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^{2}H NMR Studies on Two-Homopolypeptide Lyotropic Enantiodiscriminating Mesophases: Experimental Quantification of Solute–Fiber Affinities

Philippe Lesot,*[a] Olivier Lafon,[a] Christie Aroulanda,[b] and Ronald Y. Dong[c]

Abstract: The analytical potential and enantioselective properties of lyotropic mesophases made by mixing two chemically different chiral polypeptides are described. Here we examine the case of a mixture of poly-γ-benzyl-L-glutamate (PBLG) and poly-ε-carbobenzyloxy-L-lysine (PCBLL). We demonstrate that ^{2}H NMR spectroscopy on these chiral oriented mixtures can discriminate both enantiomers and enantiotopic directions in prochiral molecules. Moreover, in such systems, degree of enantiodiscrimination, resolution, and sensitivity can be conjointly optimized by changing the relative proportion of the two polypeptides. Therefore, these new enantiodiscriminating media provide a favorable alternative to single-polypeptide mesophases with respect to stereochemical applications. At a more fundamental level, the present work points out that solute distribution in the vicinity of each polypeptide partly governs the degree of enantiodiscrimination and NMR relaxation rates. To this end, the experimental trends of solute NMR observables ($\Delta\nu_Q$, T_1) versus the fraction of peptide units of each polymer were analyzed by using a "mean-field" model derived from that proposed for mixtures of thermotropic nematic solvents, and based on the separation of intermolecular interactions between the solute and both polypeptides. This approach allows the relative solute–fiber affinities in these lyotropic systems to be determined. To identify the factors controlling solute–polypeptide affinities, we investigated various solutes (polar/apolar, rigid/flexible, achiral/prochiral/chiral molecules) using ^{2}H NMR at natural abundance or on isotopically enriched solutes.

Keywords: enantiodiscrimination · liquid crystals · mesophases · NMR spectroscopy · polypeptides

Introduction

Nuclear magnetic resonance (NMR) spectroscopy using homopolypeptide chiral liquid crystals (CLC) as solvent is a powerful analytical tool for solving numerous stereochemical issues.[1–3] These lyotropic chiral oriented media are formed by organic solutions of polypeptide helices oriented with their long axes parallel to the static magnetic field B_o of the NMR magnet (mesophase of positive magnetic susceptibility anisotropy $\Delta\chi_m$). Hitherto, the best enantiorecognition was obtained with poly-γ-benzyl-L-glutamate (PBLG), in which the side chain is $-(CH_2)_2CO_2CH_2C_6H_5$.[1–3] When no spectral discrimination occurs in PBLG solutions, other polypeptides such as poly-ε-carbobenzyloxy-L-lysine (PCBLL) and poly-γ-ethyl-L-glutamate (PELG), in which lateral chains are $-(CH_2)_4NHCO_2CH_2C_6H_5$ and $-(CH_2)_2CO_2$-CH_2CH_3, respectively, can provide excellent results due to the chemical differences in the side chain.[4,5]

The key point for NMR in polypeptide CLCs is the ability of these mesophases to interact differently with enantiomers or enantiotopic directions in prochiral molecules, and to orient them on average differently relative to B_o.[1,2,6] The orientational behavior of solutes dissolved in these CLCs can be efficiently monitored by the ^{2}H quadrupolar interaction $\mathbf{H}_Q$, which dominates proton-decoupled deuterium (^{2}H-{^{1}H}) NMR spectra. Assuming axial symmetry for the electric field gradient along the C–D bond, the quadrupolar splitting of the deuteron is simply given by Equation (1)[2,7]

[a] Dr. P. Lesot, Dr. O. Lafon
Laboratoire de Chimie Structurale Organique
RMN en Milieu Orienté
Université de Paris-Sud (XI), ICMMO, UMR CNRS 8182
Bât. 410, 91405 Orsay cedex (France)
Fax: (+33) 169-15-81-05
E-mail: philesot@icmo.u-psud.fr

[b] Dr. C. Aroulanda
Equipe de Méthodologie RMN
Nancy-Université, SRSMC, UMR CNRS-UHP 7565
BP 239, 54506 Vandoeuvre-lès-Nancy cedex (France)

[c] Prof. R. Y. Dong
Department of Physics and Astronomy
University of British Columbia, Vancouver, BC V6T 1Z1 (Canada)

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$$\Delta\nu_Q = \frac{3}{2} C_Q \times S_{CD} \text{ where } S_{CD} = \left\langle \frac{3\cos^2(\theta_{CD}) - 1}{2} \right\rangle \qquad (1)$$

where C_Q is the ^{2}H quadrupolar coupling constant, θ_{CD} is the angle between the C–D bond direction and the B_o axis, and S_{CD} is the order parameter of the C–D direction. The notation $\langle \cdots \rangle$ denotes an ensemble average over all orientations of the C–D bond.

The ^{2}H quadrupolar splitting characterizes the average orientational ordering S_{CD} of the studied C–D bond. Additionally, ^{2}H relaxation rates (e.g., Zeeman spin–lattice relaxation rates $1/T_{1Z}$), which are governed by random fluctuations of $\mathbf{H}_Q$, can also provide important data on the way a solute can reorient or perform internal motions.[8,9] The equation defining $1/T_{1Z}$ for a spin-1 nucleus in achiral uniaxial nematic phases ($D_{\infty h}$ symmetry) remains valid for a chiral uniaxial nematic phase (D_∞ symmetry) and can be calculated from the ensemble average of such quantity, $D^{2*}_{0,n}(\tau)D^2_{0,n}(0) - |\langle D^2_{0,n}\rangle|^2$.[8,9] The elements of Wigner's matrix $D^2_{0,n}$ depend on the orientation of the C–D bond relative to the B_0 axis.[8,9]

Here we propose a novel alternative for discriminating between enantiomers or enantiotopic directions. We show that enantiodiscriminating media can indeed also be prepared by dissolving two chemically different polypeptides in an organic solvent. So far, mixtures of two polypeptides were only employed to provide achiral mesophases in which any enantiodiscrimination disappears (see Figure SI-1 in the Supporting Information).[4,10] These compensated mixtures, denoted "PBG" and "PCBL", are prepared by mixing equal amounts by weight of polypeptides of the same nature but with opposite absolute configuration, for example, PBLG and its enantiomer PBDG, or PCBLL and PCBDL.[4,10] In these compensated systems, the two enantiomers exchange rapidly, on the NMR timescale, between the vicinities of L- and D-polypeptides. This results in identical average magnetic interactions for the enantiomers, and thus no difference in their ^{2}H NMR spectra can be observed (see Supporting Information).[10]

In this context, it would be pertinent to explore oriented mixtures made by two polypeptides with the same absolute configuration but with substantial chemical differences in their lateral chains. A similar approach was explored for mixtures of two achiral thermotropic liquid crystals.[11–13] However, to the best of our knowledge, such a study has never been reported for chiral lyotropic systems.

In this work, we investigated mixtures of PBLG and PCBLL dissolved in chloroform. Solute behavior was investigated in "neat" and mixed mesophases by measuring $\Delta\nu_Q(^2H)$ and $1/T_{1Z}(^2H)$. We also briefly describe a model based on the separation of interaction mechanisms of solute toward both polypeptides to tentatively explain the ^{2}H NMR results.[11,12] Experimentally, the orientational behavior and the dynamics of a prochiral molecule, namely, 1,1-dideuterated benzylic alcohol (bza), was first studied. Then we focus our attention on the case of apolar and very polar solutes using natural-abundance ^{2}H NMR (NAD NMR). As a practical application, the study of a monodeuterated chiral molecule, namely, phenethyl alcohol (pha), in these chiral mesophases is described. This last example illustrates the advantage of having a wide range of liquid-crystalline phases available as analytical media.

Theoretical Description and Chemical Interpretation

A mean-field theoretical description of ordering of solute embedded in a mixture of two thermotropic liquid crystals has been reported by Emsley et al. and others.[11–13] That description is, however, concerned with the analysis of mixture of two achiral thermotropic mesogens of opposite $\Delta\chi_m$, which provides a so-called "magic" mixture in which the electric field gradient resulting from all the solvent molecules is almost null. Here, the investigated oriented systems strongly differ from the previous ones, since we explore the case of lyotropic mixtures, which in addition do not lead to "magic" mixtures. The mean-field theory is a general approach and the formalism proposed for mixture of thermotropic mesogens can also be applied to the case of lyotropic systems. This theoretical description can be found in the literature, and will not be repeated here.[11–13] However, the relevant formulas for analyzing the NMR results as well as the hypotheses specifically needed here are given.

