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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: -$(CH_2)_2$-CO_2-$CH_2C_6H_5$), dissolved in helicogenic weakly polar organic solvents such as $CHCl_3$, CH_2Cl_2, ..., 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 ($\Delta\sigma$) and quadrupolar splittings ($\Delta\nu_Q$) 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 ^{1}H 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 pratical 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.

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Here, we report a study of the enantioselectivity of two other synthetic polypeptides in solution, the poly-γ-ethyl-L-glutamate (noted PELG; R: $-(CH_2)_2-CO_2-CH_2CH_3$) and the poly-ε-carbobenzyloxy-L-lysine (noted PCBLL; R: $-(CH_2)_4-NHCO_2-CH_2C_6H_5$), 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 knowlegde 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 ^1H, ^{13}C, ^{13}C-{^1H} and ^2H-{^1H} NMR in natural abundance [2].

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-cristalline 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 for carbon. Because the long-term stability of our magnet, no field-frequency lock system was used. For ^2H-{^1H} 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.

RESULTS AND DISCUSSION

Figure 1(*a*) and (*b*) present the ^1H and ^{13}C NMR spectra associated with the methyl group of (±)-**1** recorded in the PELG and PCBLL/$CHCl_3$ phases, respectively. As in the case of PBLG system, the weak degree of molecular orientation in PELG and PCBLL (order parameter in the 10^{-4} to 10^{-3} range) at room temperature yields rather small residual dipolar couplings with a negative or

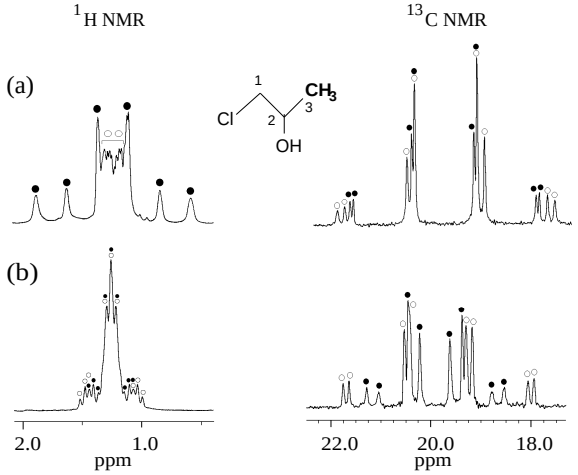

Fig. 1 *400.1 MHz ^1H and 100.6 MHz ^{13}C NMR signals associated with the methyl group of (±)-**1** recorded in PELG (a) and PCBLL (b) at 298 K by adding 64 and 1800 scans, respectively. The C-13 interferogram was acquired using a pulse angle of ~ 70°, a recycle delay of 1.5 sec and 8 k of data points.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

positive sign, and so high resolution spectra are obtained for small chiral molecules. As DOE is not directly related with the magnitude of orientational order parameters, this situation is advantageous because generally the proton and proton-coupled X spectra can be easily analysed in a similar way as for isotropic spectra. In addition, the contribution to NMR linewidths of order inhomogeneities in the sample is rather small, hence a higher signal-to-noise (S/N) ratio may be achieved. The analysis of both series of spectra shows unambiguously the doubling of signals (symbolised by white and black circles), and hence a successful chiral differentiation. In proton-coupled ^{13}C NMR, the spectral separations between enantiomers are sufficiently large to calculate the % *e.e.* by peak integration for an enriched mixture. Note that other sites of (±)-**1** exhibit a chiral differentiation and have been evidenced using ^{13}C-{^1H} and ^2H-{^1H} NMR. The results for all sites are summarised in Table 1. Due to the versatility of the DOE, the magnitude of differentiations in the three solvents varies from one site to another one, but data were not reported in the table.

As a second illustrative example, Figure 2(*a*) reports the natural abundance deuterium (NAD) NMR signals (extracted from the tilted NAD 2D *Q*-COSY spectrum) [5] of the methyl and methylene groups of (±)-4-penten-2-ol ((±)-**2**) in the phase PCBLL/$CHCl_3$. Here again, we are successful in differentiating enantiomers in both deuterated sites as two and four quadrupolar doublets

