{R or S}}) = -\frac{4\pi^2 r_{ij}^3}{h\gamma_i\gamma_j}D_{ij}^{\text{R or S}}$$

(1)

where $(\varphi_{ij}^{\alpha})^{\text{R or S}}$ and $(\varphi_{ij}^{\beta})^{\text{R or S}}$define the angles for each enantiomer, R or S, between the *ij* axes and molecule-fixed

coordinate system (x, y, z) and $D_{ij}^{\text{R or S}}$ are their respective dipolar coupling constants. Here, γ_i and γ_j are the magnetogyric ratios of the coupled nuclei, respectively, and r_{ij} is the internuclear distance between nuclei *i* and *j*. The discrimination between the spectra of the two enantiomers in a chiral phase arises because the respective average values over the anisotropic molecular reorientations are different. This effect may be due to differences in energetic (binding) values or in entropic (shape) factors for the solute solvent interactions. It is therefore of interest, in order to advance our understanding of the intermolecular interactions in these systems, to obtain the quantitative values for the discrimination between the two enantiomers. To achieve this goal, the chiral molecule, $(\pm)$-β-(trichloromethyl)-β-propiolactone (TMPL), was chosen because of its simple and rigid structure. In recent years this compound was extensively studied and used to test for the selectivity of chiral stationary phases in capillary gas chromatography of enantiomers.[8,9] The crystal structure of this molecule has been also investigated by X-rays.[10] In this paper, we report the determination and detailed analysis of the full order matrix for the two enantiomers dissolved in a polypeptide liquid-crystal solvent derived from the analysis of data of both proton and carbon-13 NMR spectra.

Experimental Section

PBLG (DP1183, Sigma) (100 mg) was weighed directly into a 5 mm NMR tube and a solution of about 70 mg of a mixture of *R(+)*-enriched (ee = 40%) TMPL dissolved in 560 mg of CD_2Cl_2 was added. The compounds were commercially obtained from Aldrich. Working with an enriched sample, the analysis of the NMR spectra and the correlation of the ^1H–^1H and ^{13}C–^1H data sets for each enantiomer are considerably simplified. The tube was degassed and sealed under vacuum in order to avoid the effects of paramagnetic oxygen and the evaporation of solvent. The tube was then centrifuged in both directions until an optically homogeneous sample was obtained. NMR experiments were performed on a Bruker AM400 high-resolution NMR spectrometer operating at frequencies of 400.13 MHz for proton and 100.16 MHz for carbon-13 and equipped with a 5 mm diameter $^1H/^{13}C$ dual probe. One component of the deuterated dichloromethane doublet provided the lock signal. The samples were spun at 20 Hz and the temperature was maintained at 300 K by the Bruker BVT 1000 system. The proton and carbon spectra were recorded using 90° pulses but for ^{13}C gated decoupling was applied during the relaxation delay

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TABLE 1: Experimental and Calculated HH and CH Dipolar Coupling Values (in Hz) of TMPL derived from the Analysis of $^1H-^1H$ and $^1H-^{13}C$ Spectra

bond	atoms[a]	J_{ij}^{iso} [b]	D_{ij}^R [b]	D_{ij}^R [c]	ΔD_{ij}^R [d]	D_{ij}^S [b]	D_{ij}^S [c]	ΔD_{ij}^S [d]
H-H	2–3	-17.06 ± 0.03	35.32 ± 0.05	35.34	-0.02	41.78 ± 0.01	41.40	0.38
	2–4	5.75 ± 0.03	-2.72 ± 0.05	-1.25	-1.47	-3.25 ± 0.01	-1.78	-1.47
	3–4	3.72 ± 0.03	4.81 ± 0.05	1.32	3.49	0.97 ± 0.01	-0.54	1.51
^{1}C-H	1–2	144.40 ± 0.05	45.75 ± 0.04	45.81	-0.06	58.70 ± 0.01	58.82	-0.12
	1–3	144.40 ± 0.05	19.80 ± 0.04	19.85	-0.05	15.32 ± 0.01	15.58	-0.26
	4–5	170.70 ± 0.05	47.88 ± 0.03	47.95	-0.07	26.43 ± 0.01	26.53	0.10
^{2}C-H	1–4	-0.50 ± 0.06	0.98 ± 0.03	0.87	0.11	-1.61 ± 0.01	-1.33	-0.28
	2–5	-2.50 ± 0.05	1.93 ± 0.04	0.20	1.73	2.34 ± 0.01	1.64	0.70
	3–5	4.20 ± 0.05	-2.17 ± 0.04	-0.78	-1.39	-0.99 ± 0.01	-2.75	1.76