The ensemble average of a solute property A, such as $\Delta\nu_Q$ or $1/T_{1Z}$, is denoted $\langle A\rangle_{mix}$ in a mixture of two polypeptides (a and b), and $\langle A\rangle_a$ or $\langle A\rangle_b$ in a mesophase containing only a single kind of polypeptide a or b. To express $\langle A\rangle_{mix}$ as function of $\langle A\rangle_a$ and $\langle A\rangle_b$, it is necessary to set some specific assumptions on the polypeptide CLC. First, we consider that the average of half the distance between the axes of two neighboring fibers $d_{fiber-fiber}/2$ is always larger than the range of intermolecular interactions in the mesophase (hypothesis 1). Indeed, $d_{fiber-fiber}$ is around 7 nm for the investigated samples (compositions are reported in Table SI-3 in the Supporting Information). Under this condition, we can assume that: 1) a solute molecule (or a co-solvent molecule) interacts only with a single polypeptide fiber a or b, 2) all solute/solute, solute/co-solvent, and co-solvent/co-solvent long-range interactions beyond the distance $(d_{fiber-fiber})/2$ as well as all interactions between fibers are neglected. Second, contrary to molecules of nonpolymeric thermotropics, such as *N*-(4-ethoxybenzylidene)-4′-*n*-butylaniline (EBBA), the polypeptide helices are assumed to be infinite molecular objects (hypothesis 2).[11,12] Thus we can disregard the contribution to $\langle A\rangle_i$ of solute molecules interacting with the ends of fibers. This assumption is justified since we use long fibers with a high degree of polymerization (DP > 700). Note also that the surface effects induced by the glass wall of the NMR tube are not considered.[14]

In the framework of hypotheses 1 and 2, the mesophase can be described by a periodic hexagonal "box" containing a finite number of molecules (solute, co-solvent, and polypeptide), as shown in Figure 1.[15] The minimal size of the

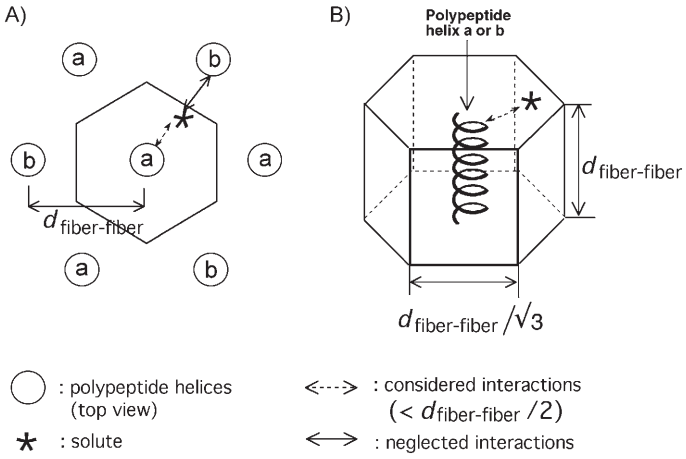

Figure 1. A) Depiction of hexagonal lattice of two polypeptide fibers a and b in the mixed mesophases. The polypeptides are randomly distributed. B) Description of the hexagonal periodic box associated with polypeptide a or b.

box is defined by the maximal range of intermolecular interactions. In accordance with hypothesis 1, we chose a minimal box whose dimensions are equal to the length $d_{\text{fiber–fiber}}$ containing a single fragment of polypeptide a or b at its center. For a "neat" mesophase (i.e., containing only a single kind of polypeptide), the periodic repetition of one box allows us to describe the whole sample. For a binary mixture of polypeptides, two boxes must be considered, one for the polypeptide a (box a) and one for the polypeptide b (box b).

Suppose $P_{\text{a}}^{\text{solute}}$ (or $P_{\text{b}}^{\text{solute}}$) is the probability that a solute is closer to α-helix a (or b, respectively) than to α-helix b (or a, respectively). These probabilities depend on the relative proportion of solute (and co-solvent) within the two boxes, that is, the molecular distribution of solute around each fiber. Clearly, this relative proportion depends directly on the affinity of the solute toward the two polypeptides, which must be related to the chemical functionality and/or structure (electronic profile) of the solute, as well as the nature of side chains in the polypeptides. Two distinct cases of distribution are considered in the following discussion: 1) identical distribution of solute in the two boxes (hypothesis 3); this case corresponds to the "random mixing" defined in reference [11]; 2) a different solute distribution towards the fibers and hence an excess of solute in the vicinity of one of the polypeptides (hypothesis 4).

Under hypothesis 3, three different situations exist. The first two correspond to the cases of rather apolar solutes (e.g., alkanes or alkenes) interacting with two polypeptides whose lateral-chain chemical nature could be regarded as either similar (e.g., PBLG and PELG) or quite different (e.g., PBLG and PCBLL). The third situation involves rather polar molecules (e.g., acids, amines, alcohols, and so on) interacting with two polypeptides whose lateral chains have relatively close chemical nature. Indeed even if the solute is polar, the similar nature of the two polypeptides should not lead to sufficient differences in the involved intermolecular interactions to produce a significant effect on the solute distribution. Hypothesis 4 applies only to polar molecules interacting with polypeptides whose chemical nature and/or polarity of the lateral chains can be considered as very different. This case could occur in a mixture of PBLG and PCBLL due to the presence of the NH group in the PCBLL side chain. Figure 2 sums up all the different cases described above.

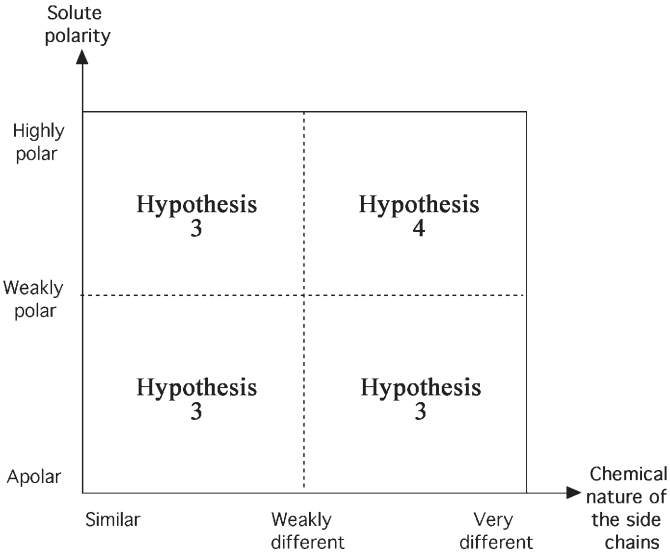

Figure 2. Application domains of hypothesis 3 (i.e., the solute would not be located preferentially near one of polypeptides), and hypothesis 4 (i.e., the solute distribution is different in the two boxes, i.e., an excess of solute in the vicinity of one of the polypeptides) versus solute polarity and the chemical nature of the side chains.

