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NMR Studies of 1-Palmitoyllysophosphatidylcholine in a Cubic Liquid Crystal with a Novel Structure

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^2H, ^{14}N, and ^{31}P nuclear magnetic resonance (NMR) studies of the cubic liquid crystalline phase in the system 1-palmitoyllysophosphatidylcholine (PaLPC) and water have been performed. This phase is located between the micellar solution and the hexagonal phase in the binary phase diagram. In contrast to other hitherto studied cubic phases this one gives rise to an NMR spectrum which is a superposition of two signals; an isotropic, narrow peak and a broad, partially averaged anisotropic peak. It is found that the line shape and the relative intensity of the isotropic and anisotropic components stay constant upon a change in the water content or the temperature of the cubic phase. It is concluded that the structure of the cubic phase consists of two different categories of closed aggregates. From an analysis of the NMR line width in terms of the molecular motion in micellar aggregates it turns out that the experimental findings are compatible with a structure model composed of rodlike micelles arranged as in solid γ-O_2 and β-F_2 at 50 K. In this structure rod-shaped aggregates, having an axial ratio of about 2, occupy the corresponding positions of the O_2 and F_2 molecules in the cubic unit cell. There is one micelle in each corner of the cell, one in the center, and two at each surface of the unit cell.

Introduction

Several lyotropic liquid crystals of cubic symmetry have been encountered both in surfactant–water systems[1,2] and in membrane lipid–water systems.[3-8] In phase diagrams of the micelle-forming amphiphiles (surfactants, soaps, etc.) cubic phases can be found[2] at different concentrations between any of the well-known micellar, normal hexagonal, lamellar, reversed hexagonal, and reversed micellar phases. For the swelling membrane lipids which are generally not forming micelles few phase diagrams exist. Cubic phases of such lipids may form at low water content and high temperatures[9] or may be in equilibrium with water.[10] The cubic phases exhibit different structures and there are two fundamentally different alternatives, namely (a) structures with continuous regions of both water and hydrocarbon chains and (b) structures composed of discrete aggregates of "oil in water" or "water in oil" type. In the pioneering X-ray work of Luzzati and co-workers some of these structures were determined.[9,11-13] In particular it was found that some cubic phases located between the hexagonal and the lamellar phases belonged to the space group *Ia3d* having a structure consisting of rod-shaped aggregates of finite length connected three by three into two three-dimensional networks, mutually interwoven and unconnected. Recently, another cubic structure occurring in the monooleoyl glyceride–water system was put forward, consisting of lipid bilayers arranged in open tetrakaidecahedra.[10] This structure determination was based on a combination of X-ray and NMR results.

It is now well established that the NMR diffusion technique provides a possibility to distinguish between cubic phases with continuous lipid and water regions and those with discrete aggregates.[3-6,8,10,14-17] Especially it has been shown[15,17] that the cubic phase located between the micellar solution and the normal hexagonal phase, in the dodecyltrimethylammonium chloride–water system,[18] is built up of closed micellar aggregates. By X-ray investigations[13,18] the structure was shown to belong to the space group *P43n* or *Pm3n*, which was also found[13] for the cubic phases at the same location in the egg yolk lysophosphatidylcholine–water system and in the sodium octanoate-*p*-xylene-water system. A structure for these phases was proposed by Tardieu and Luzzati[13] based upon a three-dimensional network of rods enclosing spherical micelles. However, it turned out to be at variance with NMR diffusion studies[15,16] which showed that no continuous network could be present. In this work we present studies of the NMR line shape of a cubic phase of 1-palmitoyllysophosphatidylcholine (PaLPC) and water located between the micellar solution and the normal hexagonal phase.[19] In contrast to previous NMR investigations of other cubic liquid crystals, no isotropic Lorentzian NMR line is obtained. The spectral line shape is shown to be in agreement with a newly proposed structure of cubic phases at this location in the phase diagram.[20]

Material and Methods

Deuterium was introduced in the α-methylene group of palmitic acid by base-catalyzed exchange.[21] Thus, sodium palmitate was mixed with ^2H_2O containing NaOH and the mixture kept at 200 °C for several days. The degree of deuteration of the [2,2-$^2H_2$]palmitic acid isolated from the reaction mixture was 85% as

