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## SIDECHAIN ORDER PARAMETERS VIA $^2\text{H}$ NMR IN POLYPEPTIDE LIQUID CRYSTALS (\*)

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**Résumé.** — La chaîne latérale du poly-L-glutamate de benzyl, PBLG, a été deutérée sélectivement. Dans les solutions de PBLG orientées magnétiquement, les bâtonnets de polymère hélicoïdal sont alignés parallèlement au champ. Le mouvement brownien de la chaîne latérale flexible du PBLG est anisotrope, donnant lieu à des dédoublements quadripolaires des résonances magnétiques nucléaires de  $^2\text{H}$ . Les paramètres d'ordre des directions de liaisons C-D dans la chaîne latérale peuvent être déterminés. Ces études donnent de nouvelles perspectives sur la nature de l'organisation des chaînes latérales à la périphérie de l'hélice  $\alpha$ .

**Abstract.** — We have selectively deuterated the sidechain of the synthetic polypeptide poly(- $\gamma$ -benzyl-L-glutamate), PBLG. In magnetically oriented nematic solutions of PBLG, the helical polymer rods are aligned parallel to the field. The Brownian motion of the flexible PBLG sidechain is anisotropic, resulting in quadrupole splittings of the  $^2\text{H}$  NMR resonances. The order parameters of C-D bond directions in the sidechain can be determined. These studies permit new insights into the nature of organization of sidechains on the periphery of the  $\alpha$ -helix.

The general features of lyotropic polypeptide liquid crystals have been reviewed recently [1]. The chirality of the  $\alpha$ -helical polymer imposes a cholesteric texture in the mesophase. However, the diamagnetic anisotropy of the helix is positive and an oriented nematic phase persists in sufficiently strong magnetic fields ( $H_c \gtrsim 5$  kOe). Solvent molecules and guest molecules added to this mesophase exercise anisotropic rotational and diffusional motion within this oriented array of hexagonally packed helices. While the details of the anisotropic motion are complex [a uniaxial dispersion force field superposed on a chemical exchange process involving a bound guest (solvent) proximate to the helix exchanging with nearly isotropic molecules removed from the helix solvation shell], it permits NMR spectroscopy experiments in liquid crystals wherein the species observed is characterized by very low orientational order parameters (usually two to three orders of magnitude smaller than thermotropic mesophases).

The low degree of order facilitates the observation of quadrupolar splittings ( $\Delta\nu_i$ ) in the  $^2\text{H}$  NMR spectra of deuterated guest (solvent) molecules [2]. Herein we describe quadrupolar splittings for specifically  $^2\text{H}$  labeled sidechains of the polypeptide component of

the liquid crystal. The magnitude of the  $\Delta\nu_i$  and their response to changes in the polymer concentration in the liquid crystal provide some general insights into the structural-dynamical organization of the sidechains on the periphery of the polypeptide helix.

Two  $^2\text{H}$  labeled derivatives of the synthetic polypeptide poly(- $\gamma$ -benzyl-L-glutamate) (PBLG), were studied: PBLG-d<sub>1</sub> with deuterium at the para position on the phenyl ring of the benzyl ester and, PBLG-d<sub>7</sub> with the benzyl ester fully deuterated. In the oriented nematic phase, the director is parallel to the spectrometer magnetic field (z-axis); the quadrupolar splitting is given by

$$\Delta\nu_i = \frac{3}{2} \frac{eQ_i V_{izz}}{h} \equiv \frac{3}{2} q_{izz} \quad (1)$$

where  $Q_i$  is the quadrupole moment and  $V_{izz}$  is the appropriate component of the electric field gradient tensor. The tensor components  $q_{\alpha\beta}$  in a molecule-fixed axis system ( $a, b, c$ ) are related to  $q_{zz}$  via the elements of the Saupe order matrix:

$$q_{zz} = \frac{2}{3} \sum_{\alpha,\beta}^{a,b,c} S_{\alpha\beta} q_{\alpha\beta} \quad (2)$$

In practice the average orientation or order parameter of the C-D bond (b-direction) can be extracted from  $\Delta\nu$  if the quadrupole coupling constant  $q_{bb}$  is known.

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(The asymmetry parameter  $\eta = (q_{aa} - q_{cc})/q_{bb}$  is assumed to be negligible.)

