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Regulation of lipid composition in biological membranes—biophysical studies of lipids and lipid synthesizing enzymes

Leif Rilfors *, Göran Lindblom

Department of Biophysical Chemistry, Umeå University, SE-90187 Umeå, Sweden

Received 7 February 2001; accepted 12 June 2001

Abstract

The study of the role played by membrane lipids, functional lipidomics, has become increasingly important in membrane biology. The physico-chemical properties of the lipids in biological membranes are subject to some fundamental requirements. In general, the acyl chains shall be in a liquid-like state to keep the membrane proteins active, and the lipids must form a bilayer structure in order for the membrane to be an insulating barrier. However, a potential ability of the lipids to form nonbilayer structures seems to be a prequisite for several membrane-associated cell processes. Therefore, organisms exposed to changes in the environmental conditions, such as temperature and uptake of fatty acids, adjust their membrane lipid composition. Examples of prokaryotic organisms that have been studied in this respect are the cell wall-less bacterium *Acholeplasma laidlawii*, the Gram-negative bacterium *Escherichia coli*, and the Gram-positive bacteria *Bacillus megaterium* and *Clostridium butyricum*, and among eukaroytic organisms are found fungi, higher plants, and poikilothermic animals. By synthesizing a proper combination of acyl chain and polar head group structures, the organisms modify the phase transition temperatures of the membrane lipids so that they are maintained in a lamellar liquid crystalline phase, and the formation of a lamellar gel phase as well as reserved nonlamellar phases is avoided. It has been shown that *A. laidlawii* and *E. coli* maintain a balance between lamellar-formed and nonlamellar-forming lipids. A growing body of evidence shows that nonlamellar-forming membrane lipids play essential roles in many aspects of membrance functioning. Short-lived nonbilayer structures are probably formed in the processes of fusion and fission of lipid bilayers, and long-lived bilayer structures with a small radius of curvature occur in several types of biological membranes (e.g. smooth endoplasmic reticulum, inner mitochondrial membrane, and prolamellar bodies). The activity of membrane-associated proteins can be modulated by adding detergents or nonlamellar-forming lipids to bilayers. Some examples are the regeneration of denatured bacteriorphodopsin, and the activities of protein kinase C, some phospholipases, and some key lipid synthases involved in the lipid metabolism of eukaryotic cells, *A. laidlawii*, and *E. coli*. The physico-chemical properties of the lipid matrix can be a direct feed-back signal on the activity of the lipid synthases. Finally, nonlamellar-forming lipids are essential for a proper cell division, and an efficient translocation of proteins across the plasma membrane, of *E. coli* cells. © 2002 Elsevier Science B.V. All rights reserved.

* Corresponding author. Tel.: +46-90-786-6345; fax: +46-90-786-7779
E-mail address: leif.rilfors@chem.umu.se (L. Rilfors).

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PII: S0927-7765(01)00310-1

Keywords: Biological membranes; Biophysical studies; Lipid composition; Lipid synthases; Non-lamellar-forming lipids

1. Introduction

An appreciation of the important advances in the understanding of biological membranes obtained during the last 20 years must be moderated by the knowledge that many fundamental questions remain incompletely answered: (i) What physico-chemical properties of the lipids are important to the operation of the membrane? (ii) Is regulation of these properties a predominant constraint controlling the lipid composition of the membrane? and finally (iii) How do these properties influence the membrane proteins? Such questions motivate research on how organisms adjust the lipid composition in their cell membranes to the environmental conditions.

It is well documented that all kinds of organisms adapt their membrane lipid composition to the prevailing environmental and physiological conditions. Three strategies seem to be utilized: (i) changes in the acyl chain structure; (ii) changes in the polar head group structure; and (iii) reshuffling of acyl chains to form new lipid molecular species without changing the average acyl chain composition [1–3]. The regulation of the membrane lipid composition by the cell wall-less bacterium *Acholeplasma laidlawii* strain A has been intensively studied in our laboratory. The organism can be grown under conditions where the regulatory changes occur predominantly in the polar head group structures. The conclusion has been drawn that the cells strive to maintain a certain balance between the lipids constituting a bilayer structure and those forming reversed nonlamellar structures [4–10]. We have also studied the metabolic regulation and the phase equilibria of the membrane lipids from wild-type cells of the Gram-negative bacterium *Escherichia coli*. The reasons for this are manifold: (i) *E. coli* is recognized as one of the foremost prokaryotic model organisms; (ii) the bacterium has only three main membrane phospholipids which occur frequently in prokaryotic as well as eukaryotic organisms; and (iii) the regulation of the lipid composition in wild-type cells is brought about by changes in the acyl chain structure, above all in the degree of unsaturation of the acyl chains [11], which is a very common response to changes in the environmental temperature among a variety of organisms [1]. On the other hand, wild-type cells of *E. coli* have a nearly constant polar head group composition under a wide range of growth conditions. In this way the organism maintains the lipids in a lamellar liquid crystalline phase and avoids the formation of both a lamellar gel phase and reversed nonlamellar phases [12].

