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ARTICLE

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Cubic topology in surfactant and lipid mixtures

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Abstract Ternary systems of palmitoyl-oleoyl-phosphatidylcholine (POPC) and the non-ionic surfactant $C_{12}EO_2$ (di-ethylene-oxide-mono-dodecyl-ether) in water have been studied with optical microscopy, NMR, DSC and X-rays from ambient temperatures to 45 °C. Below 29 °C the system is in the lamellar liquid crystalline state. Between 30 and 32 °C it transforms into a cubic Ia3d structure which converts into the cubic Pn3m phase at 39 °C. The transitions are fully reversible. An epitaxial relationship between all three phases was found, which is an elegant and convenient way to rearrange molecules from lamellar bilayers to a network of curved surfaces. The Ia3d (Q^{230}) to Pn3m (Q^{224}) transition occurs without measurable enthalpy change. This, together with the metric relation of 1.60 between the cubic lattice constants is strong evidence for a Bonnet transformation, where the structural changes occur without change in curvature. The potential significance of the cubic phases as intermediate structures for biological processes, e. g. transport across a bilayer or fusion of membranes, are discussed.

Key words Phase transition · NMR · DSC · X-ray diffraction · Bonnet Transformation

Introduction

Diacylphospholipids are the major components of cellular membranes. They usually form bilayers when dispersed in water and therefore are commonly used as model membranes. As a consequence of their amphiphilic properties the polar head groups of the lipids prefer the water interface while the hydrocarbon chains face inwards the bilayer. An important feature of these molecules is their amphotropic behaviour (lyotropic and thermotropic polymorphism) and the interactions at the membrane-water interface.

Incorporation of additives into these membranes together with temperature variation are the most common ways used to induce modifications in the membrane properties, such as hydration, stability and morphology (see e.g. Cevc and Marsh 1987; Rand and Parsegian 1989; Israelachvili and Wennerström 1992; Mädler et al. 1994). Similarly non-ionic surfactants of the oligo-(ethylene oxide)-alkyl-ether type (C_nEO_m) also show a rich variety of phases with different structure and morphology depending on the conditions they are submitted to (Mitchell et al. 1983; Funari et al. 1992, 1994). They are convenient additives because they can modify the interactions at the membrane surface without introducing electrical charges in the system, such as in the case of salt solutions. In some circumstances these modifications are so significant that phases that otherwise would not have been formed by either component in water can be induced. An example of this induction in mixtures is the formation of a hexagonal phase in the POPC/$C_{12}EO_2$/^2H_2O)[1] system (Fig. 1).

The ternary phase diagram of POPC/$C_{12}EO_2$/^2H_2O has been investigated previously (Funari and Klose 1995) in order to establish the range of stability of the lamellar phase as well as the structure of the surrounding non lamellar phases. From them, inverted hexagonal and cubic ones were identified. There is an increasing interest in these non lamellar phases because they have mesogenic units with highly curved surfaces, although the average curvature in the unit cell can be zero, as in cubic phases. Their biological importance has been established although the mechanisms where they take place are not yet fully known. In

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[1] POPC – 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine $C_{12}EO_2$-Di-ethylene-oxide-mono-dodecyl-ether