The molecular compositions of boxes a and b are identical to those of the minimal boxes used to simulate the "neat" mesophases (a or b) when hypothesis 3 is valid. Furthermore, the solute probability $P_{\text{a}}^{\text{solute}}$ (or $P_{\text{b}}^{\text{solute}}$, respectively) is equal to the molar fraction of peptide units a (or b, respectively) $x_{\text{a}}^{\text{pu}} = n_{\text{a}}^{\text{pu}}/(n_{\text{a}}^{\text{pu}} + n_{\text{b}}^{\text{pu}})$ [or $x_{\text{b}}^{\text{pu}} = n_{\text{b}}^{\text{pu}}/(n_{\text{a}}^{\text{pu}} + n_{\text{b}}^{\text{pu}})$, respectively], where n_{a}^{pu} and n_{b}^{pu} (expressed in moles) are respectively the amount of peptide units a and b, that is, $n_{\text{a or b}}^{\text{pu}} = m_{\text{polypeptide}}^{\text{a or b}}/M_{\text{a or b}}^{\text{pu}}$ and "pu" stands for "peptide unit". The use of the molar fractions of peptide units a and b instead of the molar fraction of polypeptides is imposed by the fact that each minimal box contains only a fragment of α-helix and not the whole polypeptide. Thus, $\langle A\rangle_{\text{mix}}$ is simply given by Equation (2)

$$\langle A\rangle_{\text{mix}} = x_{\text{a}}^{\text{pu}}\langle A\rangle_{\text{a}} + x_{\text{a}}^{\text{pu}}\langle A\rangle_{\text{b}} = x_{\text{a}}^{\text{pu}}(\langle A\rangle_{\text{a}} - \langle A\rangle_{\text{b}}) + \langle A\rangle_{\text{b}} \qquad (2)$$

where $x_{\text{a}}^{\text{pu}} + x_{\text{b}}^{\text{pu}} = 1$. Hypothesis 3 therefore means that the average value of any solute property should evolve linearly with x_{a}^{pu}. Since no affinity difference for the solute is expected in the PBG samples, Equation (2) is applicable and a linear variation of NMR properties with x_{a}^{pu} occurs when PBLG and PBDG polypeptides are mixed. Note that PBLG

and PBDG polymers of similar DP should be used in order to prevent the formation of biphasic system (see below). In addition, as the molar masses of the polypeptide units in PBLG and PBDG are equal (219 g mol^{-1}), the x^{pu}_{PBLG} and x^{pu}_{PBDG} percentage fractions are always equal to the weight fractions of PBLG and PBDG, respectively. This explains why mixing equal amounts by weight of PBLG and PBDG gives a compensated achiral oriented system in which no enantiodiscrimination occurs.

Under hypothesis 4, the probabilities P^{solute}_a and P^{solute}_b differ from x^{pu}_a and x^{pu}_b, and so Equation (2) is now invalid. Instead, the mole fractions in this equation must be replaced by the corresponding solute probabilities P^{solute}_a and P^{solute}_b. These latter quantities could be expressed as a function of the molar fractions of solute $k_a = c^{solute}_{a,box}/(c^{solute}_{a,box} + c^{solute}_{b,box})$ and $k_b = c^{solute}_{b,box}/(c^{solute}_{a,box} + c^{solute}_{b,box})$ in boxes a and b, as well as x^{pu}_a and x^{pu}_b (c is the concentration of solute within each box, see the demonstration in the Supporting Information). Under the ideal condition of infinite dilution, which allows solute–solute interactions to be disregarded (hypothesis 5), one simply obtains Equation (3).

$$\langle A\rangle_{mix} = \frac{k_a x^{pu}_a}{(k_a x^{pu}_a + k_b x^{pu}_b)}\langle A\rangle_a + \frac{k_b x^{pu}_b}{(k_a x^{pu}_a + k_b x^{pu}_b)}\langle A\rangle_b \quad (3)$$

Setting $K = k_a/k_b$ and $x^{pu}_a = 1 - x^{pu}_b$ gives Equation (4).

$$\langle A\rangle_{mix} = \frac{x^{pu}_a(K\langle A\rangle_a - \langle A\rangle_b) + \langle A\rangle_b}{(K-1)x^{pu}_a + 1} \quad (4)$$

As $k_a + k_b = 1$, it is easy to calculate molar fractions of solute from the K parameter: $k_a = K/(K+1)$ and $k_b = 1/(K+1)$. Equation (4) indicates that in the framework of hypothesis 4, the average value of any solute property will follow a hyperbolic evolution with maximal deviation from linear evolution [see Eq. (2)] when $x^{pu}_a = 1/(1+\sqrt{K})$. Note that Equation (4) is strictly identical to Equation (2) when $K = 1$ (i.e., when $k_a = k_b = 1/2$), which implies that the solute has the same affinity with both polypeptides (hypothesis 3).

From the viewpoint of molecular dynamics, the difference in partition of solute molecules can be seen as a difference in average residence time of the solute in the vicinity of each polypeptide. From the thermodynamic point of view, the K parameter can be seen as an equilibrium constant associated with the partition of the solute molecules between boxes a and b. Consequently, K reveals quantitative information on the relative strength of intermolecular interactions between a solute and the two polypeptides. The quantitative aspect of K should help us to classify a series of solutes as a function of their affinity toward polypeptides.

Finally, two remarks can be made. First, for a given hypothesis (3 or 4), any NMR observable of a solute should lead to the same K value. Second, since the solute distribution is a property of the whole molecule, it affects in the same way all molecular local properties, such as quadrupolar splittings and relaxation times of the different deuterons in the solute. Consequently, the K parameter can be determined directly from these local properties without the necessity of calculating the molecular ordering matrix, which requires a correct estimation of the ^{2}H C_Q values, an accurate molecular geometry, and a sufficient number of anisotropic NMR observables depending on the solute symmetry.[4,7]

Results and Discussion

NMR study on 1,1-dideuterated benzylic alcohol: The enantiodiscriminating properties of PBLG/PCBLL mixtures dissolved in chloroform were examined by ^{2}H-{^{1}H} NMR spectroscopy for the prochiral compound 1,1-dideuterated benzylic alcohol (bza). Experiments were performed with several proportions of the two polypeptides and at three temperatures (270, 300, and 330 K). Such an approach allowed the validity of the above mean-field model to be tested. We used polypeptides of similar DP, namely, PBLG with DP = 1352 ($M_w \approx 296\,000$ g mol^{-1}) and PCBLL with DP = 1100 ($M_w \approx 288\,000$ g mol^{-1}), and 50 mg of bza, that is, a solute concentration of about 10 wt%. Such a value is not common for thermotropic systems (<2%), but it is standard for lyotropic PBLG or PCBLL mesophases, particularly in numerous analytical applications (determination of enantiomeric purity by ^{13}C or ^{2}H NMR spectroscopy at natural abundance).[1,3] It could be argued that such a concentration of solute is not compatible with infinite dilution (see Supporting Information), because the solute–solute interactions could significantly modify the solute distribution and also the K value. This objection was overruled, since the K parameter obtained from the analysis of NMR data at low solute concentration (1.7 wt%) is identical to values obtained at 10 wt%. This result indicates that the proposed theoretical approach is valid for solute concentrations up to 10 wt%. The lyotropic nature of polypeptide mesophases could contribute to the absence of concentration effects of the solute.

Analysis of the ^{2}H quadrupolar splittings of bza: We studied the orientational behavior of bza by analyzing the evolution of $\Delta\nu_Q$ versus the polypeptide composition of the samples. This solute is prochiral with C_s symmetry on the NMR timescale, and it has enantiotopic C–D directions.

Figure 3 shows ^{2}H-{^{1}H} spectra of bza recorded at 300 K and various proportions of polypeptides. The PBLG and PCBLL mesophases and the mixtures of both polypeptides yield high-resolution NMR spectra showing discrimination of the enantiotopic C–D directions (at least two visible doublets). As the absolute assignment of stereodescriptors *pro-R* and *pro-S* for each doublet is unknown, notations A and B are employed on the spectra. The absence of isotropic signals or even asymmetrical peaks in ^{2}H-{^{1}H} spectra in chiral mesophases made of both PBLG and PCBLL indicates that these mixtures of two chemically different polypeptides form a macroscopically homogeneous and monophasic oriented system with a narrow distribution of the director **n** around the magnetic field direction. In addition, since no

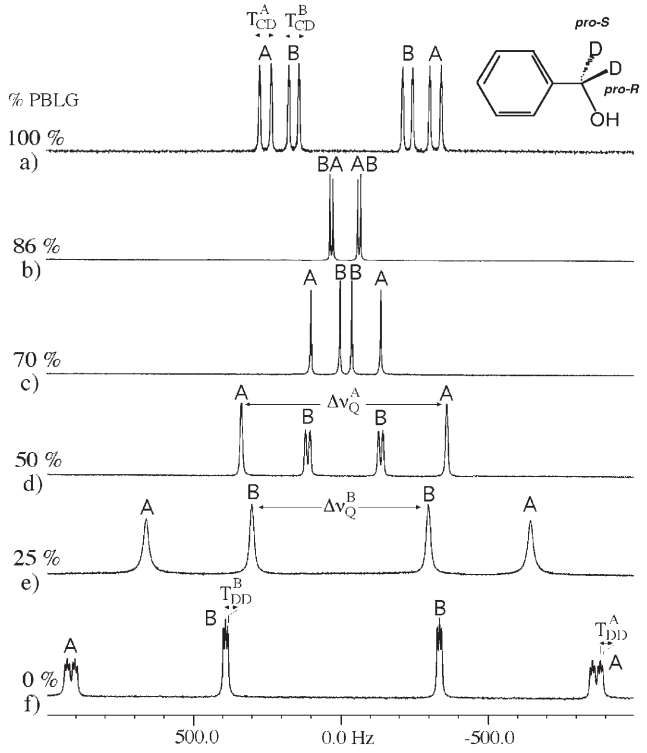

Figure 3. Evolution of 61.4 MHz $^2H\{^1H\}$ 1D spectra of bza recorded at 300 K with the amount [wt%] of PBLG in the samples. All spectra were recorded by adding 64 scans. Note that the methylene group of the solute dissolved in mixtures with 0, 50, and 100% of PBLG is isotopically enriched both in ^2H and ^{13}C, and hence ^2H–^{13}C coupling is visible in spectra a, d, and f. Spectra a and f were filtered by using a Gaussian window to measure the $^2T_{DD}$ couplings.

