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Interactions of surfactants and fatty acids with lipids

Rumiana Koynova*, Boris Tenchov

Institute of Biophysics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Abstract

The recent studies on the interaction of surfactants and fatty acids with lipids are inspired by the want of knowledge from several research fields of highest activity: identification of membrane rafts; membrane protein crystallization; formation of non-lamellar phases in membranes; membrane fusion. Detailed phase diagrams for lipid–surfactant and lipid–fatty acid mixtures, obtained in the last few years, reveal complicated mesomorphic and polymorphic behavior, including miscibility gaps and compound formation. Surfactant-induced non-lamellar to lamellar transitions in lipids and specific temperature-driven bilayer–micelle transitions represent extensions of the general three-stage model of membrane solubilization. Further research is required to construct phase diagrams as to delineate the principles of lipid–surfactant and lipid–fatty acid interactions for the variety of membrane lipid classes. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Phase diagram; Membrane solubilization; Cubic phase; Non-lamellar phase; Thermodynamic equilibrium

1. Introduction

Both surfactants and fatty acids are known for their ability to destroy the lipid lamellar structure. While surfactants solubilize lipid membranes and transform them into mixed surfactant–lipid micelles, the fatty acids have the opposite effect — they transform the lipid liquid crystalline lamellar phase into inverted hexagonal and cubic phases. A basic source of information for the equilibrium properties of such and other lipid/lipid mixtures are their phase diagrams. In excess water conditions and at constant pressure, the behavior of the mixtures is typically described by temperature-composition phase diagrams. Temperature–pressure phase diagrams at constant composition, and pressure–compositions phase diagrams at constant temperature are also eventually built up. The phase behavior of both surfactant–water systems and lipid–water systems have been frequently studied [1,2]. Only few systems comprising polar lipids and surfactants or fatty acids have been studied in sufficient detail though. Indeed, a search on lipid–surfactant mixtures in the lipid phase diagram database LIPIDAG [2] shows that practically all the entries (with very few exceptions) represent dependencies of the transition temperatures or enthalpies on the composition, but not genuine, state of the art, phase diagrams. Thus, considerable additional effort is required to fill out the gap in our knowledge in this area.

Surfactants have numerous applications, from pure and applied science to industry and everyday life. Specifically, they are widely used in the membrane biology, for biomembrane solubilization, based on

* Corresponding author. Tel.: +359-2-9713969; fax: +359-2-9712493.

E-mail address: rkoynova@obzor.bio21.bas.bg (R. Koynova).

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their ability to form mixed micelles with the membrane lipids and proteins. The membrane solubilization is an important tool for isolation and characterization of integral membrane proteins and includes a compositionally driven transformation of the membrane into mixed micelles comprising surfactant, lipids and membrane-bound proteins. Since the bilayer-to-micelle transition is reversible upon surfactant removal, the mixed bilayers formed this way are referred to as reconstituted membranes. Numerous factors regulate these transformations: the surfactant molecular structure; the membrane composition; the aqueous medium composition; and the temperature. It is again the phase diagram that would provide the basic information on the membrane–surfactant interactions.

An impressive advancement in the protein crystallization studies is the utilization of lipidic cubic phases for growing diffraction-quality crystals from integral membrane proteins [3]. Although it is not yet clear whether the bicontinuous cubic phase or the specific lipid (monoolein) plays the vital role in the process, this approach adds another context, in which the interactions in the membrane protein/lipid/surfactant system are of particular interest. Another hot topic demanding detailed knowledge on lipid–surfactant interactions is the existence of functional clusters (domains) of membrane components — the membrane rafts — which resist solubilization and which play a key role in signal transduction [4–6].

Current advances in the studies on surfactant–lipid systems were recently reviewed in a special issue of *Biochimica et Biophysica Acta* [7•], and earlier by Lasch [8•] and Lichteneberg [9•]. In the present article, and in the overall literature, the terms 'surfactant' and 'detergent' are used synonymously.