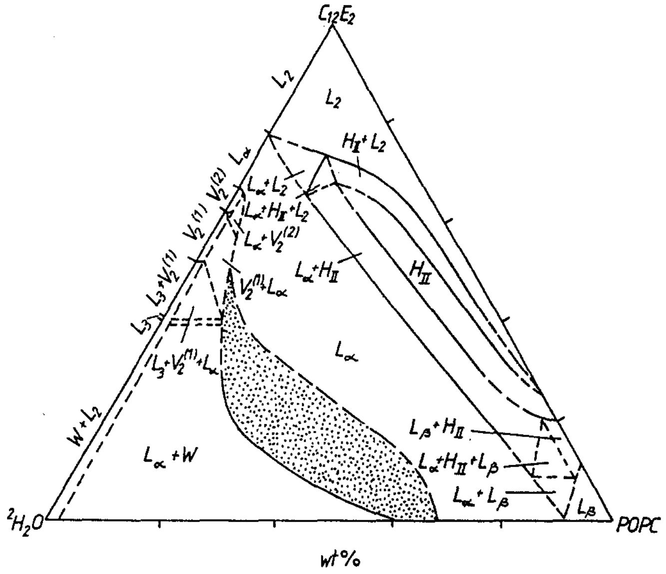

Fig. 1 Phase diagram of the ternary system POPC/$C_{12}EO_2$/^2H_2O at room temperature. Each side displays the composition of each component in weight %. The marked area corresponds to the L_α phase, although the ^2H NMR of ^2H_2O does not correspond to a Pake powder. *Dashed lines* represent phase boundaries which were not precisely determined (after Funari and Klose 1995)

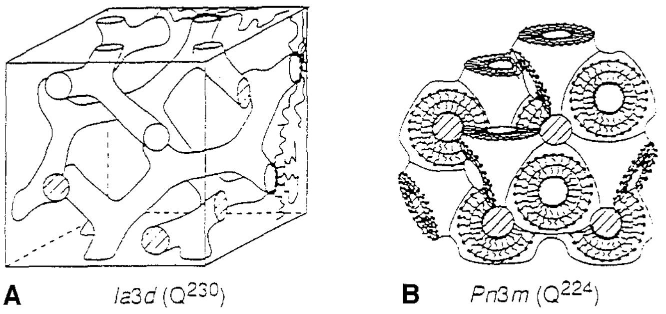
A Ia3d (Q^{230}) **B** Pn3m (Q^{224})

Fig. 2A, B Structure of two inverted bicontinuous cubic phases. **A** Gyroid type of space group Ia3d (no. 230). **B** Double diamond type of space group Pn3m (no. 224). Taken with permission from Chung and Caffrey (1994)

particular, cubic phases play an important role in physiological mechanisms as metastable intermediate structures in some metabolic processes such as fat (triglycerides) digestion into monoglycerides at acidic pH (Mariani 1988), or membrane fusion (Lindblom and Rilfors 1989). Patton (1981) observed its formation during digestion of triglycerides by pancreatic lipase. Luzzati et al. (1993) suggested that the Q^{227} cubic phase has properties of "patch-the-puncture" repairing the damage of lipolytic agents.

Cubic phases have been denoted "viscous isotropic" phases because they are non birefringent and consequently appear isotropic under the polarising microscope. The ^2H NMR spectra of samples prepared using ^2H_2O show a single and sharp Lorentzian line at zero ppm for most of them. When the amphiphile contains ^{31}P, as in the POPC lipid, the respective ^{31}P NMR spectra also show a single line at zero ppm with reference to H_3PO_4 in aqueous solution. However, X-ray diffraction patterns give clear evidence of a three-dimensional structure. Bicontinuous cubic phases have been called Q_α (oil in water) or V_2 (water in oil). They can belong to different space groups such as Ia3d or Pn3m. The Ia3d cubic phase belongs to a body-centred space group and is composed of rod-like bilayer aggregates joined three by three to form two interwoven but otherwise independent three-dimensional networks. The Pn3m phase has a primitive cubic lattice whose structure is built by rods connected four by four at tetrahedral angles (Fig. 2), forming two unconnected but also interwoven diamond lattices (Luzzati and Spegt 1967; Luzzati et al. 1968a, b; Hyde et al. 1984; Mariani et al. 1988; Chung and Caffrey 1994; Erbes et al. 1994; Czeslik et al. 1995).

These structures have also been described as bilayers centred on an *infinite periodic minimal surface* (IPMS) (Larsson 1986; Seddon et al. 1993). The Ia3d structure has been associated with IPMS of the gyroid type C_g, while the Pn3m with the diamond C_d (Larsson, 1986). These IPMSs have the property of showing zero mean curvature, i.e., concave and convex curvatures forming the rods balance each other in every point (Fontel 1990) and, therefore showing isotropic properties.