[a] See Figure 1 for the atom numbering. [b] Experimental fitted values and standard deviations obtained from the PANIC program. [c] Calculated values from the SHAPE program. [d] Difference between the experimental and calculated dipolar coupling constants.
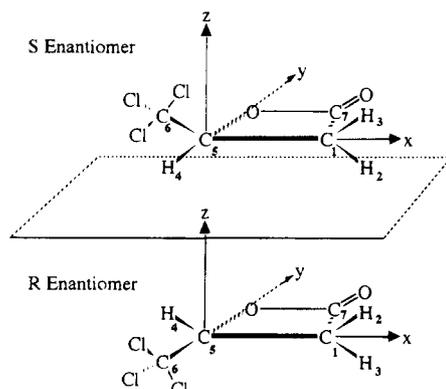

Figure 1. Numbering system of the carbons and protons and definition of the initial molecular coordinate frame for $(\pm)$-β-(trichloromethyl)-β-propiolactone (TMPL). The origin of the right-handed coordinate system is centered on carbon-5. The Ox axis is aligned with the C_5-C_1 bond.

period (4 s) to benefit from the nuclear Overhauser effect. Before the NMR spectra was measured, the sample was kept for 1 h in the magnetic field in order to achieve a good thermal equilibration.

Results and Discussions

The numbering of the atoms in $(\pm)$-β-(trichloromethyl)-β-propiolactone used in this paper is presented in Figure 1. The analysis of the dipolar NMR spectra was performed on the Bruker program PANIC using the "oriented molecule" option. In this program, the total Hamiltonian includes the chemical shift and scalar and dipolar interactions.

Analysis of Proton Spectra. The experimental and simulated spectra are presented in Figure 2 and the ^{1}H NMR data of each enantiomer are summarized in Table 1. The proton spectrum of TMPL is a typical ABX spin system. The chemical shifts of protons H2, H3, and H4 are 3.74, 3.57, and 5.04 ppm for R enantiomer and 3.75, 3.57, and 5.04 ppm for S enantiomer, respectively. The assignment of protons H2 and H3 was determined by performing a two-dimensional NOE experiment which is shown in Figure 3. The integration of the cross-peak intensities enables us to assign the cis conformation for protons H2 and H4.[11,12]

The ABX spectrum cannot be iterated by using all the nine spectral parameters. Consequently, according to the procedure of Snyder,[13] the isotropic scalar couplings were assumed equal to their anisotropic values. The relative signs of J_{HH} and D_{HH} couplings, reported in Table 1, have been determined by performing tickling experiments.[11] The final choice of the sign for J_{HH} couplings was made assuming that the geminal coupling, $^2J_{23}$, is negative.[14] With these data, the maximum deviation
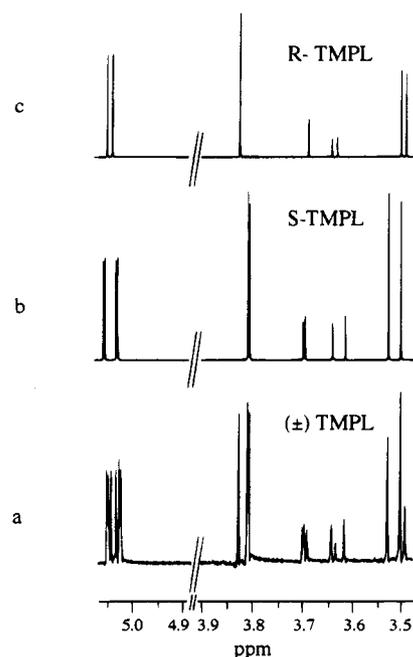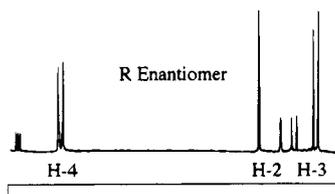

Figure 2. (a) Proton spectrum of $R(+)$-enriched (ee = 40%) TMPL in PBLG/CD_2Cl_2 at 300K. (b, c) Simulated spectra of S and R enantiomers using the fitting parameters given in Table 1. The experimental spectrum is recorded with 32 scans and 8K data points. Gaussian digital filtering and zero filling to 16K points is used to enhance digital resolution to 0.09 Hz/pt. A line width of 1.5 Hz at half-maximum was measured.

between any line in the simulated and measured spectra, for both enantiomers, was smaller than 0.04 Hz.