2. Phase behavior of lipid / surfactant mixtures

The present knowledge on the properties of the lipid/surfactant mixtures derives from observations on a limited number of lipid model systems. The majority of the model studies explore the surfactant effects on phosphatidylcholine (PC) vesicle dispersions in their liquid crystalline state. These studies substantiate a general scheme for the lipid solubilization by non-ionic and ionic surfactants, the so-called three-stage model [10,11]. This model can be summarized as follows. In the first, one-phase stage the surfactant partitions between the lipid bilayers and the aqueous phase up to a certain level where the bilayers start to break into micellar aggregates. The next stage represents a bilayer–micelle co-existence. Further increase of surfactant leads to the third, again one-phase stage where all bilayers are solubilized and only mixed micelles exist (new developments and critical assessments of this model may be found in [7•]). As it is long known, the solubilization is reversible, i.e. removal of surfactant results in a backward transformation of the mixed micelles into vesicles (see [8•,12] for reviews). A classification of the surfactants with respect to their membrane disruption effectiveness was proposed based on their partitioning between the lipid and aqueous phases [13•]. 'Strong' surfactants solubilize lipid membranes at surfactant-to-lipid molar ratios below 1:1 (e.g. alkylmaltosides, tritons). 'Weak' surfactants accommodate in the membrane up to surfactant-to-lipid ratios above 1:1 before the bilayer collapses (e.g. alkylglucosides, CHAPS). Apart from this major field of study, a noteworthy advancement has been made, referable to recent reports which highlight a rather complicated PC/surfactant mixing behavior in the gel phase of the lipid [14•,15•]. The data on other lipid systems are still scarce and the number of publications dealing with lipids different from the phosphatidylcholines is rather small. As a consequence, the solubilization parameters of major membrane lipid classes such as, for example, the phosphatidylethanolamines are at present virtually unknown. How surfactants affect the properties of the non-lamellar lipid phases is another problem of interest that has been infrequently addressed (see below).

2.1. The experimental protocol: are the lipid / surfactant mixtures at equilibrium?

A methodological issue of particular importance with regard to lipid/surfactant mixtures are the protocols for their preparation and equilibration. As is known from the studies on lipid mixtures, the standard, most appropriate protocol for producing mixed dispersions closest to equilibrium starts with dissolving the two components in a common organic solvent, so as to form a mixture on the molecular level, followed by solvent evaporation, and finally by dispersing the dried molecular mixture into the aqueous medium. An alternative, frequently used method for preparation of lipid–surfactant mixtures is the mechanical mixing of a surfactant solution with dry lipid or with lipid dispersion. It results in a co-dispersion of lipid aggregates and surfactant micelles and/or monomers. Since both substances are amphiphilic and tend to self-organize in water, and in view of the very low lipid and limited surfactant solubilities, the question how fast such mixtures reach equilibrium requires special attention. Slow changes in the phase behavior of the mechanically mixed samples have been frequently observed and for this reason they are typically allowed to equilibrate for times from hours to weeks prior to the measurements. As discussed in

detail by Lichtenberg [9•,16], the equilibration times strongly depend on the type of the lipid aggregates, e.g. uni- or multilamellar lipid vesicles, as well as on the surfactant concentration and chemical structure. With multilamellar vesicles, the access of the surfactant to the bulk of the lipid is obstructed and clearly longer equilibration times are required. For unilamellar vesicles, the access of surfactant molecules to the lipid bilayers is facilitated, but in most cases these vesicles themselves represent non-equilibrium structure and equilibration of the system may again be slow. To summarize, when equilibrium issues are under consideration, it is necessary either to follow the evolution of the lipid/surfactant system for sufficiently long time, or to compare its properties with a system of identical composition but prepared by mixing from organic solvent.

2.2. Ternary and pseudo-binary phosphatidylcholine/surfactant phase diagrams

The ternary phase diagrams are usually presented as Gibbs triangles at constant temperature and pressure. Several such diagrams for PC/surfactant/water systems are known from earlier work: egg PC/sodium cholate/water [17,18]; soybean PC/Triton X-100/water [19]; dimyristoyl PC/hexadecyltrimethylammonium bromide/water [20].

Recently, the solvent-rich parts of the ternary phase diagrams for egg PC mixtures with two cationic surfactants, hexadecyl- and dodecyltrimethylammonium chloride, in water and in 100 mM NaCl solution have been constructed [21,22]. A characteristic feature of these systems is the extensive swelling of the lipid lamellar phase that accompanies its enrichment with ionic surfactant. The observation that the swelling is not suppressed in presence of salt indicates that long-range repulsive forces of not only electrostatic origin are present in the system. The Helfrich undulation force has been invoked as a probable source of this repulsion. An important corollary of these studies is that the compositional transition between the lipid lamellar and the surfactant micellar phases does not appear to proceed as well expressed two-phase coexistence, but rather as a gradual transition involving intermediate thread-like micellar aggregates (this work is reviewed in [23]).

