Intermediate structures in the cholate-phosphatidylcholine vesicle–micelle transition

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ABSTRACT The vesicle–micelle transition of egg phosphatidylcholine (PC) and sodium cholate was described by comparing cryo-transmission electron microscopic (cryo-TEM) images of the structures formed to the associated turbidity changes. These experiments were designed to identify the morphology of the intermediates between vesicles and small spheroidal mixed micelles. With increasing cholate concentration, the vesicular structures changed size and more multilamellar vesicles were seen. Between the apparent upper and lower phase boundaries, three structures were observed: open vesicles, large bilayer sheets (twenty to several hundred nanometers in diameter), and long (150–300 nm) flexible cylindrical micelles. The cylindrical micelles evolved from the edges of the bilayer sheets. At higher relative cholate concentration, the phase boundary was sharply defined by optical clarification of the egg PC–cholate mixtures. Cryo-TEM revealed only small spheroidal mixed micelles at this transition. These results provide the first direct evidence of the structural pathway or of molecular intermediates between a lamellar and a micellar state. Understanding these specific intermediates and the transitions between them is essential to developing reconstitution protocols and properly analyzing either activity or structural data obtained from cholate-dispersed membrane proteins.

INTRODUCTION

Mixtures of phospholipids and surfactants such as the bile salts form mixed amphiphile structures, the nature of which depends on the relative amounts of phospholipid and surfactant. It is clearly established that at high surfactant-to-phospholipid ratios, small micelles are formed. At very low ratios, the predominant amphiphile, the phospholipid, forms bilayer membrane vesicles with some surfactant incorporated. However, the nature of the intermediate structures and the process by which micelles form vesicles or vesicles form micelles has been the subject of considerable investigation.

The complete phase diagram of phosphatidylcholine (PC), cholate, and water formed the basis for the original description of the transition between the micellar and vesicular structures (Small et al., 1969). The intermediate structure proposed was a discoidal mixed micelle composed of a small circular piece of phospholipid bilayer surrounded on the edges by a ring of cholate molecules. The hypothesized disk would grow if the cholate concentration decreased and shrink at higher cholate concentration, suggesting that the diameter was determined by the surfactant available to stabilize the edges. This model was refined to include cholate molecules intercalated within the disk of phospholipid (Mazer et al., 1980). Experimental evidence supporting the discoidal micelle model included quasi-elastic light scattering (QELS) determinations of the hydrodynamic radius, Rh (Mazer et al., 1980; Schurtenberger et al., 1985), negative-staining electron microscopy (Mazer et al., 1980; Fromherz and Rüppel, 1985), and x-ray diffraction (Müller, 1981). However, recent experiments have questioned the existence of discoidal mixed micelles in PC-cholate mixtures. Small angle neutron scattering of glycocholate-PC mixtures indicated that the structural evolution from solutions ranging from high to low glycocholate levels progressed from spheroidal structures, to rod-like structures, to a network, to large sheets, and finally to vesicles (Hjelm et al., 1988, 1990). In addition, at high bile salt levels, measurement of the mole ratio of PC and bile salt in the structures, combined with Rh determinations and molecular packing constraints, were fit equally well by a new capped-rod model and by the disc model for the bile salt PC mixed micelle (Nichols and Ozarowski, 1990).

One difficulty with many of these experiments is that interpreting shape from x-ray or QELS results is extremely model dependent and particularly subject to error when the samples are polydisperse. The existing electron microscopy evidence is based on images of negatively stained and dried samples, which are subject to artifact induced by the stain (Talmon, 1983), a problem acknowledged by the investigators (e.g., Mazer et al., 1980).

Cryo-transmission electron microscopy (cryo-TEM) is highly suited to studying the phase behavior of mixed

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amphiphilic systems in aqueous solutions. In brief, the sample is spread on a perforated carbon film suspended in a temperature- and humidity-controlled environment, and plunged into liquid ethane at its freezing point. No stain is used in the preparation of these vitrified specimens. Moreover, the rapid rate of cooling required for vitrification ensures that the structures dispersed in the aqueous solution are maintained in their original state (Bellare et al., 1988; Talmon et al., 1990).

Cryo-TEM analysis of PC vesicles mixed with increasing concentrations of another surfactant, octyl glucoside, revealed a region between vesicles and small spheroidal mixed micelles that contains long, flexible cylindrical mixed micelles (Vinson et al., 1989). Although hypothesized (Ollivon et al., 1988) and predicted by light scattering (Almog et al., 1990), no large discoidal structures were observed. Images of clusters of cylindrical micelles suggest that the transition occurs by a sudden rearrangement of the phospholipid and octyl glucoside from a bilayer to a cylindrical micellar aggregate at a critical R_e (i.e., the effective mole ratio of surfactant to PC in the structures).

In this study, we set out to identify the structures observed in sodium cholate–PC mixtures and correlate these to changes in bulk sample turbidity. There was no a priori reason to expect octyl glucoside and cholate interactions with phospholipid to be identical. Octyl glucoside is a nonionic surfactant with a saturated straight acyl chain, whereas cholate is an anionic molecule with a steroid "tail" that is more polar on one face than the other due to three hydroxyl moieties. R_e at the point of clarity for PC mixtures is ~3.5 for octyl glucoside (Jackson et al., 1982; Ollivon et al., 1988; Paternostre et al., 1988), but only 0.6–0.7 for sodium cholate (Lichtenberg et al., 1979; Mazer et al., 1980; Paternostre et al., 1988).

