Further evidence for closed, nonspherical aggregates in the cubic I_1 phase of lysolecithin and water

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ABSTRACT Measurements of time-resolved fluorescence quenching have been performed in the binary lauroyllysophosphatidylcholine (LaLPC)/water system. The aggregation numbers, N, are determined for the micellar solution phase ($N_{micelle} \approx 80$) and the cubic liquid crystalline I_1 phase ($N_{cub} \approx 90$) at 298–303 K. When a quencher is present, the fluorescence decays for the hexagonal phase of the LaLPC/water system and for the bicontinuous cubic phase of monooleoylglycerol/water system are nonexponential, as expected for phase structures having long-range continuous apolar regions. Nuclear magnetic resonance (NMR) measurements of the lipid translational diffusion conclusively show that the cubic I_1 phase consists of closed micelles. NMR spectra of ^{31}P obtained at 202.4 MHz of this cubic phase exhibit a characteristic line shape, which is compatible with a phase structure containing short nonspherical micelles. A comparison between electron spin resonance (ESR) spin-label spectra recorded for a micellar solution and the cubic phases of the LaLPC and monooleoylglycerol systems are also shown to support a structure of closed micelles in the cubic I_1 phase of the lysolecithin system.

INTRODUCTION

Lipids in water form a wide variety of liquid crystalline phases, with the cubic phase structures being the most complex (Mariani et al., 1988; Lindblom and Rilfors, 1989; Larsson, 1990; Fontell, 1990; Seddon, 1990; Tate et al., 1991). In recent years, a relatively large number of investigations of the cubic phases located between the aqueous micellar solution and the "normal" hexagonal liquid crystalline phase have been reported (Fontell et al., 1985; Eriksson et al., 1985; Söderman et al., 1985; Eriksson et al., 1987; Söderman and Henriksson, 1987; Johansson and Söderman, 1987; Mariani et al., 1988; Burns et al., 1990; Berggren et al., 1992). The first observation of a cubic phase with this location was made by Reiss-Husson (1967) for egg lysolecithin in water. Lysolecithins with hydrocarbon chain lengths between 12 and 16 carbons have also been shown to form this type of cubic phase (Arvidson et al., 1985; Eriksson et al., 1987), and x-ray diffraction has shown the space group to be $Pm3n/P43n$ (Balmbra et al., 1969; Tardieu and Luzzati, 1970; Fontell et al., 1985; Burns et al., 1990). However, the structure of these cubic phases has been debated (Fontell et al., 1985; Eriksson et al., 1985; Mariani et al., 1988; Charvolin and Sadoc, 1990).

The main dispute has been whether this cubic phase is bicontinuous or consists of closed micellar aggregates (Eriksson et al., 1985; Mariani et al., 1988). We are faced with a problem that cannot be solved by x-ray diffraction alone, but other methods are required (Lindblom and Rilfors, 1989). Obviously, such methods should, in some way, be sensitive to the restriction of lipid molecules localized in a limited space or aggregate in the phase structure under study. The necessary prerequisites for such investigations are indeed provided by the nuclear magnetic resonance (NMR) diffusion technique (Tanner and Stejskal, 1968), which has been frequently used by us (see, e.g., Lindblom and Rilfors, 1989). The main advantage of this method is that the translational diffusion coefficient of the lipid or water can be measured directly, i.e., no probe molecule has to be involved, and no model-dependent assumptions have to be made. The time, during which the translational motion is observed is for lipid NMR diffusion studies usually in the range of 10–100 ms, which for free diffusion in a bilayer ($D_L = 5 \cdot 10^{-12}$ m^2 s^{-1}, Lindblom et al., 1981) corresponds to a root mean square displacement of 0.5–2 μm. Thus, molecular translational motion over distances much larger than the dimension of a single micelle can be observed.

From NMR measurements of lipid diffusion in various liquid crystalline phases, it is possible to distinguish between cubic phases built up of closed aggregates or continuous hydrocarbon regions (Lindblom and Rilfors, 1989 and references therein). The simple idea behind the method is thus based on the fact that for a cubic phase consisting of micelles (I_1) the lipid molecules will be restricted to diffuse within the micelle, and therefore, the lipid can only move a very short distance on the time scale of the NMR diffusion measurement, whereas a lipid molecule in a bicontinuous phase (I_2), on the other hand, can perform translational diffusion over macroscopical distances. The rate limiting step for the lipid translational motion in the I_1 phase is the jump between adjacent micelles and because this is a relatively slow process, the observed diffusion rate will be significantly smaller for an I_1 phase than for an I_2 phase. The same basic idea can also be utilized to interpret diffusion coefficients determined by a fluorescent probe method such as fluorescence recovery after photobleaching (FRAP) (Lindblom and Rilfors, 1989; see also Cribier et al., 1990) to discriminate between the fundamentally different cubic phase structures. It should, however, be noted

