Investigation of the Enantioselectivity of Three Polypeptide Liquid-crystalline Solvents using NMR Spectroscopy

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The enantioselective potentialities of three polypeptide liquid-crystalline solutions made of poly-γ-benzyl-L-glutamate (PBLG), poly-γ-ethyl-L-glutamate (PELG) or poly-ε-carbobenzyloxy-L-lysine (PCBLL) are investigated and compared using proton, carbon-13 and deuterium NMR spectroscopy. From a practical point of view, we propose an efficient alternative to the PBLG system, which is essential when this chiral homopolymer fails in distinguishing between enantiomers or enantiotopic elements. From a theoretical point of view, this study provides new information on the role of the lateral side chain of the polypeptide in the mechanisms of enantiodiscrimination. The various experimental results reported show the extraordinary adaptability of this methodology, and so enlighten the very large potential of NMR in chiral liquid crystals in the field of enantiomeric and enantiotopic analysis.

Key words : Chiral liquid crystal, Enantiomeric and enantiotopic differentiation, NMR spectroscopy, Poly-γ-benzyl-L-glutamate, Poly-ε-carbobenzyloxy-L-lysine, Poly-γ-ethyl-L-glutamate.

INTRODUCTION

To provide an alternative to the existent isotropic NMR techniques in the field of the enantiomeric or enantiotopic analysis [1], we have developed an efficient and resourceful NMR methodology based on the use of chiral liquid crystals (CLC’s). To date the best results were obtained using a lyotropic liquid crystal made of a synthetic polypeptide, the poly-γ-benzyl-L-glutamate, (noted PBLG; R: -(CH₂)₂-CO₂CH₂C₆H₅), dissolved in helicogenic weakly polar organic solvents such as CHCl₃, CH₂Cl₂, ... as well as polar ones such as DMF, THF, ...[2]. Because of the chiral property of the solvent, organic solutions of PBLG generally generate a sufficient differential ordering effect (DOE) to distinguish between two enantiomers using multinuclear NMR spectroscopy at natural isotopic abundance level. Actually, we have demonstrated that enantiodiscrimination of chiral compounds is possible through a difference in order-sensitive NMR observables [2], namely internuclear dipolar couplings (D), chemical shift anisotropies (Δσ) and quadrupolar splittings (ΔνQ) for spins I > 1/2. This is a significant advantage compared to the NMR methods in chiral isotropic solvents that are usually based only on rather small differences in ¹H chemical shifts. In almost all cases, the spectral separation between the S and R isomers and the S/N ratio is large enough for measuring satisfactorily the enantiomeric excesses (e.e.’s) of enriched samples [2].

In spite of the numerous successful results achieved for a wide range of polar and apolar functionalised as well as non-functionalised molecules using PBLG [2,3], it was pertinent to investigate the enantioselective potential of other chiral polypeptides exhibiting liquid-crystalline properties. From a theoretical point of view, such studies may provide, for instance, fundamental information on the role of the lateral side chain of polypeptide in the mechanisms of enantiodifferentiation. From a practical point of view, it is of a great interest to have an alternative to the PBLG oriented phases, when weak or no enantiodifferentiation is obtained with this chiral polymer.
Here, we report a study of the enantioselectivity of two other synthetic polypeptides in solution, the poly-\(\gamma\)-ethyl-L-glutamate (noted PELG; R: \(-\text{CH}_2\text{CH}_2\text{CO}_2\text{-CH}_2\text{CH}_3\)) and the poly-\(\epsilon\)-carbenzoxyl-L-lysine (noted PCBLL; R: \(-\text{CH}_2\text{CH}_4\text{-NHCO}_2\text{-CH}_2\text{C}_6\text{H}_5\)) through multinuclear NMR spectroscopy. Various works on the structure and properties of these polypeptide liquid crystals were reported in literature, but to the best of our knowledge none of them investigated or described their use as chiral oriented solvents able to differentiate between enantiomers or enantiotopic groups [4]. Homopolymers are commercially available with high degree of polymerisation (DP) which ensures the existence of a large liquid-crystalline range. We illustrate our purpose through four significant examples, and in particular by investigating the case of (±)-1-chloropropan-2-ol (±)-1 for which we have reported that it was possible to differentiate its enantiomers in the PBLG/CHCl\(_3\) phase using \(^{1}\text{H},\ 13\text{C},\ 13\text{C}\{-^{1}\text{H}\}\) and \(2\text{H}\{-^{1}\text{H}\}\) NMR in natural abundance [2].

