Fu, X., Phillips, N., Jentoft, J., Tuazon, P. T., Traugh, J. A., & Leis, J. (1985) *J. Biol. Chem. 260*, 9941–9947.
Gorelick, R. J., Henderson, L. E., Hanser, J. P., & Rein, A. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8420–8424.
Haas, E., Katchalski-Katzir, E., & Steinberg, I. Z. (1978) *Biochemistry 17*, 5064–5070.
Hélène, C., Brun, F., & Yaniv, M. (1969) *Biochem. Biophys. Res. Commun. 37*, 3393–3398.
Holler, E., Bennett, E. L., & Calvin, M. (1971) *Biochem. Biophys. Res. Commun. 45*, 409–416.
Jentoft, J. E., Smith, L. M., Fu, X., Johnson, M., & Leis, J. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7094–7098.
Job, P. (1928) *Ann. Chim. 9*, 113–203.
Johnson, S., Veigl, M., Vanaman, T., & Leis, J. (1983) *J. Virol. 45*, 876–881.
Karpel, R. L., Henderson, L. E., & Oroszlan, S. (1987) *J. Biol. Chem. 262*, 4961–4967.
Katz, R. A., & Jentoft, J. (1989) *BioEssays 11*, 176–181.
Katz, R. A., Fu, X., Skalka, A. M., & Leis, J. (1986) *Gene 50*, 361–369.
Kerr, L., Olshevsky, U., Lodish, H., & Baltimore, D. (1976) *J. Virol. 18*, 627–635.
Lakowicz, J. R. (1983) in *Principles of Fluorescence Spectroscopy*, pp 305–309, Plenum Press, New York.
Lin, S. X., Baltzinger, M., & Remy, P. (1983) *Biochemistry 22*, 681–689.
Loeb, G. I., & Saroff, H. A. (1964) *Biochemistry 3*, 1819–1826.
McGinnis, J., Hizi, A., Smith, R., & Leis, J. (1978) *J. Virol. 84*, 518–522.
Méric, C., & Spahr, P.-F. (1986) *J. Virol. 60*, 450–459.
Méric, C., & Goff, S. P. (1989) *J. Virol. 63*, 1558–1568.
Méric, C., Gouilloud, E., & Spahr, P.-F. (1988) *J. Virol. 62*, 3328–3333.
Prasad, A. R. S., Luduena, R. F., & Horowitz, P. M. (1986) *Biochemistry 25*, 3536–3540.
Record, M. T., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.
Richards, F., & Wyckoff, H. (1971) in *The Proteins* (Boyer, P. D., Ed.) Vol., 4, pp 647–806, Academic Press, New York.
Richey, B., Cayley, D. S., Mossing, M. S., Kolka, C., Anderson, C. F., Farrar, T. C., & Record, M. T., Jr. (1987) *J. Biol. Chem. 262*, 7157–7164.
Roberts, W. J., Elliott, J. I., McMurray, & Williams, K. R. (1988) *Peptide Res. 1*, 74–80.
Sanchez-Pescador, R., Power, M. D., Barr, P. J., Steimer, K. S., Stempien, M. M., Brown-Shimer, S. L., Gee, W., Renard, A., Randolph, A., Levy, J. A., Dina, D., & Luciw, P. A. (1985) *Science 227*, 484–492.
Saroff, H. A., & Carroll, W. R. (1962) *J. Biol. Chem. 237*, 3384–3387.
Stryer, L. (1978) *Annu. Rev. Biochem. 47*, 819–846.
Worah, D. M., Gibboney, K. M., Yang, L., & York, S. S. (1978) *Biochemistry 17*, 4487–4492.
Wu, F. Y., & Wu, G. (1978) *Biochemistry 17*, 138–144.

An Inverse Face-Centered Cubic Phase Formed by Diacylglycerol–Phosphatidylcholine Mixtures†

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ABSTRACT: Fully hydrated unsaturated diacylglycerol–phosphatidylcholine mixtures are found to adopt an inverse face-centered cubic phase, of crystallographic cubic aspect 15. The same behavior is observed for either the 1,2- or 1,3-isomer of the diacylglycerol. This Q_{15} cubic phase, of probable space group *Fd3m* (Q^{227}), occurs between an inverse hexagonal (H_{II}) phase and an inverse micellar (L_2) solution, with increasing diacylglycerol concentration, which implies that the mean curvature of the interface is more negative than that of the H_{II} phase. This behavior is quite different from that of the more usual bicontinuous inverse cubic phases *Pn3m* (Q^{224}), *Im3m* (Q^{229}), and *Ia3d* (Q^{230}), which normally occur between the lamellar L_α and the H_{II} phases. One possible structure for the *Fd3m* cubic phase has recently been proposed (Mariani, P., Luzzati, V., & Delacroix, H. (1988) *J. Mol. Biol. 204*, 165–189), consisting of tetrahedrally arranged clusters of inverse micelles surrounded by a continuous cage of tetrahedrally connected water/lipid (inverse) channels.