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that a probe molecule may perturb the aggregate size and shape (Orädd et al., 1992), in particular this might occur with the usually large fluorescent molecules used in the FRAP experiment. Being aware of this problem, another probe method which utilizes fluorescence quenching, has great advantages in the applications to cubic phases, because with this method, the number of lipid molecules forming a closed aggregate may be determined as well as getting information about the existence of large continuous hydrophobic regions in the cubic structure. Thus, the time-resolved fluorescence (TRF) method provides a complementary way to distinguish between bicontinuous cubic phases and cubic phases built from closed aggregates. Here we have used the TRF quenching method to obtain further support for the structure of the cubic phase of lysolecithins, previously suggested to be based on slightly distorted globular micelles. ESR spin label experiments of both a bicontinuous cubic phase and of the lysolethin cubic phase are also presented. Finally, a previous (Eriksson et al., 1985) ^{31}P NMR study has been undertaken here at a higher magnetic field strength to further enhance the anisotropic characteristic of the line shape obtained for the cubic I_1 phase of lysolecithin and some further NMR diffusion data of a bicontinuous cubic phase and the I_1 phase are also presented.

MATERIAL AND METHODS

Sample preparations

Samples of the micellar solution phase (34.91 wt %), the cubic (41.85 wt %) and the hexagonal (59.95 wt %) phases were prepared from lauroyllysophosphatidylcholine (LaLPC) and water mixed in appropriate amounts. The lipid concentrations are given in the parentheses; for a phase diagram see Arvidson et al., 1985 and Eriksson et al., 1987. All samples used in the fluorescence quenching experiments were prepared with pyrene (Py) at a molar ratio of LaLPC/Py equal to about 10^5. The Py was purchased from Aldrich Chemical Co., (UK) and was recrystallized three times from ethanol. Another sample of each phase was prepared with benzophenone (BP), which was also obtained from Aldrich. The molar ratio of LaLPC/BP was about 100. For comparison, a sample containing a cubic phase of monooleoylglycerol (70 wt %) and water (30 wt %), known to have a bicontinuous structure, was prepared in a similar way as the samples with LaLPC. Monooleoylglycerol was purchased from Sigma Chemical Co. (St. Louis, MO).

The spin label, 5-doxylstearic acid was purchased from Molecular Probes, Inc. (Eugene, OR) and was used without further purification. The label/lipid molar ratio was 1/500 in all experiments.

Methods

A PRA 3000 system (Photophysical Research Associates Inc. (PRA), Canada) was used for the single-photon-counting measurements of the fluorescence decay. The excitation source was a thyratron-gated flash lamp (model 510C, PRA) filled with deuterium gas and operated at about 26 kHz. The excitation and emission wavelengths were selected by interference filters (Omega/Saven AB, Sweden) centered at 332.8 nm (HBW = 13.0 nm) and 398.8 nm (HBW = 11.4 nm). The deconvolution software (DECAY V 3.0a) was developed by PRA. The samples were not degassed but sealed in quartz capillaries or between quartz slides and thermostated to the measuring temperature to within ±1 K.

The ESR spin label experiments were performed on a Varian E-109 X-band (9 GHz) spectrometer with an E-238 (TM_{110} mode) cavity and a model V-6040 variable temperature controller. The spectrometer was interfaced to a Zenith-111-32-PC as outlined by Lipscomb and Salo (1983).

A 202.4 MHz ^{31}P NMR spectrum of the cubic phase of PaLPC and water was acquired with a Bruker AM 500 spectrometer, using a broadband 10 mm high resolution probe. High power (~5 W) proton decoupling was applied during acquisition, and the spectrum was recorded with a single pulse of length 10 μs, (30 degree flip angle). The spectral width was 100 kHz and the receiver dead-time was 8 μs. The data points in the free induction decay (FID) were sampled sequentially in the two quadrature channels, and to minimize distortions due to filter effects, the pulse phase was adjusted to give a pure dispersive signal in the real channel. The relaxation time between successive scans was 2 s. The FID was transferred to a Silicon Graphics workstation and was processed with the FTNMR software (Hare Research, Woodinwille, WA). The lipid diffusion coefficient in cubic phases of LaLPC and oleoyllysophosphatidylcholine (OlLPC) was measured with the pulsed field gradient spin echo technique (Stejskal and Tanner, 1965), using a Bruker MSL 100 spectrometer, as described previously (Eriksson et al., 1987).

RESULTS AND DISCUSSION

The fluorescence decay of fluorescent probes situated in aggregates of amphiphiles containing quenchers is affected in two ways. First, there is a proximity effect due to the presence of a quencher in the aggregate which rapidly quenches the fluorescence. Second, there is a shielding effect that hinders quenching of the excited probes residing in aggregates without quenchers. The latter effect depends on the statistical distribution of quenchers among the aggregates and is of importance when the average number of quenchers per aggregate $\langle x \rangle \leq 1$.

The possibility to distinguish between aggregates with and without quenchers forms the basis of the fluorescence quenching method used here for the determination of aggregation numbers (N) (Turro and Yekta, 1978; Atik and Singer, 1987; Infelta, 1979; Grieser, 1981; Lianos and Zana, 1980; Almgren and Löfroth, 1981).