We have studied the transition from lamellar to cubic phases, determined some geometrical parameters and discuss the bilayer's curvature in these phases. Moreover, we propose that the topology of intermediate states of biological processes such as cell fusion can be described by cubic structures.

Material and methods

POPC was purchased from Avanti Polar Lipids Inc., Alabaster, USA, with nominal purity higher than 98%, $C_{12}EO_2$ from Nikko Chemicals Co., Japan with purity >99% and 99.9% isotopic pure ^2H_2O was supplied by Isocommerz, Leipzig, Germany. These chemicals were used as received. The samples were prepared by weighing the desired amount of each component into glass tubes, sealed, mechanically agitated for homogenisation and stored at 4 °C to avoid POPC decomposition. Prior to the experiments the samples were left at room temperature for at least two days.

The nuclear magnetic resonance measurements were performed with the sample in the same tubes in which they were prepared in order to avoid contamination or changing composition due to water absorption or loss. The NMR spectra were recorded on Bruker spectrometers MSL 300 and 500 with magnetic fields of 7.04 and 11.74 T, respectively.

Phase identification using ^{31}P NMR is applicable only if no significant changes in the lipid head group conformation occur between different phases (Seelig 1978; Tilcock et al. 1986; Thayer and Kohler 1981). The structure and morphology of the mesogenic units of a phase are reflected in the line shape of the spectrum owing to the diffusion of the lipid molecules within the units in each phase. The deuterium NMR splitting of ^2H_2O is a consequence of orientational restrictions of these water molecules interacting with the surface of the mesogenic units. It depends upon the molar fraction of bound water molecules. The bound molecules are in a fast exchange with free ones and their diffusion properties are different under these two conditions (Seelig 1977; Mantsch et al. 1977; Blackburn and Kilpatrick 1992) which are reflected in the line shape of the ^2H NMR spectra.

Results from differential scanning calorimetry (DSC) were obtained using a Perkin-Elmer DSC 7 in heating and cooling modes with scan rates between 0.1 and 1 K/min. Because of the low enthalpies associated with these transitions special baseline correction and noise limiting procedures were necessary. The determination of the phase transition temperature and enthalpies followed the procedure described recently (Mädler et al. 1994).

X-ray diffraction measurements were performed on beam line X 13 of the EMBL outstation at DESY operating at a wavelength of 0.15 nm (For details see Rapp 1992). One-dimensional diffraction patterns were taken with a sealed linear detector (Gabriel 1977) as part of the standard data acquisition system (Boulin et al. 1988), which is PC controlled. Two dimensional patterns were recorded on image plate and processed with a Fuji Bas 2000 scanner. Data were analyzed using the in-house software SCACO for the 2 D patterns or the interactive data evaluation program OTOKO (Boulin et al. 1986) for 1 D data. The interplanar distances d were calculated from the reciprocal spacing $s = 1/d = (2/\lambda)\sin\theta$ (2θ: scattering angle, λ: wavelength). The instrument was calibrated using dry collagen from a rat tail tendon which has a repeat distance of 65 nm.

The samples were contained in sealed capillaries of 1 mm diameter. To eliminate artefacts due to partial orientation of the samples each capillary was spun at about 1000 rpm. During heating and cooling scans at 1 °C/min the samples were exposed for 3 s followed by a 17 s period where they were protected from irradiation by a solenoid driven shutter close to the sample.

Results and discussion

Ternary systems with slightly different compositions (w/w) of H_2O (46.3), $C_{12}EO_2$ (46.0), POPC (7.6) and ^2H_2O (45.8), $C_{12}EO_2$ (46.0), POPC (8.2) have been studied by a combination of different techniques in the temperature range from ca. 15 °C to 50 °C. The sample compositions were chosen based on the phase diagram (Funari and Klose 1995, see Fig. 1) in order to show the lamellar and cubic two phase region. They had surfactant to lipid molar ratios $R_{S/L} = 17$ and 16, respectively. The following results were similar for both sample compositions.