**RESULTS AND DISCUSSION**

Figure 1(a) and (b) present the \(^{1}\text{H}\) and \(^{13}\text{C}\) NMR spectra associated with the methyl group of (±)-1 recorded in PELG (a) and PCBLL (b) at 298 K by adding 64 and 1800 scans, respectively. The C-13 interferogram was acquired using a pulse angle of ~70°, a recycle delay of 1.5 sec and 8 k of data points. The resonances due to each enantiomer are arbitrarily labelled by (Z) and (o).

![Fig. 1](image-url) 400.1 MHz \(^{1}\text{H}\) and 100.6 MHz \(^{13}\text{C}\) NMR signals associated with the methyl group of (±)-1 recorded in PELG (a) and PCBLL (b) at 298 K by adding 64 and 1800 scans, respectively. The C-13 interferogram was acquired using a pulse angle of ~70°, a recycle delay of 1.5 sec and 8 k of data points. The resonances due to each enantiomer are arbitrarily labelled by (Z) and (o).

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**MATERIALS AND METHODS**

**Sample Preparation.** In this study, the PELG and PCBLL polymers were purchased from Sigma and used with no further purification. Their respective DP was 1707 and 992. The preparation of liquid-crystalline NMR samples made of PELG and PCBLL is similar to that described for the PBLG samples. Details on the sample preparation are reported in Ref. [2]. The exact composition of each oriented NMR sample is shown in Table 1 or given in the text.

Several qualitative studies have indicated that almost all organic co-solvents used with PBLG are also co-solvents for the PELG and PCBLL, in the same range of sample composition and temperature [2]. We have also noticed that, PELG or PCBLL was able to make soluble a larger amount of hydroxylated solute (up to 200% in case of ethanol) than PBLG, before precipitating the polymer fibers. This greater tolerance for dissolving organic compounds can be a significant advantage when large molecules are investigated.

**NMR Spectroscopy.** Proton, deuterium and carbon-13 NMR spectra were performed at 9.4 T on a Bruker DRX 400 high-resolution spectrometer equipped with a standard variable temperature unit (BVT 3000), and an inverse multinuclear probe (BBI) operating at 61.4 MHz for deuterium and at 100.6 MHz for carbon. Because the long-term stability of our magnet, no field-frequency lock system was used. For \(2\text{H}\{-^{1}\text{H}\}\) NMR, the protons were decoupled using the broad-band composite pulse sequence WALTZ-16. For the proton-coupled carbon-13 spectra, proton irradiation was applied during the relaxation delay period to benefit from the nuclear Overhauser effect. Note here that the proton decoupling of solutes embedded in these three polypeptide oriented phases does not necessitate more power than for isotropic samples because of rather small magnitude of the residual heteronuclear dipolar couplings. Specific description of the 2D natural abundance deuterium Q-COSY experiments may be found in Refs [5]. Other experimental NMR parameters or details are given in the legend of figures.
and 1600 (t/2) sites of the molecule. To take into account solvent order variations in the DOE's comparison, the results obtained with (±)-
are clearly identified (black and white symbols), respectively. Note that the quadrupolar splitting for one of the diastereotopic deuterons, 3 or 3', in one of the enantiomers is averaged to zero. This indicates that the corresponding C-D internuclear axis is fortuitously aligned in average along the magic angle (θ = 54.7°) direction. [2] The results obtained with (±)-2 can be compared with data recorded in the PBLG/CHCl₃ phase and described in ref. 5. In both experiments, the samples were prepared using the same conditions (only the DP of polymer changes). For this example, the comparison of 

![Fig. 2](image)

**Fig. 2** 61.4 MHz NAD NMR signals of the methyl and methylene groups of (±)-2 in the PCBL phase (a) and PCBL phase (b). These columns are extracted from the tilted 2D Q-COSY spectrum recorded using 320 (t1) × 1600 (t2) data points. 320 transients for each t1 increment were added, leading to a total number of 102400 scans. A gaussian filtering was applied in both dimensions. The recycle delay of the experiment is equal to 0.5 sec. The quadrupolar doublets due to each enantiomer are arbitrarily labelled by (Z) and (O).