The cryo-TEM images of the cholate–PC mixtures revealed that the vesicles initially change size (as reported by Schurtenberger et al., 1985; Almog et al., 1986), then open by detachment of small membrane patches. Cylindrical micelles appeared to form from the edges of the bilayer sheets until all the material existed in the form of cylindrical micelles. At clarity, the lipid–cholate structures were small spheroidal micelles. These images and how they correlated with prior analyses are presented below. Some of these data have been reported in preliminary form (Walter et al., 1990).

MATERIALS AND METHODS

Vesicle preparations

Vesicles were prepared from egg PC in a buffer composed of 150 mM NaCl, 10 mM Na-Hepes, 0.1 mM EDTA, and 0.02% sodium azide, pH 7.2. The lipid was deposited as a thin film on a clean glass tube from a chloroform stock solution by solvent evaporation under a stream of nitrogen gas. The lipid was held in vacuo to remove any remaining volatile organics before hydrating in buffer. Three types of vesicle preparations were used with essentially identical results. Some vesicles were prepared by repetitive freeze-thaw cycles followed by extrusion at high pressure through a stack of 0.1- and 0.2-μm nuclepore filters (Nuclepore Corp., Pleasanton, CA), essentially as recommended by the manufacturer (Extruder®; Lipex Biomembranes, Inc., Vancouver, BC, Canada). Sonicated unilamellar vesicles (SUV) were prepared in a cup-horn sonicator (model #W-380; Heat Systems Incorporated, Farmingdale, NY). Dialyzed unilamellar vesicles (DUV) were formed by dialysis from excess cholate. All three types of vesicles showed the same dependence on cholate for dissolution, and similar structures (except for the initial vesicles) were observed by cryo-TEM. Vesicles were prepared and stored under nitrogen to minimize oxidation. Total lipid phosphate determinations were carried out for all preparations (Ames and Dubin, 1960).

The phospholipids from Avanti Polar Lipids, Inc. (Birmingham, AL) were used without further purification. Cholic acid (Aldrich Chemical Co., Milwaukee, WI) was recrystallized twice from ethanol (Kagawa and Racker, 1971). Stock solutions of the sodium salt (100 or 200 mM) were prepared in our standard buffer by titrating cholic acid with NaOH until a stable pH (7.2) was attained.

Turbidity of the cholate–PC mixtures

Light scattering was monitored during continuous cholate addition to 9 mM egg PC SUVs (Fig. 1) as described in detail previously (Ollivon et al., 1988). The sodium cholate was added from a 100 mM stock solution at a rate of 5 μl/min (i.e., ~0.25 mM cholate/min). Scattering

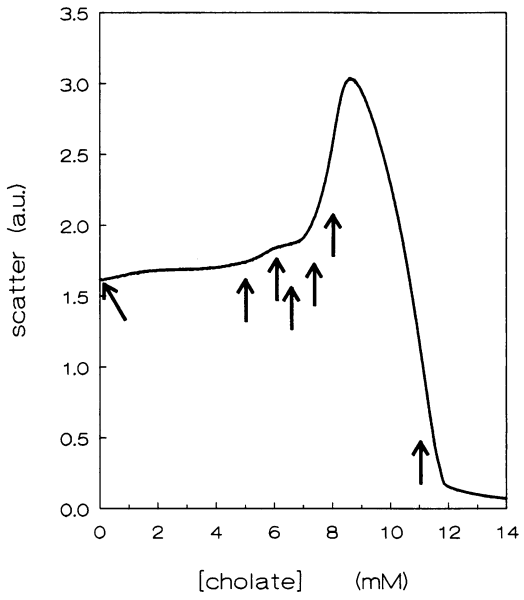


FIGURE 1 Turbidity, as an indirect indicator of the microstructural changes of the lipid-containing aggregates, was measured by light scattering during the continuous addition of concentrated cholate (100 mM at 5 μl/min) to a well-stirred thermojacketted cuvette containing 9 mM egg PC sonicated vesicles at 25°C. The arrows indicate the points shown in the cryo-TEM images.

was followed at 470 nm using a spectrofluorometer (DMX-1000; SLM Instruments Inc., Urbana, IL).

Cryo-TEM

Egg PC–cholate mixtures were prepared by adding the requisite amount of cholate from a stock solution (usually 100 mM Na-cholate, pH 7.2) to preformed vesicles. The samples were allowed to equilibrate for at least 30 min at room temperature.

Samples of the egg PC–cholate mixtures were prepared for cryo-TEM in a controlled environment vitrification system (CEVS) to ensure that the temperature and water activity (98–99% RH) remained constant during the sample preparation before vitrification. A bulk sample was equilibrated in the CEVS to 25°C, then a few microliters were applied to a holey carbon film on an EM copper grid that was held by tweezers mounted on a spring-loaded shaft above a shutter separating the CEVS from the liquid ethane bath below. The sample was blotted with clean filter paper to distribute the liquid across the 2–8-μm holes of the carbon film to a thickness of 10–500 nm. Immediately after blotting, a mechanism opened the shutter and activated the spring, plunging the sample into the liquid ethane held at its freezing point by a bath of liquid nitrogen. The rapid change in temperature assures vitrification, meaning there was no change in the phase. Thus, there was no damage to the structures due to water crystallization, nor were the molecular arrangements of the lipid–surfactant structures perturbed. The vitreous specimen was transferred under liquid nitrogen to a liquid nitrogen Dewar for storage at 77 K.

