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REVIEW

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Cubic phases in membrane lipids

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Abstract On the basis of data obtained by time-resolved X-ray diffraction, we consider in the present article the occurrence and formation pathways of inverted bicontinuous cubic phases, or bilayer cubic phases, Q_{II}^{B}, in diluted dispersions of lipids representing major biomembrane lipid classes [phosphatidylethanolamines (PEs), mixtures of PEs and phosphatidylcholines (PCs) with other lipids, glycolipids]. We show that Q_{II}^{B} formation proceeds much more easily upon cooling from the H_{II} phase than upon heating or isothermal conversion from the L_{α} phase, thus identifying an indirect but faster route for Q_{II}^{B} phase induction in lipids. The data collected consistently show that the ability to convert into cubic phase upon temperature cycling appears to be a general property of all lipids exhibiting an $L_{\alpha} \leftrightarrow H_{II}$ phase transition. Admixtures of charged phospholipids, both anionic and cationic, strongly facilitate Q_{II}^{B} formation in PEs. Their effect may be attributed to increased electrostatic repulsion between the lipid bilayers that reduces the unbinding energy and facilitates the dissipation of the L_{α} phase required for its conversion into bilayer cubic phase.

Special Issue: Scattering techniques in biology—Marking the contributions to the field from Peter Laggner on the occasion of his 68th birthday.

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Introduction

The lipids found in biological membranes constitute an assorted and important group of biomolecules. Most of the membrane lipids are amphiphilic and behave as lyotropic liquid crystals. In the presence of water, they self-assemble in a variety of phases with different structures that transform into one another via highly cooperative phase transitions. Their major function is to serve as building blocks of biological membranes. However, although the basic structural unit of biomembranes is the liquid-crystalline lipid bilayer, a significant fraction of membrane lipids are able to form nonlamellar phases as well, most often represented by the inverted hexagonal H_{II} phase and occasionally by a number of inverted cubic Q_{II} phases (Luzzati 1968; Seddon and Templer 1995; Lewis et al. 1997); For example, many phosphatidylethanolamines (PEs)—the second most common phospholipid class after the phosphatidylcholines (PCs)—easily transform from lamellar into H_{II} phase in aqueous dispersions (Seddon et al. 1984). Glycolipids can also form H_{II} and Q_{II} phases (Hinz et al. 1991; Koynova and Caffrey 1994a; Mannock and McElhaney 2004). Under specific conditions, such as low pH or the presence of divalent cations, H_{II} phases can also be induced in phosphatidylserines, cardiolipins, and phosphatidic acids (Lindblom and Rilfors 1992). Moreover, biomembrane lipid extracts and membrane-mimicking lipid compositions also form nonlamellar phases if heated above physiological temperatures, dehydrated, or treated with divalent cations (Mouritsen and Andersen 1998; Rilfors and Lindblom 2002; Koynova and MacDonald 2007; Mariani et al. 1990).

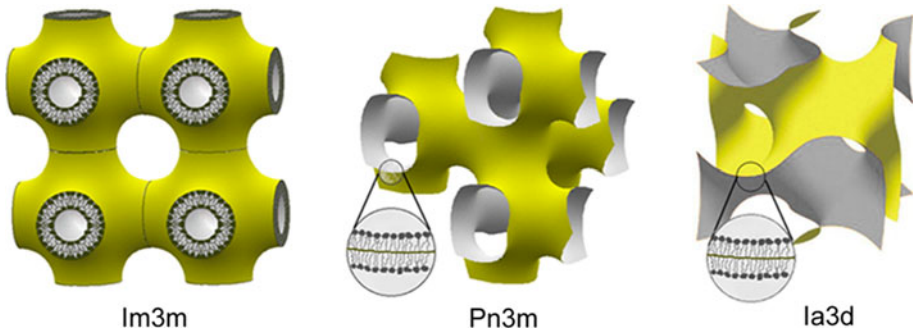

Fig. 1 Structure of the bilayer cubic phases Q^{229} (Im3m), Q^{224} (Pn3m), and Q^{230} (Ia3d), corresponding to the diamond D, primitive P, and gyroid G infinite periodic minimal surfaces, respectively

The ability of lipids to form cubic phases in aqueous dispersions has been known for a long time (Luzzati 1968; Luzzati et al. 1960; Luzzati and Reiss-Husson 1966). Seven cubic phases so far identified in lipids and various surfactants include three micellar type I phases with space groups Q^{223} (Pm3n), Q^{225} (Fm3m), and Q^{229} (Im3m), one inverse micellar (type II) phase Q^{227} (Fd3m) (Luzzati et al. 1996; Luzzati 1997), and three bicontinuous inverted cubic phases with space groups Q^{224} (Pn3m), Q^{229} (Im3m), and Q^{230} (Ia3d) (Lindblom and Rilfors 1992; Luzzati et al. 1997; Seddon 1990b). Another cubic phase, Q^{212} ($P4_332$), has been found to form in a lipid/water/protein mixture (Mariani et al. 1988). An additional sponge phase, L_3, can be viewed as a melted cubic phase, because it shares the properties of bicontinuous cubic phases but does not have long-range order (Engblom and Hyde 1995). According to a well-known geometrical representation of their structure, the bicontinuous inverted cubic phases, also termed bilayer cubic phases, Q_{II}^{B}, consist of single, contiguous lipid bilayers folded into infinite periodic minimal surfaces (IPMS), which partition the aqueous space into two disjointed compartments (Seddon and Templer 1995; Anderson et al. 1988; Charvolin and Sadoc 1988, 1996; Lindblom and Rilfors 1989). In IPMS representation, the three bilayer cubic phases Q^{224} (Pn3m), Q^{229} (Im3m), and Q^{230} (Ia3d) exhibit morphologies corresponding to the diamond D, primitive P, and gyroid G surfaces, respectively (Fig. 1).