extra resonance line is observed, we can exclude the existence of slow exchange (on the NMR timescale) between the solute and the two polypeptides, which would have entailed the presence of two kinds of signals in the spectrum, one for each polypeptide. This spectral situation is similar to that observed in PBG mixtures. The substructures observed for some quadrupolar doublets (spectra a, d, and f in Figure 3) arise from the $^1J_{CD}$ and $^1D_{CD}$ couplings due to ^{13}C isotopic enrichment of the methylene group. Other extra small splittings (three-iso-intense lines) originate from ^2H–^2H total geminal coupling $^2T_{DD}$ between enantiotopic deuterons.

Strikingly, the magnitude of enantiodiscrimination varies with polypeptide composition. Table 1 lists the values and the signs of $\Delta\nu_Q$ and $^1T_{CD}$ at 300 K. The sign of $\Delta\nu_Q$ is deduced from the magnitude of the one-bond ^{13}C–^2H dipolar coupling $^1D_{CD}$, which can be calculated from the ^{13}C–^2H total spin–spin coupling $^1T_{CD}$ ($^1T_{CD}=^1J_{CD}+2^1D_{CD}$) measured on the ^2H spectra of bza by assuming $^1J_{CD}=+22$ Hz.[16] As the ratio $\Delta\nu_Q/^1D_{CD}$ is independent of S_{CD} and approximately equal to -80, we can identify the correct solutions for the magnitude and the sign of $^1D_{CD}$, and hence deduce the sign of $\Delta\nu_Q$.[16]

Figure 4a shows the trend of quadrupolar splittings for the outer and inner doublet of bza versus x^{pu}_{PBLG} at 300 K. Clearly, the variation of $\Delta\nu_Q$ cannot be fitted linearly with Equation (2). The maximal deviation between experimental and expected values based on hypothesis 3 is observed for sample 5 (i.e., $x^{pu}_{PBLG}=54.5\%$) and is equal to 166 and 90 Hz for the outer and inner doublets, respectively. Such deviations cannot be reasonably explained by wrong preparation of the sample (inaccuracy in the weights, inhomogeneity of the mesophase, etc.). Conversely, the experimental data are correctly fitted by Equation (4) when $\langle A\rangle$ is $\Delta\nu_Q$ and the subscripts a and b are replaced by PBLG and PCBLL, thus giving Equation (5)

$$\langle\Delta\nu_Q\rangle_{mix}=\frac{x^{pu}_{PBLG}[K(\Delta\nu_Q)_{PBLG}-(\Delta\nu_Q)_{PCBLL}]+(\Delta\nu_Q)_{PCBLL}}{(K-1)x^{pu}_{PBLG}+1} \quad (5)$$

where $K=k_{PBLG}/k_{PCBLL}$. The K, k_{PBLG}, and k_{PCBLL} parameters for doublets A and B are listed in Table 2 together with the K values calculated from fitting the trend of the average quadrupolar splittings. This latter case would correspond to the situation in which the mesophase is achiral.

At 300 K, the values of k_{PBLG} and k_{PCBLL} indicate that the fraction of bza is larger in the vicinity of PCBLL compared to PBLG (ca. 20% more). This reveals stronger intermolecular affinity between bza and PCBLL compared to bza and PBLG. In this case, the presence of an NH group in the lateral chains of PCBLL could be the origin of this effect by either promoting direct hydrogen bonding with the hydroxyl groups or increasing (compared to PBLG) the negative charge of the carboxyl group if this group is involved. This specific interaction could also explain the significant increase of $\Delta\nu_Q$ in the "neat" PCBLL mesophase compared to the "neat" PBLG mesophase and the change in the sign of $\Delta\nu^A_Q$ and $\Delta\nu^B_Q$ as well. As discussed below, this will not be observed for rather apolar solutes such as toluene. Note that the NH group of the α-helix of the polypeptide is not accessible to the solute and cannot be involved in hydrogen bonding.

In addition, as $(\Delta\nu_Q)^A_{PBLG}<(\Delta\nu_Q)^B_{PBLG}$ while $(\Delta\nu_Q)^A_{PCBLL}>(\Delta\nu_Q)^B_{PCBLL}$, it is possible to determine the exact composition in mass of polypeptide that produces a sample in which the quadrupolar splittings for deuterons A and B are equal ($(\Delta\nu_Q)^A_{mix}=(\Delta\nu_Q)^B_{mix}$) and thus the prochiral enantiotopic discrimination is negated. This particular sample could be called a cryptochiral mixture (CM) because the enantiotopic discrimination vanishes while the oriented medium is still chiral, in contrast to the case of PBG mixture, in which no spectral discrimination is possible. This situation corresponds to equating Equation (5) for the two doublets A and B to give Equation (6).

$$x^{pu}_{PBLG}(CM)=\frac{(\Delta\nu_Q)^A_{PCBLL}-(\Delta\nu_Q)^B_{PCBLL}}{K[(\Delta\nu_Q)^A_{PBLG}-(\Delta\nu_Q)^B_{PBLG}]-(\Delta\nu_Q)^A_{PCBLL}+(\Delta\nu_Q)^B_{PCBLL}} \quad (6)$$

In this example, the $\Delta\nu_Q$ are equal when $x^{pu}_{PBLG}=89.1\%$ (i.e., 87.2 wt% of PBLG). Graphically, this abscissa corresponds

Table 1. Spectral ^{2}H data for bza recorded at 270, 300, and 330 K

NMR sample	w/w ($x^{pu}_{PBLG}/x^{pu}_{PBLG}$)[b]	T [K]	$\Delta\nu_Q^A$ [Hz]	$\Delta\nu_Q^B$ [Hz]	$\lvert\Delta\nu_Q\rvert$ [Hz]	$\lvert{}^2T_{DD}\rvert$ [Hz]	$^1T_{CD}^A$ [Hz]	$^1T_{CD}^B$ [Hz]	T_{1Z}^{av} [s][e]
1[a]	0/0	a) 270	0	0	0	0	0	0	0.216
	(0/0)	b) 300	0	0	0	0	0	0	0.538
		c) 330	0	0	0	0	0	0	0.1080
2[a]	100/0	a) 270	643±8	−459±8	184	<LW[c]	−39	−34	0.119
	(100/0)	b) 300	527±4	−345±6	182	3	−36	−31	0.327
		c) 330	−357±3	−200±2	157	3	−31	−27	0.775
3	86/14	a) 270	−41±4	−18±3	23	<LW	–[d]	–	0.98
	(88.0/12.0)	b) 300	−93±2	−118±2	25	<LW	–	–	0.283
		c) 330	−49±1	−41±1	8	<LW	–	–	0.689
4	70/30	a) 270	+405±9	+20±7	385	<LW	–	–	0.86
	(73.6/26.4)	b) 300	+236±4	+40±3	196	<LW	–	–	0.243
		c) 330	+132±3	+83±3	49	<LW	–	–	0.662
5[a]	50/50	a) 270	–	–	–	–	–	–	
	(54.5/45.5)	b) 300	+692±4	+233±4	459	3	+3	+15	0.215
		c) 330	+418±2	+230±2	188	<LW	+10	+14	0.608
6	25/75	a) 270	+2080±40	+450±20	1630	<LW	–	–	0.059
	(28.5/71.5)	b) 300	+1285±20	+540±10	745	<LW	–	–	0.189
		c) 330	+805±15	+446±8	359	<LW	–	–	0.530
7[a]	0/100	a) 270	+2676±8	+617±6	2059	<LW	−30	<LW	0.053
	(0/100)	b) 300	+1787±6	+728±4	1059	8	−27	<LW	0.166
		c) 330	+1127±4	+592±3	535	5	−9	−5	0.500
8[a]	50/50[f]	a) 270	+1108±15	+109±10	1009	<LW	<LW	+17	0.070
	(54.5/45.5)	b) 300	+718±4	+231±3	487	3	+4	+15	–