Analysis of Carbon-13 Spectra. Kumar *et al.* have shown that the measurements of carbon-proton dipolar couplings can be used to obtain a full order matrix when the number of proton-proton dipolar constants is insufficient[15] and this approach applied here. We have recently presented an experimental study which describes the visualization of enantiomers in the PBLG/CD_2Cl_2 liquid-crystalline system through natural abundance carbon-13 NMR.[7] This solvent offers two advantages over thermotropic nematic or cholesteric solvents. Firstly, the ordering is small[16] and consequently the $^{13}C-^1H$ spectra of solutes are essentially first order and similar to isotropic spectra. Secondly, the carbon-13 signals arising from the solvent appear as a noninterfering background to the spectra of the dissolved molecules. Hence, elimination of several data points in the beginning from the FID prior to the Fourier transformation is not needed and the optimal signal to noise ratio can be obtained.[15]

The experimental and simulated spectra for the C_5 and C_1 carbons of each enantiomer are displayed in Figure 4a,b and

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R Enantiomer

H-4   H-2 H-3

In the determination of the $^1D_{CH}$ couplings we assumed that the value of the scalar coupling $^1J_{CH}$ is positive.[17] The choice of signs for the scalar couplings, $^2J_{CH}$, will be justified later herein. The maximum deviation, for any line, between the simulated and experimental ^{13}C spectra were 0.2 and 0.1 Hz for the R and S enantiomers, respectively. It might be noted that the assignment of the lines for each enantiomer is facilitated

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TABLE 2: Five Independent Elements, S_{ab}, of the Order Tensor for the R and S Enantiomers

parameter[a]	enantiomer S	enantiomer R
S_{xx}	0.000 27	0.000 18
S_{xy}	−0.000 52	−0.000 37
S_{xz}	0.000 57	−0.000 04
S_{yz}	0.000 30	0.000 13
S_{zz}	−0.001 92	−0.001 64

[a] The $S_{\alpha\beta}$ values are reported with respect to the molecule fixed Cartesian coordinate system (see Figure 1).

TABLE 3: Elements, $S_{\alpha'\beta'}$, of the Diagonalized Order Matrix for the R and S Enantiomers

parameters[a]	enantiomer S	enantiomer R
$S_{x'x'}$	0.000 28	0.000 08
$S_{y'y'}$	0.001 83	0.001 57
$S_{z'z'}$	−0.002 11	−0.001 65

[a] The $S_{\alpha'\beta'}$ values are associated with the axes, x',y',z', of the principal molecular frame (see Figure 5).

The inputs of the SHAPE program was suitably modified to discard those dipolar coupling constants which could not be measured experimentally ($^{13}C-^{13}C$ couplings). Nevertheless, these constants could be obtained by back calculation from the $S_{\alpha\beta}$ parameters evaluated through SHAPE. This method also enabled us to determine the signs of $^2D_{CH}$, and of $^2J_{CH}$, from the one bond dipolar couplings, $^1D_{ij}$, using the calculated values of $S_{\alpha\beta}$.

The five calculated independent elements of the ordering matrix $S_{\alpha\beta}$ are listed in Table 2. The value of the ratios ($h\gamma_i\gamma_j/4\pi^2$) used in the calculations are 120 067 Hz Å^3 and 30 190 Hz Å^3 for pairs of protons and carbon−proton, respectively. The
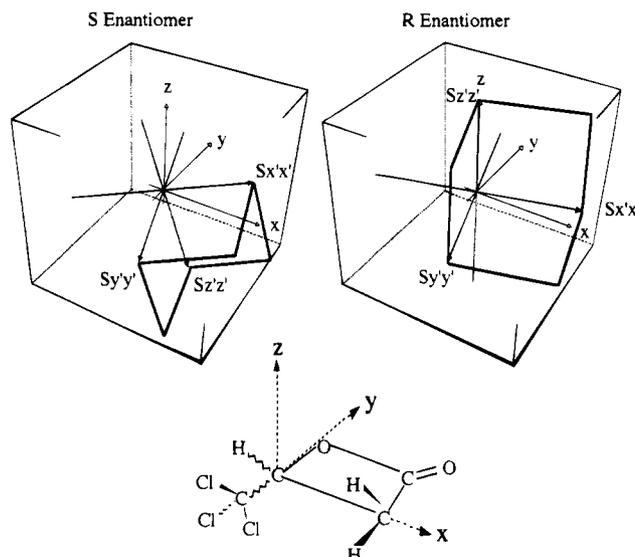