For observation, specimens were transferred under liquid nitrogen to the cryo-TEM cold stage (model 626; Gatan Inc., Pleasanton; CA), which was inserted into the microscope (JEM 2000FX; JEOL U.S.A. Inc., Peabody, MA, or CM30; Philips Electronic Instruments Co., Mahwah, NJ). Temperature was maintained at 100–105° K throughout specimen observation. The specimen was imaged at 100 kV accelerating voltage and a nominal underfocus of 4 μm, and recorded on Kodak SO-163 film that was developed with full-strength D-19 developer (Eastman Kodak Co., Rochester, NY).

RESULTS

Our goal in this project was to identify the structural intermediates that occur as cholate is added to PC vesicles to form a mixed micellar solution. It was clear from the changes in turbidity or scatter that the macromolecular assembly underwent one or more rearrangements before small mixed micelle formation (Fig. 1). It is assumed that the scatter profile depicted in Fig. 1 represents equilibrium or extremely long-lived metastable structures, because a very similar curve is generated by adding PC to discrete concentrations of cholate and waiting for up to 24 h: previous experiments indicate that the transitions approach completion at the rate of cholate addition used in the experiment shown. The shape of the scatter curve with increasing cholate concentration was similar for all PC concentrations (not shown), which made it possible to identify several discrete points that were assumed to represent a unique assembly of macromolecular structures. The position of these points was dependent on two factors, i.e., the aqueous or monomeric cholate concentration (C_{aq}) in equilibrium with the structures, and the molar ratio of cholate to PC in the structures (R_e). Using these relationships as a guideline, a set of samples was prepared including the ones indicated by the arrows in Fig. 1. These were examined by cryo-TEM.

Structures observed in the vesicular-to-micellar regions of the egg PC–cholate phase diagram

Egg PC forms bilayer structures when dispersed in aqueous solution at high water content. Examples of unilamellar vesicles prepared by two different protocols are shown in Fig. 2. Small unilamellar vesicles formed by extensive sonication were extremely small with diameters ranging from 15 to 40 nm (Fig. 2 *a*). There were a few multilamellar vesicles remaining in this preparation with many closely spaced lamellae. The cholate dialysis vesicles (DUV) are unilamellar spheres, most of which range in diameter from 40 to 60 nm, with an occasional larger or bilamellar vesicle (Fig. 2 *b*). The vesicles prepared by extrusion through nuclepore filters were larger (~100 nm in diameter), and many of these were multilamellar or appeared nonspherical (data not shown).

Vesicular structure was maintained after the addition of low levels of sodium cholate, but the morphology was altered (Fig. 3). The image shown in Fig. 3 *a* (5 mM cholate, 9 mM PC) was from the SUV preparation. Comparison of Fig. 3 *a* with Fig. 2 *a* showed a population of vesicles with diameters from 30 to 80 nm (mode ~45 nm) coexisting with the tiny starting vesicles. In another area of the same sample (Fig. 3 *b*), exceedingly large vesicles (200–400 nm in diameter) were observed coexisting with smaller vesicles. One vesicle appeared to be open (*X*) and another one (*Y*) appeared to have lost the normal integrity of the bilayer as indicated by its angular and rippled surface. Many bi- and trilamellar vesicles with widely spaced lamellae were seen in some images in this concentration region (not shown). These data indicated that cholate addition changed the energetically favored diameter of the vesicles (*a*), and suggested that the structures opened and closed to permit the formation of the loosely packed multilamellar vesicles (*b*).

The idea that cholate promoted transient but metastable openings was supported by images of vesicles containing openings (Fig. 4 *a*). The openings in the bilayer sheet must appear with either significant frequency or lifetime to be captured in the vitrification process. These were seen consistently at just slightly higher cholate concentrations than the images in Fig. 3. Surfactant-induced pores or holes in egg PC vesicles have been observed by