How do *E. coli* cells manage to keep the polar head group composition of the membrane lipids nearly invariant? Since extensive overproduction of the enzymes synthesizing these lipids has little effect on the lipid composition [13], it has been assumed that the regulation of the lipid composition occurs through an adjustment of the relative activity of the enzymes and not at the level of gene expression and the amount of the enzymes [14,15]. Nearly all of the membrane lipid synthesizing enzymes in *E. coli* have been purified, characterized, and sequenced. However, studies of the influence of the composition and physico-chemical properties of the lipid bilayer on the activity of these lipid synthases are very sparse, and they have predominantly been performed as in situ studies [15,16]. Investigations of the activity and membrane binding of one of the key lipid synthases in *E. coli* have, therefore, been initiated [17,18].

In this review we will also give examples of the roles played by nonlamellar-forming lipids for the activity of some membrane-associated proteins and for membrane fusion. The review consequently emphasizes the important involvement of nonlamellar-forming lipids in several aspects of membrane functioning.

2. Regulation of lipid composition

2.1. Acholeplasma laidlawii

A. laidlawii has been a tool for a large number of investigations of the physico-chemical properties of biological membranes. The reason for this is the combination of mainly two features: (i) the ability of introducing controlled changes in membrane acyl chain and sterol composition; and (ii) the ease with which pure membranes free from contaminants can be obtained. Examples of investigations, in which *A. laidlawii* membranes were used to establish fundamental properties that are common for a majority of biological membranes, are: (i) DSC studies showed that the lipids in the membrane of *A. laidlawii* exhibit a reversible gel-to-liquid crystalline phase transition [19]; (ii) low- and wide-angle X-ray diffraction studies showed that a bilayer structure is formed by the lipids in the intact *A. laidlawii* membrane [20,21]; (iii) ^{2}H NMR spectroscopy of deuterated acyl chains of lipids in the intact membrane of *A. laidlawii* showed that the orientational order profile in this membrane is very similar to the corresponding order profile in lamellar liquid crystalline phases of amphiphiles [22].

A. laidlawii strain A synthesizes seven membrane lipids (Fig. 1) forming liquid crystalline phases. Three of the lipids are able to form nonlamellar phases, monoglucosyldiacylglycerol (MGlcDAG); monoacyl-MGlcDAG (MAMGlcDAG); and monoacyldiglucosyldiacylglycerol (MADGlcDAG) [4–6,9,10]. The lamellar-forming lipids are phosphatidylglycerol (PG), diglucosyldiacylglycerol (DGlcDAG), glycerophosphoryl-DGlcDAG (GPDGlcDAG), and monoacylbisglycerophosphoryl-DGlcDAG (MABGPDGlcDAG) [4,6,9,23]. The chemical structures of these lipids have been determined by multidimensional NMR [24–27].

A. laidlawii can be grown under conditions where fatty acids cannot be endogenously synthesized, and the cells are, therefore, forced to incorporate the exogenously supplied fatty acids into its membrane lipids. The cells respond by adjusting the composition of the polar head groups to the incorporated acyl chains, and the polar head group composition is regulated in a coherent way. Generally, the fraction of the lipids forming reversed nonlamellar structures decreases when the length and the unsaturation of the acyl chains are increased (Fig. 2) [9]. It is well known that an increase in length and unsaturation of the acyl chains favors the formation of nonlamellar phases by membrane lipids. Therefore, the regulation of the ratio between the lipids forming lamellar and nonlamellar phases is expected to yield phase transition temperatures from a lamellar to a nonlamellar phase within a rather narrow interval for total lipid extracts. In fact, a number of experimental investigations have shown that this hypothesis is valid (Fig. 3) [3,4,7,8,10,28].