Models for analyzing fluorescence quenching data from micellar systems have been developed (Infelta et al., 1974; Infelta, 1979; Atik et al., 1979; Tachiya, 1975; Van der Auweraer et al., 1981; Almgren and Löfroth, 1982; Almgren et al., 1988). These models are based on the assumptions that the micelles are monodisperse and, that the distribution of the probes, *Py*, and quencher molecules, BP, over the micelles is Poissonian. For stationary quenchers it has been shown that the fluorescence decay is then given by (Infelta, 1979; Atik et al., 1979)

$$F(t) = F(0) \exp[-t/\tau_f - \langle x \rangle \{1 - \exp(-k_q t)\}], \qquad (1)$$

where k_q is the rate constant of quenching, τ_f is the fluorescence lifetime of *Py* in the absence of quenchers and $\langle x \rangle$ is the mean number of BP molecules per micelle. Eq. 1 implies that the tail of the fluorescence decay is expo-

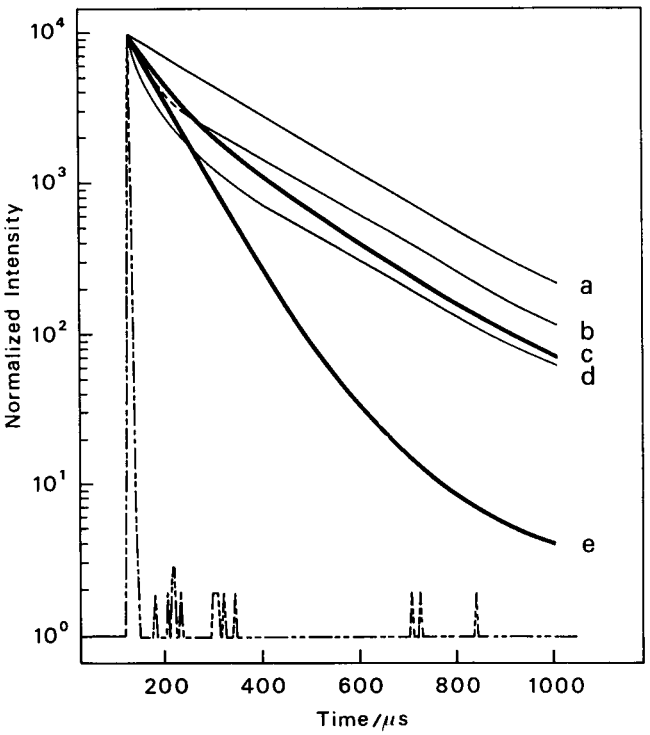

FIGURE 1 Time-resolved fluorescence decay curves (from single photon counting experiments) of pyrene solubilized in: (*a*) the micellar solution phase of lauoryllysophosphatidyl-choline (LaLPC) in the absence of benzophenone (BP); (*b*) in the presence of BP in the micellar solution (34.9 wt % LaLPC); (*c*) a hexagonal H_1 phase of LaLPC (59.95 wt %) and water containing BP; (*d*) the cubic phase (I_1) of LaLPC and water in the presence of BP (41.85 wt % LaLPC), and (*e*) Py and BP solubilized in a cubic phase of monoolein (70 wt %) and water. The dotted curve is the excitation pulse of the system. The reconvoluted functions are shown (that is, a sum of exponentials convoluted with the excitation response function) which have typical χ^2 values of 1.0–1.2.

nential with the rate of the unquenched decay, i.e., $1/\tau_f$. With the total concentrations of BP and LaLPC, and with $\langle x \rangle$ determined from Eq. 1, the aggregation number, N, can be calculated from

$$N = \langle x \rangle([\text{LaLPC}] - \text{c.m.c.})/[\text{BP}], \qquad (2)$$

where c.m.c. is the critical micelle concentration of LaLPC, i.e., $2 \cdot 10^{-4}$ M (Weltzien et al., 1977).

If the quenchers migrate, the tail of the fluorescence decay is still exponential but at a rate faster than $1/\tau_f$, Eq. 1 is no longer valid, but the appropriate equations for this case have been derived (Malliaris, 1988). We find that the tails of the fluorescence decays in the presence and in the absence of BP are parallel in micellar and cubic phases of LaLPC (Fig. 1). Then, Eq. 1 is applicable and a graphical evaluation of the data according to this equation was performed. Thus, we obtain that aggregation numbers for the micellar aqueous solution and the cubic I_1 phases are $N_{\text{micelle}} = 77 \pm 15$ and $N_{\text{cubic}} = 89 \pm 20$ at 298–303 K.