Table 1: **1H**, **13C** and **2H** sites of (±)-1 showing unambiguously a chiral discrimination at 298 K

<table>
<thead>
<tr>
<th>Polymer</th>
<th>DP</th>
<th>MW</th>
<th>Composition¹</th>
<th>T / K</th>
<th><strong>1H</strong> NMR</th>
<th><strong>13C</strong> NMR</th>
<th><strong>2H</strong>-{<strong>1H</strong>} NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBLG</td>
<td>562</td>
<td>123000</td>
<td>100/100/350</td>
<td>298</td>
<td>1.3</td>
<td>1.3</td>
<td>1.1,c,2,3</td>
</tr>
<tr>
<td>PELG</td>
<td>1707</td>
<td>268000</td>
<td>100/100/400</td>
<td>295</td>
<td>3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>PCBLL</td>
<td>992</td>
<td>260000</td>
<td>100/100/350</td>
<td>298</td>
<td>3</td>
<td>1.3,2,3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

¹ Amount in mg of solute/polymer/co-solvent. ² Increase of the linewidths due to an unresolved peak separation.

The numbering 1 and 1' refers to the diastereotopic deuterons of the molecule.

Table 2: **DOE** for calculated all deuterated sites of (±)-2 in the PBLG/CHCl₃ phases and the PCBLL/CHCl₃ phases at 298 K

<table>
<thead>
<tr>
<th>Polymer</th>
<th>DP</th>
<th>Composition</th>
<th>D-1⁵</th>
<th>D-2</th>
<th>D-3</th>
<th>D-3'</th>
<th>D-4</th>
<th>D-5</th>
<th>D-5'</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBLG</td>
<td>562</td>
<td>100/100/350</td>
<td>0.74</td>
<td>0.08</td>
<td>0.09</td>
<td>0.18</td>
<td>0.06</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>PCBLL</td>
<td>992</td>
<td>100/100/350</td>
<td>1.11</td>
<td>0.13</td>
<td>0.64</td>
<td>2.00</td>
<td>1.53</td>
<td>0.50</td>
<td>1.11</td>
</tr>
</tbody>
</table>

⁵ DOE = \[\Delta \nu_\alpha - \Delta \nu_\beta \] \[/\] \[\Delta \nu_{\text{DOE}} \]. To take into account solvent order variations in the DOE’s comparison of (±)-2 in PCBLL [5], the Δν_α’s measured in this phase were weighted by the ratio (Δν_α of CDCl₃ in PBLG / Δν_α of CDCl₃ in PCBLL). ⁶ Deuterated sites of the molecule.

NAD spectra indicates that the magnitude of quadrupolar splittings in PCBLL is smaller than in PBLG (except for one site). However the measured differences, \[\Delta \nu_\alpha - \Delta \nu_\beta \], between enantiomers are much larger than in the PBLG phase. To describe quantitatively this observation, Table 2 presents the DOE’s for all non-equivalent deuterons in the molecule. As DOE is related to the difference in the order parameters of the CD bond (S_{CD}) for both isomers, the systematic increasing of DOE values in this CLC evidences a better enantioselectivity of PCBLL helices for this chiral compound. Such a result suggests therefore substantial prospects when organic solutions of PBLG give rather poor results or fail in discriminating between enantiomers.