In the excess water range the properties of the system become independent of the water content thus allowing to reduce the ternary phase diagram into a pseudo-binary phase diagram. The latter diagrams are usually given in temperature–composition coordinates and represent the phase behavior of the mixture in both the fluid and solid phase regions. Detailed phase diagrams of that kind have been published for the dimyristoyl PC/$C_{12}E_4$ mixture (Fig. 1) and earlier

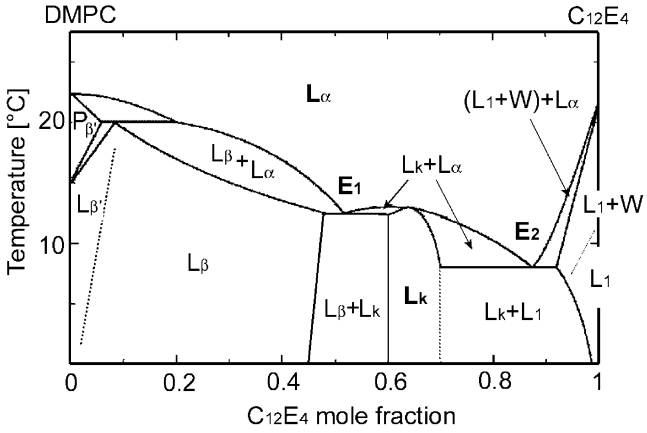

Fig. 1. Dimyristoyl PC/$C_{12}E_4$ pseudo-binary phase diagram. Phase notation: L_α, lamellar liquid crystalline phase; L_β, lamellar gel phase with non-tilted hydrocarbon chains; $L_{\beta'}$, lamellar gel phase with tilted chains; $P_{\beta'}$, ripple gel phase; L_k, lamellar gel compound complex; L_1, homogeneous micellar phase; $(L_1 + W)$, heterogeneous micellar/water phase (clouded); E_1, E_2, eutectic points (adapted from [15•] with permission).

for the dipalmitoyl PC/$C_{12}E_4$ mixture [14•,15•]. The diagram in Fig. 1 illustrates the complicated polymorphic behavior of the lipid/surfactant mixture at low temperatures. It is dominated by two eutectics at surfactant mole fractions of 0.55 and 0.85, and compound formation at lipid/surfactant molar ratio of approximately 1:2. A micellar transition (cloud point) precedes the formation of lamellar aggregates as the surfactant-rich mixtures are heated.

These studies clearly demonstrate the strong dependence of the lipid–surfactant interactions on the lipid phase state. While a fluid bilayer accommodates surfactant molecules up to a level where it starts to disintegrate, the gel phases tend to expel 'foreign' molecules much earlier and are thus more resistant to solubilization (see also [24]). Taking into account the large differences in their chemical structures (packing parameters, chain lengths, etc.), it is not surprising that lipid and surfactant molecules have a limited affinity for each other and that their mixtures would display large miscibility gaps in the solid lamellar phases.

A specific issue of interest concerns the surfactant-induced transitions from lamellar into micellar phase at reduced hydration levels. Reports on dimyristoyl PC/$C_{12}E_8$ [25] and palmitoyl-oleoyl PC/$C_{12}E_2$ [26,27] mixtures provide detailed characterizations of the hexagonal phases (H_I in the former and H_{II} in the latter system) mediating the lamellar to micellar transformations.

2.3. Surfactant-induced non-lamellar to lamellar phase transformations in lipids

Except for lamellar phases, lipids in aqueous dis-

persions can also form a variety of non-lamellar mesomorphic structures typically represented by the inverted hexagonal H_{II} phase and several phases of cubic symmetry [28–30]. Recent work consistently shows that surfactants tend to disrupt the non-lamellar lipid phases and to induce their transformation into lamellar liquid crystalline phase.