On the other hand, the fluorescence decay in the hexagonal phase with BP is clearly nonexponential, in contrast to the behavior of the isotropic phases (Fig. 1). This finding also pertains to the bicontinuous phase with MO discussed below. A biexponential fit of the data obtained for the hexagonal phase with BP does no longer yield the lifetime of *Py* (~200 ns), which is otherwise obtained in the absence of BP in this phase (Fig. 1). This is an expected result, because the aggregates in the hexagonal phase are continuous and BP and *Py* can move a long distance along the aggregate by translational diffusion. Fig. 1 also shows the quenched decay of *Py* solubilized in a bicontinuous cubic liquid crystalline phase composed of MO and water. The fluorescence decay of *Py* in the absence of quencher is exponential with a lifetime of 190 ns. Clearly, the fluorescence quenching is more efficient in the cubic phase of the MO system than in the hexagonal phase of the LaLPC system. Such a rapid quenching is expected for a bicontinuous cubic phase, because the translational mobility of both *Py* and the quencher molecules occurs in three dimensions in the bicontinuous phase compared to only one dimension in the hexagonal phase.

The N values determined for the cubic and micellar phases of the LaLPC system are only slightly larger than those obtained for the corresponding phases of C_{12}TAC (Johansson and Söderman, 1987). Assuming that the aggregates are spherical, the radius can be estimated from the aggregation number and the van der Waals volume of the lipid, i.e., using group contributions to the volume of the C_{12} chain, viz. $V_{CH3} = 49$ Å^3 and $V_{CH2} = 28$ Å^3 (Jönsson, 1981; Gallot and Skoulios, 1966). The radii thus calculated for the globular aggregates in the micellar solution and the cubic phase of the LaLPC/water system are obtained to be 25.1 Å (35 wt-% LaLPC) and 26.3 Å (42 wt-% LaLPC). This is in agreement with the micellar radius, 27.0 Å, determined by NMR diffusion (Eriksson et al., 1987).

When the observed aggregation number of a micelle becomes larger than a value possible for a spherical aggregate, the actual micelles, of course, have to be nonspherical and limited in at least one dimension by the length of the hydrocarbon chain. Thus, cylindrical and disclike structures are the only conceivable ones. For LaLPC with increasing concentration, the I_1 phase is followed by an H_1 phase, indicating a tendency to form a rodlike micellar structure in the I_1 phase. The aggregation number for the micellar solution is comparable with the value obtained for the I_1 phase, strongly indicating that the cubic structure consists of short rodlike micelles (Eriksson et al., 1985; Johansson and Söderman, 1987).

The NMR diffusion data (Eriksson et al., 1987), conclusively show that the cubic I_1 phase with 60 wt % heavy water in the LaLPC system is built from closed micelles. Fig. 2 *B* shows the decay of the spin-echo in the NMR diffusion experiments for this I_1 phase (*circles*) and for