The effect of diacylglycerols (DG)[1] on membrane structure is currently a topic of great interest because of their role in signal transduction in cells. In response to stimulation of various membrane receptors, diacylglycerols are formed from phosphatidylinositols, and they then activate the membrane-bound enzyme protein kinase C (Berridge, 1984; Berridge & Irvine, 1984; Nishizuka, 1983, 1986). In addition, diacylglycerols enhance the hydrolysis by phospholipases C and A_2 of phospholipids such as PC (Dawson et al., 1983). This was found to be correlated with the appearance of an isotropic component in the ^{31}P NMR spectrum, which appeared when the amount of incorporated DG exceeded 15 mol % (Dawson et al., 1984). Further addition of DG caused the appearance of a hexagonal-type line shape, and by 50 mol % DG the

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[1] Abbreviations: PC, phosphatidylcholine; egg PC, phosphatidylcholine from egg yolk; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DG, 1,2- or 1,3-diacylglycerol; egg DG, 1,2-diacylglycerol derived from egg PC; 1,2-DOG, 1,2-dioleoylglycerol; 1,3-DOG, 1,3-dioleoylglycerol; c_{DOG}, weight fraction of dioleoylglycerol; L_1, normal micellar solution; L_2, inverse micellar solution; L_α, fluid lamellar (bilayer) phase; H_{II}, inverse hexagonal phase; *Fd3m* (Q^{227}), cubic phase of crystallographic space group number 227.

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mixtures were in a pure inverse hexagonal H_{II} phase. This was associated with a total loss of enzyme activity. It thus seems that the activity of the phospholipases is correlated with structural perturbations of the membrane.

Monolayer studies have indicated that both 1,2- and 1,3-dioleoylglycerol (1,2-DOG and 1,3-DOG) form stable complexes with 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) at a mole fraction of DOG of 0.25 (Smaby & Brockman, 1985). A calorimetric study of dispersions of mixtures of 1,3-DOG with 1-stearoyl-2-oleoylphosphatidylcholine (SOPC) also found evidence for this stoichiometry, showing that complex formation is not restricted to monolayers (Cunningham et al., 1989). The transition temperature of the complex was determined to be 9.4 °C, the increase compared to the value of 5.3 °C for pure SOPC being attributed to a stabilizing effect on the membrane structure due to the complex formation. Evidence was presented to show that metastable effects occur for larger mole fractions of DOG. These authors found that the bilayer morphology was maintained until a DOG mole fraction of 0.8 was reached, when a nonlamellar phase appears. Freeze-fracture electron microscopy of this phase showed aggregates with a surface having an irregular, nonplanar hexagonal array. The aggregates appeared to consist of small particles, possibly arranged as dodecahedra, with diameters in the range 270–1200 Å, the majority being 360 Å.

An X-ray diffraction study of fully hydrated egg PC–egg DG mixtures found a region of coexisting lamellar phases, before a pure H_{II} phase appeared at DG contents greater than 30 wt % (Das & Rand, 1984, 1986). Additional unidentified phases were observed in this region of the pseudobinary (i.e., with a large excess of water—taken to be a constant amount—also present) phase diagram. For DG contents between 30 and 70 wt %, a pure H_{II} phase was found, with a repeat spacing of d_{10} = 61.5 Å, which value, surprisingly, was constant over this entire range of composition. Between 70 and 75 wt % DG, a transition to a cubic phase occurred, which was identified as being space group $Pn3m$ (Q^{224}), with a lattice parameter of 114.4 Å (Das & Rand, 1986).

There are four principal locations in the phase diagram where lyotropic cubic phases may be found [for recent reviews, see Lindblom and Rilfors (1989), Larsson (1989), Fontell (1990), and Seddon (1990)]. Normal topology (oil-in-water) cubic phases may be found either adjacent to the micellar solution (often between the normal micellar solution (L_1) and the hexagonal H_I phases) or between the H_I phase and the lamellar L_α phase. In the former case the structures probably consist of anisotropic micellar aggregates (Fontell et al., 1985; Eriksson et al., 1985; Charvolin & Sadoc, 1988). In the latter case the structures appear invariably to be bicontinuous. Cubic phases may also be found between the L_α and H_{II} phases. In this case, the structures are inverse (water-in-oil) and are also invariably bicontinuous. Most of our knowledge about the structures of bicontinuous cubic phases stems from the pioneering X-ray diffraction studies of Luzzati and co-workers (Luzzati, 1968; Mariani et al., 1988). Cubic phases occurring in the fourth likely location of the phase diagram, namely, between the H_{II} phase and the inverse micellar solution (L_2), have so far never been clearly identified (Lindblom & Rilfors, 1989; Fontell, 1990).