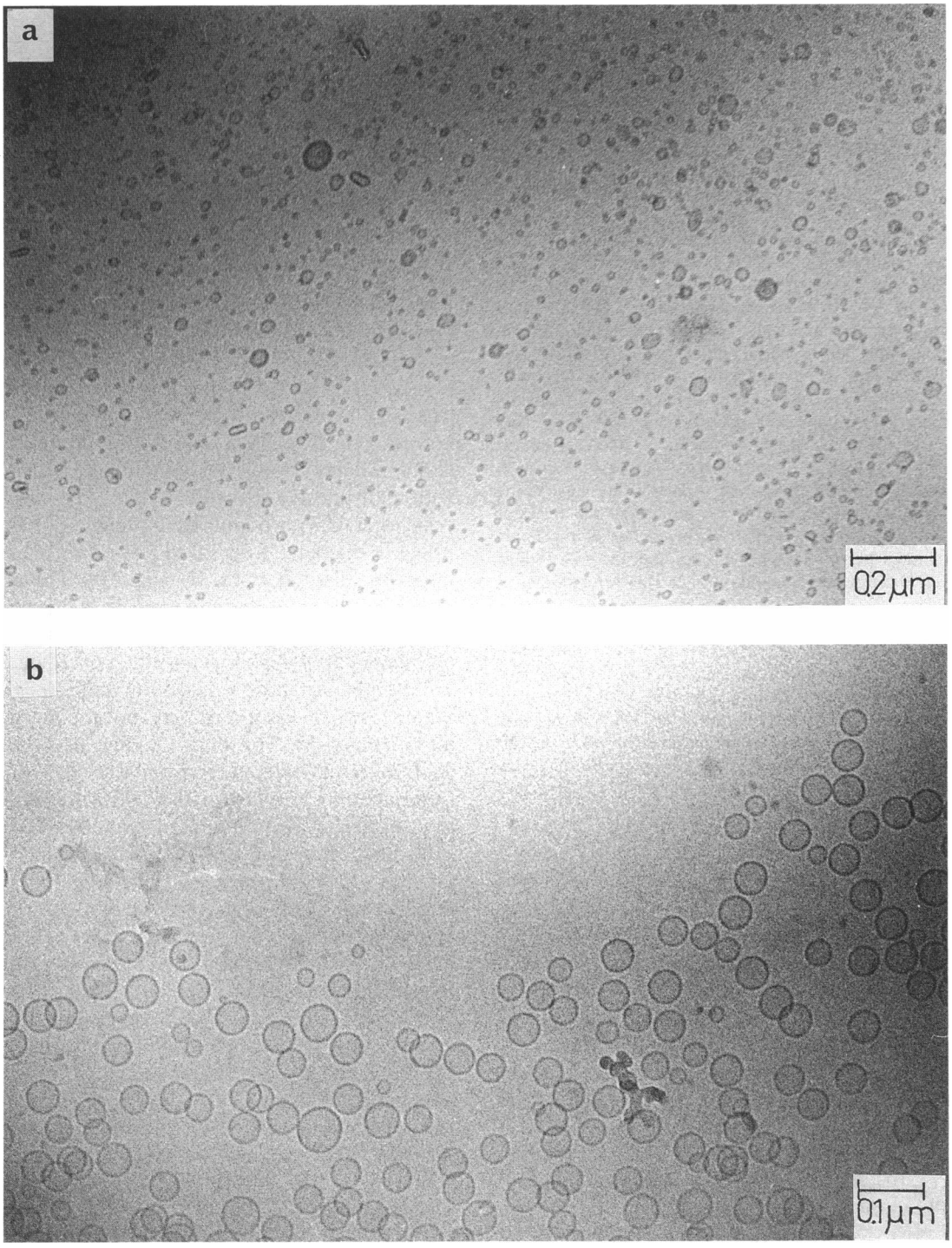


FIGURE 2 Two types of vesicle preparations imaged by cryo-TEM. (*a*) Sonicated egg PC forms small unilamellar vesicles. The mode diameter for this dispersion is ~20 nm. The preparation was not centrifuged and some multilamellar structures remain. (*b*) Egg PC vesicles prepared by dialysis from sodium cholate. These are unilamellar, spherical, and fairly uniform in diameter, with a range of ~40–60 nm.