2.2. Escherichia coli

Phosphatidylethanolamine (PE), PG, and diphosphatidylglycerol (DPG) are the main membrane lipids synthesized by wild-type cells of *E. coli*. PE has the strongest propensity to form reversed nonlamellar phases, and this propensity is profoundly influenced by the length and the degree of unsaturation of the acyl chains. In contrast to *A. laidlawii*, *E. coli* cells synthesize all

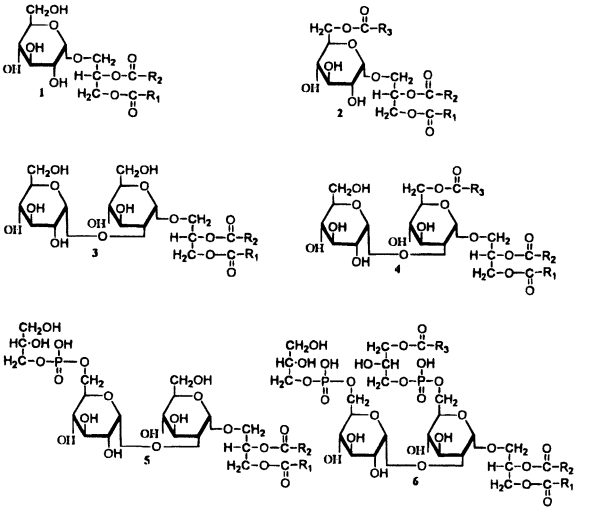

Fig. 1. Structures of the glucolipids and the phosphoglucolipids synthesized by *A. laidlawii* strain A. 1, MGlcDAG; 2, MAMGlcDAG; 3, DGlcDAG; 4, MADGlcDAG; 5, GPDGlcDAG; 6, MABGPDGlcDAG.

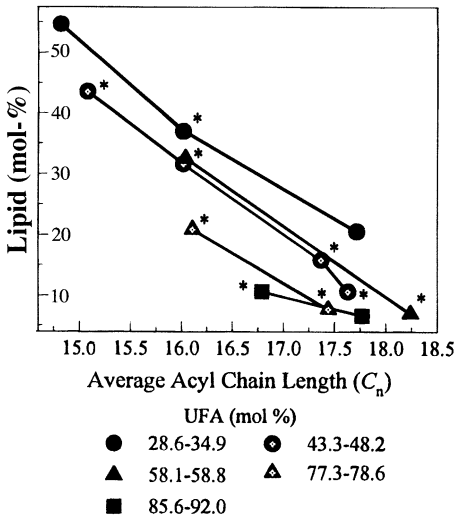

Fig. 2. Fraction of *A. laidlawii* membrane lipids able to form reversed nonlamellar phases as a function of the acyl chain length and the degree of unsaturation of the acyl chains (UFA). The fraction consists of MGlcDAG, MAMGlcDAG, and MADGlcDAG. The data points marked with '*' represent an average value obtained from two or more lipid extracts with similar acyl chain composition. Adapted from [9].

their fatty acids themselves and these are consequently not incorporated from the growth medium. When the growth temperature of *E. coli* is changed, the polar head group composition remains practically constant, while the unsaturation of the acyl chains is adjusted (Fig. 4) [12]. Generally, an increased temperature shifts the membrane lipid phase equilibria from lamellar toward reversed cubic and/or reversed hexagonal (H_{II}) phases. Wild-type *E. coli* cells respond to higher growth temperatures by incorporating shorter and more saturated acyl chains into their membrane lipids. These changes decrease the ability of PE to form reversed nonlamellar phases, thus counteracting the increase in temperature. Consequently, the lipid extracts have a nearly equal ability to form nonlamellar phases at equal elevations above the growth temperature (Fig. 5).

A lipid biosynthetic mutant of *E. coli* has also been used to study the polymorphic regulation of the membrane lipid composition [29,30]. This mutant lacks the ability to synthesize PE, which constitutes about 75–80 mol% of the membrane lipids in wild-type *E. coli* cells. Thus, only anionic lipids are present in the membrane of the mutant, which has an absolute growth requirement for high concentrations of some divalent cations. It was suggested that DPG together with the cations can replace the role of PE, and that the *E. coli* mutant also exhibits a polymorphic regulation of its membrane lipid composition [29–31].