[a] Solute enriched in ^{13}C in the methylene group. [b] Weight fractions in PBLG/PCBLL and molar fractions in PBLG/PCBLL peptide units; see also Table SI-3 in the Supporting Information. As the peptide units for PBLG (M_{pu}=219 g mol^{-1}) and PCBLL (M_{pu}=262 g mol^{-1}) are similar, the w/w and x^{pu} values are very similar. [c] The $^2T_{DD}$ or $^1T_{CD}$ coupling is less than the linewidth (LW). [d] No experimental data. [e] Averaged values over all separated components of the doublets. Exponential filtering was used when ^{2}H–^{2}H coupling was observed. [f] Compared to sample 5, sample 8 was prepared with PBLG and PCBLL with DPs of 782 and 778, respectively.

to the crossing point of two curves $\Delta\nu_Q^A = f(x^{pu}_{PBLG})$ and $\Delta\nu_Q^B = f(x^{pu}_{PBLG})$ in Figure 4a. This value is close to x^{pu}_{PBLG} of sample 3 and explains the small spectral enantiodiscrimination observed in Figure 3b.

At 330 K, we obtain high-resolution ^{2}H spectra whatever the sample, and enantiotopic directions are discriminated regardless of polypeptide composition. As expected at high temperature, $\Delta\nu_Q$, $^1T_{CD}$, and $^2T_{DD}$ splittings are globally smaller in magnitude than those at room temperature due to the higher mobility of the solute (see Table 1). However, as before the variation of $\Delta\nu_Q$ with x^{pu}_{PBLG} can be described by Equation (5) (see Table 2). Here again the fraction of solute molecules is larger in the vicinity of PCBLL compared to PBLG (ca. 20% more), in confirmation of the previous results.

At 270 K, except for sample 5, we also obtained high-resolution ^{2}H spectra showing enantiotopic discrimination and larger splittings due to reduced solute mobility at low temperature (see Table 1). For sample 5 (50% of PBLG and PCBLL), observation of more ^{2}H resonances than expected revealed the presence of an inhomogeneous oriented phase. In fact, low-resolution ^{2}H-{^{1}H} spectra with broad resonances (>50 Hz) were recorded even at 280 K. This result emphasizes the complexity of the phase diagram for lyotropic systems whose phase stability subtly depends on temperature, relative concentrations of components, nature of the co-solvent, DP of polypeptides, and so on. To understand this unexpected result, we recorded the ^{2}H spectrum of bza at 300 and then 270 K by using a mixture of PBLG and PCBLL with lower DPs of 782 and 778, respectively (sample 8), while keeping the same concentration of polymer and solute. At 300 K, the quadrupolar and dipolar splittings (Table 1, entry 8b) are very close to the values measured for sample 5 at the same temperature (Table 1, entry 5b), that is, the DP of polypeptide has a rather limited effect on the molecular orientational ordering of the solute. Contrary to sample 5, sample 8 remains homogeneous at 270 K, since a high-resolution ^{2}H spectrum is still observed (Table 1, entry 8a). Note that the values of $\Delta\nu_Q$ measured on sample 8 will be used for determining the K parameter during the fit of the experimental data at 270 K. As above, the variation of quadrupolar splittings with x^{pu}_{PBLG} leads again to the fraction of solute molecules being larger in the vicinity of PCBLL compared to PBLG (ca. 20% more).

Analysis of data at three temperatures shows that results are relatively self-consistent. Indeed, whatever the temperature, it appears that the fraction of solute molecules is larger around PCBLL fibers compared to PBLG fibers. Experimentally, the variation of K with T for bza seems insignificant, probably due to the experimental errors in the measurement of quadrupolar splittings as well as the small range of temperature explored (60 K). With the hypotheses in the model, the approach proposed can be applied either in an achiral or chiral mesophase. As a consequence, it is rather puzzling to obtain different K parameters from $\Delta\nu_Q^A$ and $\Delta\nu_Q^B$ values (or the average of $\Delta\nu_Q$) for the three temperatures. Indeed, at a given temperature, such a difference is a priori not expected for a prochiral molecule, because the K

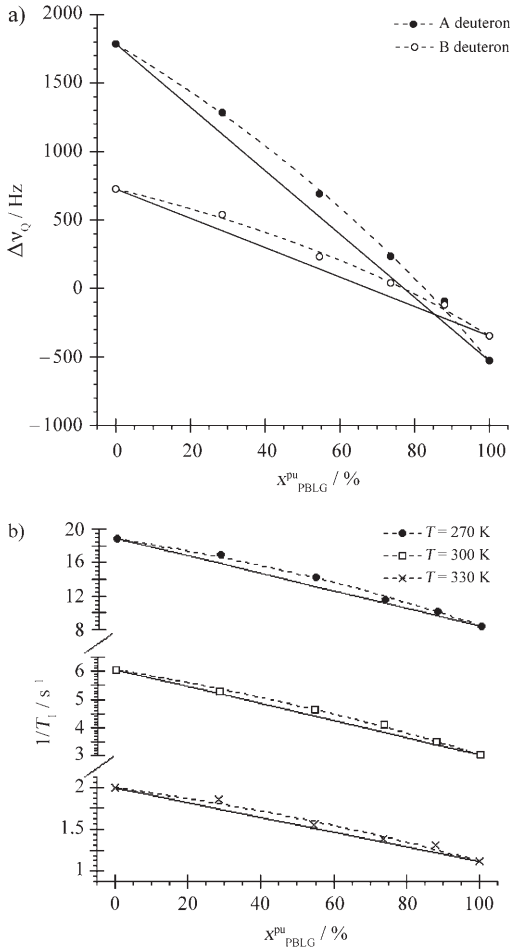

Figure 4. Theoretical and experimental evolution of a) $\Delta\nu_Q$ for the outer and inner doublets of bza with x^{pu}_{PBLG} at 300 K and b) average relaxation rates $1/T_{1Z}$ versus x^{pu}_{PBLG} at 270, 300, and 330 K. In a) and b), the continuous lines correspond to the fit of data to Equation (2) when $\langle A\rangle = \Delta\nu_Q$ and $1/T_{1Z}$, respectively. In b, the y scale is split for a better view. The dashed lines in a) and b) correspond to the fit of experimental points with Equation (4) where $\langle A\rangle = \Delta\nu_Q$ and $\langle A\rangle = 1/T_{1Z}$, respectively.

values should be strictly identical for all deuterium sites. In contrast, this assertion could be invalid for enantiomers, since two independent molecules interact with the two polypeptides in this case (see below). Thus, we attribute the difference in K for the enantiotopic deuterons of bza to experimental errors in the measurement of $\Delta\nu^A_Q$ and $\Delta\nu^B_Q$. These experimental errors increase with line broadening, and this explains the larger difference in K parameters at 270 K. In fact, as we discuss below, such discrepancies in the K values associated with nonequivalent deuterium sites in achiral compounds (where no enantiodiscrimination exists) have also been found.

Analysis of the ^{2}H relaxation rates of bza: We also examined the dynamics of bza at 270, 300, and 330 K by determining T_{1Z} for the *pro-R* and *pro-S* deuterons. The measurements were performed with the well-known inversion–recovery pulse sequence.[7] As significant differences in T_{1Z} values for *pro-R* and *pro-S* deuterons in prochiral solutes have never been observed in these polypeptide mesophases, T_{1Z} values reported in Table 1 correspond to the average of T_{1Z} values measured for both enantiotopic deuterons. Whatever the temperature, the values of T_{1Z} in isotropic liquid are much larger than those measured in CLC, due to reduced solute mobility in a mesophase. In addition, the T_{1Z} values decrease with decreasing temperature due to slowing down of the molecular dynamics, both in isotropic and anisotropic phases. The trends of $1/T_{1Z}$ versus x^{pu}_{PBLG} for the three temperatures are plotted in Figure 4b. As can be seen, this variation is nonlinear and is only fitted correctly by using Equation (4) in which $\langle A\rangle$ is replaced by $1/T_{1Z}$ and a and b by PBLG and PCBLL, respectively. The data associated with the fitting of $1/T_{1Z}$ are given in Table 2.