The fact that the sequence of phases observed for PC–DG mixtures with increasing DG concentration was lamellar–H_{II}–cubic (Das & Rand, 1986) suggests that this cubic phase is not in the normal location expected for an inverse bicontinuous cubic phase and casts doubt on the identification of the space group as $Pn3m$.

Materials and Methods

Chemicals. All lipids used were 99% purity. DOPC and egg PC were obtained from Lipid Products (South Nutfield, Surrey, U.K.). 1,2-DOG and 1,3-DOG were obtained from Larodan Fine Chemicals, Malmö, Sweden. Identical results were obtained by using 1,3-DOG, substantially free of 1,2-isomer, obtained from Sigma Chemical Co., Poole, Dorset, U.K. The purity of the lipids was checked before and after the X-ray measurements by thin-layer chromatography in a solvent system of $CHCl_3/CH_3OH/33\%$ NH_3 (65/35/5 v/v), with staining for phosphate groups by molybdenum blue, followed by sulfuric acid charring. In addition, a solvent system of petroleum ether/diethyl ether (70/30 v/v) was used to check the isomeric purity of the diacylglycerols. For the single-isomer samples, any contamination by the other isomer was estimated to be less than 2%. The water was triply distilled.

Polarizing Microscopy. Optical observations were carried out by using a Nikon Labophot polarizing microscope equipped with a Linkam heating stage.

X-ray Diffraction. Diffraction patterns were obtained by using either a Guinier camera (R. Huber, 8211 Rimsting, West Germany) fitted with a quartz crystal monochromator, set to isolate the Cu $K\alpha_1$ (λ = 1.5405 Å) radiation, or with a Franks camera, with Ni-filtered Cu $K\alpha$ radiation (λ = 1.5418 Å) from the point-focus window of an Elliott GX 20 rotating-anode generator fitted with a 0.1-mm focus cup (Marconi Avionics, Borehamwood, U.K.). The Guinier camera, which was operated in vacuum in order to reduce air scatter, has a fixed sample–film distance of 114.6 mm; the Franks camera has a variable sample–film distance between 35 and 160 mm, depending on the settings of the mirrors. The diffraction patterns were recorded on stacks of up to six sheets of Kodak DEF 392 X-ray film, to ensure that both the strongest and the weakest peaks could be measured, and subsequently scanned with a Joyce-Loebl Mark IIIC microdensitometer. Exposure times were typically in the range 1–24 h. Temperature regulation of the sample (±1 °C) was by electrical heating, employing an electronic controller, the temperature probe being embedded in the brass sample mounting block in the X-ray camera, as close as practicable to the sample (within 1 cm).

Weighed amounts of the lipids were codissolved in chloroform/methanol, the solvent was blown off under a stream of nitrogen, and the lipids were then stored overnight in a vacuum desiccator (pressure <100 Pa) over phosphorus pentoxide. The samples were then mixed with water, employing a fine spatula, and the stiff lipid pellets sealed with excess water in purpose-built metal X-ray holders with thin mica windows. The samples were then equilibrated at room temperature for up to 5 days, the uniformity/homogeneity being assessed by monitoring the diffraction patterns.

Results

A sample of DOPC–1,3-DOG (c_{DOG} = 0.70) was examined at 25 °C by polarizing microscopy before and after hydration with an excess water phase. Before hydration, the sample was an optically isotropic liquid, presumably an inverse micellar solution (L_2 phase). Following hydration, the sample transformed into a viscous, optically isotropic mesophase.

The X-ray diffraction pattern obtained from this mixture in excess water at 30 °C, obtained by using a point-focus Franks camera, is shown in Figure 1. The spottiness of the pattern indicates the presence of relatively large (>1 μm) monodomains of the (cubic) phase in the sample (Lipson & Steeple, 1970). The broader reflection, third from the center,

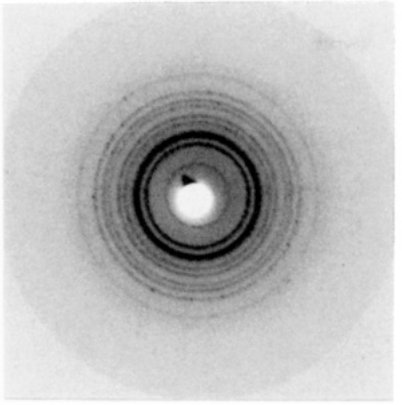

FIGURE 1: Diffraction pattern obtained with a point-focus Franks camera from a DOPC–1,3-DOG mixture (c_{DOG} = 0.70) in excess water at 30 °C. The sample–film distance was 120 mm. The lattice parameter of the *Fd*3*m* cubic phase is a = 153 ± 2 Å.