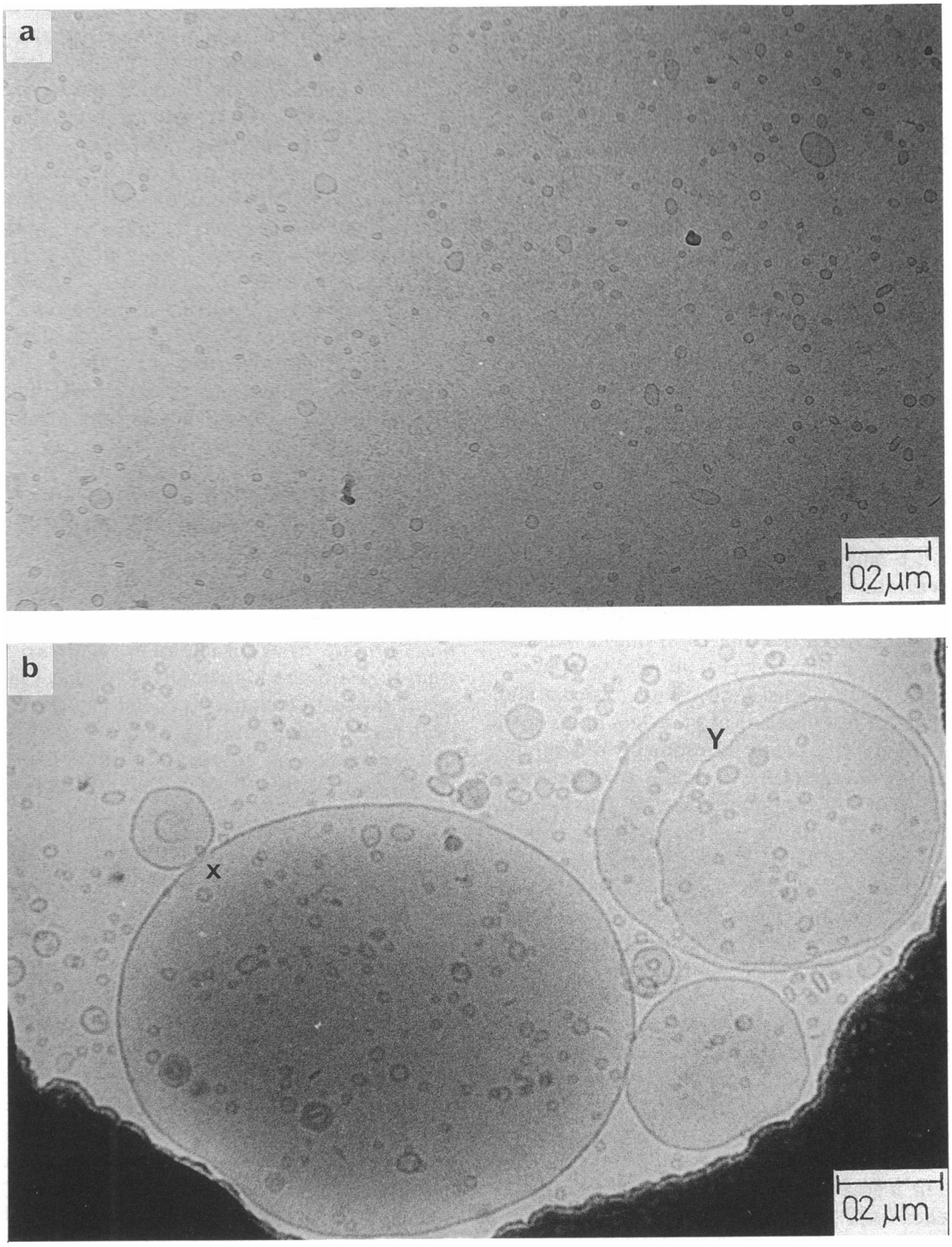


FIGURE 3 PC vesicles with very low levels of cholate. (*a*) Cryo-TEM image of a mixture of egg PC (9 mM) and cholate (6 mM) that was prepared by adding cholate to sonicated lipid vesicles. The diameter of the vesicles has increased 30–80 nm from the original sonicated preparation (cf. Fig. 2 *a*). (*b*) Some exceptionally large vesicles coexist with smaller vesicles similar to those observed in *a*. The vesicle at *x* appears to be open and the bilayer at *Y* is rippled in such a way as to indicate that the normal bilayer structure is perturbed.

cryo-TEM at low levels of the nonionic surfactants Triton X-100 and octyl glucoside (Edwards et al., 1989; Vinson et al., 1989).

Dense linear projections, patches of single lipid bilayers, and long flexible cylindrical structures coexisted with the open vesicles over a wide range of cholate concentrations (Fig. 4 *a*). The dense structures (*Z*) in Fig. 4 *b* must be edge-on views of the patches of membrane: an example of an oblique view (*V*) clearly showed that these dense linear images and the patches were the same structures. The very low electron density of the patches indicated that they were only one bilayer thick, whereas the high electron density of the shorter structures was consistent with these representing edge-on views of a membrane, which from that angle would be many molecules thick.

The bilayer patches had uneven edges, from which short cylindrical micelles projected (Fig. 4, *b* and *c*). The cylindrical structures appeared to arise from the edge of the patch: no images indicated cylinders from the central portion. Many cylindrical micelles evolved simultaneously from a single patch of bilayer. At somewhat higher concentrations of cholate (Fig. 5 *a*), the predominant structures observed were flexible cylindrical micelles. It was clear from the images of bilayer patches (Fig. 4) that the cylindrical micelles formed from material at the edges. This is consistent with the concept that cholate concentration is greater at the edges of an open bilayer sheet than in the center. Presumably this configuration is more energetically favorable than uniform mixing because the hydrophobic face of the cholate molecule can interact with the egg PC acyl chain region. This interaction would reduce hydrocarbon contact with the aqueous phase, while both the cholate polar head region and the relatively hydrophilic hydroxyl face are in contact with water. It is probable that patches of membrane detach from a vesicle along lines of high local cholate concentration.

The images of open vesicles, collapsed vesicles, bilayer patches, and cylindrical mixed micelles were observed over a range of relative cholate concentrations, forming a coexistence region of bilayer and micellar phases that corresponds to the region where the scatter profile is changing substantially (Fig. 1). As the cholate concentration increased, the proportion of cylindrical mixed micelles increased (Fig. 5 *a*). The cylindrical micelles appeared flexible due to the random bending in three dimensions. The dark "dots" observed in these images were probably end-on views of the cylindrical micelles, as well as spheroidal micelles. It is difficult to locate the ends of these micelles due to the extensive intertwining. However, from several micelles that could be measured, the lengths ranged from 100 to 300 nm or more, and their diameters were ~3–5 nm.

If enough cholate was added to a suspension of egg PC vesicles, the dispersion became clear, indicating that the structures were small. Fig. 5 *b* is an image of an almost clear mixture of egg PC and cholate (9 and 11 mM, respectively). Most of the structures appeared as uniform tiny dots, indicative of spheroidal micelles. The diameters cannot be resolved accurately but are ~4 nm. These are similar to images of spheroidal mixed micelles (SMM) at higher relative cholate concentration.