Recently, another physico-chemical property of the membrane was suggested to be subjected to regulation by *E. coli* [32]. These authors studied the diffusion of a fluorescent probe by FRAP in multilayers of total lipid extracts from *E. coli*. The measured diffusion coefficient showed an anomalous maximum around the growth temperature of the cells. The anomaly is attributed to the transformation from multilamellar dispersion to uni-lamellar vesicles at a so-called critical temperature T^*. These authors also proposed that the remarkable anomaly is a consequence of the adjustment of the membrane lipid composition to the growth temperature. They suggested that membrane bilayers are in a fluidlike critical state at, or very near, the physiological growth temperatures of the cells, thereby keeping the translational diffusion rates of embedded macromolecules at a critical value. This suggestion implies that adjustments of dynamic properties of the bilayer are of crucial importance for membrane function, and is in conflict with our model for the regulation of the lipid composition, which implies that the packing of the lipids is crucial for a functioning membrane. The new data by Jin et al. [32] prompted us to measure the lateral diffusion coefficient for total lipid extracts from *E. coli* membranes by a noninvasive NMR method [33]. No anomaly in the diffusion coefficient for the lipid extracts was observed at the growth temperatures, but it increases monotonically with the temperature. Consequently, we conclude that the lipid dynamics is not involved in the adjustment of the membrane lipid composition, and our model involving lipid structural properties still seems to be appropriate (Orädd, G., Lindblom, G., Rilfors, L., and Morein, S., to be published).

2.3. Eukaryotic organisms

An important conclusion drawn from the studies of *A. laidlawii* and *E. coli* is that the cells always seem to adjust the membrane lipid composition so that a lamellar liquid crystalline phase is maintained, thus avoiding the formation of both a gel phase and nonlamellar liquid crystalline phases (Figs. 3 and 5). The relationship between the membrane lipid composition, and the physicochemical properties of the lipids, has been explored in other prokaryotic organisms, and *Clostridium butyricum* and *Bacillus megaterium* seem to regulate their membrane lipid composition in analogy with *A. laidlawii* and *E. coli*, respectively, [7,34,35]. Also eukaryotic organisms, like fungi, higher plants, and poikilothermic animals, often change their membrane lipid composition in response to changes in the ambient temperature [1,36,37]. It has been suggested that the lipid regulation in these organisms takes place according to a model having the same features as ours; it is implied that the temperature acclimation or adaptation modifies the phase transition temperatures of the lipids so that a lamellar phase is always stable and the ambient temperature is confined to a 'window' bounded by the gel and nonlamellar phases [36].

One example of the regulation of membrane lipid composition occurring in plants will be given. Freezing intolerance of the plasma membrane of oat and rye leaves is primarily a consequence of membrane destabilization resulting from freeze-induced dehydration [38]. The lipid composition of the plasma membrane isolated from leaves of spring oat was found to be vastly different from that of winter rye. The plasma membrane of spring oat contains large fractions of phospholipids, cerebrosides and acylated sterylglucosides, while that of winter rye contains a greater proportion of phospholipids, and a lesser fraction of cerebrosides, but larger fractions of