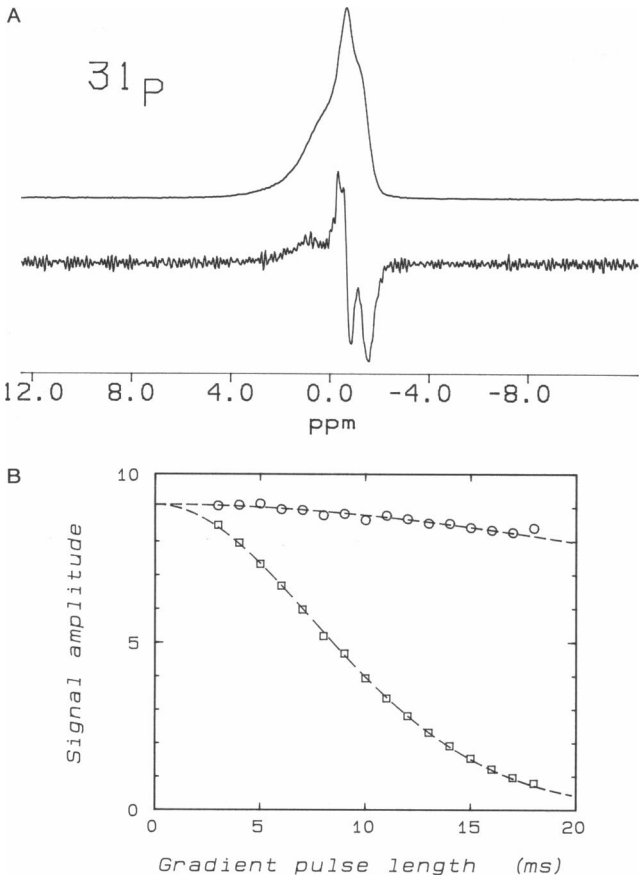

FIGURE 2 (*A*) 202.4 MHz ^{31}P spectrum (above) of the cubic phase of PaLPC and water at 31°C. In the derivative spectrum (below) the two components of the lineshape are clearly distinguished. Sample composition: 56.4 wt % $^{1}H_2O$ (deuterium depleted) 43.6 wt % palmitoyllysophosphatidylcholine (PaLLPC). (*B*) The decay of the ^{1}H spin echo amplitude with gradient pulse length (δ), in an NMR diffusion experiment on cubic phases of lauoryllysophosphatidylcholine (LaLPC) (*circles*) and oleoyllysophosphatidylcholine (OlLPC) (*squares*). Sample compositions: 40 wt % LaLPC, 60 wt % $^{2}H_2O$ and 80 wt % OlLPC, 20 wt % $^{2}H_2O$, respectively. Experimental parameters: spin echo time (t) 40 ms, time between gradient pulses (Δ) 50 ms, gradient pulse strength (g) 1.19 T/m, and the number of scans is 8. The diffusion coefficient, D, calculated from a fit of the Stejskal-Tanner equation, $E = E_0 \times \exp\{-(\gamma\delta g)^2(\Delta - \delta/3)D\}$ to the echo decay (the hatched lines in the figure), is $1.6 \cdot 10^{-12}$ m^2 s^{-1} for OlLPC and $6 \cdot 10^{-14}$ m^2 s^{-1} for LaLPC. The cubic phase of OlLPC is bicontinuous and the cubic phase of LaLPC is built from closed aggregates.

the bicontinuous cubic phase (*squares*) of oleoyllysophosphatidylcholine (OlLPC) with 20 wt % heavy water. The slow translational diffusion of LaLPC between the micelles in the cubic phase structure results in a very small measured diffusion coefficient ($D = 6 \cdot 10^{-14}$ m^2 s^{-1}), whereas for the bicontinuous cubic phase of OlLPC, the translational motion along the continuous hydrocarbon aggregates results in a measured diffusion coefficient ($D = 1.6 \cdot 10^{-12}$ m^2 s^{-1}) of the same order of magnitude as in the lamellar phase ($D = 6 \cdot 10^{-12}$ m^{2}s^{-1}) (Eriksson et al., 1987).

Besides the NMR diffusion results, the extraordinary line shapes of ^{2}H, ^{14}N and ^{31}P NMR spectra obtained for the I_1 phase of PaLPC (Eriksson et al., 1985) are very important, giving further information on the structure of this cubic phase. Due to complete motional averaging of all anisotropic interactions, the NMR spectra of a cubic phase usually consist of narrow symmetric lines (see Lindblom and Rilfors, 1989). However, for the I_1 phase of PaLPC the NMR lineshapes of these nuclei consist of a superposition of a narrow line due to isotropic motion and a line typical for partially averaged anisotropic interactions (Eriksson et al., 1985). This feature is accentuated even more in the ^{31}P spectrum at higher magnetic field (11.75 T; 202.4 MHz), obtained in this study (Fig. 2 *A*). In particular, it is evident from the derivative spectrum in Fig. 2 *A*. Thus, it was concluded that this cubic phase structure must consist of two categories of closed aggregates, one for which motional averaging on the NMR time scale (ms) of the anisotropic interactions is complete and one for which a residual anisotropy remains.

To reconcile the NMR data with x-ray data from the I_1 phases, a structural model was proposed (Fontell et al., 1985). In this model, which contains eight rodlike micelles per unit cell, two of the micelles are occupying the corners and the center of the unit cell, while the remaining six micelles are located in pairs at the faces of the unit cell. Inherent in this model is the assumption that the micelles in the corners and in the center experience an isotropic environment resulting in an isotropic reorientation and a complete motional averaging, while the micelles on the faces experience an anisotropic environment leading to an anisotropic reorientation and an incomplete motional averaging of the static spin interactions. This model is based on the assumption that the micelles have a nonspherical shape, which is confirmed by the TRF quenching measurements in this study.

Of crucial importance is the sign of the chemical shift anisotropy in the ^{31}P NMR spectrum of the I_1 phase, which is opposite to the sign of the chemical shift anisotropy obtained for the hexagonal phase (Fig. 2 *A* and Eriksson et al., 1985). This indicates that the nonspherical micelles at the surface of the unit cell are performing a rotational diffusion, which is restricted to occur in a plane. As previously discussed (Eriksson et al., 1985) the structural model for the I_1 phase (Fontell et al., 1985) is able to account for this. Note that in the hexagonal phase the anisotropic interactions are motionally averaged by local reorientational motions and by translational diffusion around the cylindrical (infinite) aggregates. The translational diffusion gives rise to the well known change in the sign of the ^{31}P shift anisotropy upon a transition from a lamellar to a hexagonal phase. Thus, for a (hypothetical) sample of immobilized rodlike micelles in a cubic structure, the chemical shift anisotropy

would have the same sign as the hexagonal phase, but with a decreased magnitude due to the chemical exchange with the lipids on the ends of the aggregates. The structural model for the I_1 phase suggests that the pairs of rodlike micelles at the faces of the unit cell are free to rotate about one of the short axes. This leads to a motional averaging about an axis, which is perpendicular to the long axis of the micelle about which translational motion occurs. The rotational motion about one of the short axes of the aggregates therefore gives a change in the sign of the anisotropic interaction relative to the hexagonal phase as observed for ^{31}P NMR of the I_1 phase.