In contrast to the type I cubic phases, which form at limited water contents and dissipate into micellar solutions upon dilution, the bilayer cubic phases, Q_{II}^{B}, are typically stable in excess of water. As is clear from their spatial geometry and large internal aqueous volumes (Fig. 1), for their formation these phases require water contents that well exceed the so-called excess water limit corresponding to full hydration of the lipid polar head groups. Even more, cubic phases induced in PE dispersions by heating–cooling cycles require H_2O contents as high as 80–90 wt.% for their unhindered development (Tenchov et al. 1998).

Much of the progress in studies of inverted cubic phases has been achieved with a limited number of single-chain lipids that are not present in biomembranes. These include 18:1 monoacylglycerols, most notably monoolein and monoelaidin, which spontaneously form bicontinuous cubic phases in broad ranges of water content and temperature (Lutton 1965; Hyde et al. 1984; Caffrey 1987; Larsson 2000; Kulkarni et al. 2010). In spite of their potential for studies on cubic phases, the monoglycerides do not represent a practicable biomembrane model in view of the significant molecular structure differences between them and biomembrane lipids. As nonlamellar lipid structures are believed to be involved in various cellular processes (Mouritsen and Andersen 1998; Rilfors and Lindblom 2002; Hyde et al. 1997), characterizing the ability of membrane lipids to form such structures has been a topic of long-standing interest. The present article is focused on the occurrence and formation pathways of bilayer cubic phases Q_{II}^{B} in diluted dispersions of lipids representing the major biomembrane lipid classes (PEs, mixtures of PEs and PCs with other lipids, glycolipids). It is based on data obtained by time-resolved small-angle X-ray diffraction studies carried out at DESY, Hamburg, and APS, Argonne, using low-angle X-ray setups described in previous work (Tenchov et al. 1998, 2006). Due to its low acquisition times and high low-angle resolution, the latter method is particularly well suited for real-time studies on the formation of structures with large lattice constants, reaching values of 35–45 nm as in some of the membrane lipid Q_{II}^{B} phases.

Cubic phases on the temperature scale

Temperature, water content, and pressure are primary variables in lipid–water systems, responsible for their polymorphism. In diluted dispersions at ambient pressure, a generalized sequence of temperature-induced phase transitions including all lamellar and nonlamellar phases may be written as (Tenchov 1991):

$$(L_c \leftrightarrow L_\beta) \leftrightarrow L_\alpha \leftrightarrow Q_{II}^{B} \leftrightarrow H_{II} \leftrightarrow Q_{II}^{M}. \quad (1)$$

At low temperatures, the lipids reside in lamellar crystalline (subgel) L_c and gel L_β phases; on heating,

these phases undergo a melting transition into the lamellar liquid-crystalline L_α phase. With further increase of temperature, lipids able to form nonlamellar phases exhibit a series of mesomorphic phase transitions resulting in the transformation of L_α into bilayer cubic Q_{II}^B, inverted hexagonal H_{II}, and inverted micellar cubic Q_{II}^M phases. Whenever present, the Q_{II}^B phases are always located between the L_α and the H_{II} phases on the temperature scale. Thus, Q_{II}^B may form either by heating from L_α or by cooling from the H_{II} phase.

Bilayer cubic phase formation from the L_α phase

$L_\alpha \rightarrow Q_{II}^B$ transitions in heating scans are mainly observed in short-chain PEs and glycolipids (Koynova and Caffrey 1994a, b). While longer-chain representatives of these lipid classes exhibit direct $L_\alpha \rightarrow H_{II}$ phase transitions, skipping the Q_{II}^B phase, decrease of the chain length to 12 carbon atoms results in the appearance of intermediate Q_{II}^B in ether-linked lipids. Thus, didodecyl phosphatidylethanolamine (DDPE) exhibits the sequence (Seddon et al. 1990b):

$$L_\alpha \xrightarrow{91\,^\circ C} Q^{229} \xrightarrow{110\,^\circ C} Q^{224} \xrightarrow{135\,^\circ C} H_{II}. \quad (2)$$

Similarly, didodecylglucosylglycerol (12-Glc) exhibits the sequence (Hinz et al. 1991)

$$L_\alpha \xrightarrow{57\,^\circ C} Q^{224} \xrightarrow{88\,^\circ C} H_{II}. \quad (3)$$