It could be argued that the assignment of the quadrupolar doublets associated with the two diastereotopic deuterons is not trivial since the difference of chemical shifts between them is very small [3]. To overcome this problem, we have recorded the NAD 2D Q-COSY spectrum of (±)-2 in a racemic mixture made of PBLG and its enantiomer, PCBDL (DP=1296, MW=234000, also commercially available from Sigma), in CHCl₃, and denoted hereafter PCBL. As in the case of racemic mixtures made of PBLG and PBGD (enantomer of PBLG), the S and R isomers in a PCBL phase are in fast exchange limit by diffusing very rapidly, on the NMR time scale, from the vicinity of PBLG and PCBDL [6]. Consequently, we observe only an average of these situations, thus eliminating all chiral discriminations. Note that this two-step analytical strategy allows the relative sign of quadrupolar doublet in the chiral phase to be determined. Indeed disregarding the solvent effects, the quadrupolar splittings measured in the achiral oriented phase correspond to the algebraic average of Δν_α and Δν_β.

measured in the CLC [6]. Figure 2(b) displays the NAD signals of the methyl and methylene groups in the racemic mixture. As expected, the enantiomddifferentiation is lost because one and two quadrupolar doublets (instead of one) are detected for each non-equivalent deuteron. We are now able to assign the two doublets for each of the diastereotopic deuterons in the PBLG phase as shown in...
enantiotopic discrimination of deuterons in the methylene group of $\text{PCBLL}$ at 298 K. As expected, the $^2\text{H}$ spectrum in the PCBLL system (reported in Figure 4(b)) exhibits a single quadrupolar doublet for the two enantiotopic deuterons of $\text{PCBLL}$. Note here that the sign of splittings for deuterons 1 and 3,3' is the same, namely either all positive or negative.

Another useful application of NMR in chiral liquid crystalline phases is the possibility of differentiating between enantiotopic nuclei in rigid molecules of $C_5$, $C_{2v}$, $D_{2d}$ and $S_4$ symmetry [7,8]. To investigate the enantiotopic selectivity of PELG and PCBLL polymers dissolved in CHCl$_3$, we have recorded the proton coupled carbon-$^{13}$C spectra and deuterium spectra of two $C_5$ symmetry molecules, the ethanol (3) and the ethylbenzene-$^3$-d$_{10}$ (4) [5] at 298 K. The sample compositions (given in mg) used for solutes 3 and 4 were 100/100/420 and 15/100/400, respectively. In Figure 3, we can see that the proton-coupled carbon-$^{13}$C spectral pattern associated with the methylene group of 3 cannot be considered as an $A_2X$ spin system, but as an $AA'X$ ($X$ is the carbon, A and A’ are the two protons). Indeed the presence of further peaks in the spectrum (labelled by black circles) indicates that protons H-1 and H-2 are no longer magnetically equivalent because the $^{13}$C-1H dipolar couplings are nonequivalent here. In this example, the difference of dipolar couplings between the prosterogenic carbon and the pro-R and pro-S protons is equal to $[^{13}\text{C}-^1\text{H}]$ Hz. Actually, this result, also observed using the PBLG phase, [7] reveals indirectly the $\text{Si} / \text{Re}$ facial differentiation of 3 in PELG.

The same conclusion can be formulated from the very straightforward analysis of the $^2\text{H}$ spectrum of 4 given in Figure 4(a). Indeed, the observation of two quadrupolar doublets associated with the CD$_2$ group means clearly that enantiotopic deuterons D-1 and D-2 are also not magnetically equivalent. In this case, the difference of quadrupolar splittings associated with the pro-R and pro-S deuterons is equal to $[^{13}\text{C}-^1\text{H}]$ Hz. As in case of enantiomers, we have checked that the enantiotopic discrimination of deuterons in the methylene group of 4 was eliminated in an achiral oriented solvent made of a racemic mixture of PCBLL and PCBDL (15/50+50/400) at 298 K. As expected, the $^2\text{H}$ spectrum in the PCBL system (reported in in Figure 4(b)) exhibits a single quadrupolar doublet for the two enantiotopic deuterons of 4.
understanding of the mechanisms of enantiodifferentiation. Finally, the various results, reported here, open new and substantial prospects, and so point out that this non-familiar tool for organic chemists is a unique and very general methodology in the field of enantiomeric and enantiotopic analysis. New analytical applications in stereochemistry using these three polypeptidic liquid-crystalline phases are currently underway.

NOTES AND REFERENCES

‡ The notation is explained in the legend of Table 1.