In the polarizing microscope the samples were birefringent at room temperature showing an oily streak texture which is characteristic of the lamellar L_α structure. Application of slight pressure showed a high mobility of the sample indicative of low viscosity. Upon heating the samples became non birefringent (isotropic) at 32 °C and their viscosity increased substantially. Slow cooling gave it a "pale white" appearance, its birefringence and low viscosity were readily restored at room temperature.

From the increasing viscosity observed when the samples were heated one can eliminate the possibility that this isotropic phase is L_2 or L_1 micelles. The binary phase diagram of $C_{12}EO_2/^2H_2O$ (Conroy et al. 1990) shows that samples with surfactant concentration similar to ours should have bicontinuous inverted cubic (V_2) properties.

NMR spectra of ^2H and ^{31}P taken at 29 °C indicated the coexistence of two phases with lamellar and cubic structures. The ^2H NMR spectra of ^2H_2O showed a doublet with a splitting of 1.2 kHz indicative of a lamellar phase and a single line at zero Hz indicative of a cubic phase. The ^{31}P NMR spectra showed a single line at zero ppm (cubic phase) and a line at –17 ppm which is attributed to an oriented lamellar phase. On heating the sample to 32 °C only a single line remained in both spectra (^2H and ^{31}P NMR) which is evidence of a transition to a single cubic phase. Upon cooling the NMR spectra indicated again the lamellar plus cubic biphasic region at 29 °C.

The phase transition temperatures and their enthalpies were measured with DSC. Only one sharp and reproducible peak with its maximum at 32 °C was detected when heating and cooling between 25 to 45 °C. No significant hysteresis was observed in cooling or heating at scan rates between 0.1 to 1 K/min. The transition temperature does not change significantly which is an indication that it occurs under conditions where each of the two phases are in thermodynamic equilibrium. The enthalpy of 1.06 kJ/mol is typical for a transition from L_α to a non-lamellar phase (Marsh 1990; Mädler et al. 1994). The slight DSC peak asymmetry may be associated with a rearrangement of water molecules (large heat capacity c_p) interacting with the membrane surface during the phase transition from L_α to cubic.

A sequence of X-ray diffraction patterns obtained during a temperature scan is shown in Fig. 3. Despite the high amount of water present in the samples they have a remarkable number of peaks, which allows phase identification. At 28 °C a diffraction pattern with first and second order reflections typical for a lamellar structure with an interplanar distance of 5.4 nm is observed. Upon heating reflections corresponding to the Ia3d cubic phase evolve, initially as a biphasic region. Further heating leads to the Pn3m phase, also via a two phases region, although with a much smaller temperature interval. The intensities and respective indexing of the reflections are summarized in Table 1. Different cubic phases could be unambiguously determined by the ratios of the reciprocal spacings of the observed reflections and by the absence of certain reflections. Detailed analysis of the diffraction patterns taken at 35 °C shows that the observed reflections have spacings with ratios of $\sqrt{6}:\sqrt{8}:\sqrt{14}:\sqrt{20}:\sqrt{22}$, corresponding to the (211), (220), (321), (402) and (332) planes respectively. The appearance of reflections with this sequence is typical for an Ia3d cubic phase (Lindblom and Rilfors 1989, Seddon 1990). Above 39 °C a different structure appears

Table 1 Structural parameters derived from X-ray diffraction data. Temperature, phase structure, lattice parameter **a**, lattice planes, interplanar distance corresponding to the diffraction peaks and their respective intensities are summarized. *vvs* = very very strong; *vs* = very strong, *s* = strong and *w* = weak