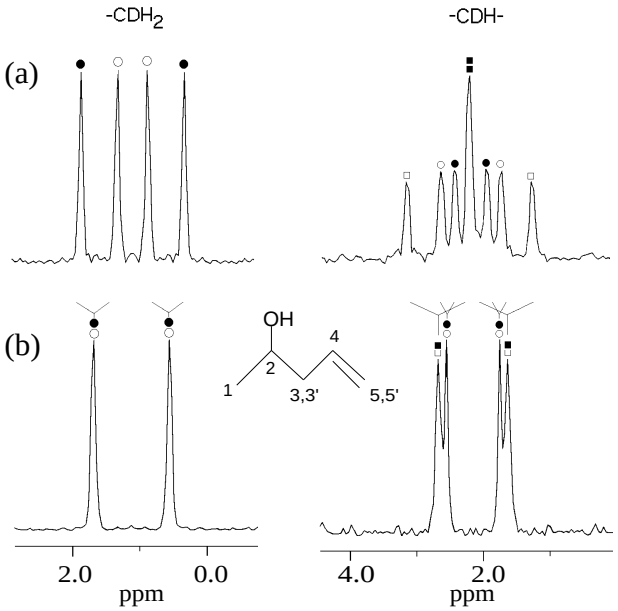

Fig. 2 *61.4 MHz NAD NMR signals of the methyl and methylene groups of (±)-**2** in the PCBLL phase (a) and PCBL phase (b). These columns are extracted from the tilted 2D Q-COSY spectrum recorded using 320 (t_1) × 1600 (t_2) data points. 320 transients for each t_1 increment were added, leading to a 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 (**1**) and (o).*

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/$CHCl_3$ 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 NAD spectra indicates that the magnitude of quadrupolar splittings in PCBLL is smaller than in PBLG (except for one site). However the measured differences, $\left|\Delta\nu_Q^R - \Delta\nu_Q^S\right|$, 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 PCBLL and its enantiomer, PCBDL (DP=1296, MW=234000, also commercially available from Sigma), in $CHCl_3$, and denoted hereafter PCBL. As in the case of racemic mixtures made of PBLG and PBDG (enantiomer 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 PCBLL 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 $\Delta\nu_Q^S$ and $\Delta\nu_Q^R$ 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 enantiodifferentiation is lost because one and two quadrupolar doublets (instead of two and four) are detected for each non-equivalent deuteron. We are now able to assign the two doublets for each of the diastereotopic deuterons in the PCBLL phase as shown in

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

Polymer	DP	MW	Composition[a]	T / K	^{1}H NMR	^{13}C NMR	^{13}C-{^{1}H} NMR	^{2}H-{^{1}H} NMR
PBLG	562	123000	100/100/350	298	1,3	1,3	2,3	1,1'[c],2,3
PELG	1707	268000	100/100/400	295	3	1,3	1[b],2,3	3
PCBLL	992	260000	100/100/400	298	3	1,3[b]	1[b],3	1(or 1'), 2,3

[a] *Amount in mg of solute/polymer/co-solvent.* [b] *Increase of the linewidths due to an unresolved peak separation.*
[c] *The numbering 1 and 1' refers to the diastereotopic deuterons of the molecule.*

Table 2 DOE[a] calculated for all deuterated sites of (±)-**2** in the PBLG/$CHCl_3$ phases and the PCBLL/$CHCl_3$ phases at 298 K

Polymer	DP	Composition	D-1[b]	D-2	D-3	D-3'	D-4	D-5	D-5'
PBLG	562	100/100/350	0.74	0.08	0.09	0.18	0.06	0.07	0.03
PCBLL	992	100/100/350	1.11	0.13	0.64	2.00	1.53	0.50	1.11

[a] DOE $= \left|\Delta\nu_Q^R - \Delta\nu_Q^S\right| / \left(\left|\Delta\nu_Q^R + \Delta\nu_Q^S\right| / 2\right)$. *To take into account solvent order variations in the DOE's comparison of (±)-**2** in PCBLL [5], the $\Delta\nu_Q$'s measured in this phase were weighted by the ratio ($\Delta\nu_Q$ of $CDCl_3$ in PBLG / $\Delta\nu_Q$ of $CDCl_3$ in PCBLL).* [b] *Deuterated sites of the molecule.*

Figure 2. 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_s, 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 spectra and deuterium spectra of two C_s symmetry molecules, the ethanol (**3**) and the ethylbenzene-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 spectral