Recently, a quantitative lineshape analysis of the ^2H, ^{14}N and ^{31}P NMR spectra of lysolecithin in the cubic phase was performed (Berggren et al., submitted for publication). The line shape calculation, using a stochastic Liouville approach, was based on the dynamical model suggested previously (Eriksson et al., 1985). The slowest dynamics of the micelles was found to be in the slow-motion regime, and was described by a Brownian rotational diffusion model. The line shape calculations confirm that the cubic I_1 phase consists of two dynamically different types of aggregates with relative weight 2:6.

Recently, Charvolin and Sadoc (1990) suggested that all the lyotropic structures can be described in the same geometrical terms, as periodically ordered systems of fluid films separated by interfaces. The structures of these films must reconcile constant interfacial distances and curvatures. When the conditions are such that the interfaces become curved, a typical geometrical frustration arises, a problem which is solved by introducing structure of disclinations. In their model for the cubic phase in question an assembly of two dodecahedra and six tetrakaidecahedra is thus obtained in which the micelles reside. There is a striking similarity between the structure model we use and the one proposed by Charvolin and Sadoc (1990). Furthermore, to be consistent with the ^{31}P NMR line shape the micelles in their model also have to either perform rotational motions as described above or the aggregates have to have different geometrical shapes, i.e. spherical and nonspherical micelles have to coexist in the cubic phase.

Finally, the ESR spin label experiments (Fig. 3) show spectra that for the naked eye are almost identical for the micellar solution and the cubic phase of the LaLPC/water system, again indicating that the micelles in the two phases are similar. In a previous study (Wikander et al., 1990) a simple two-step model was used to describe the dynamics in the micelles. This model appears to be insufficient for larger aggregates like those formed by LaLPC and PaLPC. It has been found that the rapid local anisotropic rotation has to be explicitly introduced in the dynamical model to accurately describe the molecular motion of a spin label within the lipid micelles. We are currently working on such models (Liang et al., unpublished results) and it can be expected that a simulation of

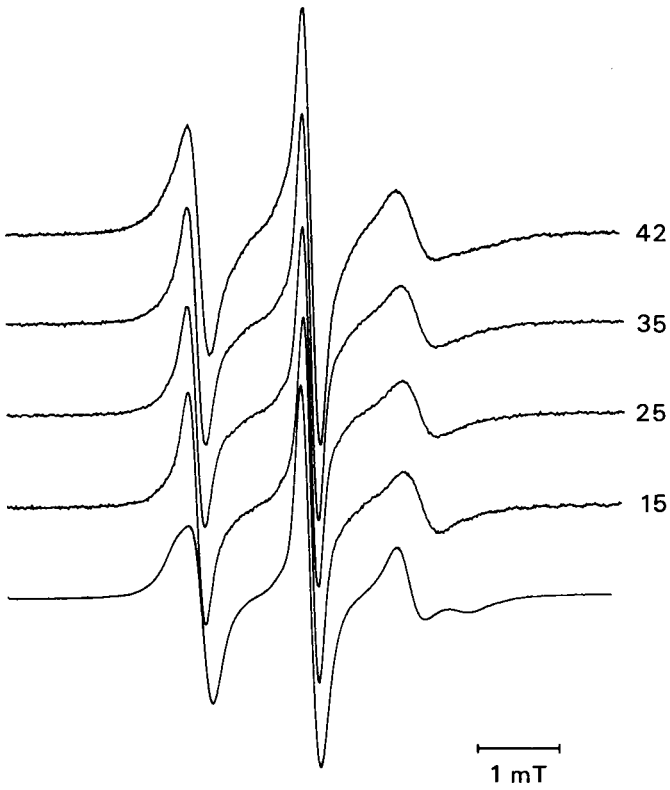

FIGURE 3 ESR spectra of 5-doxylstearic acid at 45°C solubilized in the micellar solution and the cubic liquid crystalline phase of the lauroyllysophosphatidylcholine (LaLPC)/water system and with the spin label solubilized in the bicontinuous cubic phase of monooleoylglycerol (70 wt %) and water (*bottom spectrum*). The compositions (in w/w % LaLPC) are given next to each spectrum. The total scan range is 8 mT.

ESR spectra using such a model will give information of not only the dynamics of the spin label but also about the shape of the micelles. However, further support for that closed aggregates build up the cubic phase of LaLPC is provided also by ESR from a comparison of the spectrum from a bicontinuous cubic phase structure with that from an cubic I_1 phase. Therefore, an ESR spectrum of 5-doxylstearic acid solubilized in the bicontinuous cubic phase of monooleoylglycerol and water is included in Fig. 3. It is apparent that the lineshape of the spinlabel in this phase is in the "slow motion" region (cf. the extra minimum at high field and the broadening of the low field peak). Such a lineshape is due to an incomplete motional averaging of the static magnetic interactions of the spinlabel. Furthermore, it should be noted that the lipid diffusion in the cubic phase of monooleoylglycerol is approximately 200 times larger than the lipid diffusion in the LaLPC (Lindblom et al., 1979; Eriksson et al., 1985). Hence, such a small measured diffusion coefficient of LaLPC in a cubic phase is not compatible with a bicontinuous structure of this phase (see e.g., Lindblom and Rilfors, 1989), because the observed ESR lineshape shows very little anisotropy in the LaLPC cubic phase.