Temp./°C	Phase	*a*/nm	hkl	dist./nm	intensity/a.u.
28	L_α	5.4	001	5.4	vs
			002	2.6	s
33	Ia3d	15.0	211	6.2	vvs
			220	5.3	vvs
			321	4.1	w
			400	3.8	w
			402	3.4	s
			332	3.2	w
39	Pn3m	9.3	110	6.6	vvs
			111	5.4	vvs
			200	4.7	s
			211	3.8	vs
			220	3.3	w
			221	3.1	w

showing six reflections with spacing ratios $\sqrt{2}$:$\sqrt{3}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{9}$ which are associated with the (110), (111), (200), (211) (220) and (221) planes of a Pn3m structure. The two cubic structures are illustrated in Fig. 2. The thermal evolution of the diffraction patterns is most clearly seen in Fig. 4. Note that at $s = 1/d = 0.19$ nm^{-1} there is one strong reflection present in each phase.

The positions of the observed reflections of the two lattices plotted vs. $(h^2+k^2+l^2)^{1/2}$ are shown in Fig. 5. For both phases they fall onto straight lines passing the origin which proves that the assignment is correct. Each phase observed shows a line whose slope is proportional to the lattice parameter. The Ia3d cubic phase appearing at lower temperature has a lattice parameter a = 15.0 nm, and the length of a rod was calculated according to Luzzati et al. (1968 b) to $l = a/\sqrt{8} = 5.3$ nm. Considering that there are 24 rods per unit cell, we calculated their radius to be

$$r = (a^2\sqrt{8}/24\pi)^{1/2} = 2.9 \text{ nm}.$$

In the Pn3m cubic phase the lattice parameter is 9.3 nm. The length of a single rod can be calculated using $l = a\sqrt{3}/2 = 8.1$ nm. Knowing that there are 4 rods per unit cell the diameter of each one can be calculated as

$$r = (a^2/2\pi\sqrt{3})^{1/2} = 2.8 \text{ nm}.$$

One should note that the diameter of the rods is similar in both phases, i. e., the transition between the two cubic structures is primarily a spatial reorganization of the rods where their length changes but the hydrophobic and hydrophilic cores remain unaltered.

The sequence of phases and transition temperatures which were observed in the X-ray diffraction scans (Fig. 3 and 4) can be summarized as follows:

$$L_\alpha \xleftrightarrow{29°C} L_\alpha + \text{Ia3d}(V_2) \xleftrightarrow{32°C} \text{Ia3d} \xleftrightarrow{38°C} \text{Ia3d} + \text{Pn3m} \xleftrightarrow{39°C} \text{Pn3m}(V_2)$$

Although neither the results from DSC nor from NMR measurements indicated a further phase transition above 32 °C, the X-ray diffraction data clearly show two different cubic structures. The transition from the lamellar L_α to the cubic Ia3d structure is much wider in temperature than the Ia3d to Pn3m cubic to cubic transition occurring within one degree. It is adiabatic since there was no DSC peak or Δc_p shift observable at 39 °C. As shown in Fig. 3 substantial changes and reorganization of the mesogenic units occur during this thermally narrow transition. It is consistent with a Bonnet transformation in the system where no change in total curvature occurs and which proceeds without heat exchange (Hyde et al. 1984; Larsson 1985). It is most likely due to a reorganization of the rods resulting in a structure with lower 'rod density' in the unit cell, even though the volume of the unit cells between the two cubic structures decreases by a factor of four. For the Ia3d we obtain $24/a^3 = 7 \cdot 10^{-3}$ rod/nm^3 while for the Pn3m we obtain $4/a^3 = 5 \cdot 10^{-3}$ rod/nm^3.