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determined by NMR. This acid was used for the preparation of 1,2-[2,2-$^2H_2$]dipalmitoyl-*sn*-glycero-3-phosphocholine as described by Gupta et al.[22] Deacylation with phospholipase A_2 according to Chakrabarti and Khorana[23] then yielded 1-[2,2-$^2H_2$]palmitoyl-*sn*-glycero-3-phosphocholine. The lysophosphatidylcholine was purified by column chromatography on Malinckrodt Silicic Acid CC-7 on which no acyl migration is reported to occur.[24] Thin-layer chromatography showed that the contamination of the lysophosphatidylcholine with other lipids was <1%. The samples were prepared by mixing appropriate amounts of vacuum-dried lipid and doubly distilled water in glass tubes. The tubes were flame sealed and mixing was accomplished by centrifuging back and forth repeatedly. The samples were allowed to equilibrate at 25 °C for at least a day before measurements were performed. At measurements the sample tubes were placed inside the NMR tubes.

The ^2H and ^{14}N NMR spectra at 38.40 and 18.06 MHz, respectively, were obtained with a Bruker WM 250 FT spectrometer equipped with a 5.9-T wide-bore superconducting magnet, using tunable wide-band probes with sample diameters of 10 and 15 mm, respectively. The quadrupole echo technique[25] was applied with full phase cycling of the pulses and quadrature detection was used. 90° radio frequency pulses of 25 (^2H) and 122 μs (^{14}N) length with an interpulse spacing of 50 (^2H) and 250 μs (^{14}N) were used. The long interpulse spacing time for ^{14}N was necessary in order to eliminated effects from probe ringing. The time between pulse sequences was 0.3 (^2H) and 0.2 s (^{14}N). The width of the spectrum 40 (^2H) and 20 kHz (^{14}N) and typically 3000 (^2H) and 30000 (^{14}N) transients were recorded. The temperature was controlled by a heated air stream around the NMR tube and measured with a thermocouple placed beneath the tube.

The ^{31}P NMR spectra at 148.5 MHz were obtained with a Nicolet NM-360 FT NMR spectrometer equipped with a 8.6-T wide-bore superconducting magnet in the Department of Physical Chemistry 2 at the University of Lund. High-power broad-band proton decoupling was applied and 300–400 transients were recorded with 0.5 s between pulses. 90° pulses of 20 μs length were used and the spectrum width was 20 kHz.

Results and Discussion

Usually the NMR spectra of optically isotropic cubic liquid crystalline phases consist of isotropic Lorentzian lines[26] indicative of fast isotropic molecular reorientations which averages out all static interactions in the system. In order for motional averaging to occur the *isotropic* motion, characterized by a correlation time τ_c, must be fast relative to any static interaction left unaveraged by fast *anisotropic* motions in the system, i.e., $\tau_c\omega_1 << 1$, where ω_1 is the static interaction expressed in angular frequency units.[27] In the limit of very slow motions, i.e., when $\tau_c\omega_1 >> 1$, the spectrum consists of a superposition of spectra from all possible orientations. In the intermediate region, when $\tau_c\omega_1 \sim 1$, the line shape is complex and very sensitive to the rate and type of motion.[28]

The structure model for cubic phases belonging to space group *Ia3d*, with rodlike aggregates or with lamellar units,[10] are consistent with the observed isotropic NMR lines. In these structures the molecular reorientation is fast due to fast lipid lateral diffusion through all positions in the cubic unit cell.

The cubic phase of PaLPC and water is located in the phase diagram between the isotropic micellar solution and the normal hexagonal phase[19] (see Figure 1). Figure 2 shows NMR spectra of liquid crystalline samples of [2,2-$^2H_2$]PaLPC and water. The