The small-molecule analogue toluene- $d_8$  provides a set of  $q_{bb}$ . The  $q_{bb}$  are derived from the  $^2\text{H}$  NMR spectrum of toluene- $d_8$  oriented in the PBLG liquid crystal (Fig. 1). The orientation of the toluene can

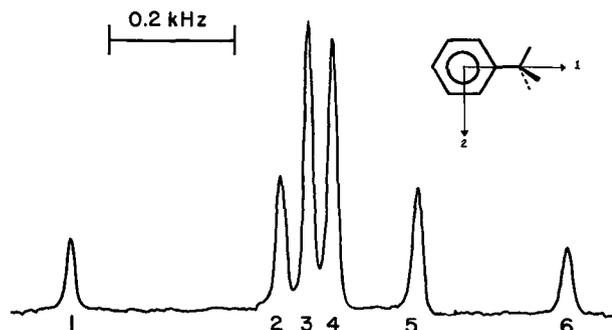


FIG. 1. — The  $^2\text{H}$  NMR spectrum of toluene- $d_8$  dissolved in an oriented PBLG- $\text{CH}_2\text{Cl}_2$  solution (30 % by wt. polymer).

be specified with two order matrix elements,  $S_{11}$  and  $(S_{22}-S_{33})$ . The former is obtained from the dipolar splittings in the  $^1\text{H}$  NMR spectrum of toluene methyl group. The results are based on the assumption of identical ordering and geometries for toluene and toluene- $d_8$  in the liquid crystal (Table I). There is good agreement with earlier determinations of the  $q_{bb}$  [3].

Next we contrast the ordering of the *free* probe molecule benzyl alcohol- $d_7$  dissolved in the polypeptide liquid crystal with the *bound* probe, the benzyl ester in PBLG- $d_7$ . It is readily apparent from the difference in the magnitude of the  $\Delta\nu_i$ 's (Fig. 2), that the deuterated probe covalently attached to sidechain is considerably more constrained than the *free* probe. The former exhibits  $\Delta\nu$ 's spanning a spectral width of nearly 25 kHz whereas the spectrum of the latter is limited to  $\sim 5$  kHz. Also, labeling the sidechain in itself does not appreciably influence the orientation; spectra of PBLG- $d_1$  and PBLG- $d_7$  superpose (Fig. 3).

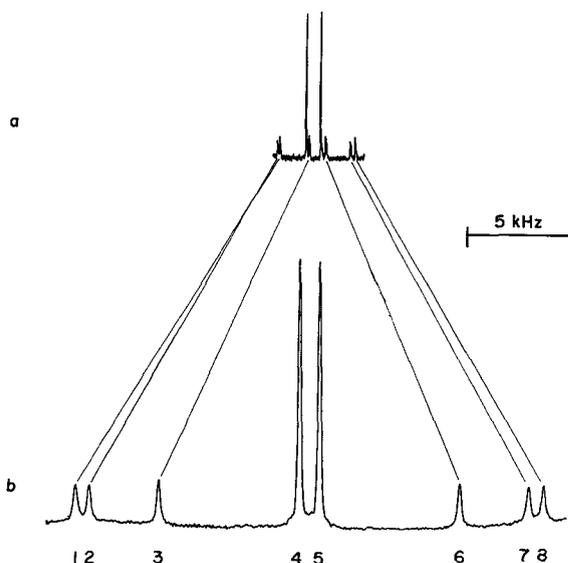


FIG. 2. — *a*) The  $^2\text{H}$  NMR spectrum of benzyl alcohol- $d_7$  dissolved in an oriented PBLG- $\text{CH}_2\text{Cl}_2$  solution. *b*) The  $^2\text{H}$  NMR spectrum of oriented PBLG- $d_7$ ; the solvent is  $\text{CH}_2\text{Cl}_2$  (30 % by wt. polymer); Resonance 1, 3, 6, and 8 are from the benzyl- $\text{CD}_2^-$ ; resonances 4 and 5 are from the ortho and meta deuterous and resonances 2 and 7 are for the para deuterium.

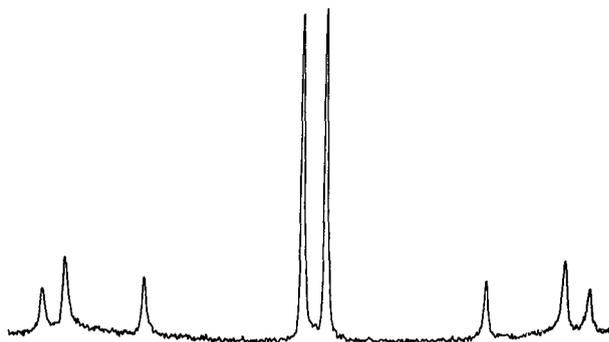


FIG. 3. — The  $^2\text{H}$  NMR spectrum of a 50/50 mixture of PBLG- $d_1$  and PBLG- $d_7$  in the liquid crystalline phase; the increased intensities of the superposed para deuterium resonances are apparent.