Using X-ray diffraction, Angelov et al. [31] examined the effects of octylglucoside, an effective and frequently used surfactant in the biomembrane research, on the H_{II} phase of dioleoylphosphatidylethanolamine (DOPE) and the inverted bicontinuous cubic Pn3m (Q^{224}) phase of monoolein. Addition of octylglucoside at a DOPE/surfactant molar ratio of 1:1.55 results in destabilization of the DOPE H_{II} phase and its transformation into lamellar phase in a broad temperature range. Similarly, octylglucoside at a monoolein/surfactant molar ratio of 1.36:1 eliminates the Pn3m phase of monoolein and again transforms it into lamellar liquid crystalline state. It remains unclear, however, what are the threshold octylglucoside concentrations required to trigger these transformations.

Further evidence for a surfactant-induced destabilization of the monoolein cubic phases and their transformation into lamellar phase provide the ternary phase diagrams obtained for monoolein mixtures with cetyltrimethylammonium bromide and sodium cholate in water and 100 mM NaCl solution [32,33]. At solvent concentrations in the range of 50–60 wt.%, about 1 surfactant molecule per 10 molecules of monoolein is sufficient to induce a cubic to lamellar phase transition. Another surfactant, *n*-dodecyl-β-D-maltopyranoside, was also shown to promote a cubic-to-lamellar transition in hydrated monoolein [34].

These findings, which are of certain relevance to the membrane protein reconstitution and crystallization studies, suggest that surfactants should typically suppress the non-lamellar lipid phases in favor of the lamellar liquid crystalline phase. They appear to induce a transition from the non-lamellar to lamellar state at concentrations well below the concentrations required to solubilize a membrane and to convert it into a solution of mixed micelles. The surfactant-induced non-lamellar to lamellar lipid phase transitions may be thus correlated with the three-stage model of membrane solubilization as illustrated in Fig. 2.

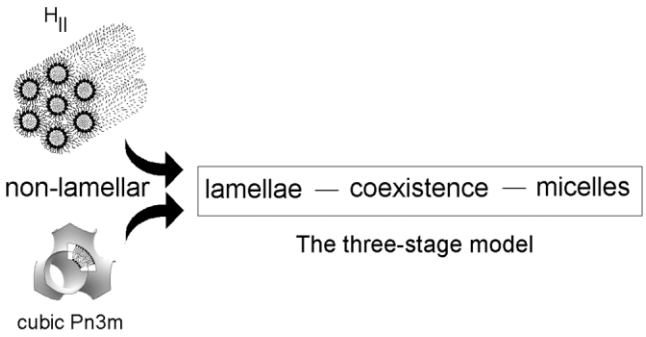

Fig. 2. An extension of the three-stage model, accounting for the surfactant effects on the non-lamellar lipid phases.

2.4. Temperature-induced bilayer–micelle transformations

It is worth noting that, except for driven by composition (at increasing surfactant concentration), a bilayer-to-micelle transformation can be also thermally induced, at constant lipid and surfactant concentrations. Such transformation has been recorded earlier in the egg PC/octylglucoside system at increasing temperature [35]. The driving force for that transformation is supposed to be the strong decrement of the octylglucoside cmc upon temperature increase. Most interestingly, an opposite, micelle-to-bilayer transformation has been recently detected with temperature increase in mixtures of saturated PCs with bile salt [36•,37,38•,39] and with $C_{12}E_8$ [40•], at specific lipid/surfactant molar ratios (2:1 for PC/$C_{12}E_8$; between 3:1 and 7:1 for PC/bile salt). This transformation is supposed to proceed via two possible structural pathways: (1) from discoidal mixed micelles to long rod-like micelles forming network-like structures, and then to multilayer membrane structures; and (2) from discoidal micelles to membrane fragments and finally to unilamellar vesicles [37,38•]. It appears to reflect an increased flexibility (reduced elastic constants) of the surfactant-containing membrane [41]. Temperature-sensitive systems like these are convenient for studying particular stages of the micelle to vesicle transformation since the thermal transition is completely reversible and can be halted at any step. An open question is how it relates to the chain-melting transition of the lipids. It seems that the micelle-to-vesicle transition on heating is more likely a mesomorphic transformation triggered by the chain-melting transition. It may thus take place almost simultaneously or closely above, but not necessarily at the temperature of the latter transition. Analogously, the reverse vesicle to micelle transition, taking place on cooling, may be triggered by the lipid fluid–gel transition, due to the lower ability of the gel bilayers to accommodate surfactant molecules [9•]. It is worth noting, however, that an opposite lamellar-to-micellar transformation has been followed upon heating in a similar system, Na-taurocholate/DPPC, and at similar lipid/surfactant ratios [42]. Clearly,

further studies are required in order to clarify the issues of thermodynamic equilibrium and kinetic effects in the temperature-driven bilayer–micelle transformations.