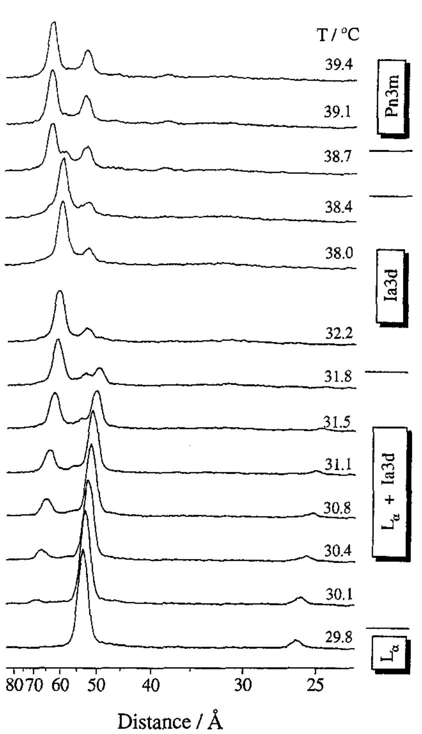

Fig. 3 X-ray diffraction patterns of a "powder" sample at different temperatures during a heating scan and the assignment to the respective phases. Sample composition in wt%: $^2H_2O(45.8)$; $C_{12}EO_2(46.0)$, POPC(8.2), $R_{S/L} = 16$

The transition from L_α to cubic Ia3d via a biphasic region has also been observed by Rançon and Charvolin (1988) in the $C_{12}EO_6/^2H_2O$ binary system. An epitaxial relationship between the (001) plane of the L_α phase and the (211) plane of the normal Ia3d phase was shown. Upon further cooling a hexagonal phase H_α evolves which again has an epitaxial relation with the cubic one. Based on the finding that planes exist with the same spacing in all three phases, i. e., $d_{001}(L_\alpha) = d_{211}(Q_\alpha) = d_{10}(H_\alpha)$ they concluded that these planes have a high density of matter which are the characteristics of an epitaxial relation.

The transition between cubic phases belonging to the space groups Ia3d and Pn3m has been observed by Caffrey

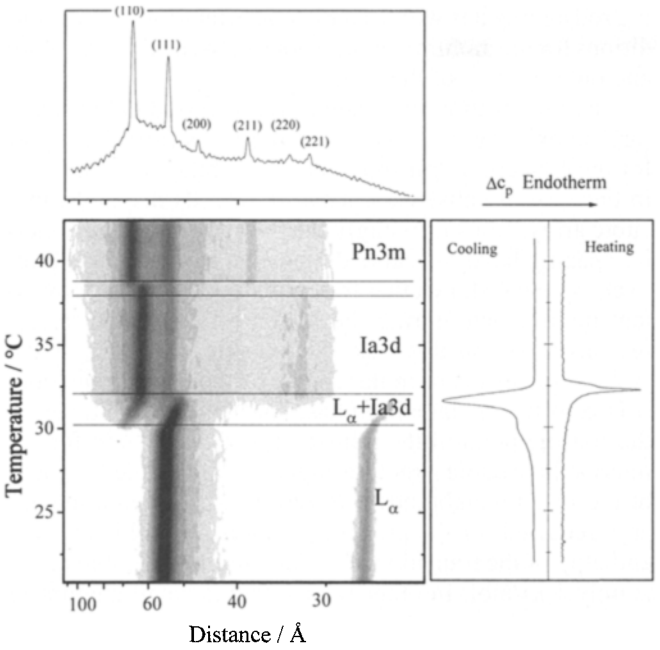

Fig. 4 Contour plot of the 1 D X-ray diffraction patterns obtained during an entire heating scan from 18 to 45 °C at 0.5 °C/min. There is one reflection in either phase which arises from an interplanar distance of 5.4 nm and, thus showing the epitaxial relation between the respective lattices. A sector integration from a 2 D powder diffraction pattern of the Pn3m cubic phase obtained at 46 °C on image plate is presented on the top. The DSC graphs shown on the right display only the L_α to Ia3d phase transition. No sign of a transition is seen at 39 °C where the Ia3d cubic phase transforms to the Pn3m phase. This adiabatic transition is evidence for a Bonnet transformation. Sample composition (in wt%): H_2O(46.3), $C_{12}EO_2$(46.0), POPC(7.6), $R_{S/L}=17$

(1987) studying monoacylglycerides and also by Larsson (1988) in the sunflower oil monoglyceride-water system. The phase diagram shows that this transition is concentration driven. It occurs at ~38 wt% H_2O and is almost temperature independent in the range between 35 and 55 °C. Upon increasing water content it transforms from Ia3d to Pn3m. However, no biphasic region was shown although it would be expected. The phase diagram shows that upon heating the Pn3m or Ia3d phases they transform to hexagonal phases. Evidence for an epitaxial relation between the different structures has also been found since the spacing of the (211) plane of the Ia3d and the (111) plane of the Pn3m phase are very close to that of the first diffraction peak of the hexagonal phases.