TABLE I

*Toluene + toluene- $d_8$  in PBLG liquid crystals*

$^1\text{H}$ NMR	nucleus	$\Delta\nu$ (Hz)	$S_{11} (\times 10^3)$ (*)
	CH <sub>3</sub>	$90 \pm 1$	2.82
$^2\text{H}$ NMR	nucleus	$\Delta\nu$ (Hz)	$q_{bb}$ (kHz)
	CD <sub>3</sub>	$221 \pm 4$	$157 \pm 4$
	papara C-D	$795 \pm 5$	$188 \pm 4$
	ortho, meta C-D	$40 \pm 1$	188
			$(S_{22} - S_{33})$ (***)
			$1.32 \times 10^{-3}$

(\*) Using  $\Delta\nu = -(3\gamma_{\text{H}}^2 h/4\pi r_{\text{HH}}^3) P_2(\cos 90^\circ) S_{22}$  with the valence angle in the methyl  $\alpha = 70.5^\circ$ ;  $r_{\text{HH}} = 1.78 \text{ \AA}$ .

(\*\*) Using  $S_{11} = 2.82 \times 10^{-3}$ ;  $\Delta\nu_{\text{para}} = \frac{3}{2} q_{bb} S_{11}$ ;  $\Delta\nu_{\text{CD}_3} = \frac{3}{2} q_{bb} P_2(\cos \alpha) S_{11}$ .

(\*\*\*) Assuming  $q_{bb} = 188 \text{ kHz}$ ,  $S_{11} = 2.82 \times 10^{-3}$ ;  $\Delta\nu_{\text{o,m}} = \frac{3}{2} q_{bb} [P_2(\cos \beta) S_{11} + \frac{1}{2}(S_{22} - S_{33}) \sin^2 \beta]$  with  $\beta = 60^\circ$ .

In an analysis of the origin of the constraints affecting the *bound* probe PBLG-d<sub>7</sub>, one can readily identify two general influences : 1) the anisotropic dispersion forces present in the void *between* the rigid, helical cores of the polymer rods and 2) the conformational restrictions arising from the valence and dihedral angles in the sidechain. And, on the basis of the results in figure 2, one might suppose that the dispersion force contribution to the orientational order is roughly 20 % of the total. This contribution may vary, however, with the volume fraction of polymer in the liquid crystals ; the *intermacromolecular* space will decrease when the volume fraction of polymer,  $\Phi$ , increases.

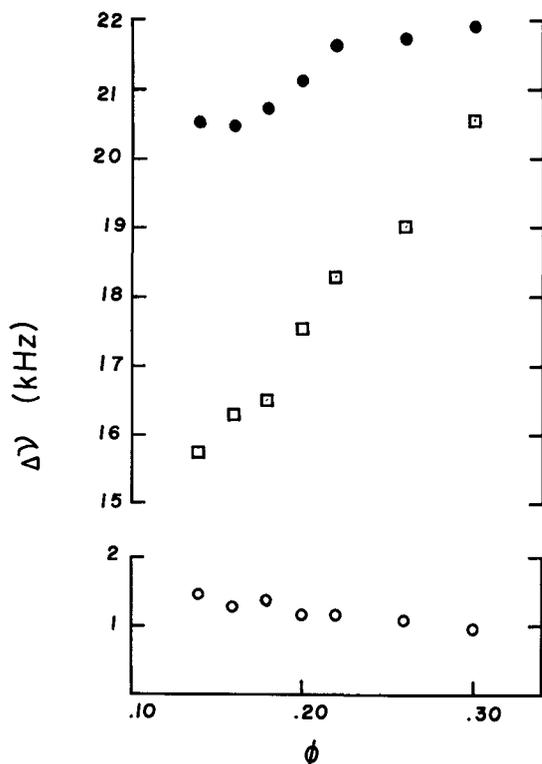


FIG. 4. — The concentration dependence of the quadrupole splittings in PBLG-d<sub>7</sub>; ○ ortho and meta deuterium, □ para deuterium, ● a benzyl-CD<sub>2</sub>.