is actually a closely spaced doublet. This is clearly seen in the X-ray diffraction patterns obtained from the same sample by using a line-focus Guinier camera, shown in Figure 2. A total of 11 Bragg peaks are observed, which index as the 111, 220, 311, 222, 400, 331, 422, 511/333, 440, 533, and 711/551 reflections of a cubic phase of cubic aspect 15 (Hahn, 1983). This cubic aspect is characterized by Bragg reflections whose reciprocal d spacings are in the ratios ($\sqrt{3}$, $\sqrt{8}$, $\sqrt{11}$, $\sqrt{12}$, $\sqrt{16}$, $\sqrt{19}$, $\sqrt{24}$, $\sqrt{27}$, $\sqrt{32}$, $\sqrt{35}$, $\sqrt{36}$, $\sqrt{40}$, $\sqrt{43}$, $\sqrt{44}$, $\sqrt{48}$, $\sqrt{51}$, $\sqrt{56}$, ... There are only two space groups possible for this cubic aspect, namely, *Fd*3*m* (Q^{227}) and *Fd*3 (Q^{203}). In accordance with the suggestion of Luzzati and co-workers (Mariani et al., 1988), we assume that the more symmetrical space group, *Fd*3*m* (Q^{227}), is the correct one. The difference between *Fd*3*m* and *Fd*3 is that for a reflection with $h \neq k \neq l$, $I(hkl)$ and $I(khl)$ are equal for the former space group but different for the latter one. In order to measure these intensities separately, it would be necessary to have an aligned sample of the cubic phase. However, none of the observed reflections actually fit the criterion of $h \neq k \neq l$, and so even if a monodomain sample were available, it would only permit the discrimination to be made if further reflections such as 531, 620, or 642 could be detected.

Densitometer scans of the diffraction patterns of parts a and b of Figure 2 are shown in parts b and a of Figure 3, respectively. The indexing of the diffraction data was assessed by plotting the reciprocal d spacings ($1/d_{hkl}$) of the 11 observed reflections versus $m = (h^2 + k^2 + l^2)^{1/2}$. For a cubic phase, such a plot should pass through the origin and be linear with a slope of $1/a$, where a is the cubic unit cell lattice parameter. The data index perfectly (plot not shown) as cubic aspect 15 (space group *Fd*3*m* or *Fd*3) with a lattice parameter of a = 153 ± 2 Å, with no unobserved reflections below hkl = 531.

By diffraction, the *Fd*3*m* cubic phase was observed to be stable between 25 and 40 °C; however, examination by polarizing microscopy indicated that a viscous isotropic phase (presumably, but not necessarily, the *Fd*3*m* cubic phase over the entire temperature range) was stable up to at least 90 °C. For a DOPC–1,3-DOG mixture (c_{DOG} = 0.65), which formed the *Fd*3*m* cubic phase with a lattice parameter of 130 Å in excess water, a sample that was probably not fully hydrated was found to give rise to sharp peaks whose reciprocal spacings were in the ratios $\sqrt{10}$, $\sqrt{12}$, $\sqrt{14}$, and $\sqrt{17}$. This suggests that another phase–conceivably a different cubic phase—might be formed at low hydration for certain mixtures.

In order to see whether the formation of the *Fd*3*m* cubic phase was dependent on the isomeric form (1,3 or 1,2) of the diacylglycerol, mixtures of DOPC with 1,2-DOG were also studied. For a mixture with a composition c_{DOG} = 0.77, the same *Fd*3*m* cubic phase was observed in excess water at 30 °C, with a lattice parameter of a = 131 Å (data not shown).