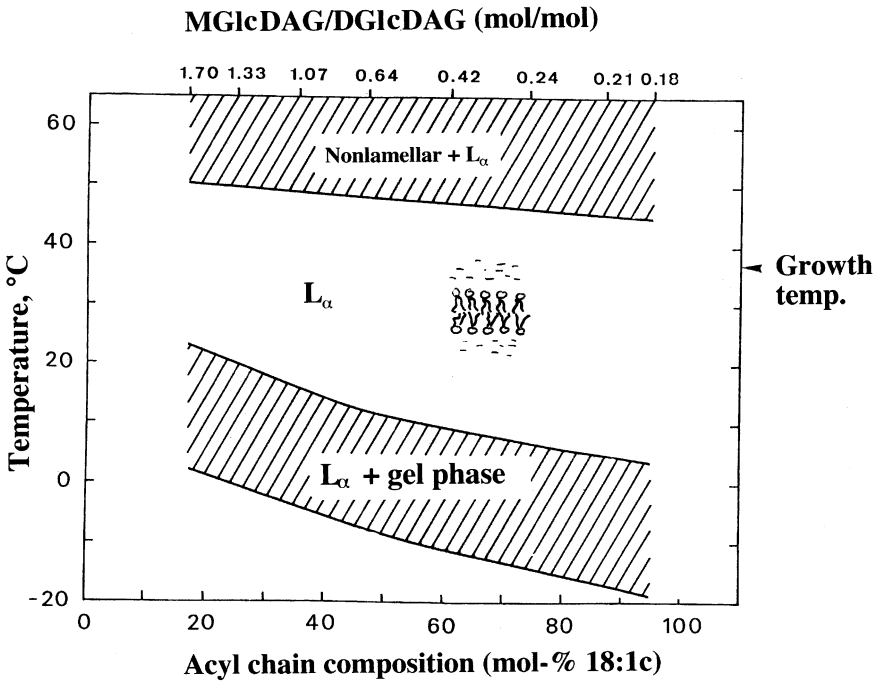

Fig. 3. Phase equilibria of total lipid extracts, containing different fractions of palmitoyl and oleoyl chains (lower x-axis), from membranes of *A. laidlawii* strain A grown at 37 °C. Water contents were 20 wt.%. The upper x-axis shows the metabolically obtained MGlcDAG/DGlcDAG ratios. The lower hatched area denotes the gel–liquid crystalline phase transition interval, and the upper hatched area denotes the appearance of H_{II} and/or reversed cubic phases in the lipid mixtures. Adapted from [4].

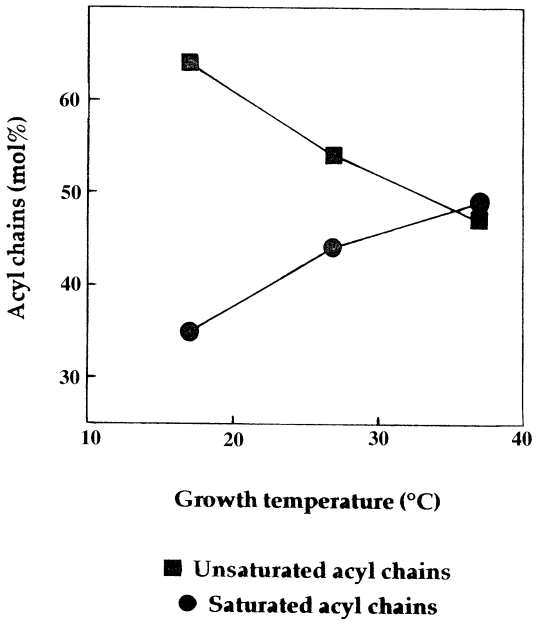

Fig. 4. Acyl chain composition of total lipid extracts from the inner membrane of *E. coli*. The cells were grown at 17, 27, and 37 °C. Data from [12].

free sterols. However, for both the organisms, cold acclimation results in an increase of the fraction of the phospholipids, and a decrease in the cerebroside component. It was shown that the lipids form an H_{II} phase in the freezing injury. The incidence of the H_{II} phase correlates with the lethal injury to both protoplast and leaf tissue as indicated by loss of osmotic responsiveness of protoplasts and leakage of the intracellular contents of leaves. The temperature dependence for the onset of the freeze-induced formation of the H_{II} phase is significantly different for winter rye and spring oat and it is, not surprisingly, associated with the differences in the lipid composition of the plasma membranes. However, freeze-induced formation of the H_{II} phase does not occur after cold acclimation in either of the organisms because of the strong decrease in the cerebroside fraction in the plasma membrane. Such an adaptation can be understood, since cerebrosides promote the formation of the H_{II} phase at low temperatures [39]. Cerebroside has a low hydration of its polar head group, resulting in a more wedge-shaped molecule, which easily pack to an H_{II} structure. Therefore, the propensities of the plasma membranes of rye and oat to undergo the lamellar to H_{II} phase transition during freeze-induced dehydration appears to be a consequence of the physico-chemical properties of the membrane lipids, including bilayer surface hydration and lipid packing.

3. Role of nonlamellar-forming lipids for membrane function

The lamellar liquid crystalline phase with its multibilayer structure has long been used as a model for biological membranes, and single-bilayer vesicles are frequently utilized in for example pharmaceutical applications. However, the awareness of the formation of nonlamellar structures, occurring for a number of membrane lipids, has gradually changed the view on the functional role played by membrane lipids in cell processes; surprisingly, this knowledge still cannot be found