Table 2. Values of K, k_{PBLG}, k_{PCBLL}, and χ determined from the fit of data for bza using Equations (4) and (5).

Fitted data	Eq.	Parameters	Temperature 270 K	300 K	330 K
$\Delta\nu^A_Q$	5	K[a]	0.71	0.71	0.70
		k_{PBLG}/k_{PCBLL}[b]	41.5/58.5	41.5/58.5	41.2/58.8
		χ[c]	0.998	0.999	0.998
$\Delta\nu^B_Q$	5	K[a]	0.54[f]	0.63	0.64
		k_{PBLG}/k_{PCBLL}[b]	35.1/64.9	38.7/61.3	39.0/61.0
		χ[c]	0.995	0.998	0.999
$\Delta\nu^{av}_Q$[d]	5	K[a]	0.67	0.68	0.68
		k_{PBLG}/k_{PCBLL}[b]	40.1/59.9	40.5/59.5	40.5/59.5
		χ[c]	0.998	0.999	0.998
T_{1Z}	4[e]	K[a]	0.68	0.71	0.69
		k_{PBLG}/k_{PCBLL}[b]	40.5/59.5	41.5/58.5	40.8/59.2
		χ[c]	0.998	0.999	0.993

[a] The fit uncertainties lead to an error of ± 0.02 in the K value. [b] Both values are given in percent. [c] The χ value is the standard deviation for the fit. [d] Average of experimental quadrupolar splittings ($\Delta\nu^{av}_Q = (\Delta\nu^A_Q + \Delta\nu^B_Q)/2$). [e] Equation (4) where A is replaced by $1/T_{1Z}$ and a, b by PBLG, PCBLL. [f] The discrepancy in K values derived from $\Delta\nu^A_Q$ and $\Delta\nu^B_Q$ splittings at low temperature originates from larger errors in the measurement of quadrupolar splittings due to the broadened lines.

Our results show that 1) Equation (4) is applicable whatever the observable considered ($\Delta\nu_Q$ or T_{1Z}), 2) the values of K determined from $1/T_{1Z}$ are in full agreement with K values obtained from $\Delta\nu_Q$ within the experimental errors, and 3) the range of temperature is not large enough to see a significant change of K as a function of T. The good agreement between the two sets of K parameters seems to validate the theoretical model proposed here.

Analysis of isotopically unmodified molecules: The study on bza proved that solute distribution strongly influences the degree of orientation and enantiodiscrimination in PBLG/PCBLL mesophase. It would therefore be relevant to identify the factors controlling the solute distribution in such systems. To assess the importance of polarity for solute distribution, we analyzed the natural-abundance deuterium (NAD) spectra of toluene (tol) and phenol (phe) dissolved in PBLG/$CHCl_3$ and PCBLL/$CHCl_3$ phases as well as in a 50/50 (w/w) mixture of these two polypeptides (see Table

SI-3 in the Supporting Information). Natural-abundance deuterium NMR experiments are advantageous because we can simultaneously examine all nonequivalent ^{2}H sites in a molecule without any isotopic labeling.[2,3,17] Besides, recording the proton-coupled ^{13}C spectrum allows the determination of the magnitude of one-bond ^{13}C–^{1}H couplings, and also the sign of dipolar couplings, assuming that the $^1J_{CH} > 0$. Indeed, as the two-order parameters S_{CH} and S_{CD} can be assumed equal, then the ratio $|\Delta\nu_Q/^1D_{CH}|$ lies approximately in the range 10–13.[16] Here an average value of 12 was adopted in order to get estimates of the magnitude of $^1D_{CH}$ in toluene and phenol. Comparing the magnitudes of $^1D_{CH}$ calculated from $\Delta\nu_Q$ with those of $^1D_{CH}$ obtained from $^1T_{CH}$ measured on the first-order carbon signals ($^1D_{CH=}$ ($^1T_{CH}-^1J_{CH}$)/2) provides the signs of $^1D_{CH}$, and subsequently the sign of $\Delta\nu_Q$.

Although toluene and phenol are neither chiral nor prochiral molecules, these aromatic compounds are good model solutes. The former is characterized by the lack of reactive, polar group (aaplication domain of hypothesis 3), whereas the latter has a hydroxyl group susceptible to forming strong hydrogen bonds due to activation by the electron-withdrawing properties of the phenyl ring (application domain of hypothesis 4).

For this series of experiments, we used PBLG with DP = 782 and PCBLL with DP = 778, that is, average molecular weights of 171300 and 204000 g mol^{-1}, respectively. The NAD spectra are presented in Figure 5 and the ^{2}H spectral data are summarized in Table SI-1 in the Supporting Information. While the assignment of doublets for the aromatic deuteron in *para* (p) position and the methyl group are rather trivial, the assignment of quadrupolar components of *meta* (m) and *ortho* (o) aromatic deuterons requires the use of *Q*-resolved Fz 2D experiments (see inset of Figure 5a).[3,18] The presence of a low-intensity doublet centered at $\delta = 7.3$ ppm and featured with extra broad components and $|\Delta\nu_Q| \approx 1800$ Hz observed in the PBLG and MIX phases is assigned to the aromatic deuterons of the side chains of the polypeptides (Figure 5a). This result was confirmed by recording the NAD 1D spectrum of pure PBLG dissolved in $CHCl_3$. Compared to deuterons in the rigid α-helix, aromatic deuterons have longer relaxation times due to the high mobility of the phenyl ring in the side chain.[19]

In the three mesophases, the global feature of the NAD spectra for the signal of toluene and the co-solvent is very similar. Examination of all ^{2}H data for toluene shows that its average molecular orientation is rather similar in the PBLG and PCBLL mesophases, in spite of the opposite signs for the splittings of *ortho* and *meta* deuterons. In fact, these are small, and not sufficient to reveal a strong orientation of the solute in the two different mesophases. The variations of ^{2}H quadrupolar splittings of toluene with x^{pu}_{PBLG} are shown in Figure 6a, and were fitted by using Equations (2) and (5). The corresponding best-fit parameters for Equation (5) are given in Table 3. As expected, the *K* parameter is indeed very close to unity, that is, distributions of solute molecules around both polypeptides are similar.

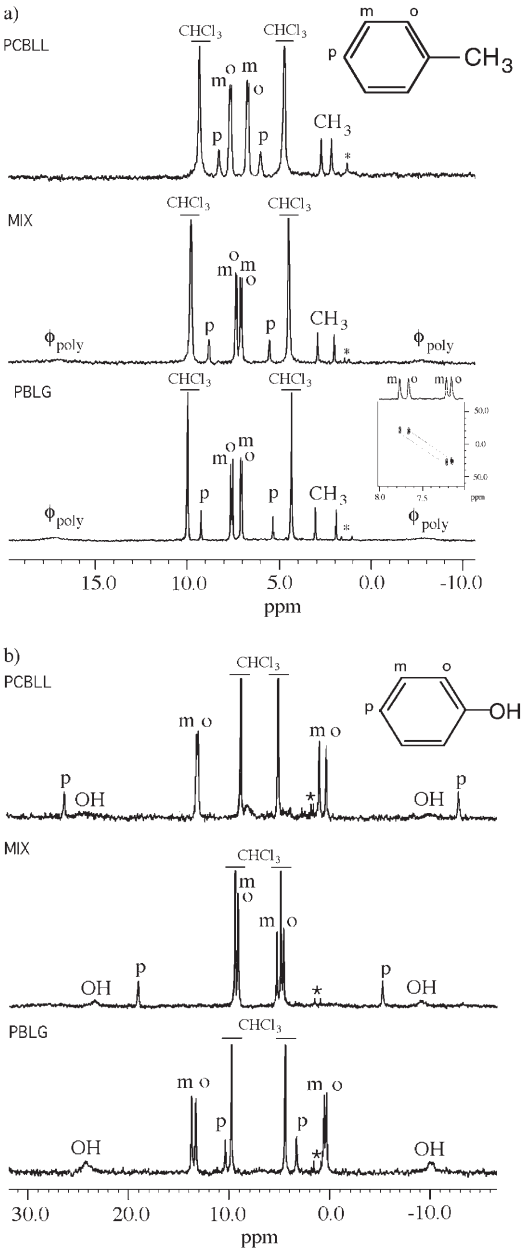

Figure 5. 92.1 MHz ^{2}H{^{1}H} spectra at natural abundance of toluene (a) and phenol (b) dissolved in PBLG (bottom), PCBLL (top), and the MIX phases (middle) at 300 K. The 1D spectra were recorded by adding 5000 scans. Exponential filtering (LB = 2 Hz) was applied. Signals marked by an asterisk correspond to the methyl group of ethanol that is used for stabilizing the chloroform. The inset displays a zoom of the NAD 2D *Q*-resolved Fz map of toluene showing the assignment of doublets associated with the *ortho* and *meta* aromatic deuterons.