2.5. Identification and stability of membrane rafts

The usefulness of detergent solubilization as a method for extraction and characterization of membrane constituents and integral membrane proteins has been long recognized and intensely explored. A novel surfactant application in the biomembrane research requires, however, special emphasis.

A vast number of studies on lipid mixtures show that the polar lipids, with few exceptions, do not mix ideally but, depending on the differences in their chemical structures, form lateral compositional clusters (domains) or phase separate in the membrane plane. Recently, much attention has been paid to the concept that sphingolipids and cholesterol organize into functional domains (rafts) which are able to move in the membrane and serve as attachment sites for specific proteins involved in the signal transduction pathways [4–6]. This view derives much of its support from the evidence for the existence of detergent (Triton X-100)-insoluble domains in the cellular membranes. Detergent-resistant membrane patches, enriched in sphingolipids and cholesterol, have been isolated from a variety of cell types (for reviews see, e.g. [5,6,43]).

The physical origin of the membrane insolubility by Triton X-100 has been investigated using model membranes (vesicles) having lipid compositions similar (with respect to PCs, sphingomyelin and cholesterol) to that found in the detergent-resistant membranes [44–47•]. An interesting conclusion of these studies is that both the model and native detergent-resistant membranes reside in a specific, less susceptible to solubilization lipid phase state. Sphingomyelin and cholesterol tend to demix from the PCs and to organize into domains which are in a state intermediate between the gel and the liquid crystalline phases, the so-called liquid-ordered state. Similarly to the lipid gel phases, the liquid-ordered state of the model membranes was found to be more resistant to detergent solubilization than the liquid crystalline phase. These studies suggest that the detergent insolubility is an inherent property of the lipids and emphasize the important role of cholesterol for the formation of detergent-resistant, liquid-ordered patches in the membranes. In general support of these findings, the liquid-ordered states induced by cholesterol in DPPC [46] and DMPC [48] aqueous dispersions were also found to resist solubilization.

3. Lipid / fatty acid mixtures

Fatty acids are able to transform the lipid liquid crystalline lamellar phase into inverted hexagonal [49,50] and cubic [51,52] phases. The interest in these mixtures is in part due to the emerging view that the non-lamellar lipid phases are biologically relevant [28,53]. Various proposals referring to the biological role of the non-lamellar lipid patterns have been forwarded. It is believed that such patterns are part of the processes of cell fusion, transport and secretion of macromolecules across membranes, cell–cell and cell–virus interactions, fat digestion. The lipid/fatty acid mixtures, most often their 1:2 (mol/mol) preparations, are currently used as model systems for studying the mechanism and kinetics of the non-lamellar phase formation in lipid membranes [54,55•,56•, 57•,58•,59,60•,61].

3.1. Temperature–lipid composition phase diagrams

Thorough phase diagrams were recently constructed for the dimyristoyl PC (DMPC)/myristic acid (MA) and dilauroyl PC (DLPC}/lauric acid (LA) hydrated mixtures [56•]. These mixtures were found to display miscibility gaps about the 1:2 lipid/fatty acid stoichiometry, not only in the solid gel and subgel phases, but also in the liquid crystalline range. In the latter range, a phase separation between lamellar and inverted hexagonal phases of different compositions is observed. This fluid–fluid miscibility gap well accounts for the unusual temperature and pressure dependence of the H_{II} phase lattice constant observed by Winter et al. [60•] and Erbes et al. [61]. The liquid crystalline regions of the lipid/fatty acid phase diagrams in their fatty acid-rich parts are dominated by non-lamellar phases. Upon increasing the fatty acid content in the DLPC/LA mixture, they arrange in the sequence: bilayer cubic (Ia3d, Pn3m, Im3m) → inverted hexagonal (H_{II}) → micellar cubic (Fd3m) → micellar isotropic (M) (Fig. 3). For the widely explored 1:2 PC/fatty acid preparations, a lamellar liquid crystalline L_{α} phase is observable in a narrow temperature interval above the melting transition for the DLPC/LA mixture. That phase is eliminated, however, in the longer-chain mixtures where the melting proceeds directly into non-lamellar phase (bilayer cubic for DMPC/MA and inverted hexagonal for longer chain mixtures).