In longer-chain PEs, an intermediate cubic phase may be induced by methylation of the PE amino group. Headgroup methylation has similar effect to that of reducing chain length on the PE phase behavior. It results in expansion of the L_α range to higher temperatures and appearance of additional phase located between the L_α and H_{II} phases. Such intermediate (although not identified) phase has been observed in monomethylated ditetradecyl PE (Seddon et al. 1983). Monomethylation of dioleoyl PE (DOPE) results in the appearance of a well-resolved Q_{II}^B between the L_α and H_{II} phases. While DOPE exhibits an $L_\alpha \rightarrow H_{II}$ transition at 5–8 °C, the H_{II} existence range of its monomethylated analog, DOPE-Me, is shifted to >60 °C with a thermodynamically stable cubic phase intervening in the range 55–60 °C (Gruner et al. 1988; Siegel and Banschbach 1990; Cherezov et al. 2003; Siegel and Tenchov 2008). The $L_\alpha \rightarrow Q_{II}^B$ transformation is rather slow and requires either very slow rates of temperature increase (Siegel and Banschbach 1990) or isothermal incubation (Siegel and Tenchov 2008; Cherezov et al. 2003). The type of the emerging cubic phase depends on the concentration of the DOPE-Me dispersion. Concentrated dispersions (~30 wt.% of lipid) form the Pn3m phase (Cherezov et al. 2003), while diluted dispersions (~15 wt.% and less) form the Im3m cubic phase (Siegel and Tenchov 2008), in accordance with the behavior of other PEs (Tenchov et al. 1998). In our experiments on isothermal incubation of 10 wt.% DOPE-Me dispersions in the 55–60 °C range, we consistently observed formation of an Im3m phase with lattice constant in the range 35–40 nm (Fig. 2a). This figure illustrates the process of isothermal $L_\alpha \rightarrow$ Im3m transformation in DOPE-Me, which involves gradual disordering (unbinding) of the L_α phase with concomitant formation of Im3m phase. The driving force of this transformation is the elastic energy gain associated with the Im3m formation, which, at the incubation temperature, obviously exceeds the unbinding energy associated with the dissipation of the L_α phase of DOPE-Me (Siegel and Tenchov 2008).

Bilayer cubic phase formation from the H_{II} phase

Our X-ray diffraction studies show that Q_{II}^B formation in PEs proceeds much more easily upon cooling from the H_{II} phase than upon heating from the L_α phase (Tenchov et al. 1998; Siegel and Tenchov 2008), thus identifying an indirect but rather fast route for cubic phase induction in lipids. DOPE-Me is a good example of this behavior: while prolonged incubation is required to transform the lamellar L_α phase into Im3m cubic phase, the latter phase readily forms upon relatively fast cooling of the inverted hexagonal H_{II} phase (Fig. 2b; Siegel and Tenchov 2008). The $H_{II} \rightarrow$ Im3m transition turns out to be very fast, one to two orders of magnitude faster than the $L_\alpha \rightarrow$ Im3m transition. As a result of the $H_{II} \rightarrow$ Im3m transition, the 10 wt.% DOPE-Me dispersion is fully converted into Im3m phase with lattice parameter of ~40 nm, without any traces of reappearing L_α phase. It is thus clear that a lamellar to cubic phase transformation can be rapidly induced in diluted DOPE-Me dispersions by a single heating–cooling cycle ($L_\alpha \rightarrow H_{II}$ in heating, followed by $H_{II} \rightarrow$ Im3m in cooling). Remarkably, the two methods for induction of Q_{II}^B phase illustrated in Fig. 2a, b (slow isothermal conversion versus heating–cooling cycle, respectively) result in formation of Im3m phases with identical X-ray patterns and similar lattice constants. As determined by data obtained for several independent sample preparations with DOPE-Me, the Im3m lattice spacings are typically in the range 35–40 nm (data not shown). We thus reach the important conclusion that, similarly to constant-temperature incubation, the cooling $H_{II} \rightarrow$ Im3m transition also results in formation of an equilibrium Im3m cubic phase, even though the cooling scans have been performed at relatively high scan rates of 1–5 °C/min. Once formed at 55–60 °C, the Im3m phase does not change upon further cooling to

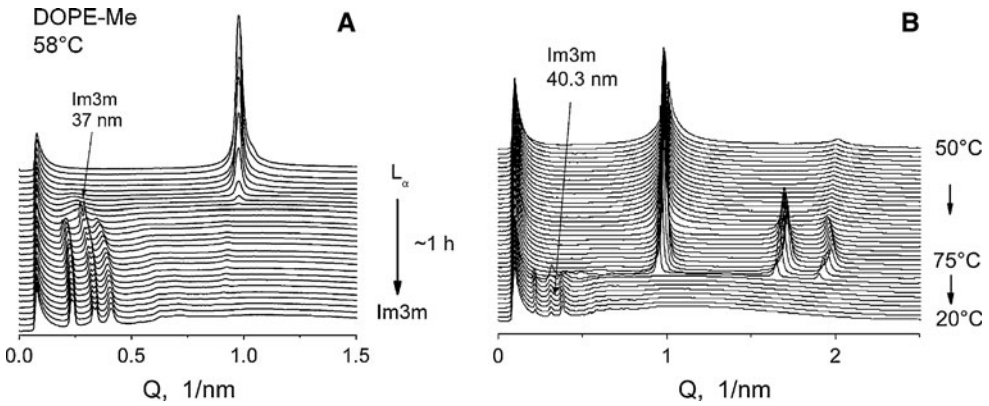

Fig. 2 **a** Isothermal conversion of the DOPE-Me lamellar L_α phase into bilayer cubic Im3m phase upon incubation at 58 °C. The final Im3m lattice constant is 37 nm. Consecutive diffraction frames of 1 s exposure were recorded every 15 min. The sample was a 10 % (w/v) DOPE-Me dispersion in phosphate-buffered saline (PBS) pH 7.2. Reproduced from (Siegel and Tenchov 2008) with permission from Elsevier. **b** Conversion of an initial lamellar L_α phase into Im3m cubic phase in DOPE-Me dispersion in a single heating–cooling cycle $L_\alpha \rightarrow H_{II} \rightarrow$ Im3m. The Im3m lattice constant is 40.3 nm. Heating rate 1 °C/min; cooling rate 5 °C/min. Diffraction frames of 1 s exposure were recorded every 1 min. $L_\alpha \rightarrow H_{II}$ transition onset at 67.5 °C. $H_{II} \rightarrow$ Im3 m transition onset ~55 °C. The sample was a 10 % (w/v) dispersion of DOPE-Me in PBS pH 7.2 (Siegel and Tenchov 2008)

20 °C and exists for indefinitely long time at room temperatures (although it might actually be metastable with respect to the L_α phase at the latter temperatures). DOPE-Me thus provides a phospholipid system that appears to achieve an equilibrium cubic phase on an experimentally feasible timescale using a time-efficient temperature cycle protocol.