Comparison of the data for toluene and phenol is interesting. First, the magnitude of $\Delta\nu_Q$ of phenol in the three mesophases is much larger than that of toluene, and this implies a significant increase in the molecular order parameters. Second, the magnitude and the sign of the doublets for the aromatic deuterons of phenol differ in PBLG and PCBLL. The ratio of the $\Delta\nu_Q$ values in the two mesophases for the *para* deuteron of phenol is about 7, while the $\Delta\nu_Q$ values are

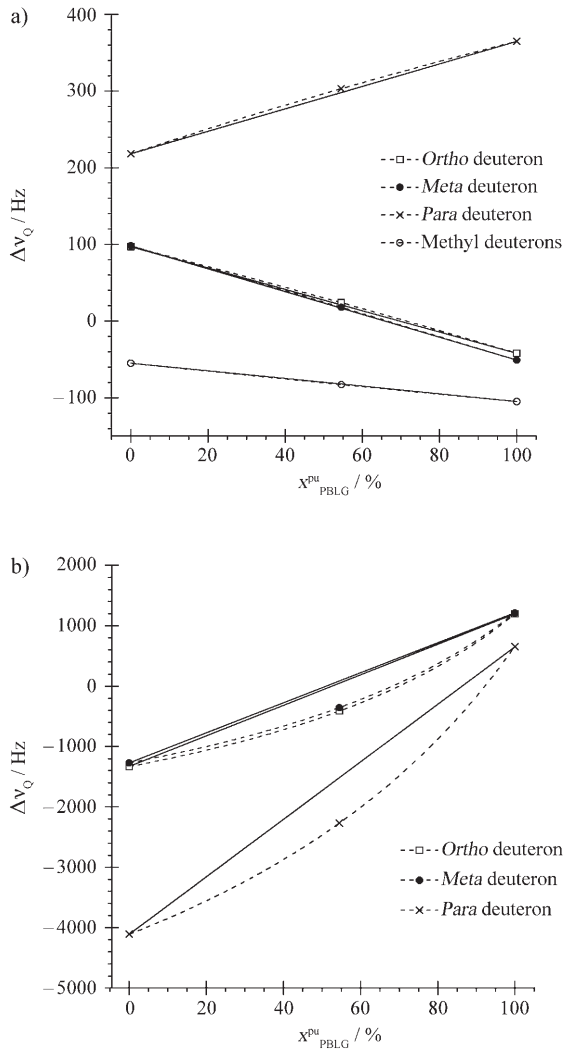

Figure 6. Theoretical and experimental evolution of ^{2}H quadrupolar splittings for toluene (a) and phenol (b) with x^{pu}_{PBLG}. In both plots, the continuous and dashed lines correspond to the fit of data with Equation (2) when $\langle A\rangle = \Delta\nu_Q$ and Equation (5), respectively.

similar for chloroform. Clearly the experimental data (Figure 6b) can only be fitted by using Equation (5). The values of K, k_{PBLG}, and k_{PCBLL} for phenol are also listed in Table 3. The magnitude of K (ca. 0.5) implies that strong specific interactions occur between this solute and one of the two polypeptides, namely, PCBLL, since $k_{PCBLL} > k_{PBLG}$. The K value found for phenol is much lower than that of benzylic alcohol and thus indicates stronger affinity towards PCBLL than bza. The k_{PCBLL} parameter for the three solutes at the same temperature follows the trend $k_{PCBLL}(phe) > k_{PCBLL}(bza) > k_{PCBLL}(tol)$. This trend may be related to the polarity and polarizability properties of solute, since the dielectric permittivity constants of bza, tol, and phe at 300 K ($\varepsilon(phe) = 12.4 > \varepsilon(bza) = 11.9 > \varepsilon(tol) = 2.38$) also evolve in the same sense.[20] In this series of examples, the evolution of k_{PCBLL} agrees with simple arguments based on the chemical properties of solutes. Thus, the distribution coefficients k_{PBLG} and k_{PCBLL} seem to provide reliable indicators to quantitatively evaluate and compare the relative strength of solute–polypeptide interactions.

Table 3. Values of K, k_{PBLG}, and k_{PCBLL} for toluene and phenol from data fitted at 300 K by using Equation (5).

	Parameters	Solute	
		toluene	phenol
ortho	K[a,b]	0.92	0.47
	k_{PBLG}/k_{PCBLL}[c]	47.9/52.1	32.2/67.8
meta	K[a,b]	0.97	0.49
	k_{PBLG}/k_{PCBLL}[c]	49.2/50.8	32.9/67.1
para	K[a,b]	1.08	0.53
	k_{PBLG}/k_{PCBLL}[c]	51.9/48.1	34.7/65.3
methyl	K[a,b]	1.06	–
	k_{PBLG}/k_{PCBLL}[c]	51.5/48.5	–
average for all	K[a,b]	1.01	0.50
^{2}H sites	k_{PBLG}/k_{PCBLL}[c]	50.2/49.8	33.4/66.6

[a] As the fit was made for only three experimental points, the χ parameter is equal to unity. [b] The fit uncertainties lead to an error of ± 0.02 in K values. [c] Value in percent.

Applications: analysis of an enantiomer mixture: The development of NMR spectroscopy in polypeptide CLCs was initially motivated by the possibility to spectrally discriminate the signal of enantiomers, which provides a useful tool for measuring enantiomeric excess in scalemic mixtures. As a practical application, we investigated the case of the monodeuterated chiral molecule (±)-2-deuterophenethyl alcohol (pha, see Figure 7). Although the model described above involved a single type of solute, it clearly also applies to the case of enantiomers. However, the distribution of each enantiomer in the mesophase may a priori be different.

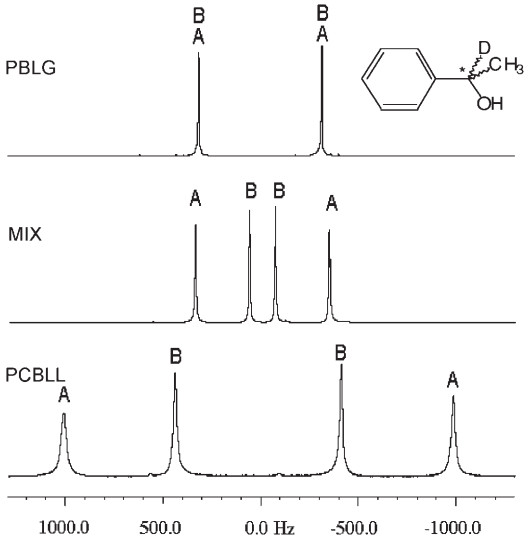

Figure 7. 92.1 MHz ^{2}H–{^{1}H} spectrum of monodeuterated phenethyl alcohol dissolved in PBLG ($\Delta\nu_Q^A = \Delta\nu_Q^B = -586$ Hz), PCBLL ($\Delta\nu_Q^A/\Delta\nu_Q^B = +1994/+853$ Hz), and MIX mesophases ($\Delta\nu_Q^A/\Delta\nu_Q^B = +685$ Hz/$+130$ Hz) at 300 K. The signs of $\Delta\nu_Q$ were determined using the procedure previously described. The 1D spectra were recorded by adding 64 scans. Exponential filtering (LB = 1 Hz) was applied.