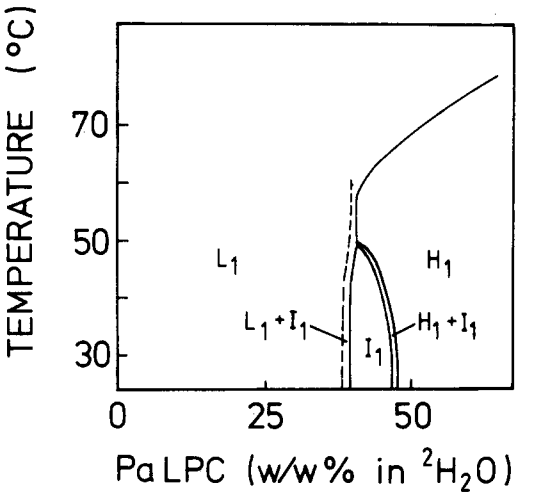

Figure 1. Part of the phase diagram of PaLPC and ^2H_2O showing the location of the cubic phase (I_1) between the micellar solution (L_1) and the hexagonal phase (H_1). Redrawn from ref 19.

spectral line shapes of the cubic phase (A, C, and E) show marked deviations from an isotropic Lorentzian form indicating a not fully averaged anisotropic interaction. The spectra appear to be a superposition of two signals, one narrow (isotropic) and one broad (partially averaged anisotropic) signal. Variation of the water content or the temperature within the cubic phase area has no significant effect on the line shape or the relative intensity of the narrow and broad components. The cubic phase samples were optically isotropic when viewed between crossed polarizers. On raising the temperature (D and F) or lowering the water content (B) the samples become a mixture of cubic and hexagonal phases (cf. Figure 1). This is clearly seen in the NMR spectra where the spectrum from the cubic phase is superimposed on a quadrupole splitting or on a chemical shielding anisotropy with a high-field shoulder and a low-field peak typical of a hexagonal phase.[29] The ^2H and ^{14}N line shapes were insensitive to a change of the pulse separation in the quadrupole echo sequence and a single pulse with a short dead time (for ^{14}N recorded at 25.9 MHz) gave essentially the same spectrum as the quadrupole echo sequence.

Structure of the Cubic Phase. As the line shape of the spectra obtained from the cubic phase appears to consist of two components it is reasonable to assume that the phase structure is composed of *two categories of aggregates*, in one of which the static interactions are fully averaged by fast isotropic motions, and in one of which any isotropic motion is too slow to fulfill $\tau_c\omega_1 << 1$. Furthermore, the structure model must be compatible with the observed phosphorus NMR spectra of the cubic phase (E) showing residues of a low-field shoulder as in a lamellar phase.[29] From the location of the cubic phase (see Figure 1) it seems, however, less probable that this phase could be composed of disklike or lamellar aggregate units.

Previously a structure model composed of spherical micelles entrapped within a cage of rodlike aggregates has been proposed by Tardieu and Luzzati for the cubic phase of lyso egg yolk phosphatidylcholine and water.[13] Since egg yolk LPC mainly consists of PaLPC this cubic phase probably has a structure identical with the one studied in this work. The appearance of two components in the NMR spectra could at first sight perhaps be taken as support for the former structure: the isotropic component arising from the spherical micelles and the anisotropic component from the cage of rods. The reorientation brought about by lipid lateral diffusion along the curved rods should be too slow, so that anisotropic interactions are left unaveraged. The static quadrupole interaction left by fast anisotropic motions (local molecular reorientations and diffusion around the rods) can be taken as $\omega_1 = 2\pi(4/3)\Delta_{hex}$, where Δ_{hex} is the quadrupole splitting in the hexagonal phase. A rough estimate of the correlation time,