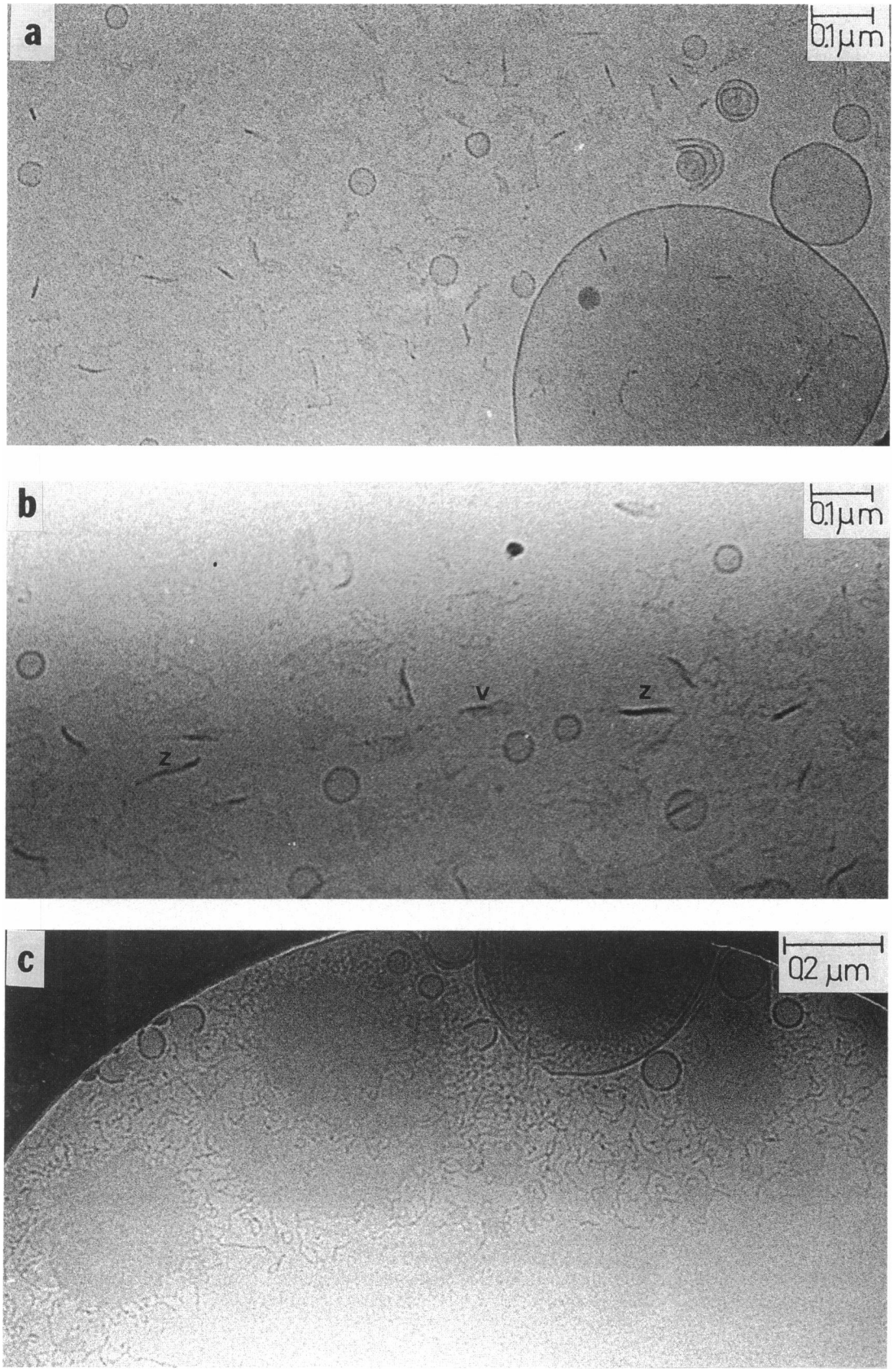
Correlation between the observed structures and the cholate–egg PC phase diagram

As noted in Fig. 1, the transition from vesicles to spheroidal micelles is readily monitored by observing the turbidity of the sample by scattered light. The transition structures are observed primarily in the region where the scatter changes (i.e., the small hump and large peak region, between R_e 0.25 and 0.67; data not shown), and the spheroidal micelles were the only structure as the solution cleared (R_e of 0.67 and greater).

DISCUSSION

Cryo-TEM, a technique uniquely suited for observing structures in complex fluids such as mixed micellar dispersions, was used to identify the structural changes associated with the addition of the surfactant sodium cholate to PC vesicular dispersions. A structural richness not expected from earlier studies was revealed, and at least one new transitional state was identified, namely, the formation of cylindrical (threadlike) micelles from the edges of lamellar sheets. The structural changes correlate with changes in the turbidity of the sample, and as we will discuss below, are consistent with previous