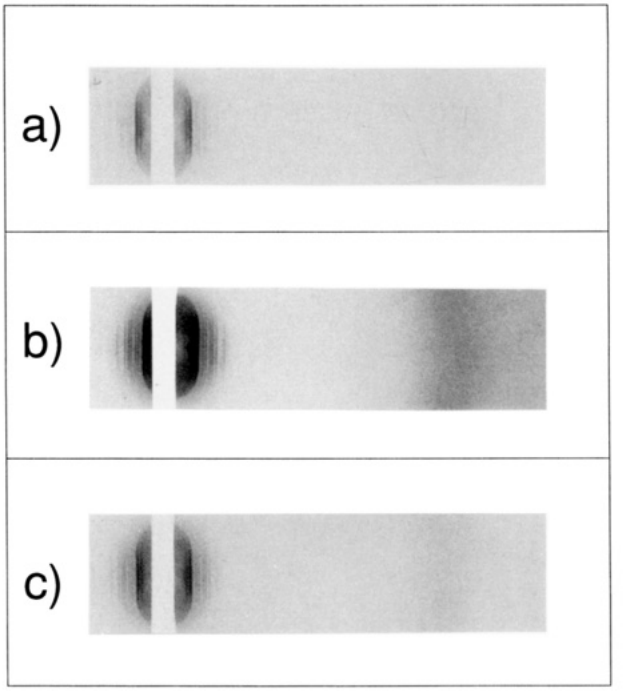

FIGURE 2: Diffraction patterns obtained from the same sample as in Figure 1 but with a line-focus Guinier camera, with continuous sample rotation around the incident beam. The temperature was (a) 30, (b) 33, and (c) 40 °C. The exposure time was greatest for the pattern shown in (b). The 311 and 222 reflections are clearly resolved in patterns (a) and (c).

To establish that the *Fd*3*m* cubic phase was not specific to DOPC, mixtures of 1,3-DOG with egg PC were also studied. A mixture with a composition of c_{DOG} = 0.64 formed the *Fd*3*m* cubic phase in excess water at 25 °C, with a lattice parameter of a = 159 Å (data not shown).

A fully hydrated mixture of egg PC–1,3-DOG with a lower diacylglycerol content of c_{DOG} = 0.47 was observed to form a birefringent phase, found by diffraction to be predominantly an H_{II} phase, with a d spacing of 58 Å (lattice parameter of 67 Å). This is in agreement with previous results for egg PC–egg DG mixtures, in this range of composition (Das & Rand, 1986).

Discussion

There are three quite independent arguments against the identification as *Pn*3*m* (Q^{224}) and in favor of *Fd*3*m* (Q^{227}) of the cubic phase observed in fully hydrated PC–DG mixtures at high DG contents (greater than approximately 60 wt % DG). First, the location of this phase in the pseudobinary phase diagram (in the presence of an excess aqueous phase) does not appear to lie between L_α and H_{II}, which to date appears to be the usual location for the *Pn*3*m* cubic phase (it has so far only been found with an inverse, type-II topology). Rather, the evidence is that the PC–DG cubic phase has a location between the H_{II} phase and an inverse micellar solution (L_2): X-ray diffraction of pure DG in water shows a diffuse maximum in the region of $1/d$ = 28 Å^{-1} (Das & Rand, 1986), indicative of micellar aggregates, undoubtedly of inverse topology. A strong argument in support of this view is that the effect of incorporating the weakly amphiphilic DG into the strongly amphiphilic PC should be to reduce the effective hydrophilicity of the lipid headgroup region and hence to tend

to reduce the hydration. Reducing hydration will tend to drive the phase equilibrium toward inverse phases of increasingly negative interfacial mean curvature (i.e., curvature of the interface toward the aqueous region). The fact that the cubic phase appears for *higher* concentrations of the weakly amphiphilic component than the H_{II} phase implies that *the average interfacial mean curvature of the cubic phase must be more negative than that of the H_{II} phase* and must therefore lie "beyond" the H_{II} phase, rather than between the lamellar and the H_{II} phases.

Second, the indexing of the diffraction data as *Pn3m* (Das & Rand, 1986) is not convincing. Although the indexing given as 110, 211, 220, 221, 222, 321, 411/330, 420, 422, 441/522 by Das and Rand is indeed reasonably consistent with their observed spacings, reflections 111, 200, 310, 311, 400, 322, 331, 421, 332, 510/431, 511/333, 432, 521, and 440 would all have to be unobserved, although permitted by the space group. When the fact that (in particular) the second and third allowed reflections would both have to have zero intensity is taken into account, the indexing as *Pn3m* becomes most unlikely. However, indexing the reflections as *Fd3m* accounts perfectly for all of the observed reflections up to the ninth one, with all permitted reflections of this space group being observed up to 440. The tenth reflection is probably 533 (although we cannot completely rule out the 622 reflection, which is very close in spacing), with reflections 531, 442, and 620 being too weak to be observed (it should be emphasized that, for liquid-crystalline phases, the Bragg reflections always tend to become very weak for high *hkl* indices, because of the short-range disorder inherent in fluid phases). The last observed reflection corresponds to 711/551, with (622) and 444 being unobserved.