Cubic phase formation by temperature cycling

As was first shown for DOPE dispersions, a cubic phase can also be induced by extensive temperature cycling through the $L_\alpha \leftrightarrow H_{II}$ phase transition of the lipid/water system (Shyamsunder et al. 1988). Cycling through its $L_\alpha \leftrightarrow H_{II}$ transition several hundred or thousand times resulted in partial conversion of this system from L_α into cubic phase (Shyamsunder et al. 1988; Erbes et al. 1994). The large number of cycles required shows that only a small fraction of the lipid, of the order of 0.1 %, rearranged from lamellar into cubic structure during each cycle. The resistance of the lipid to such rearrangement is thought to be associated with a large kinetic barrier imposed by the different geometries of the two phases. It has been further reported that the cubic phase formation in PEs upon temperature cycling can be strongly accelerated by the presence of salts and other solutes such as disaccharides in the aqueous phase (Tenchov et al. 1998). Thus, for dielaidoyl PE (DEPE) aqueous dispersions (10 wt.% of lipid at 1 M solute concentration), 20–25 temperature cycles are typically sufficient for complete conversion of the L_α phase into cubic phase (Figs. 3, 4). Similarly accelerated conversion of L_α into cubic phase upon temperature cycling was observed in all studied PEs. It resulted in the formation of Im3m phase in dihexadecyl PE (DHPE), dipalmitoyl PE (DPPE) (Tenchov et al. 1998), DOPE, and stearoyl-oleoyl PE (SOPE), and of Pn3m phase in dipalmitoleoyl PE (DPoPE) (Fig. 5). Detailed examination of the cubic phase evolution during the consecutive heating–cooling cycles shows that the cubic phases basically form during the cooling stages of the cycles (Tenchov et al. 1998), in agreement with the kinetics of Q_{II}^B phase formation in DOPE-Me. In conclusion, we may note that, in our experience, the ability to convert into cubic phase upon temperature cycling appears to be a general property of all lipids exhibiting an $L_\alpha \leftrightarrow H_{II}$ phase transition. The cubic phases induced in this way replace the L_α phase over its whole existence range and may exist for times of the order of several days, although they may actually be metastable with respect to the L_α phase.

Induction of cubic phases by charged lipids

Admixtures of charged phospholipids, both anionic and cationic, strongly facilitate Q_{II} formation in PEs. An example is the mixture of dipalmitoleoyl PE (DPoPE) with 2 mol% dimyristoyl phosphatidylserine (DMPS). In this mixture, a Pn3m phase fully develops upon isothermal incubation after cooling from the H_{II} phase range (Fig. 6). Another example of charged lipids facilitating Q_{II} formation in PEs is presented by the DPoPE mixtures with cationic ethylphosphatidylcholines (ePCs; Tenchov et al. 2008). Some of the cationic ePCs were able to eliminate the direct $L_\alpha \rightarrow H_{II}$ transition of DPoPE and to promote the formation of Q_{II} phase (Fig. 7). Remarkably, the ability of

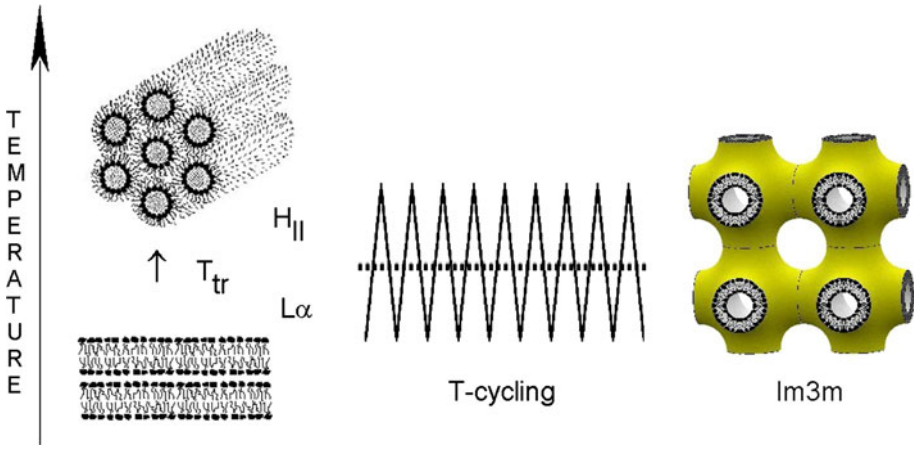

Fig. 3 Schematic presentation of cubic phase formation upon T-cycling through the L_α–H_{II} phase transition