FIGURE 4 Membrane patches with cylindrical mixed micelles emerging from the periphery. (*a*) Large vesicles coexist with short dense projections and en face views of pieces of bilayer. Some of the vesicles appear to have openings (6 mM cholate, 9 mM PC). (*b*) Vesicles and patches of bilayer seen on edge indicated by *z* and en face. The structure at *v* appears to be a twisted piece of bilayer as indicated by a linear electron-dense region appearing to unfurl into a piece of bilayer. The projections emerging from the patches appear to be the origin of cylindrical mixed micelles (7.25 mM cholate, 9 mM PC). (*c*) Large patches of membrane with many cylindrical mixed micelles emerging from the edges simultaneously (6.5 mM cholate, 9 mM PC).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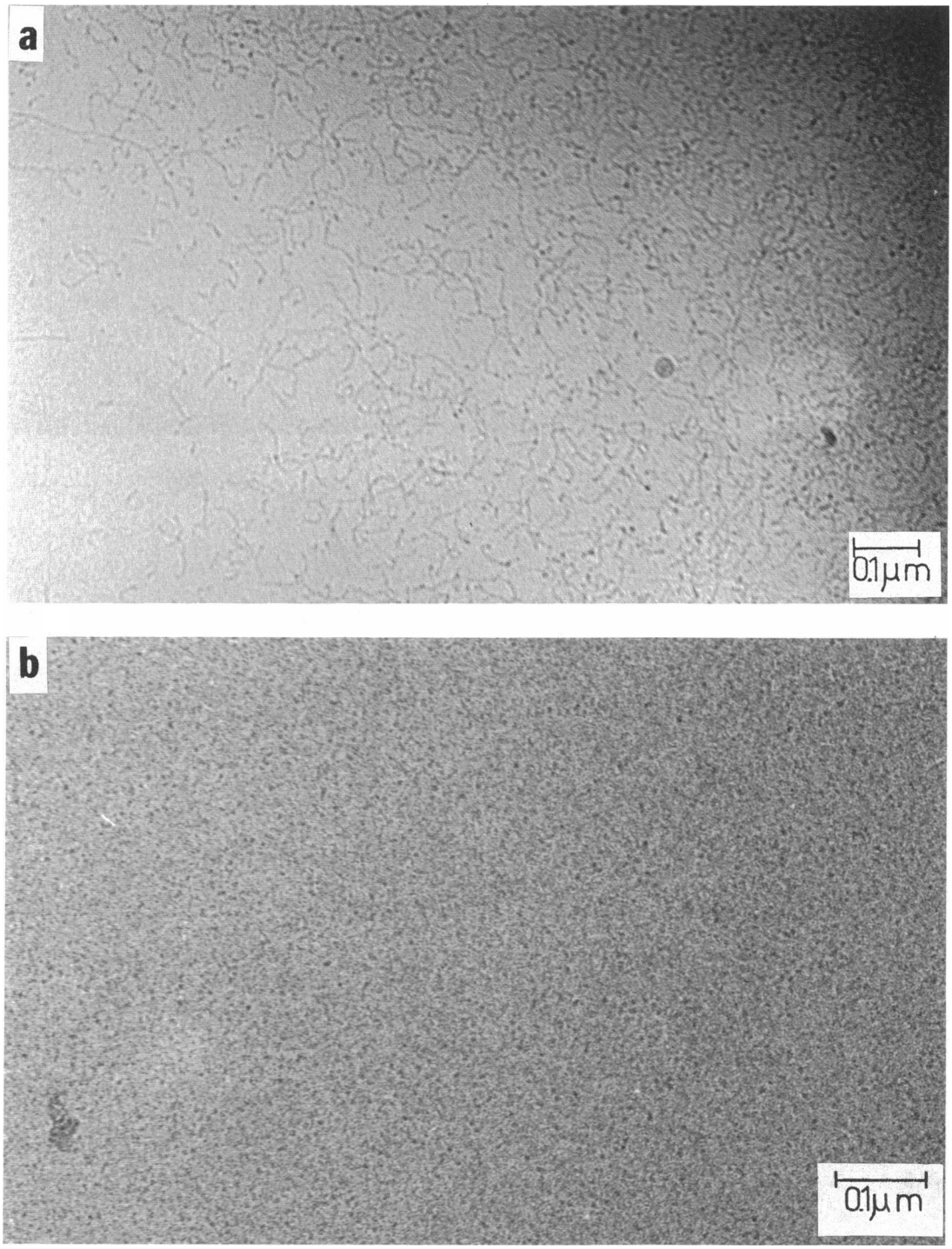

FIGURE 5 Cholate–egg PC mixed micelles. (*a*) Cylindrical mixed micelles observed by cryo-TEM at cholate concentrations of 8 mM cholate and 9 mM egg PC. The images indicate that the micelles are flexible and can bend in three dimensions. (*b*) Small mixed micelles from a relatively clear preparation of egg PC and cholate just before the break shown in Fig. 1 (11 mM cholate and 9 mM PC).

probes of the transition region, but not with all previous interpretations of the data. Direct observation by cryo-TEM demonstrated that flexible cylindrical micelles are the micellar intermediate between vesicles and small spheroidal micelles: these structures are similar to the so-called "rod-like" micelles proposed by Hjelm et al. (1988, 1990), and are not like the mixed discoidal micelles of increasing diameter previously described (e.g., Mazer et al., 1980; Müller, 1981; Schurtenberger et al., 1985; Carey, 1988). Moreover, in general, the structures observed in PC-cholate mixtures are very similar to those seen in the octyl glucoside–PC system despite the vast differences in R_e at each transition and the surfactant chemistry.

Vesicle–micelle transition: pore formation, bilayer sheets, and cylindrical mixed micelles

An increase in permeability to aqueous solutes by a pathway that acts like a water-filled channel is observed at low cholate concentrations (Abramson and Shamoo, 1979). Leakage of small molecules occurs at lower total cholate than leakage of larger molecules, indicating transient pores (Schubert et al., 1986). Small molecules such as raffinose and carboxyfluorescein begin to leak at an R_e of ~ 0.1 (Schubert et al., 1986; Paternostre et al., 1988). The relatively large openings observed in this study (Fig. 4) may be considered a continuum of this pore-like phenomenon. Similar pores are observed with the addition of Triton X-100 or octyl glucoside to phospholipid vesicles (Edwards et al., 1989; Vinson et al., 1989).

Breaks in the vesicle membrane occur at discrete spots and result in large pieces or sheets of bilayer membrane (30 nm or greater in diameter) that relax from a curved to a flat configuration (Fig. 4). These observations corroborate the suggestion of sheets from small angle neutron scattering (Hjelm et al., 1990). The emergence of cylindrical mixed micelles from the rims makes it seem probable that the edges of these sheets are "stabilized" by a higher cholate concentration than that of the central part of the bilayer, as was suggested for the smaller diameter disk models (Small et al., 1969; Mazer et al., 1980). Furthermore, these sheets appear to be the intermediate between vesicular and cylindrical micellar structures because the cylindrical micelles originate from the sheets' edges (Fig. 4). Many micelles may peel off simultaneously, giving the appearance of a bilayer unraveling from its edge. The peculiar distribution of polarity on the cholate molecule with a polar/charged headgroup and one relatively polar face makes it likely that satisfying the hydrophobic requirements of the molecule will make up the entropic difference associated with its high concentration on the edge compared with the center of a disk. Although we cannot definitively rule out the possibility of a similar structure in other surfactant systems, it was not seen in octyl glucoside–PC mixtures (Vinson et al., 1989). Instead, the vesicles in that system appear to suddenly form cylindrical micelles directly. From molecular structure and composition arguments, this difference makes sense, as octyl glucoside has a single polar head group and an eight carbon acyl chain that can lie parallel to the phospholipid acyl chains.