A third argument against the indexing as *Pn3m*, and in favor of the indexing as *Fd3m*, is the distribution of intensity among the various Bragg reflections (i.e., the structure factors). An obvious yet important principle of structural analysis of lipid phases is that if two similar lipid systems of comparable electron density distributions form the same cubic phase, with comparable water contents, then the intensity distribution of the various Bragg reflections must be qualitatively similar. The first four observed reflections from the PC–DG cubic phase have intensities that are relatively weak, strong, very strong, and medium (see Figure 3). This distribution, if assigned to the 110, 211, 220, and 221 reflections of *Pn3m*, is quite unlike that observed in a variety of lipid systems forming cubic phases of this space group (Longley & McIntosh, 1983; Mariani et al., 1988; J. M. Seddon, unpublished data). On the other hand, such an intensity distribution, assigned to reflections 111, 220, 311, and 222 of *Fd3m*, is quite similar to that observed from other systems forming the cubic phase *Fd3m* (Mariani et al., 1988, 1990).

It may be concluded that the observed reflections, taken together, unambiguously identify the lattice type as face-centered cubic, with cubic aspect number 15; only two space groups have this aspect: *Fd3* (No. 203) and *Fd3m* (No. 227). In accordance with the suggestion of Luzzati and co-workers (Mariani et al., 1988), it can be assumed that the more symmetrical space group is the correct one (in fact, it would probably not greatly alter the structure if the other possibility was considered). This novel cubic phase was first discovered in a lipid extract from *Pseudomonas fluorescens* (Tardieu, 1972). Only recently, however, has a structure been proposed, on the basis of a crystallographic analysis (Mariani et al., 1988, 1990). Two further examples of this phase have been found: in hydrated monoolein/oleic acid mixtures (Mariani et al., 1988) and in oleic acid buffered to pH 7 (J. M. Seddon, unpublished data). The system PC–DG is thus the fourth known example of this phase and there is every reason to believe that it will be found in the future in other systems.

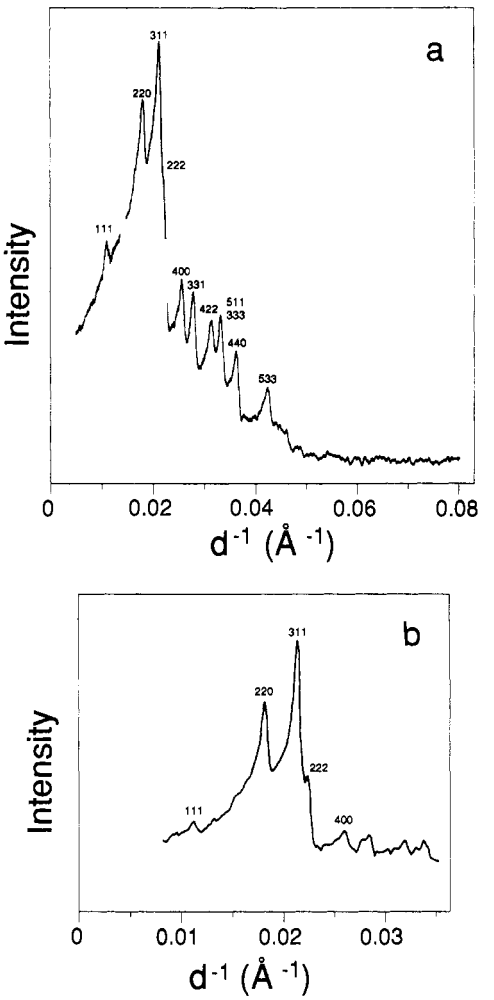

FIGURE 3: (a) Densitometer trace of the diffraction pattern of Figure 2b. For the overexposed 220, 311, and 222 peaks, the tracing was measured from the second film (attenuation factor of approximately 3). (b) Densitometer trace of the diffraction pattern of Figure 2a, with the 311 and 222 reflections resolved.

A noteworthy feature of this *Fd3m* cubic phase is that, like the inverse bicontinuous cubic phases *Pn3m* and *Im3m*, it can coexist with an excess aqueous phase and thus may be of more direct relevance to biological systems than those cubic phases that have a normal (type I, oil-in-water) topology and are not normally stable in the presence of an excess aqueous phase.

It is interesting to note that diacylglycerol–phosphatidylcholine mixtures represent the first system found to adopt the *Fd3m* cubic phase without a free fatty acid component being present. It seems likely that the requirement to form this phase is for an amphiphilic component that is weakly hydrophilic yet is able to interact (probably via hydrogen-bonding) with the phospholipid headgroups, effectively reducing their hydration.