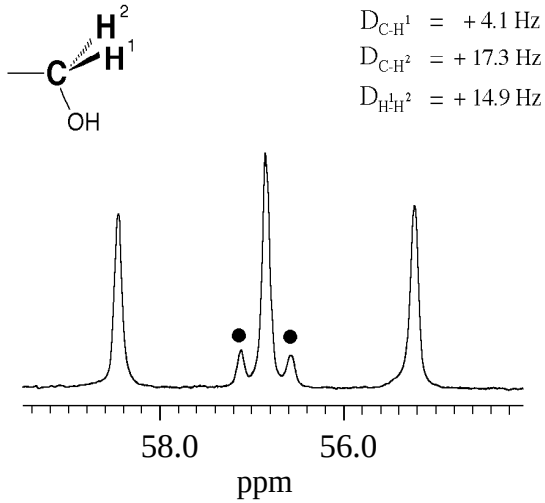

Fig. 3 *100.6 MHz ^{13}C spectral pattern of prochiral carbon of **3** in PELG/$CHCl_3$ phase at 298 K by adding 7000 scans. The C-13 interferogram was recorded using the same experimental conditions as those given in legend of Figure 1. The numbering 1 and 2 associated to the ^{13}C-^{1}H dipolar couplings is arbitrary. The value of D_{C-H} and D_{H-H} couplings were calculated with the program PANIC.*

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 inner 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-^{1}H dipolar couplings are nonequivalent here. In this example, the difference of dipolar couplings between the prostereogenic carbon and the *pro-R* and *pro-S* protons is equal to $|13.2|$ Hz. Actually, this result, also observed using the PBLG phase, [7] reveals indirectly the *Si / Re* facial differentiation of **3** in PELG.

The same conclusion can be formulated from the very straightforward analysis of the ^{2}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 $|19.2|$ 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}H spectrum in the PCBL system (reported in in Figure 4(*b*)) exhibits a single quadrupolar doublet for the two enantiotopic deuterons of

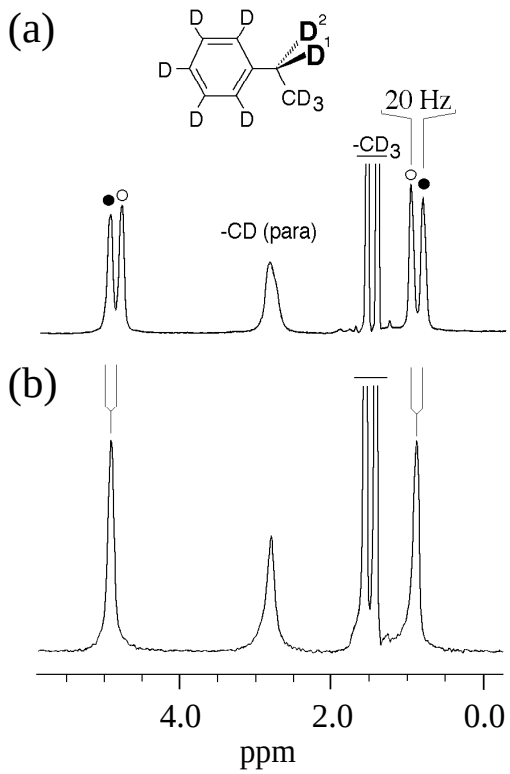

Fig. 4 *61.4 MHz ^{2}H signals of enantiotopic deuterons of **4** in the PCBLL phase (a) and the PCBL phase (b) by adding 256 scans at 298 K. A gaussian filtering was applied to enhance the spectral resolution. The numbering 1 and 2 associated to the ^{13}C-^{1}H dipolar couplings is arbitrary.*

this C_s symmetry molecule. Finally, it should be noted that to date NMR in the PBLG phase has failed to distinguish between the enantiotopic nuclei of this unsaturated hydrocarbon. This result proves the obvious interest to prospect new enantioselective oriented solvents which can be complementary to the PBLG system or provide an efficient alternative.

CONCLUSION

In this short paper, we have shown that organic solutions of PCBLL or PELG provide suitable enantioselective partially ordered media, and so can appear to be a convenient alternative when PBLG systems give poor results. This point suggests therefore that the nature of groups attached to the α-carbon plays a non-negligeable role in the enantiodifferentiation mechanisms in these uncharged chiral homopolymers. However, the magnitude of differential ordering effect between two enantiomers embedded in these various chiral polypeptide-solvent systems is not fully predictable yet, and would require various systematic studies. Additional experimental investigations of the role of the α-helical conformation of the polymer compared with that of chemical groups in the side chains should hopefully provide a better

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.

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