In summary, this work give further evidence for a structure of the cubic I_1 phase in the lysolecithin/water system to consist of nonspherical micellar aggregates, probably having a rodlike shape.

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REFERENCES

Almgren, M., and J.-E. Löfroth. 1981. Determination of micelle aggregation numbers and micelle fluidities from time-resolved fluorescence quenching studies. *J. Colloid Interface Sci.* 81:486–499.

Almgren, M., and J.-E. Löfroth. 1982. Effects of polydispersity on fluorescence quenching in micelles. *J. Chem. Phys.* 76:2734–2743.

Almgren, M., J. Alsins, E. Mukhtar, and J. van Stam. 1988. Fluorescence quenching dynamics in rodlike micelles. *J. Phys. Chem.* 92:4479–4483.

Arvidson, G., I. Brentel, A. Khan, G. Lindblom, and K. Fontell. 1985. Phase equilibria in four lysophosphatidyl-choline/water systems. Exceptional behaviour of 1-palmitoyl glycerophosphocholine. *Eur. J. Biochem.* 152:753–759.

Atik, S. S., and L. A. Singer. 1978. Nitroxyl radical quenching of the pyrene fluorescence micellar environments. Development of a kinetic model for steady-state and transient experiments. *Chem. Phys. Lett.* 55:519–524.

Atik, S. S., and L. A. Singer. 1979. Spectroscopic studies on small aggregates of amphiphilic molecules in aqueous solution. *J. Am. Chem. Soc.* 101:6759–6761.

Balmbra, R. R., J. S. Clunie, and J. F. Goodman. 1969. Cubic mesomorphic phases. *Nature (Lond.).* 222:1159.

Burns, J. L., Y. Cohen, and Y. Talmon. 1990. Structure of cubic mesomorphic phases determined by low-temperature transmission electron microscopy and small-angle x-ray scattering. *J. Phys. Chem.* 94:5308–5312.

Charvolin, J., and J. F. Sadoc. 1990. Cubic phases as structure of disclinations. *Colloid Polym. Sci.* 268:190–195.

Cribier, S., L. Bourdieu, R. Vargas, A. Gulik, and V. Luzzati. 1990. Modulated fringe pattern photobleaching applied to lipid-water cubic phases: structural information. *J. Physique (Colloq.).* C7-51:105–108.

Ekwall, P. 1975. Composition, properties and structures of liquidcrystalline phases in systems of amphiphilic compounds. *Adv. Liq. Cryst.* 1:1–142.

Eriksson, P.-O., G. Lindblom, and G. Arvidson. 1985. NMR studies of 1-palmitoyllysophosphatidylcholine in a cubic liquid crystal with a novel structure. *J. Phys. Chem.* 89:1050–1053.

Eriksson, P.-O., G. Lindblom, and G. Arvidson. 1987. NMR-studies of micellar aggregates in 1-acyl-sn-glycero-phosphocholine systems. The formation of a cubic liquid crystalline phase. *J. Phys. Chem.* 91:846–853.

Fontell, K. 1990. Cubic phases in surfactant and surfactant-like lipid systems. *Colloid Polym. Sci.* 268:264–285.

Fontell, K., K. Fox, E. Hansson. 1985. On the structure of the cubic phase I_1 in some lipid-water systems. *Mol. Cryst. Liquid Cryst.* 1:9–17.

Gallot, B., and A. Skoulios. 1966. Electrical interactions in the mesomorphic phases of the amphiphile-water system: Role of water content, of the paraffin chain length, of the nature of the cation and of the temperature. *Kolloid Z. Z. Polym.* 208:37–43.

Grieser, F. 1981. Nitrite quenching of terbium luminiscence in sodium dodecylsulphate solutions. *J. Phys. Chem.* 85:928–932.

Infelta, P. P. 1979. Fluorescence quenching in micellar solutions and its applications to the determination of aggregation numbers. *Chem. Phys. Lett.* 61:88–91.

Infelta, P. P., M. Grätzel, and J. K. Thomas. 1974. Luminiscence decay of hydrophobic molecules solubilized in aqueous micellar systems. A kinetic model. *J. Phys. Chem.* 78:190–195.

Johansson, L. B.-Å, and O. Söderman. 1987. The cubic phase (I_1) in the dodecyltrimethylammonium chloride/water system. A fluorescence quenching study. *J. Phys. Chem.* 91:7575–7578.

Jönsson, B. 1981. The thermodynamics of ionic amphiphile-water systems. A theoretical analysis. Ph.D. thesis, University of Lund, Sweden.

Larsson, K. 1989. Cubic lipid-water phases-structures and biomembrane aspects. *J. Phys. Chem.* 93:7304–7314.