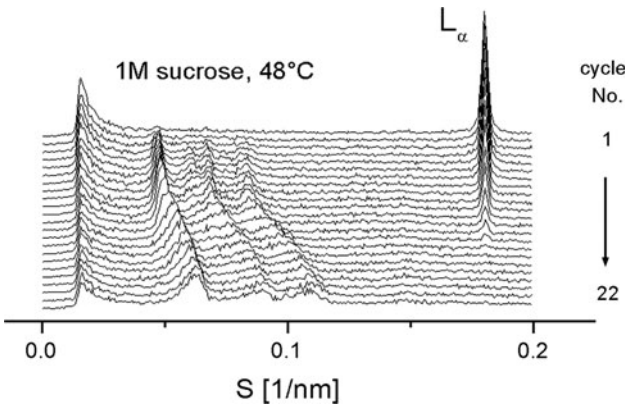

Fig. 4 Conversion of 10 wt.% DEPE dispersions in 1 M sucrose solution from lamellar into cubic phase upon temperature cycling through the L_α–H_{II} phase transition executed by laser-flash temperature jumps. The diffraction patterns shown were recorded immediately before the next T-jump (see Tenchov et al. 1998 for details). Reproduced from (Tenchov et al. 1998) with permission from Elsevier

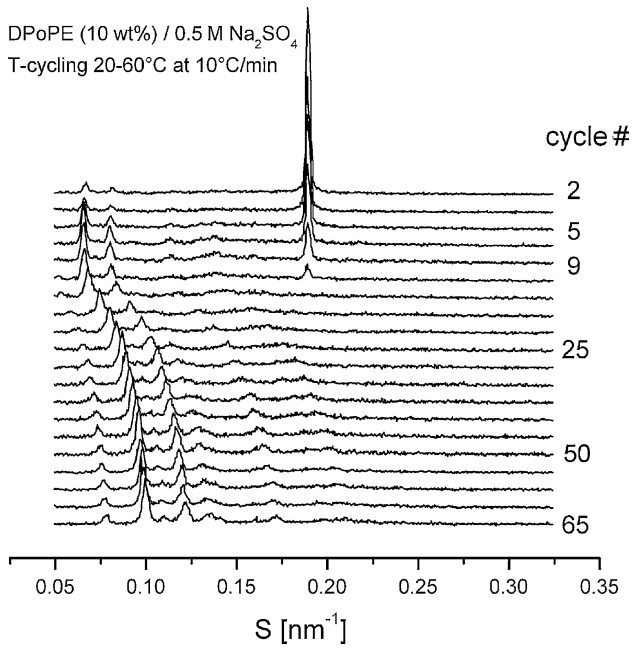

Fig. 5 Conversion of 10 wt.% DPoPE dispersion in 0.5 M Na_2SO_4 solution from lamellar into cubic phase upon temperature cycling (20–60 °C at 10 °C/min) through the L_α–H_{II} phase transition at 43 °C. The Pn3m phase obtained in this way is long-living and does not convert back to L_α upon storage at room temperature

the cationic ePCs to eliminate the direct $L_\alpha \rightarrow H_{II}$ transition and to induce the formation of Q_{II} phase in DPoPE correlated well with their efficiency to transfect DNA (Tenchov et al. 2008; Koynova et al. 2009). We assume that the charged lipids facilitate the transformation into cubic phase by increasing the electrostatic repulsion between the lipid bilayers and reducing in this way the unbinding energy required for dissipation of the L_α phase prior to its conversion into bilayer cubic phase.

Other notable examples of charged lipid mixtures forming bicontinuous cubic phases include the cationic/anionic lipid mixtures important from the viewpoint of the application of cationic lipids in drug delivery and their interactions with negatively charged membrane lipids (Lewis and McElhaney 2000; Tarahovsky et al. 2004). Figure 8a illustrates a Pn3m cubic phase formed in a mixture of ethylphosphatidylcholine with the membrane lipid phosphatidylglycerol (Koynova et al. 2006).

Polyethylene glycol (PEG)–lipid conjugates are another class of anionic lipids widely used in drug delivery. Low amounts of dimyristoylphosphatidylethanolamine (DMPE)-PEG550 have been reported to induce cubic phase in phosphatidylethanolamine (Fig. 8b; Koynova et al. 1997a, 1999).

Cubic phases in PC mixtures with other lipids

Membrane PCs are bilayer-forming lipids unable to transform into nonlamellar phase even at very high temperatures. However, formation of inverted cubic phases has been observed in PC mixtures with other lipids, which alone also