Structure of the Cubic Phase Fd3m (Q^{227}). A structure has recently been proposed for the *Fd3m* cubic phase by Luzzati and co-workers, based upon a crystallographic analysis (Mariani et al., 1988, 1990). This structure is derived from that of the bicontinuous inverse cubic phase *Pn3m* (Q^{224}), which consists of a double-diamond arrangement of water channels, the two independent networks being separated by a continuous lipid bilayer. In the *Fd3m* structure, one of the

tetrahedral networks of water channels is replaced by inverse micelles at positions halfway along each water channel segment (inverse water/lipid rod). A tetrahedral cluster of four inverse micelles is thus embedded within a surrounding cage of 12 (nearest-neighbour) tetrahedrally connected water channel segments, there being 16 inverse micelles per unit cell. If the continuous tetrahedral network had the same dimensions as each of the two networks in the cubic phase *Pn3m*, this would lead to an effective doubling of the lattice parameter; in practice, the lattice parameters for *Fd3m* seem to be of the order of 1.6 times those of comparable *Pn3m* cubic phases. Symmetry requirements mean that the shape of the inverse micelles could be oblate or prolate, as well as spherical, as long as the symmetry axes of the micelles are aligned along the 3-fold axes. However, space-filling considerations suggest that the first possibility is unlikely.

It has been suggested by Luzzati and co-workers that the lyotropic cubic phases can be divided into two families. One of these is based upon a *bilayer* interfacial film; the bicontinuous cubic phases *Pn3m* (Q^{224}), *Im3m* (Q^{229}), and *Ia3d* (Q^{230}) are examples of this family. They can be either normal, type I (oil in water), or inverse, type II (water in oil). The second family of cubic phases is proposed to be based upon a *monolayer* interfacial film, normally in conjunction with closed micellar or globular aggregates. A type I example of this is supposed to be the cubic phase *Pm3n* (Q^{223}), altough this view has been disputed (Fontell et al., 1985; Eriksson et al., 1987). The cubic phase *Fd3m* (Q^{227}) is suggested to be an example of an inverse (type II) member of this second family. In fact, Luzzati and co-workers have identified a further type II member of this family, of space group $P4_332$ (Q^{212}), which is the first chiral lyotropic cubic phase to be discovered (Mariani et al., 1988).

Although the crystallographic evidence for the "monolayer/inverse micelle" structure proposed for *Fd3m* (Q^{227}) by Luzzati and co-workers is quite compelling for the particular systems they studied, it should be pointed out that different lipid systems could conceivably adopt cubic phases having this same space group *yet with entirely different structures*.

The most plausible such alternative structure for Q^{227} is a cubic packing of anisotropic inverse micelles. Such a structure has been implicitly considered in a topological/geometrical study of the possible structures formed by periodic systems of frustrated fluid films (Charvolin & Sadoc, 1988). When the films are constrained to meet so that the dihedral and edge angles stay close to 120° and 109°28′, which are the optimal values to balance the film tensions, then only two space-filling solutions are found that have cubic symmetry. These two structures are analogous to those of the water clathrates, consisting of close-packed assemblies of two types of slightly distorted polyhedra. One has space group *Pm3n* (Q^{223}), and the other has space group *Fd3m* (Q^{227}). A type I (oil-in-water) version of a *Pm3n* (Q^{223}) cubic phase has in fact been observed in the region of the binary phase diagram adjacent to the normal micellar solution, in systems such as lysophosphatidylcholine (Tardieu & Luzzati, 1970; Eriksson et al., 1985, 1987). In the geometrical view of Charvolin and Sadoc, the "frustrated interfacial film" in this case would correspond to the locus of midpoints of the aqueous regions separating two sets of nonequivalent micelles (two of one type and six of the other) within the cubic unit cell (Charvolin & Sadoc, 1988). It is very interesting that the other possibility has space group *Fd3m*: this suggests a possible structure for an *Fd3m* cubic phase, made up entirely from two sets of nonequivalent aggregates (8 of one type and 16 of the other). Preliminary results of a crystallographic analysis indicate that the *Fd3m* cubic phase of diacylglycerol–phosphatidylcholine mixtures may have a structure analogous to this, *consisting purely of inverse micelles* (V. Luzzati and J. M. Seddon, unpublished results). It is interesting to note that the fact (as discussed in the Introduction) that freeze-fracture electron microscopy of stearoyloleoylphosphatidylcholine–dioleoylglycerol mixtures with a DOG mole fraction of 0.8 shows aggregates of very small particles (Cunningham et al., 1989) could be consistent with an *Fd3m* cubic phase structure consisting purely of closed (inverse micellar) aggregates.