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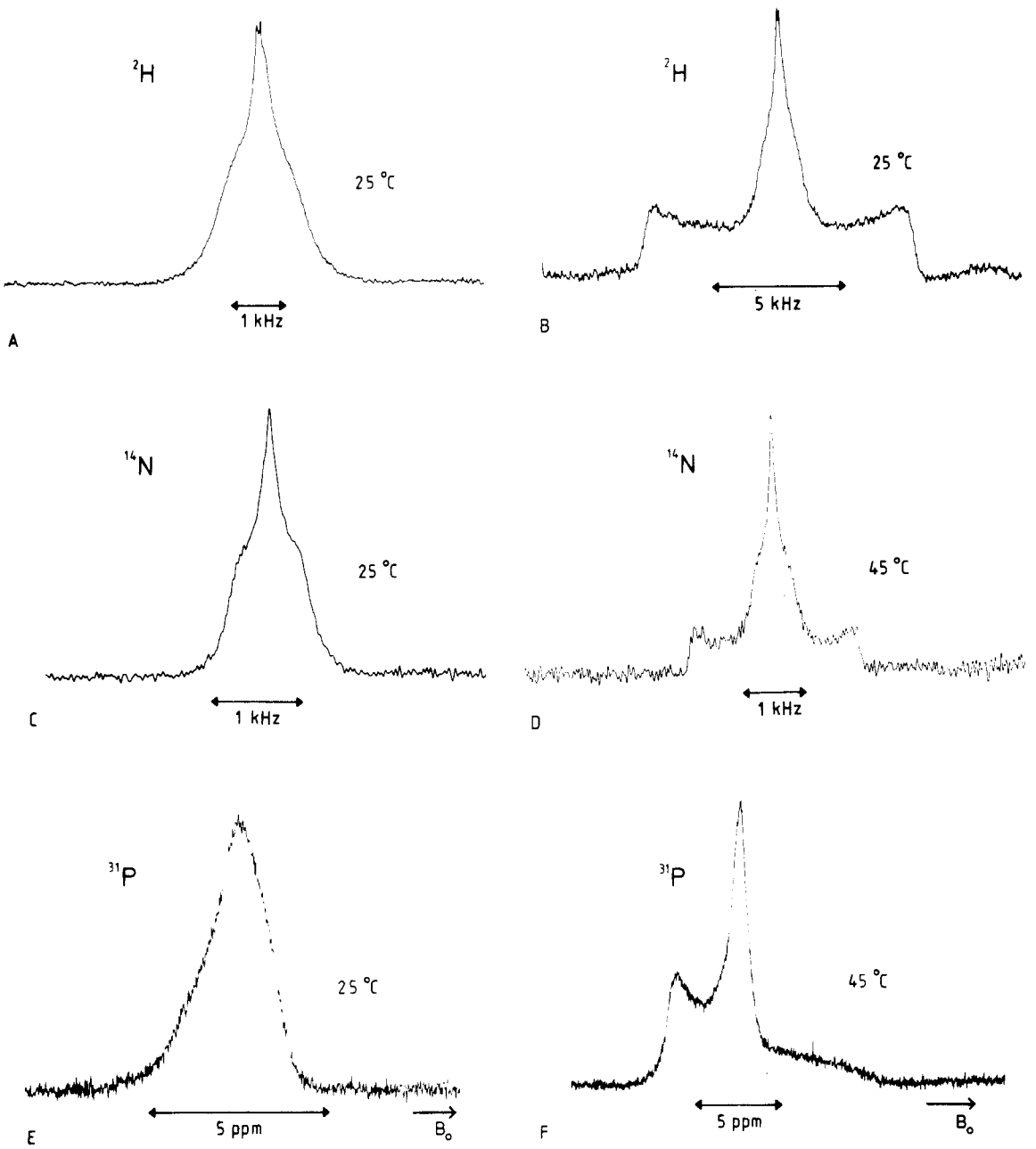

Figure 2. NMR spectra of liquid crystalline samples of [2,2-$^2H_2$]PaLPC and H_2O. (A), (C), and (E): pure cubic phase. (B), (D), and (F): mixtures of cubic and hexagonal phases. Sample compositions: (A), (C), and (E) 46 w/w % PaLPC, (B), (D), and (F) ~50 w/w % PaLPC.

τ_c, for the isotropic motion can be obtained[17] by considering lateral diffusion on a sphere with a diameter equal to the length of the cubic unit cell, a. Then $\tau_c = (a/2)^2/6D_L$ where D_L is the lateral diffusion coefficient of the lipid. In the lamellar phase of 1-oleoyl-*sn*-glycero-3-phosphatidylcholine[19] and water $D_L = 2 \times 10^{-12}$ m^2 s^{-1} at 25 °C[30] which is taken as an estimate of D_L in the cubic phase of egg yolk LPC. With $\Delta = 3$ kHz as for ^{14}N in the hexagonal phase and $a = 15$ nm as determined by X-ray diffraction,[13] $\omega_1\tau_c = 0.12$. Thus an isotropic NMR signal should be obtained which is in contrast to the experimental observation. Moreover, the fraction of lipids contained in the spheres is about 0.05 which is not in agreement with the relative intensity estimated for the isotropic NMR signal. Furthermore, NMR diffusion studies of the cubic phase of PaLPC and water (P.-O. Eriksson, G. Lindblom, and G. Arvidson, to be submitted for publication) showed that this phase is composed of closed amphiphile aggregates. A structure with continuous network of rods is thereby ruled out.