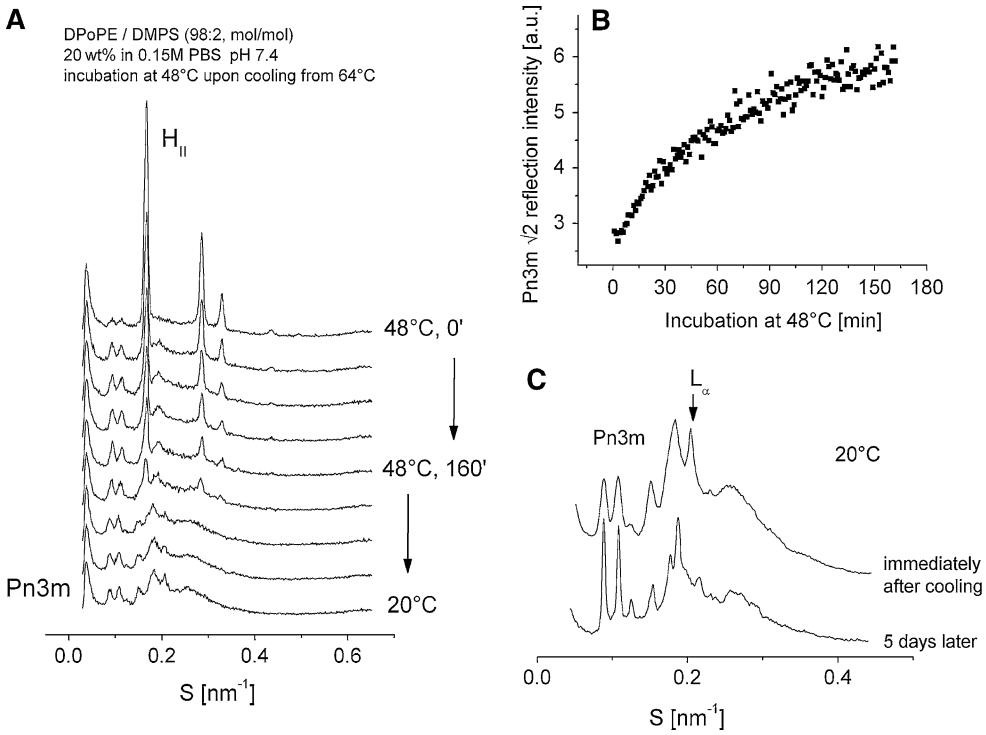

Fig. 6 Development of a Pn3m cubic phase during isothermal incubation at 48 °C of a DPoPE/DMPS 98:2 mol/mol mixture after cooling from H_{II} phase range; the Pn3m phase obtained in this way is stable and does not convert back to L_α upon storage at room temperature. **a** Diffraction patterns recorded form 20 wt.% dispersion of DPoPE/DMPS (98:2, mol/mol) in 0.15 M phosphate buffer (pH 7.4) upon isothermal incubation at 48 °C and subsequent cooling to 20 °C; **b** cubic phase reflection intensity growth upon the incubation; **c** diffraction patterns from the same sample, recorded immediately after cooling to 20 °C and after 5 days storage at that temperature

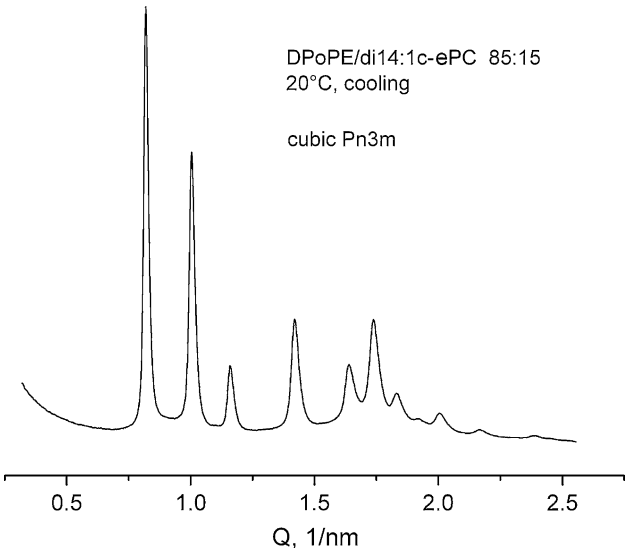

Fig. 7 X-ray diffraction pattern of a Pn3m phase in the mixture DPoPE/di14:1c-ePC (85:15) recorded at 20 °C after a 20 °C–90 °C–20 °C heating–cooling scan (Tenchov et al. 2008)

do not form a cubic phase. Prominent examples are the PC/fatty acid binaries [dilauroyl PC/lauric acid (DLPC/LA), dimyristoyl PC/myristic acid (DMPC/MA), dipalmitoyl PC/palmitic acid (DPPC/PA), and distearoyl PC/stearic acid (DSPC/SA)], which have been found to form all known inverted cubic phases at specific PC/fatty acid molar ratios (Koynova et al. 1997b; Templer et al. 1998; Winter et al. 1999), as well as some PC/cholesterol mixtures at high cholesterol contents (Tenchov et al. 2006).

Inverted micellar cubic phase Fd3m

A frequently observed inverted micellar cubic phase in lipids is of space group Q^{227} (Fd3m). It is located at temperatures above the H_{II} phase in the general phase sequence (1). This phase consists of two types of spherical inverse micelles of different diameters packed in an ordered array of cubic symmetry (Fig. 9, inset; Delacroix et al. 1996). It has been observed mainly in mixtures of a strongly polar lipid such as PC with a very weakly polar amphiphile such as diacylglycerol or a fatty acid. The only reported examples of a single lipid forming the Fd3m phase are the dialkyl xylopyranosylglycerols of chain length C16–19 (Seddon et al. 1996) and diphytanylglucosylglycerol (Minamikawa and Hato 1998).

Examples of lipid mixtures forming the Fd3m micellar cubic phase include monoacyl glycerol/fatty acid (Mariani et al. 1988), PC/diacylglycerol (Seddon 1990a; Takahashi et al. 1996; Tyler et al. 2011), oleic acid/sodium oleate (Seddon et al. 1990a), PE/diacylglycerol (Luzzati et al. 1992), PC/fatty acid (Koynova et al. 1997b; Luzzati et al. 1992; Fig. 9), PC/fatty alcohol (Huang et al. 1996), PC/phosphatidylinositol (Mulet et al. 2008), and *Pseudomonas fluorescens* lipid extract (Mariani et al. 1990).