Figure 7 shows the 92.1 MHz proton-decoupled deuterium spectrum of pha in PBLG/$CHCl_3$ at 300 K. Surprisingly, no enantiomeric discrimination is observed in the spectrum, the linewidth of which is smaller than 3 Hz. This situation arises when the C–D bonds in both isotopically enriched enantiomers fortuitously have the same orientation on average. It corresponds to a differential ordering effect (DOE) factor equal to zero.[3] Note that DOE factor is an empirical criterion for evaluating the quality of the deuterium spectral enantiodiscrimination, and so allows simple comparisons between different ^2H spectra or inequivalent ^2H sites. Changes in temperature and/or sample composition (with the same component) can improve the discrimination, but the result is never guaranteed. In a more general context, it is preferable, as indicated in the Introduction, to change the nature of the polypeptide.[8,9] The results obtained in PCBLL/$CHCl_3$ mesophase are convincing, because a strong spectral discrimination occurs in this system ($|\Delta\nu_Q^A| - |\Delta\nu_Q^B| = 1141$ Hz), that is, a DOE factor of 0.8. This large discrimination is, however, accompanied by large magnitudes of quadrupolar splittings (853 and 1994 Hz) and severe line broadening (17 and 28 Hz). In fact, the line broadening related to the increased splitting reflects a slight deviation from ideally homogeneous alignment among the solute molecules. This spectral situation could be unsuitable for analyzing perdeuterated molecules due to the large number of deuterium doublets when high resolution is required. In addition, such line broadening significantly reduces the signal-to-noise (S/N) ratio and thus limits the use of NAD NMR for measuring enantiomeric excess. Here the alternative of using a mixture of two polypeptides is advantageous because one can favorably expect to combine chiral discrimination for pha with smaller quadrupolar splittings and linewidths than those obtained in PCBLL systems. This situation is effectively demonstrated in a 50/50 (w/w) mixture of PBLG and PCBLL. In this example, the spectral difference ($|\Delta\nu_Q^A| - |\Delta\nu_Q^B| = 555$ Hz) is indeed reduced by a factor of around two compared to PCBLL, while the DOE factor is improved (1.4) and the linewidth are significantly reduced (6 and 9 Hz). From the viewpoint of sensitivity, the benefit of mixing two polypeptides is clearly seen. Indeed, the S/N ratio is increased by a factor of about 4.5 for the signals of the enantiomers A and B, when comparing the S/N ratios in PCBLL phase ($(S/N)^A/(S/N)^B = 220/340$) with the mixture ($(S/N)^A/(S/N)^B = 1100/1437$). This experimental example illustrates the analytical potential of NMR spectroscopy with a mixture of two polypeptides.

As in the previous analyses, the evolution of quadrupolar data can be fitted by using Equation (5). The K values for enantiomers A and B of pha are 0.85 and 0.84, respectively (see Figure SI-2 in the Supporting Information). This discrepancy cannot be considered as truly significant because the difference is within experimental errors, mainly due to the linewidths. Hence, we cannot conclude on a difference of affinity between enantiomers towards both polypeptides. Other attempts to detect such effect are currently underway. Using an averaged K value, we obtained (46 ± 1) and (54 ± 1) % for k_{PBLG} and k_{PCBLL}, respectively. The K value for pha is slightly larger than the value obtained for bza ($K = 0.71$) but remains relatively close. This result is consistent when considering that the polarities of the two compounds are rather similar. The small difference could result from the presence of the methyl group around the stereogenic carbon atom. Indeed the bulk of this group compared to a single hydrogen atom, as well as its donor effect, could affect the strength of the hydrogen bonds toward the PCBLL, and so reduce the affinity of pha toward the PCBLL.

Analysis of mixtures with a large difference in DP: To assess the valid domain of hypothesis 2, we studied mixtures of two polypeptides with a large difference in DP (DP ratio of ca. 10). We found that such peculiar lyotropic mixtures form not homogeneous but biphasic systems consisting of both isotropic and anisotropic domains. For analytical purposes, such a situation is clearly unsuitable and demonstrates the necessity of using chemically different polypeptides with very similar DPs. A complete description of this study is given in the Supporting Information.

Conclusion

Organic solutions made of the two chemically different polypeptides PCBLL and PBLG of similar DP do indeed provide suitable enantioselective ordered media for discriminating between enantiotopic directions in prochiral molecules or enantiomers and hence offer an efficient alternative to single-polypeptide chiral mesophases. Furthermore, varying the relative proportions of polypeptides in these novel CLCs provides a new degree of freedom for optimizing the NMR parameters relevant to analytical applications, that is, the magnitude of spectral discrimination, sensitivity, and resolution. Finally, these new enantiodiscriminating mesophases are stable over a large range temperature and accommodate large amounts of solutes, whatever their polarity or flexibility. Such stability and versitility are both desirable in analytical applications.

Besides practical applications, this work provides valuable insights into enantiodiscrimination mechanisms in polypeptide liquid crystals. In particular we have established that the magnitude of enantiodiscrimination in two-polypeptide chiral systems strongly depends on the solute distribution within the mesophase. This molecular distribution can be characterized by analyzing solute NMR data ($\Delta\nu_Q$, T_{1Z}) with respect to the relative proportion of PBLG and PCBLL. Such a simple method allows the relative affinity of small solute molecules toward both distinct polypeptides to be quantified experimentally. In addition, identification of factors controlling the solute distributions can lead to specific solute–polypeptide interactions, which play a leading role in chiral-discrimination mechanisms. The comparison of results obtained for polar (phenol and benzylic alcohol) and apolar (toluene) solutes reveals that specific van der Waals interactions and/or hydrogen bonds between solute and polypep-

tides can strongly modify the solute distribution within the mixture. In addition, it is noteworthy that the lyotropic nature of the current solvent systems leads to the absence of solute concentration effects (up to 10 wt%) on the solute distribution.

Numerous extensions of this work are possible. First, it would be interesting to refine our model by taking into account, for instance, long-range interactions at more than half the interfiber distance.[12] Second, the investigation of polar solutes which cannot be involved in hydrogen bonds could supply valuable information on the contribution of this specific interaction to the solute distribution and especially the K parameter. Third, studying mixtures of PBLG and PELG could quantify the effect of a benzyl group compared to ethylene group in the lateral chains on the K parameter for polar or apolar solutes. This should provide new information on the strength of π-stacking interactions between an aromatic solute and PBLG helices. All these investigations are currently underway.

Experimental Section

Polypeptides PBLG and PCBLL are commercially available from Sigma Corp. Preparation of fire-sealed samples was similar to the procedure described previously.[1,3] All samples of benzylic alcohol (1–8) were made by adding about 50 mg of solute, about 100 mg of polypeptide (neat or mixture), and about 350 mg of $CHCl_3$. For samples of toluene and phenol (9–14), we used the same amount of solute and polypeptide, but about 554 mg of $CHCl_3$. For samples of (±)-phenethyl alcohol (15–17), we used about 10 mg of solute, about 100 mg of polypeptide, and about 540 mg of $CHCl_3$. For samples 1–7 of bza, the polypeptide DPs were 1352 (PBLG) and 1100 (PCBLL). For tol, phe, pha, and sample 8 for bza, the polypeptide DPs were 782 (PBLG) and 778 (PCBLL). Table SI-3 in the Supporting Information summarizes the exact composition of each sample. $CHCl_3$ was chosen as co-solvent because 1) it is an aprotic and weakly polar solvent that can dissolve the three solutes investigated here, and 2) it is the best co-solvent for NAD NMR spectroscopy, as it has a high molecular weight and a single deuterium site.[2,3]

From the point of view of liquid-crystalline properties, the mixture of polypeptides leads to birefringent NMR samples that are chemically stable over time (at least more than one year), like the "neat" mesophases. However, preparation of oriented phases formed by two polypeptides required more attention than for "neat" mesophases. In particular, it is crucial to obtain highly homogeneous phases in which a statistical distribution of each kind of fibers over the whole sample exists. When this equilibrium state is not reached, evolution of the molecular ordering of the solute is possible with time. To limit this effect, sample preparation must be performed at least 24 h before recording the NMR spectra. The ^2H NMR spectra of bza were recorded on a 9.4 T Bruker Avance spectrometer equipped with a 5 mm ^2H BBO probe. All NAD spectra as well as the ^2H NMR spectra of pha were recorded on a 14.1 T Bruker Avance II spectrometer equipped with a selective ^2H 5 mm cryogenically cooled probe.[21] The WALTZ-16 pulse sequence was used to decouple protons. As the gain in sensitivity compared with a standard selective ^2H probe is greater than a factor of 5–6, we can record NAD spectra with a good S/N ratio in a short time (about 40 min).

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