A similar situation occurs in the system we have studied. It is shown in Fig. 3 and 4 that all phases observed give a diffraction peak at $s = 1/d = 1/5.4\ \mathrm{nm}^{-1}$, corresponding to the same interplanar distance and thus relating the (001) plane of the L_α to the (220) of the Ia3d and the (111) of the Pn3m phases, i.e., $d_{001}(L_\alpha) = d_{220}(\mathrm{Ia3d}) = d_{111}(\mathrm{Pn3m})$. This observation shows that also in this system an epitaxial relationship exists between different phases. It is interesting to note that in the samples we have measured no hexagonal phase has been observed and hence, such an epitaxial relationship can also occur in the absence of a hexagonal phase. In the phase transition between the two cubic phases the rods' (cylindrical mesogenic units) spatial distribution are the necessary condition for the occurrence of such a relationship.

The epitaxial relation described by Rançon and Charvolin (1988) during phase transitions could be understood in terms of hydrophobic regulation (oil in water), where the thickness of the paraffinic core is a regulation factor. For example, in the $C_{12}EO_6$ system it is represented by the diameter of the rods from the hexagonal and cubic phases and the lamellar thickness. In our case it corresponds to the relation between the bilayer thickness and the hydrophobic core surrounding the rods in the different cubic phases. Because these phase transitions relate planes with high mass density it suggests that these transitions should be thermally narrow or involve a low enthalpy due to a smaller mass transport when compared with transitions where this relationship is not present. Such an epitaxial relation of the neighbour phases would facilitate reversibility of the transition and therefore no or only a small hysteresis occurs. An example where such relation is not present are the lamellar–cubic–hexagonal phase transitions in DOPE (Shyamsunder et al. 1988; Erbes et al. 1994) which are not readily reversible and extend over a large temperature range.

Figure 4 shows that in the L_α+Ia3d two phase region the spacing corresponding to the most intense peak decreases for both phases. In this region we have two distinct morphologies of the aggregate units with different curvatures. The lamellar phase has layered structures while the cubic phase is formed of rods with a cylindrial cross section of the bilayer. This is not the case for the transition between the two cubic phases, where only the organization of the rod units changes but the average curvature remains the same. The latter is indicative of a phase transition corresponding to a Bonnet transformation.

A transition between two cubic phases of the gyroid (e.g. Ia3d) and diamond (e.g. Pn3m) type has been observed in monoolein systems (Hyde et al. 1984; Czeslik et al. 1995). No enthalpy could be measured and it was concluded that no change in net curvature occurs during the transition. Making use of metric relations between the IPMSs associated with these transitions it was shown that the phase transition is a Bonnet transformation.

In the present lipid/surfactant ternary system we also have an adiabatic phase transition between two cubic structures which is another evidence of a Bonnet transformation. Moreover, the metric relation found for the lattice parameters of these two phases, i.e. 15.0 nm/9.3 nm = 1.61, is close to the theoretical value of $1.11\sqrt{2}=1.57$ (Hyde and Anderson 1985) for a transformation from a cubic phase of the gyroid type to another of the diamond type, $C_g \rightarrow C_d$. In our case the difference between the theoretical and experimental values can be attributed to the fact that the lattice parameters for the two cubic phases were obtained at different temperatures. In the temperature range of our measurements the lattice parameters are not temperature dependent which makes an extrapolation of the lattice parameter to a common temperature impossible.