Cubic–cubic phase transitions

Membrane lipids and lipid mixtures able to form inverted cubic phases can often form more than one Q_{II}^{B} phase,

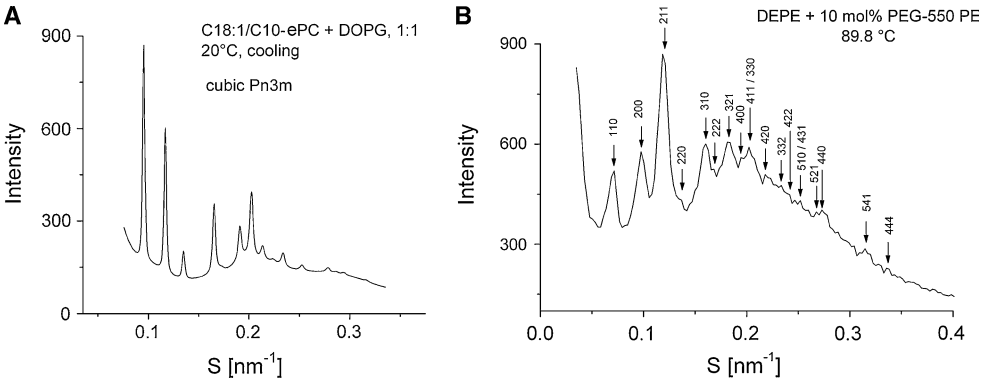

Fig. 8 X-ray diffraction pattern of: **a** Pn3m phase in the cationic/anionic lipid mixture C18:1/C10-ePC/dioleoylphosphatidylglycerol (1:1, mol/mol) at 20 °C (Koynova et al. 2006); **b** mixture of DEPE + 10 mol% DMPE-PEG550 at 89.8 °C [reproduced from (Koynova et al. 1997a) with permission from Elsevier]

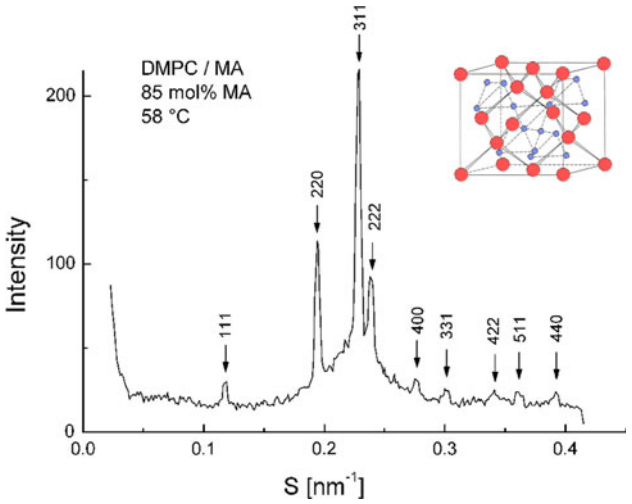

Fig. 9 X-ray diffraction pattern of the micellar cubic Fd3m phase in DMPC/myristic acid (15:85, mol/mol) mixture at 58 °C [reproduced from Koynova et al. (1997b) with permission from Elsevier]; *inset* schematic presentation of the Fd3m phase structure

which transform between each other via temperature-induced phase transitions. Our studies on cubic–cubic transitions indicate that these transitions are typically facile, cooperative, and characterized by very small latent heats. We will consider these transformations in more detail in a subsequent publication and only mention here several lipid systems suitable for studies on such transitions. As shown in sequence (2) above, DDPE dispersions exhibit a Q^{229}(Im3m) → Q^{224}(Pn3m) transition upon heating, at 110 °C (Seddon et al. 1990b). Transitions between cubic phases also take place in certain glycoglycerolipids (Koynova and Caffrey 1994a). For example, 1,2-*sn*-didodecyl-*β*-D-glucopyranosyl glycerol exhibits a Q^{230}(Ia3d) → Q^{224}(Pn3m) transition on heating, at ∼64 °C (Mannock et al. 1992).

A rich variety of cubic–cubic transitions have been found in PC/fatty acid mixtures at certain stoichiometric ratios. The DMPC/MA (1:2 mol/mol) dispersion displays a reversible $Q^{229} \leftrightarrow Q^{224}$ transition (Koynova et al. 1997b). The DLPC/LA (1:2 mol/mol) mixture can form all three bicontinuous cubic phases, and displays a variety of cubic–cubic transitions (Koynova et al. 1997b; Templer et al. 1998; Winter et al. 1999). Examination of the data on the latter mixture appears to suggest that the temperature sequence of the cubic phases may depend on the lipid concentration in the dispersions.

The cubic phase formed by PEs is most often Im3m (Q^{229}). With increase of temperature, a cooperative Im3m → Pn3m transition may take place, transforming the initial Im3m phase into a mixture of coexisting Pn3m (Q^{224}) and Im3m phases (Fig. 10). Noteworthily, the Im3m/Pn3m lattice parameter ratio of the coexisting Im3m and Pn3m phases is equal with good accuracy to 1.28, in compliance with the value predicted on the basis of the representation of the Im3m and Pn3m phases with the primitive (P) and diamond (D) IPMS, respectively, and the Bonnet transformation between these surfaces (Hyde et al. 1984). However, the Bonnet transformation between P and D requires intermediate self-intersecting minimal surfaces in the sequence P ↔ G ↔ D, whereas we observe a cooperative, seemingly direct Im3m → Pn3m transition with no evidence for an intermediate Ia3d phase (the G surface). A possibility for direct Im3m → Pn3m transformation has been proposed by Sadoc and Charvolin (Sadoc and Charvolin 1989). It can be visualized as pulling apart the octahedral tunnel joint of an Im3m unit cell into two tetrahedral joints of Pn3m cells. Such reshaping of an Im3m node into two Pn3m nodes has the advantage of not requiring bilayer self-intersections (Seddon and Templer 1995). It is consistent with the 1.28 Im3m/Pn3m lattice parameter ratio. With a lattice parameter ratio of 1.28, the lipid bilayer enclosed within one Im3m cell equals that enclosed within exactly two Pn3m cells.