The cylindrical mixed micelles are clearly formed from the edges of the bilayer sheets, indicating that this is the structural pathway from the lamellar to the micellar states for this mixture of amphiphiles. This particular pathway is not obviously reversible. Thus, an important remaining issue is to determine how the cylindrical mixed micelles coalesce to form lamellar sheets and vesicles.

Cylindrical mixed micelles

Cryo-TEM images show long cylindrical micelles. These are 200–300 nm in length and similar in thickness to that of a phospholipid bilayer (Fig. 5). In general, this observation is consistent with the rods proposed from small angle neutron scattering of 16 nm to "infinite" in length with a 2.7-nm radius (Hjelm et al., 1988; 1990). Although Hjelm and co-workers describe these micelles as rods, it is important to note that the structures observed here must be very flexible, since they bend in three dimensions (Fig. 5). A single structure can be seen to have a curve in the plane of the photomicrograph and also turn 90° upward with its ends in the image plane. The apparent flexibility suggests an uneven and fluid distribution of the two molecular components. The lipid–lipid interactions in the cylindrical micellar region appear bilayer-like on the basis of resonance energy transfer efficiency between lipid probes (Walter, A., manuscript in preparation), thermal activity (Spink and Manley, 1990), and ^{31}P-NMR (Paternostre et al., 1988). These parameters would be similar to bilayer lipids if the lipid was organized in the modified cylindrical packing as proposed for the hexagonal phase of PC and cholate (Ulmius et al., 1982). The proposed radial orientation of lipid is possible due to the effect of a very small amount of cholate on the optimal radius of curvature. It is worth noting that similar structures in the octyl glucoside–PC system require an R_e of ~ 2, whereas the R_e for cholate-PC cylindrical micelles is ~ 0.4.

Small spheroidal mixed micelles

In the region where the light scattering decreases precipitously in cholate-PC mixtures, small mixed micelles are formed. In addition, based on cryo-TEM, this boundary represents the complete transformation of the mixed micellar population from cylindrical to small spheroidal micelles as shown by comparing Fig. 5, *a* and *b*. The dimensions of these small mixed micelles could be consistent with the interpretation of a distinctive small spheroidal micelle 6.2 nm in diameter as determined by small angle x-ray analysis (Müller, 1981), or with the 4–7-nm diam disks suggested by Small et al. (1969). In the small mixed micellar region, hydrodynamic radii (R_h) ranging from 2–3 nm at high cholate concentrations to 12–20 nm at the phase boundary, have been reported (Schurtenberger et al., 1985; Mazer et al., 1980). The latter higher values certainly do not correspond to the observed spheroidal micelles.

One explanation for these morphometric differences could be the type of bile salts, tauro- and glycocholate vs. cholate. However, no disks were reported in glycocholate-PC mixtures examined by small angle neutron scattering (Hjelm et al., 1988, 1990). It is not clear that the optical evidence for disks is very strong. The choice of a disk structure over a rod-like shape was possible only by introducing considerable polydispersity into the model (Mazer et al., 1980). When the relative cholate and PC composition data were combined with Rh determination by gel filtration chromatography of smaller structures (high R_e) was equally consistent with space filling models of short rods (10–20 nm long) or disks (Nichols and Ozarowski, 1990).

Ambiguity in defining structures from scattering data may explain the much larger discoidal micelles reported in the coexistence region between R_e of ~0.3 and 0.7 (Mazer et al., 1980, Müller et al., 1981). Several shapes were used to analyze the QELS and x-ray data cited, but the only rod or prolate structures tested were straight; flexible cylinders were not considered. Rh of 20 nm is equivalent to a disk diameter of 50 nm or a straight rod 120 nm long and 5 nm wide (following the Perrin equations). The flexible cylindrical micelles observed are at least 200 nm long, but do not extend fully due to the amount of bending. In terms of material volume, the cylindrical micelles are close to the previously reported disks. Thus, it is plausible that the discrepancy in the structural interpretation is due to the ambiguity of indirect analysis.

It is also possible that some of those very large discs in the coexistence region could be equivalent to the sheets of bilayer seen in Fig. 4. The molecular structure of the sheets is likely to be that proposed for the mixed disk model. However, the sheets observed in Fig. 4 are clearly not part of a continuum of small to large disk micelles and should be categorized as lamellar rather than micellar lipid.

Conclusions

We have corroborated by scattering and cryo-TEM, previous estimates of the phase boundaries marking the vesicular–micellar transition regions for the highly hydrated mixed cholate–egg PC system under conditions to make this mixture a reasonable physiological model for bile. Several controversies over the structure of the macromolecular aggregates in this region were examined. We show that, at the upper boundary, all the structures are small spheroidal mixed micelles. We identify a new micellar structure for this system, a flexible cylindrical mixed micelle, and propose that it is the logical extension of the hexagonal phase rods in the highly hydrated region of the phase diagram. The initial step in vesicle solubilization is associated with pore formation and reorganization of the bilayer lipid. The region of transient pore formation is followed by the formation of large bilayer sheets. It is assumed that the hydrophobic edges of these sheets are stabilized by a band rich in cholate molecules. The high edge activity of cholate then induces the formation of cylindrical mixed micelles that appear to "peel" from the bilayer sheet. With a clear picture of the structural evolution in this surfactant–phospholipid system, it is possible to begin to model the formation of vesicles, the behavior of protein, and the effects of additional amphiphiles such as cholesterol in this important biochemical and physiological system.

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