The findings presented here emphasize the peculiar problems associated with structural studies of lyotropic cubic phases, which can very easily lead to misinterpretation of the diffraction patterns. The most significant problems may be reiterated as follows:

1. Usually rather few lines are detected, due to the liquidlike short-range order characteristic of liquid-crystalline phases.
2. Certain of the diffraction lines may be quite close together, so a moderately high resolution X-ray apparatus is required to resolve them adequately.
3. It is common for the patterns to be intermediate between "powder" and "single-crystal" ones, due to the tendency for growth of large monodomains (see Figure 1). This creates severe problems when line-focus cameras are employed and requires various procedures, such as continuous sample rotation, to overcome them.
4. Some of the diffraction lines, although permitted by the space group, may nontheless be too weak to be observed, due to the underlying continuous transform of the structure being close to zero at the position of the reflection.
5. Pronounced metastable effects are frequently encountered with cubic phases, which means that the sample may not be at equilibrium, leading to spurious lines due to the residual presence of other phases. If any doubt exists, it is necessary to explore over a range of water contents, to ensure that the spacings of all of the lines shift consistently with each other.
6. Cubic phases in excess water frequently exhibit large temperature dependences of the lattice parameters (Seddon et al., 1984; Caffrey, 1987), presumably mainly due to strongly changing limiting hydrations with temperature. This can lead to a broadening of the diffraction lines, if the temperature of the sample is not carefully regulated during the diffraction experiment.

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REFERENCES

Berridge, M. J. (1984) *Biochem. J. 220*, 345–360.
Berridge, M. J., & Irvine, R. F. (1984) *Nature 312*, 315–321.
Caffrey, M. (1987) *Biochemistry 26*, 6349–6363.
Charvolin, J. (1985) *J. Phys. Colloq. 46* (C3), 173–190.
Charvolin, J., & Sadoc, J. F. (1988) *J. Phys. 49*, 521–526.
Cunningham, B. A., Tsujita, T., & Brockman, H. L. (1989) *Biochemistry 28*, 32–40.
Das, S., & Rand, R. P. (1984) *Biochem. Biophys. Res. Commun. 124*, 491–496.
Das, S., & Rand, R. P. (1986) *Biochemistry 25*, 2882–2889.
Dawson, R. M. C., Hemington, N. L., & Irvine, R. F. (1983) *Biochem. Biophys. Res. Commun. 117*, 196–201.

Dawson, R. M. C., Irvine, R. F., Bray, J., & Quinn, P. J. (1984) *Biochem. Biophys. Res. Commun. 125*, 836–842.
Eriksson, P. O., Lindblom, G., & Arvidson, G. (1985) *J. Phys. Chem. 89*, 1050–1053.
Eriksson, P. O., Lindblom, G., & Arvidson, G. (1987) *J. Phys. Chem. 91*, 846–853.
Fontell, K., Fox, K., & Hansson, E. (1985) *Mol. Cryst. Liq. Cryst. (Lett.) 1*, 9–17.
Fontell, K. (1990) *Colloid Polym. Sci. 268*, 264–285.
Hahn, T., Ed. (1983) *International Tables for Crystallography*, Vol. A, D. Reidel, Dordrecht, Holland.
Larsson, K. (1989) *J. Phys. Chem. 93*, 7304–7314.
Lindblom, G., & Rilfors, L. (1989) *Biochim. Biophys. Acta 988*, 221–256.
Lindström, M., Ljusberg-Wahren, H., Larsson, K., & Borgström, B. (1981) *Lipids 16*, 749–754.
Lipson, H., & Steeple, H. (1970) *The Interpretation of X-ray Powder Diffraction Patterns*, Macmillan, London.
Luzzati, V. (1968) in *Biological Membranes* (Chapman, D., Ed.) Vol. 1, pp 71–123, Academic Press, London.
Mariani, P., Luzzati, V., & Delacroix, H. (1988) *J. Mol. Biol. 204*, 165–189.
Mariani, P., Rivas, E., Luzzati, V., & Delacroix, H. (1990) *Biochemistry 29*, 6799–6810.
Nishizuka, Y. (1983) *Philos. Trans. R. Soc. London B 302*, 101–112.
Nishizuka, Y. (1986) *Science 233*, 305–312.
Seddon, J. M. (1990) *Biochim. Biophys. Acta 1031*, 1–69.
Smaby, J. M., & Brockman, H. L. (1985) *Biophys. J. 48*, 701–708.
Scriven, L. E. (1977) in *Micellization, Solubilization, and Microemulsions* (Mittal, K. L., Ed.) Vol. 2, pp 877–893, Plenum Press, New York.
Tardieu, A., & Luzzati, V. (1970) *Biochim. Biophys. Acta 219*, 11–17.
Winsor, P. A. (1968) *Chem. Rev. 68*, 1–40.