Lipid Molecules in Lyotropic Liquid Crystals With Cylindrical Structure (A Spin Label Study)

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Lipid Molecules in Lyotropic Liquid Crystals With Cylindrical Structure (A Spin Label Study)

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Spin labeled fatty acids are used to investigate a lyotropic mesophase in which the amphiphilic molecules are arranged in cylindrical rods. A homogeneous ordering of the cylinders is obtained by sucking the liquid crystalline phase in glass capillaries. This considerably simplifies the epr spectra. The lipid molecules in the cylinders undergo fast rotations around their long axes with a correlation time of approximately 10^{-10} sec and slower rotations around the axis of the cylinder. The latter effect is responsible for distinct differences between the spin label spectra of cylinder and bilayer phases. The hydrocarbon chains in the cylinders are flexible and the order parameter decreases from the polar surface towards the interior of the cylinders. The microviscosities of both phases are about equal and amount to approximately 0.05 poise.

Spin labels[1] have opened a new way in which to probe the properties of smectic liquid crystals. In previous reports we have primarily been concerned with *lamellar* mesophases since they are of importance in the membranes of living systems[2-7], but spin labels should be equally applicable to any other kind of lyotropic liquid crystalline structure. We have therefore studied a so-called *hexagonal* phase, in which the amphiphilic molecules are arranged in rods of cylin-

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drical cross section surrounded by water. The spin label spectra of this system exhibit some unusual features which will be compared here with the corresponding spectra of a liquid crystalline bilayer.

The *hexagonal* phase has the following chemical composition: sodium caprylate 43 wt. %, decanol 8 wt. %, water 49 wt. %. Its geometry is known from X-ray studies of Ekwall et al.[8,9] The diameter of the cylinder amounts to ~26 Å, and the area per polar group is ~40 Å^2. The *lamellar* liquid crystals have a similar composition, namely: sodium caprylate 28 wt. %, decanol 42 wt. %, water 30 wt. %. The thickness of this bilayer is ~23 Å and the area per polar group ~24 Å^2.

As spin labels we have used fatty acids of the general structure I (m, n):

$$CH_3-(CH_2)_m-C-(CH_2)_n-COOH$$

$$O-N \quad O \qquad\qquad I(m,n)$$
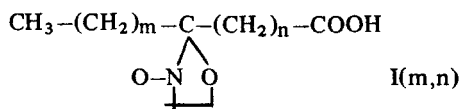
The total length and the position of the label group on the chain have been varied. The concentration of spin label in the liquid crystalline material was less than 0.1 wt. %. If not stated otherwise, all experimental conditions are the same as described previously.[4,7] The measurements were made at room temperature (~22°C) with a Varian E-9 spectrometer.

Most experiments were performed with spin label I (4.2). This molecule has a chain length of 9 carbon atoms which lies between that of caprylic acid (8 C atoms) and decanol (10 C atoms). The epr spectra of I (4.2) in statistical distributions of hexagonal and lamellar liquid crystals are shown in Figure 1a and 1b, respectively. (These random distributions are obtained by filling the material in sample tubes of approximately 4 mm diameter). The bilayer spectrum 1b is well understood by now.[7] Its molecular origin are fast anisotropic rotations of the bilayer molecules around their long molecular axes coupled with trans-gauche isomerisations of carbon-carbon bonds. The line shape analysis yields a correlation time of approximately 2×10^{-10} sec for the rotational morion of the hydrocarbon chains. From the outer and inner hyperfine splittings it is also possible to determine the degree of order S_3 of the spin labeled chain segment. which in the case of I (4.2) amounts to $S_3 = 0.59$ (based on an isotropic hyperfine splitting constant $a_N = 14.7$ G).

The spin label spectrum 1a of the hexagonal phase differs appreciably from that of the bilayer. It shows less fine structure and the outer extrema are smeared out to some extent. On the other hand, the linewidth of the central peak is practically the same in both cases. Since this linewidth is a measure of the velocity of the movements, it immediately follows that the rotation of the hydrocarbon chains in the cylinders must be as rapid as that in the bilayer.

How can we then account for the observed spectral differences? The answer

FIGURE 1   Spin label I (4.2) in random distributions of lyotropic liquid crystals at 22°C.
a) Cylindrical rods
b) Bilayers

to this question comes from experiments with *oriented cylinders*. As we have shown previously it is possible to orient lamellar liquid crystals either between two optically flat glass plates[2] or in glass capillaries.[7] We have therefore tried both techniques with the hexagonal phase. No homogeneous orientation could be obtained using planar glass plates, but glass capillaries proved to be successful and produced a rather perfect ordering of the amphiphilic cylinders, such that the rods were aligned parallel to the long axis of the capillary.