Nonlamellar lipid phases and biological membranes

Recent developments show that the ability of lipids to form nonlamellar structures seems to be a prerequisite for

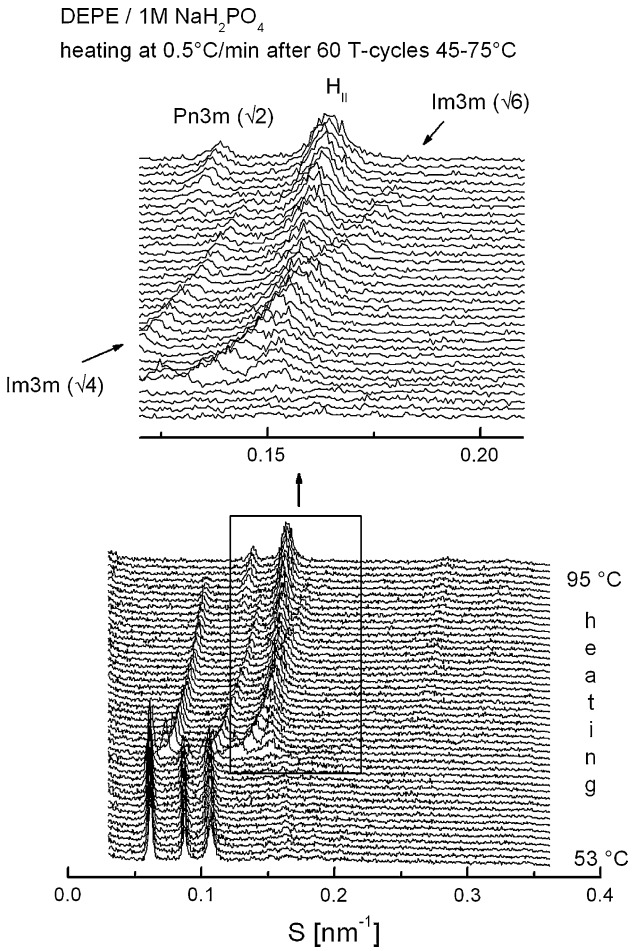

Fig. 10 Temperature evolution of the Im3m phase induced by temperature cycling in 10 wt.% DEPE dispersion (Tenchov et al. 1998). Upper panel: expanded view of the Im3m → Pn3m transition taking place at ~85 °C. Scan rate 0.5 °C/min

important membrane-associated cell processes (Mouritsen and Andersen 1998). It has been demonstrated, for example, that prokaryotic organisms maintain a delicately adjusted balance between lamellar-forming and non-lamellar-forming lipids (Rilfors and Lindblom 2002). Growing evidence suggests that non-lamellar-forming membrane lipids play essential roles in many aspects of membrane functioning. Short-lived nonbilayer structures are supposed to mediate the processes of fusion and fission, and long-lived bilayer structures with small radius of curvature occur in some types of biological membranes (e.g., endoplasmic reticulum, inner mitochondrial membrane, prolamellar bodies). Membrane phase transitions take place in the course of some cellular processes (Biltonen 1990; Bloom et al. 1991; Hazel 1995; Heimburg and Jackson 2007); For example, the action of anesthetic agents is believed to correlate with a lamellar–cubic transition in membranes (Larsson 1988). The prolamellar bodies in the etioplasts of dark-grown seedlings are organized into a cubic lipid phase; they undergo a light-induced phase transition to lamellar phase—the thylakoid membranes of chloroplasts. Cubic patterns have been inferred from the electron micrographs of many cytomembranes (Landh 1995; Almsherqi et al. 2006). Thus, study of the roles played by membrane lipids, i.e., functional lipidomics, is becoming increasingly important in membrane biology. In particular, the transformation from lamellar into bilayer cubic phase examined in the present work may be considered as a cooperative act of multiple fusion events, whereby a set of initially separate, parallel bilayers fuse into a single bilayer of cubic topology (Siegel 2005).

Conclusions

The data collected in our X-ray diffraction studies provide grounds for several important conclusions about the properties of bilayer cubic phases that may be relevant to lipid behavior in membranes.

1. As is particularly clear from the results on DOPE-Me dispersions (Fig. 2), Q_{II}^{B} formation proceeds much faster upon cooling from the H_{II} phase than upon isothermal conversion from the L_{α} phase. The Im3m phases reached in the $L_{\alpha} \rightarrow Q_{II}^{B}$ and $H_{II} \rightarrow Q_{II}^{B}$ transformations have practically identical parameters, indicating that both routes lead to the same, presumably equilibrium, Im3m phase in diluted DOPE-Me dispersions.
2. The measurements on several long-chain PEs and various lipid mixtures consistently show that the ability to convert into cubic phase upon temperature cycling appears to be a general property of all lipids exhibiting an $L_{\alpha} \leftrightarrow H_{II}$ phase transition. The induced cubic phases replace the initial L_{α} phase and may exist for indefinitely long times, although actually they may be metastable in the range of the L_{α} phase.
3. Admixtures of charged phospholipids, both anionic and cationic, strongly facilitate the formation of Q_{II}^{B} phase in PEs. Their effect is likely due to increased electrostatic repulsion between the lipid bilayers that facilitates the dissipation of the L_{α} phase preceding its conversion into bilayer cubic phase.

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