Figure 5. Space representation of the orientational principal axis system of both enantiomers. The eigenvectors associated to the eigenvalues of the diagonal order matrix are orthonormal.

fact that the orientation in a liquid crystal is apolar.[1] Consequently, the choice of the final eigenvectors for the two molecular frames must be determined. In doing this we take into account that the principal molecular coordinate systems for the enantiomers should have opposite handedness (not superimposable) and the symmetry plane (defined to visualize the two enantiomers, in our case the xy plane) must be shared by the two coordinate systems. Under these conditions, we have chosen a right-handed and a left-handed coordinate system for

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due to specific interactions of the enantiomers in the chiral media The spectral discrimination between the enantiomers originates from this difference.

Conclusions

In this work, we have been able to quantify the differential ordering effect of two enantiomers in PBLG/CD$_2$Cl$_2$ liquid crystal. For the first time, all the five independent elements of the Saupe order matrix have been measured for two optical isomers. They were derived from nine independent ^{1}H$-^1$H and ^{1}H$-^{13}$C dipolar couplings, the geometry being assumed from X-ray results.

Furthermore, diagonalizing the order matrix allowed for the determination of the principal axis system for orientation in each of the enantiomers. The main conclusion is that in this chiral liquid-crystal solvent, the principal axis systems of the R and S enantiomers are not images of each others. We demonstrated that those frames are tilted by an amount that we have expressed through the three Euler angles ($\theta = 16.02°$, $\phi = -0.41°$, and $\chi = -2.56°$) necessary to rotate one of the principal axis systems to coincide with the image of the other. This results allows us to draw the following conclusions about the differential ordering effect of enantiomers in chiral liquid crystals:

Order parameters $S_{ij} = \langle 3 \cos^2 \theta_{ij}^z - 1 \rangle/2$, are composite quantities as they depend both on the angle θ_{ij}^z and on the motional averaging. Consequently, whenever two order pa-

SHAPE geometries and X-ray structures might also provide information about possible deformations of enantiomers in chiral liquid crystalline phases.

References and Notes

(1) Emsley, J. W.; Lindon, J. C. In *NMR Spectroscopy Using Liquid Crystal Solvents*; Pergamon Press: Oxford, U.K., 1975.

(2) For a recent application of ^{13}C NMR, see: Hagemeyer A.; Tarroni R.; Zannoni C. *J. Chem. Soc, Faraday Trans.* **1994**, *90*, 3433 and references cited therein.

(3) Bayle J. P.; Courtieu J.; Gabetty E.; Loewenstein, A.; Péchiné J. M. *New J. Chem.* **1992**, *16*, 837.

(4) Canet, I.; Meddour, A.; Courtieu, J. *J. Am. Chem. Soc.* **1994**, *116*, 2155.

(5) Meddour, A.; Canet, I.; Loewenstein, A.; Péchiné J. M.; Courtieu, J. *J. Am. Chem. Soc.* **1994**, *116*, 9652.

(6) Canet, I.; Meddour, A.; Loewenstein, A.; Péchiné, J. M.; Courtieu, J. *J. Am. Chem. Soc.* **1995**, *117*, 6520.

(7) Lesot, P.; Merlet, D.; Meddour, A.; Loewenstein, A.; Courtieu, J. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1371.

(8) Wynberg, H.; Staring, E. G. J. *J. Am. Chem. Soc.* **1982**, *104*, 166.

(9) Koenig, W. A. *J. High Resolut. Chromatogr.* **1993**, *16*, 569

(10) Barasan, R.; Dou, S.; Weiss, A. *J. Mol. Struct.* **1991**, *249*, 127.

(11) Ernst, R. R., Bodenhausen, G.; Wokaun, A. In *Principle of Nuclear Magnetic in One and Two Dimensions*; Clarendon Press: Oxford, U.K., 1987.

(12) Keepers, J. W.; James, T. L. *J. Magn. Reson.* **1984**, *57*, 404.