Figure 4 shows the change in  $\Delta\nu$ 's with  $\Phi$  for the deuterated sidechain in PBLG-d<sub>7</sub>. The para deuterium quadrupolar splitting,  $\Delta\nu_{\text{para}}$ , increases by 5 kHz over the concentration range considered. This reflects, undoubtedly, a decrease in the sidechain orientational freedom. There is an apparent nonlinearity of the  $\Delta\nu$  vs.  $\Phi$  plot localized in the region of  $\Phi = 0.2$ . This peculiarity may result from intercalation of sidechains from adjacent polypeptides. Early X-ray studies [4] of this liquid crystal showed that the interhelix separation,  $d$ , is proportional to  $\Phi^{-2}$ . Using reasonable values for the solvated helix diameter,  $D = 20\text{-}30 \text{ \AA}$ , the X-ray data suggests that the sidechains on adjacent polypeptides interpenetrate one another for  $\Phi \gtrsim 0.2$  ( $d$ - $D$  becomes negative).

Although there is the evidence for *extramolecular* dispersion force contributions to the sidechain orientation, it is instructive to consider the magnitude of the observed orientation in terms of *intramolecular* conformational constraints. We consider a cylindrically symmetric rod (polypeptide) capable of exercising free rotation about the rod major axis ( $h$ -axis) with a C-D bond constrained at some orientation relative to  $h$ . In this scheme, we can investigate the plausibility of sidechain conformations. We will focus on  $\Delta\nu_{\text{para}}$  and attempt to get order of magnitude agreement between calculated and observed values of  $\Delta\nu_{\text{para}}$ .

*Free rotation model.* — This model of the sidechain conformation assumes rapid, uniform rotation about each bond in the polypeptide sidechain (all values of the six dihedral angles in the sidechain are equally probable). With a value for the average orientation of the  $h$ -axis with respect to the nematic director,  $S_h$ , and conventional values for the five valence angles  $\theta_i$  ( $i = 1\text{-}5$ ) in the sidechain we find (where  $\theta_0 = 121.6^\circ$  is the angle the  $^{\alpha}\text{C}-\beta\text{C}$  bond makes with the  $h$ -axis)

$$\Delta\nu_{\text{para}} = \frac{3}{2} q_{bb} \prod_{i=0}^5 P_2(\cos \theta_i) S_h. \quad (3)$$

For  $q_{bb} = 188 \text{ kHz}$  and  $S_h = 0.75$  from X-ray studies [5], eq. (3) yields a value  $\Delta\nu_{\text{para}} = 20 \text{ Hz}$ , some three orders of magnitude smaller than that observed.

*Static sidechain model.* — In this description, the two low-energy sidechain conformations calculated for PBLG by Scheraga *et al.* [6] yield the orientation of the para C-D bond with respect to the  $h$ -axis : for the  $Rl(+)$  conformation the para direction makes an angle  $\delta = 24.9^\circ$  with the  $h$ -axis and for the  $Rt(-)$  conformation  $\delta = 125.6^\circ$ . In the general scheme we have adapted to calculate  $\Delta\nu_{\text{para}}$ , we obtain

$$\Delta\nu_{\text{para}} = \frac{3}{2} q_{bb} P_2(\cos \delta) S_h. \quad (4)$$

This yields quadrupole splittings of 155 kHz and 54 kHz for the  $Rl(+)$  and  $Rt(-)$  conformations, respectively, factors of 3 and 10 larger than the observed splitting.

*Intermediate models.* — If one employs a static, low-energy sidechain conformation out to some atom in the sidechain, allowing free rotation beyond that point, one can obtain a reduction in the calculated values. For example, if free rotation is permitted about the ester O-CH<sub>2</sub> bond, then eq. (4) is reduced by the factor  $P_2(\cos \theta_5)$ . In the case of the  $Rt(-)$  conformation, since the valence angle in the benzyl CH<sub>2</sub> group is approximately tetrahedral, eq. (4) is reduced by a factor of 1/3, giving very reasonable agreement between the calculated and the observed  $\Delta\nu_{\text{para}}$ .

While we have in fact good agreement between

calculated and observed values for  $\Delta\nu_{\text{para}}$  with the above model of the sidechain, the model is not unique. Reasonable calculated values of  $\Delta\nu_{\text{para}}$  can also be achieved for both the  $Rl(+)$  and  $Rl(-)$  static conformations in which free rotation is allowed about the preceding ester bond to the carbonyl carbon. However, with a judicious use of chemical intuition

about the feasibility of unrestricted rotation in the sidechain, contrasting the  $^2\text{H}$  NMR measurements with calculations offers a unique approach to structural-dynamical investigations of polymers in solution.

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