3.2. Temperature–pressure phase diagrams

Such phase diagrams were built for the PC/fatty acid mixtures at 1:2 stoichiometry, with chain length

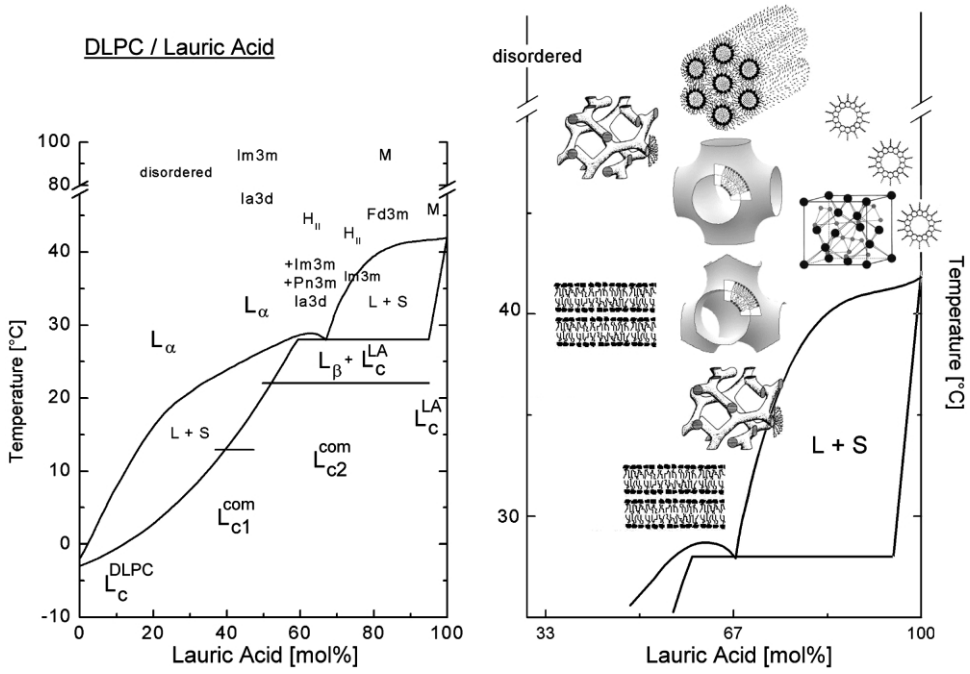

Fig. 3. DLPC/LA pseudo-binary phase diagram (left). Data form [56•] (L_c, lamellar crystalline (subgel) phase; L_c^{com}, compound lamellar subgel phase; S, solid phase; L, liquid phase). Liquid crystalline phases at high fatty acid contents (right).

of 12, 14, 16, and 18 C-atoms, over the range 1–1000 bar [60•]. Increasing pressure in that range produces linear increase of the phase transition temperatures in the mixtures, but does not affect their propensity to form bicontinuous cubic phases.

3.3. Temperature–water content phase diagrams

The phase diagrams of (1:2 PC/fatty acid)/water mixtures of chain lengths 12–20 C-atoms revealed that the L_α phase is suppressed at chain length >12 not only at full hydration but at all water contents [55•,57•]. Direct gel L_β–H_{II} transition takes place at chain length >14 at any water content, with the limiting hydration of the gel phase exceeding that of the inverted hexagonal phase. Interestingly, the phase diagram of the (1:2 DLPC/LA)/water mixture shows hexagonal phases at both low and high hydrations in the temperature range $\sim$30–50°C, with intervening lamellar and cubic phases at intermediate hydrations [57•,58•]. This behavior is reminiscent of the H_{II}–L_α–H_{II} transition upon hydration/dehydration (reentrant H_{II} phase) observed in the DOPE/water system [62].