Recently, Fontell et al.[20] suggested a novel structure for cubic phases located between the micellar solution and the normal hexagonal phase. They proposed that these liquid crystalline cubic phases, belonging[13] to the space groups *P43n* or *Pm3n*, have the same structure as solid γ-O_2 and β-F_2 at 50 K[31] and N_2 at room temperature and 49 kbar.[32,33] In this structure rod-shaped aggregates with an axial ratio around 2 occupy the corresponding positions of the N_2, F_2, and O_2 molecules in the cubic unit cell, cf. Figure 3A. There is one rod-like micelle in each corner of the unit cell, one in the center, and two at each surface of the cell.

If we represent a rod-shaped micelle as a cylinder with two half-spheres at the ends and assume a micellar radius of $r = 2.5$ nm and a cubic unit cell dimension $d = 14.1$ nm as determined for PaLPC,[19] and a specific volume of the lipid[34] $v = 0.921$ cm^3

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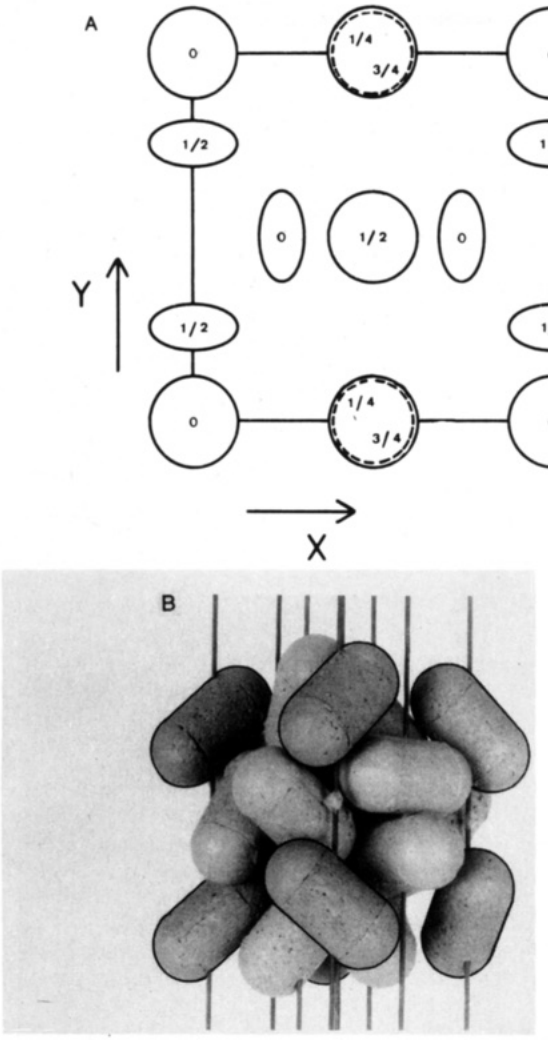

Figure 3. (A) Structure of the cubic liquid crystalline phase of PaLPC and water, based upon the crystal structure of β-F_2 and γ-O_2 at 50 K.[31] Rod-shaped micelles occupy the corresponding positions of the F_2 and O_2 molecules. The micelles at positions 000 and $^1/_2\ ^1/_2\ ^1/_2$ are approximately spherically disordered. The micelles at $^1/_4\ ^1/_2\ 0$, $^3/_4\ ^1/_2\ 0$, $^1/_2\ 0\ ^1/_4$, $^1/_2\ 0\ ^3/_4$, $0\ ^1/_4\ ^1/_2$, and $0\ ^3/_4\ ^1/_2$ are sterically hindered to reorient around one of the minor axis, thereby attaining an oblate spheroidal distribution. (B) A scaled model of one unit cell built of spherocylindrical bodies with an axial ratio of 2. The model illustrates the different motional constraints of the spherically disordered (with contours) and the cylindrically disordered micelles.

g^{-1}, a weight fraction of lipid $w = 0.48$ in ^2H_2O corresponds to a micellar axial ratio of 2.1 and an area per polar head group of 0.72 nm^2. A scaled model of the cubic phase built from spherocylindrical bodies with axial ratio of 2 is shown in Figure 3B. It illustrates that the micelles in the corners and in the center of the unit cell are able, by a concerted motion of adjacent micelles, to take on all orientations in the cell (spherically disordered), while the micelles at the surfaces are considerably more hindered to rotate around one of the short axis than around the other two (cylindrically disordered).