Evidence for this assertion is found in the epr spectra shown in Figure 2 and Figure 4. In Figure 2 the magnetic field is applied along the axis of the capillary. The observed signals are very similar for the lamellar and the hexagonal phase and consist of three sharp lines with a hyperfine splitting constant of approximately 10 G. The spectra in Figure 2 must be interpreted as time-averaged spectra in which each individual spin label contributes exactly the same signal to

the overall spectrum. The rather small hyperfine splittings indicate further that the motion is anisotropic and that the magnetic field is essentially perpendicular to the nitrogen $2p\pi$ orbital in both cases. Since this orbital is extended along the backbone of the hydrocarbon chain two conclusions can be drawn from Figure 2.: (1) the hydrocarbon chains in the bilayers and the cylinders are oriented perpendicular to the axis of the capillary (2) In both cases the molecules must rotate freely around their long molecular axis. A cross section of the glass capillary can therefore be described as is shown schematically in the inset of Figure 2. The individual hydrocarbon chains must be thought to lie in the plane of the paper. The fundamental difference between the two distributions should be noted. In the lamellar phase the bilayers are arranged in more or less
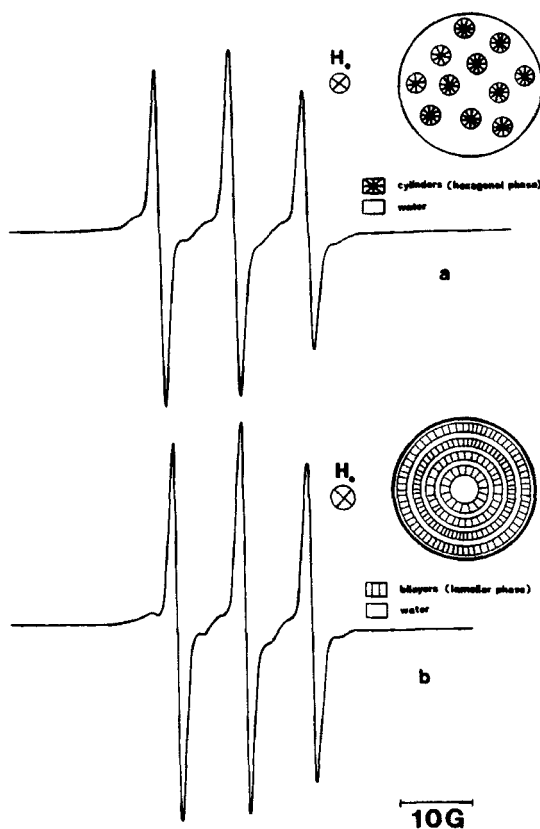

FIGURE 2   Spin label I (4.2) in liquid crystals oriented in a glass capillary. 22°C
Magnetic field H_0 *parallel* to the capillary axis.
a) Cylindrical rods
b) Bilayers
Inset: schematic diagram (cross section) of the orientation of cylinders and bilayers in the capillary.

concentric shells around the axis of the capillary, while in the hexagonal phase we deal with compact rods of small diameter ($\sim$ 26 Å).

This difference becomes important if we take into account the translational diffusion of the lipid molecules. The line shape analysis of the epr spectra yields a correlation time τ_c of roughly 2.5×10^{-10} sec, which is related to the microviscosity η according to eq. (1)

$$\tau_c = \frac{4\pi r^3 \eta}{3kT} \tag{1}$$

Assuming a radius of 3.6 Å for the spin labeled hydrocarbon chain we estimate a microviscosity of $\eta \sim 0.05$ poise and a translational diffusion constant $D \sim 1.5 \cdot 10^{-6}$ cm^2/sec[7]. In an interval of 10^{-8} sec, a time which still is comparable with the correlation time, the spin labeled lipid molecule will therefore diffuse a distance of roughly 17 Å in the bilayer or the cylinder. If we consider again the cross section of the glass capilary, this time drawn on a larger scale (figure 3) we see that the radius of curvature of the cylindrically oriented bilayers is much larger than that of the compact rods. This has the important consequence that a diffusion along a distance of 17 Å may change the direction
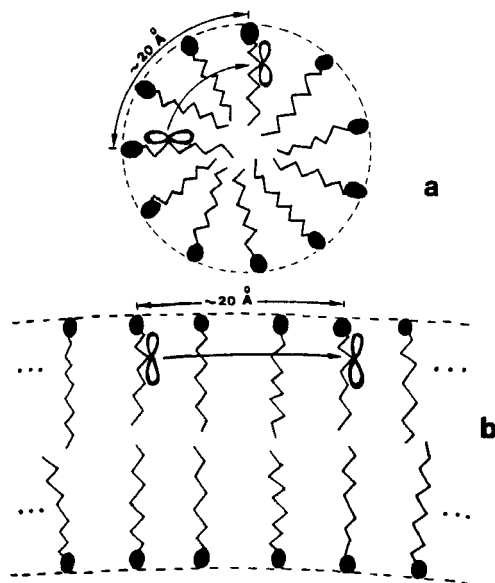

FIGURE 3 Enlarged view of the capillary cross section. Comparison between the curvature of the bilayer and the cylindrical rods.
a) Cylinder (hexagonal phase)
b) Bilayers (lamellar phase)

of the nitrogen $2p\pi$ orbital by as much as 90° in the cylindrical rods but will virtually not affect its orientation in the bilayer.