Lianos, P., and R. Zana. 1980. Use of pyrene excimer formation to study the effect of NaCl on the structure of sodium dodecyl sulfate micelles. *J. Phys. Chem.* 84:3339–3341.

Lindblom, G., and L. Rilfors. 1989. Cubic phases and isotropic structures formed by membrane lipids. Possible biological relevance. *Biochim. Biophys. Acta.* 988:221–256.

Lindblom, G., L. B.-Å. Johansson, and G. Arvidson. 1981. Effect of cholesterol in membranes. Pulsed NMR measurements of lipid lateral diffusion. *Biochemistry.* 20:2204–2207.

Lindblom, G., K. Larsson, L. Johansson, K. Fontell, and S. Forsen. 1979. The cubic phase of monoglyceride-water systems. Arguments for a structure based upon lamellar bilayer units. *J. Am. Chem. Soc.* 101:5465–5470.

Lipscomb, J. D., and R. W. Salo. 1983. Electron paramagnetic resonance spectrometer data accumulation and reduction system for microcomputers. *Computer Enhanced Spectroscopy.* 1:11–15.

Lutton, E. S. 1965. Phase behaviour of aqueous systems of monoglycerides. *J. Am. Oil. Chem. Soc.* 42:1068–1070.

Malliaris, A. 1988. Fluorescence probing in aqueous micellar systems. An overview. *Int. Rev. Phys. Chem.* 7:95–121.

Mariani, P., V. Luzzati, and H. Delacroix. 1988. The cubic phases of lipid-containing systems. Structure analysis and biological implications. *J. Mol. Biol.* 204:165–189.

Orädd, G., G. Wikander, G. Lindblom, L. B.-Å. Johansson, and P.-O. Eriksson. 1992. Effect of hydrophobic molecules on N,N'-dimethyl dodecylamine oxide micelles in water. *J. Phys. Chem.* 96:5170–5174.

Reiss-Husson, F. 1967. Structure des phases liquid-cristallines de differents phospholipides, monoglycerides, sphingolipides, anhydres ou en presence d'eau. *J. Mol. Biol.* 25:363–382.

Seddon, J. M. 1990. Structure of the inverted hexagonal (H_{II}) phase, and nonlamellar phase transitions of lipids. *Biochim. Biophys. Acta.* 1031:1–69.

Stejskal, E. O., and J. E. Tanner. 1965. Spin diffusion measurements: spin echoes in the presence of time-dependent field gradients. *J. Chem. Phys.* 42:288–292.

Tanner, J. E., and E. O. Stejskal. 1968. Restricted self diffusion of protons in colloidal systems by the pulsed gradient, spin-echo method. *J. Chem. Phys.* 49:1768–1777.

Söderman, O., H. Walderhaug, U. Henriksson, and P. Stilbs. 1985. NMR relaxation in isotropic surfactant systems ^2H, ^{13}C and ^{14}N NMR study of the micellar and cubic (I_1) phases in the doecyltrimethylammonium chloride/water system. *J. Phys. Chem.* 89:3693–3697.

Söderman, O., and U. Henriksson. 1987. ^{2}H and ^{13}C nuclear magnetic relaxation studies of the cubic liquid-crystalline phase I_1 in the sodium octanoate-octane-water system. *J. Chem. Soc. Faraday Trans.1.* 83:1515–1529.

Tachiya, M. 1975. Application of a generating function to reaction kinetics in micelles. Kinetics of quenching of luminescent probes in micelles. *Chem. Phys. Lett.* 33:289–292.

Tardieu, A., and V. Luzzati. 1970. Polymorphism of lipids. A novel phase—a cagelike network of rods with enclosed spherical micelles. *Biochim. Biophys. Acta.* 219:11–17.

Tate, M. W., E. F. Eikenberry, D. C. Turner, E. Shyamsunder, and S. M. Gruner. 1991. Nonbilayer phases of membrane lipids. *Chem. Phys. Lipids.* 57:147–164.

Turro, N. J., and A. Yekta. 1978. Luminescent probes for detergent solutions. A simple procedure for determination of the mean aggregation number of micelles. *J. Am. Chem. Soc.* 100:5951–5952.

Van der Auweraer, M., J. C. Dederen, E. Gelade, and F. C. De Schryver. 1981. Fluorescence quenching in micelles: a theoretical model for the intramicellar first order quenching rate constant. *J. Chem. Phys.* 74:1140–1147.

Weltzien, H. U., B. Arnold, and R. Reuther. 1977. Quantitative studies on lysolecithin-mediated hemolysis. Use of ether-deoxy lysolecithin analogs with varying aliphatic chain-lengths. *Biochim. Biophys. Acta.* 466:411–421.

Wikander, G., P.-O. Eriksson, E. E. Burnell, and G. Lindblom. 1990. ESR lineshapes in lyotropic systems. The micellar and liquid crystalline phases of the dodecyltrimethylammonium chloride/water system. *J. Phys. Chem.* 94:5964–5972.