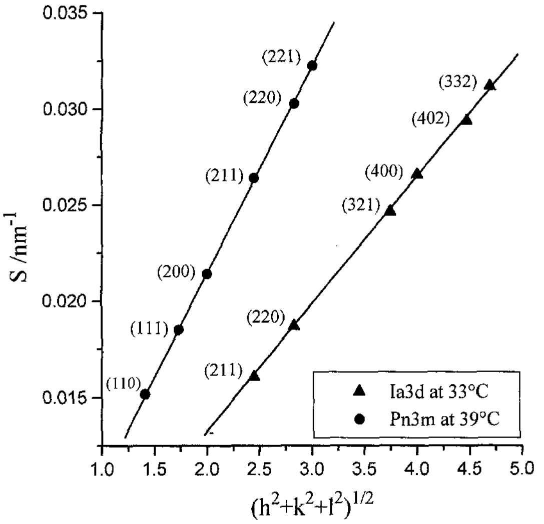

Fig. 5 Reciprocal spacing of the two cubic lattices vs. $(h^2+k^2+l^2)^{1/2}$. The straight lines are linear regressions. The lattice parameters obtained from the slope are a = 15.0 nm for the Ia3d and a = 9.3 nm for Pn3m structures

Finally, we argue on the role of the phase transitions discussed above in biological processes and suggest that during fusion between membranes the intermediate state shows a cubic topology. Such evidence has also been shown in fusion of liposomes from DOPE-Me and DOPE/DOPC mixtures (Ellens et al. 1989). The importance of curved surfaces from non lamellar phases during cell fusion has been already demonstrated (Brandenburg et al. 1990; Chung and Caffrey 1994). A three stage process, adhesion, joining and fission/fusion involving curved surfaces has been illustrated by Verkleij (1991). After joining, the two cells can either split again or fuse with consequent mixing of their aqueous compartments.

Further evidence comes from lipopolysaccharides, that consist of a sugar portion covalently linked to a lipid moiety, called lipid A. Brandenburg et al. (1993) have shown a direct correlation between non lamellar inverted structures and endotoxicity. This corresponds to a particular organization of lipid A aggregates in physiological fluids causing biological activity and leading to disturbances at or around the binding site at the cell surface, where lipid A bilayers should have high curvature. For natural lipid A from *E. coli,* a cubic structure was found to be predominant, similar to lipid A from *S. minnesota* (Brandenburg et al., 1993) where the lipids are incorporated into a lamellar arrangement with the backbone exposed.

More evidence can be found in protein binding to membranes (Lelkes and Pollard 1991). A possible interrelationship between the lipid composition and the state of assembly of the adjacent microfilaments was suggested. However, a direct correlation between the state of assembly of these microfilaments and the regulation of transmembrane ion fluxes has also been shown. The enhancement of the microfilament system caused a concomitant increase in calcium and sodium influxes. This shows the association of curvature and ion transport. The cubic topology containing rod aggregates with water inside offers alternative conditions for the mobility of these ions between the inner core and outer moiety of the cell.

The observation of lamellar and inverted cubic structures coexisting as a two phase region is further support for the concept of transport across the bilayer (membrane in the case of cells) via a network of rods displaced in a cubic array. Not surprisingly the rod length and the lamellar spacing have the same dimension, i.e. 5.4 nm. Moreover, we have shown that the cubic topology is a convenient intermediate during the fusion process for different reasons. First, in this case the average curvature of the system remains zero in the course of the entire transition. It is zero for the lamellar phase, or membrane bilayer, at the starting point of the transition; it remains zero for the intermediate cubic structure and in the final lamellar phase of the fused membranes it is zero again. Second, the energy required for the process is small. As said before the enthalpy of the transition from lamellar to the cubic phase is only 1 kJ/mol. In other words, only a small amount of energy is necessary to induce the transition process. This energy is given back to the system after the formation of the fused membrane or after the two membranes separate again going back to the initial situation. Third, the temperature range where the L_α to cubic transition occurs in this system is close to physiological temperatures.

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