With this structure model the complex NMR line shape can be understood; the narrower, isotropic component arises from the spherically disordered micelles, which can rotate around three axes averaging all static interactions, while the broader, anisotropic component is ascribed to the cylindrically disordered micelles at the surfaces, which are sterically hindered to rotate isotropically. There are two spherically disordered and six cylindrically disordered micelles per unit cell, which is in agreement with a rough estimate of the relative intensities in the ^2H and ^{14}N spectra.

A quantitative treatment of the partial averaging of the static interactions for the cylindrically disordered micelles can be made as follows. For infinitely long, inflexible cylindrical micelles, where the exchange of lipids between adjacent micelles is negligible, the quadrupole splitting and the chemical shift anisotropy will be the same as in the hexagonal phase, Δ_{hex} and $\Delta\sigma_{hex}$, respectively. The phosphorus spectra will show a low-field peak and a high-field shoulder. In a micelle of finite length the lipid lateral diffusion will further average the interaction. Representing the micelle with a cylinder with two half-spheres at each end the averaging process can be viewed as a fast chemical exchange between a sphere and a cylinder. The resulting quadrupole splitting will be $\Delta = p_{cyl}\Delta_{hex}$ where p_{cyl} is the relative amount of lipids in the cylindrical part of the micelle. The same holds true for the ^{31}P chemical shift anisotropy. For a micelle with an axial ratio equal to two, $p_{cyl} = 0.6$. The *spherically disordered* micelles rotate freely around all three axes and the residual interaction is averaged out completely and an isotropic NMR peak appears. The *cylindrically disordered* micelles at the surfaces of the unit cell rotate freely around only *one* of its short axes. The interaction is then lowered[29] by a factor of $^1/_2[3\cos^2(\pi/2) - 1] = -^1/_2$ giving $\Delta = -^3/_{10}\Delta_{hex}$ and $\Delta\sigma = -^3/_{10}\Delta\sigma_{hex}$. Note that the phosphorus chemical shift anisotropy changes sign and a powder pattern with a low-field shoulder and a high-field peak analogous to the lamellar phase is expected to show up in the NMR spectrum. This is also observed in the ^{31}P NMR spectrum (see Figure 2E). With $\Delta(^2H) = 10$ kHz, $\Delta(^{14}N) = 4$ kHz, and $\Delta\sigma(^{31}P) = 12$ ppm in the hexagonal phase the fast averaging motions just described yield $\Delta(^2H) = -3$ kHz, $\Delta(^{14}N) = -1.2$ kHz, and $\Delta\sigma(^{31}P) = -3.6$ ppm which are compatible with the width of the broad component in the spectra from the cubic phase. As a result of further averaging motions with a time scale comparable to the interactions left by the fast motions, i.e., 10^{-5}–10^{-4} s, the powder pattern of the anisotropic component of the spectra is destroyed and a broad structureless band shape is obtained (cf. ref 25). There are several possible candidates for this slow motion: exchange of lipid molecules between adjacent micelles, rotation of the micelle around the third sterically hindered axis, and collective translation of whole micelles between different positions in the lattice. It should be noticed that a free rotation of the micelles at the surfaces of the unit cell around all three axes would in principle not lead to an isotropic NMR signal, since the micellar environment is anisotropic.

Concluding Remarks

The following observations support a structure model of the cubic phase studied consisting of rod -shaped micelles occupying two nonequivalent sites as illustrated in Figure 3: (1) the occurrence of two components, one isotropic and one anisotropic, in the NMR spectra with relative intensities in agreement with the structure model; (2) the relative sign of the residual ^{31}P chemical shielding anisotropy in the cubic phase; (3) the width of the anisotropic spectral components compatible with the motion of the micelles.

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