3.4. General features of PC / fatty acid phase behavior

1. With increasing *water content*, the non-lamellar mesophases appear in the order: $H_{II} \rightarrow$ Ia3d $\rightarrow$ Pn3m $\rightarrow$ Im3m in the system 1:2 DLPC/LA [57•,58•].
2. In that system, increasing the *temperature* produces the same order of appearance of the bicontinuous cubic phases, but with the inverted hexagonal phase dominating at highest temperature: Ia3d $\rightarrow$ Pn3m $\rightarrow$ Im3m $\rightarrow H_{II}$ [56•].
3. In the 1:2 PC/fatty acid preparations, increasing the *chain length* eliminates the inverse bicontinuous cubic phases [55•,56•,58•,63]. Their destabilization follows a similar order to that with increasing the temperature: the Ia3d is present at chain length of 12 C-atoms but is eliminated at chain length of 14 C-atoms; all cubic phases disappear from the phase diagrams at chain lengths of 16 and 18 C-atoms.
4. With increasing the *LA / DLPC molar ratio*, the liquid crystalline phases arrange in the order: lamellar $\rightarrow$ bilayer cubic (Ia3d $\rightarrow$ Pn3m $\rightarrow$ Im3m) $\rightarrow$ inverted hexagonal (H_{II}) $\rightarrow$ micellar cubic (Fd3m) $\rightarrow$ micellar isotropic (M) [56•,60•].

3.5. Phosphatidylserine / fatty acid mixtures

In contrast to hydrated 1:2 PC/fatty acid mixtures, a recent study on phosphatidylserine/fatty acid mixtures [64] shows that at their 1:2 (mol/mol) preparations, the lamellar liquid crystalline phase is preserved in a wide temperature range at neutral pH. The

stabilizing effect for the lamellar architecture has been attributed to the anionic nature of phosphatidylserine.

3.6. Ceramides / stearic acid

This mixture was examined in view of the participation of both components in stratum corneum, as to get some insight into the molecular interactions of this complex biological system [65]. Ceramides type IV and stearic acid are immiscible in the solid state and form an eutectic mixture, with eutectic point at ~90 mol% stearic acid, at a temperature which is by 25°C below the melting transition of the ceramides and by 5–6°C below the melting transition of the fatty acid.

3.7. Monoolein / oleic acid

At full hydration at pH 7, increasing the oleic acid/monoolein molar ratio from 0 to 1:10 was found to result in a $Pn3m \rightarrow Im3m$ transition [66]. At the 1:10 ratio, addition of 100 mM NaCl reverts, however, the system back into Pn3m phase. Lowering the pH results in an $Im3m \rightarrow Pn3m \rightarrow H_{II}$ phase sequence. These effects have been attributed to electrostatic interactions.

3.8. Relation to membrane permeability

An interesting recent observation concerns the ability of low amounts (up to 5 mol%) of fatty acids to modulate the lipid bilayer permeability at the gel–liquid crystalline transition [67]. The unsaturated oleic but not the saturated stearic acid was found particularly effective in neutralizing the increase of the membrane ion permeability through domain boundaries upon their melting transition.

4. Conclusions

The major advancements regarding the phase behavior of lipid/surfactant and lipid/fatty acid aqueous mixtures in the last years comprise: (i) construction of detailed pseudo-binary and ternary phase diagrams of lipids with surfactants, revealing complicated polymorphic behavior; (ii) initial studies on the effect of surfactants on non-lamellar lipid phases demonstrating that surfactants tend to transform the latter phases into lamellar phase; (iii) specific temperature effects on lipid–surfactant phase behavior — induction of reversible micelle–vesicle transformation upon heating, at constant surfactant content; (iv) construction of comprehensive temperature–composition phase diagrams of PC–fatty acid mixtures revealing the formation of a large variety of non-lamellar structures and liquid-phase miscibility gaps, in addition to a complicated polymorphic behavior; (v) establishment of the phase behavior of PC/fatty acid (1:2) mixtures as a function of water content, temperature and pressure; construction of the respective phase diagrams.

A deficiency in this field is the lack of sufficient number of comprehensive phase diagrams outlining the principles of the lipid–surfactant and lipid–fatty acid interactions. Another shortage of knowledge follows from the fact that the greater part of studies refer to phosphatidylcholines and their lamellar liquid crystalline phases. Much additional work is required to decipher the surfactant and fatty acid effects on other major classes of membrane lipids.

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