As long as the magnetic field is oriented along the axis of the glass capillary, i.e. perpendicular to the plane of Figure 3, this translational diffusion has no influence on the observed epr signals, neither in the bilayer nor in the hexagonal phase. In this case the direction of the nitrogen $2p\pi$ orbital, i.e. the z-axis of the NO group, is immaterial. Only the averaged x and y components of the g- and T-tensors are seen. This explains the similarity between Figure 2a and Figure 2b.

However, if the magnetic field is applied perpendicular to the glass capillary, we encounter two different situations with respect to the epr experiment (Figure 4). Although we have a cylindrical distribution of nitrogen $2p\pi$ orbitals in
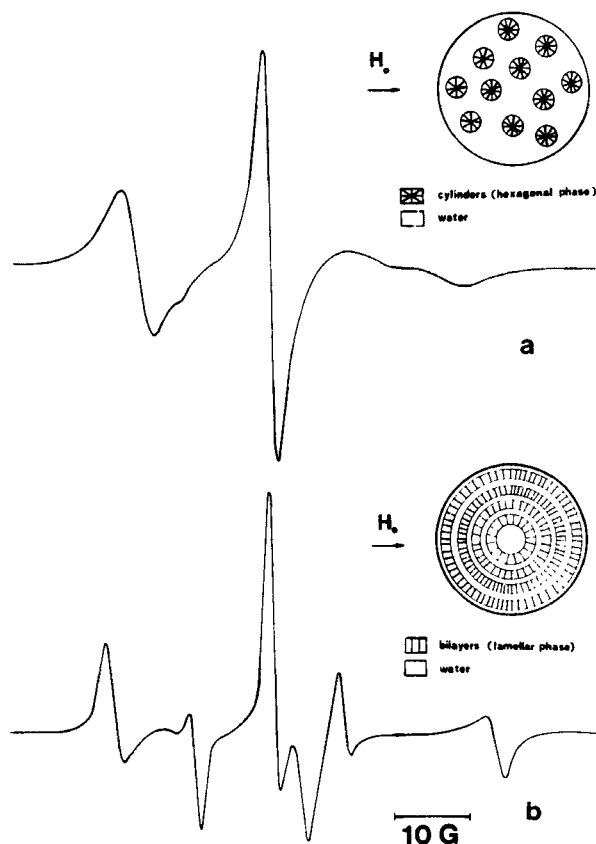

FIGURE 4   Spin label I (4.2) in liquid crystals oriented in a glass capillary. 22°C Magnetic field H_0 *perpendicular* to the capillary axis.
a) Cylindrical rods
b) Bilayers

both phases, this must be regarded as a *static* distribution for the bilayer and as a *dynamic* distribution for the hexagonal phase. The epr signal of the bilayer phase arises from a superposition of spin labels having various orientations with respect to the magnetic field, but during the time of the epr experiment the angular orientations of the individual spin labels remain unchanged. Assuming a *static* cylindrical distribution of spin labels it is then possible to simulate the bilayer spectrum 4b.[7] In the hexagonal phase the orientation of a specific spin label is not fixed due to the rotational diffusion described above. This motion is slower than the rotation around the long axis of the hydrocarbon chain, but nevertheless leads to an additional averaging of the g- and T-tensor (Figure 4a). This effect must be even more pronounced in the case of a random distribution of cylindrical rods (Figure 1a).

Although we have not yet obtained computer simulated spectra of the hexagonal phase, it is possible to extract quantitative information on the structure of this phase by means of Figure 2a. If we assume the same polarity for label I (4.2) in the hexagonal phase is observed in the bilayer ($a_N = 14.7$ G), then the hyperfine, splitting of Figure 2 yields an order parameter $S_3 = 0.48$. This is smaller than the corresponding order parameter of the bilayer ($S_3 = 0.59$), but can easily be explained by the specific geometry of the hexagonal phase. The surface area per polar group is much larger in the cylinders than in the bilayer, which, in turn, gives more motional freedom to the hydrocarbon chains. The movements of the hydrocarbon chains become also more random towards the center of the cylinders. This can be deduced from quantitative measurements with I (3.2) and I (2.3) which yielded order parameters of $S_3 = 0.42$ and $S_3 = 0.35$, respectively, and also from qualitative results with other labels I (m, n).

Comparing our present data of the hexagonal phase with those of the bilayer we may conclude therefore that in spite of the different geometries the physical structure of both systems is similar to a large extent. Both types of liquid crystals have viscosities of $\eta \approx 0.05$ poise: the movements of the hydrocarbon chains are anisotropic, i.e. fast rotations around the long molecular axes with correlation times of the order of 10^{-10} sec; this anisotropy is characterized by order parameters of similar magnitude; both phases exhibit an increasing randomization of the chain motion towards the central part of the lipid region. Finally both phases show translational diffusion, but this is only a lateral diffusion in the case of the bilayer, while in the case of the cylinders lateral diffusion along the cylinder axis is combined with a rotational diffusion around this axis.

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