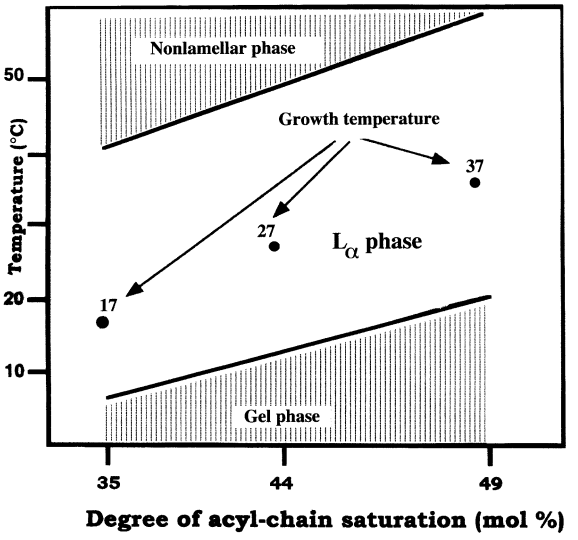

Fig. 5. Phase equilibria of total lipid extracts from *E. coli* cells grown at 17, 27, and 37 °C. The water content was 20 wt.%. The *x*-axis shows the fraction of saturated acyl chains in the lipids. The line of the lower hatched area denotes the temperatures at which a gel phase appears in the lipid mixtures, and the line of the upper hatched area denotes the temperatures at which an H_{II} phase appears in the lipid mixtures. Data from [12].

in general biochemistry textbooks. There are now a great deal of experimental evidence showing that the lipids actively participate in many important functions of the cell, and in this review our intention is to touch upon a few areas where nonlamellar-forming lipids are believed to be involved.

3.1. Membrane structures

One reason for cells to synthesize nonlamellar-forming membrane lipids, and to maintain a given balance between these lipids and the lamellar-forming ones, is that nonlamellar-forming lipids are needed to form either nonbilayer structures, or bilayer structures having a small radius of curvature. Nonbilayer structures are suggested to be formed in the processes of fusion and fission of lipid bilayers (see Section 3.3 for details). Bilayer structures having a small radius of curvature occur in several types of biological membranes, such as the endoplasmic reticulum, the inner mitochondrial membrane, and the grana stacks of thylakoid membranes in chloroplasts. In particular, it has been shown by analysis of transmission electron micrographs of the smooth endoplasmic reticulum and the inner mitochondrial membrane that these membranes resemble bicontinuous cubic structures [40]. A highly ordered, branched tubular membrane structure called the prolamellar body is present in etioplasts. These organelles are found in leaves of plants grown in the dark. After the exposure to light, the etioplasts transform into chloroplasts, and the prolamellar body develops into the thylakoid membranes of the chloroplast. The three-dimensional arrangement of the tubules in the prolamellar body is similar to the arrangement of the structural elements in a bicontinuous cubic phase [41].

3.2. Activity of membrane-associated proteins

Several investigations from the 1980s showed that the efficiency of protein incorporation during the reconstitution of membrane proteins, and the activity of membrane-bound proteins and enzymes, is enhanced in the presence of lipids forming nonbilayer structures, or by the incorporation into the membrane of molecules known to destabilize the bilayer structure ([3] and references therein). More recent studies have revealed that the activity of the phosphatidylcholine (PC)-specific phospholipase C from *B. cereus* is enhanced by the presence in the bilayer of lipids that destabilize the lamellar phase [42]. However, the activation is attributed to a packing stress in the lipid bilayer ('frustration'), rather than to the actual formation of reversed nonlamellar phases. These results fall in line with the hypothesis suggested by Kinnunen [43] that 'frustrated' lipid bilayers play a role in the anchorage and activation of peripheral membrane proteins. For protein kinase C it has been shown that both the partitioning of the enzyme to a membrane, and the activity of the membrane-bound form of the enzyme, is increased in the presence of nonlamellar-forming lipids [44,45]. The peptide alamethicin exhibits a voltage-dependent ion channel, and the states of higher conductance are more probable when the fraction of a nonlamellar-forming lipid in the bilayer is increased, or, expressed in an alternative way, when the spontaneous curvature of the two monolayers is increased [46]. Finally, by using the lipid biosynthetic mutant of *E. coli* discussed in Section 2.2, two important membrane-associated processes were shown to be dependent on the membrane lipid composition: firstly, nonlamellar-forming lipids are essential for an efficient translocation of proteins across the plasma membrane of the cells [47]; and secondly, *E. coli* cells completely deficient in PE are defective in cell division [48].

One example can be given where the introduction of a nonlamellar-forming lipid into a lipid bilayer has a restraining effect on a membrane protein. Curran et al. [49] studied the regeneration of SDS-denatured bacteriorhodopsin in PC/PE lipid vesicles, and they found an inverse relationship between the regeneration yield of this integral membrane protein and the fraction of PE in the vesicles. The authors interpretation of the results is that the introduction of the smaller PE headgroup increases the intrinsic desire for the monolayers to curve towards the water region, and thus increases the lateral pressure in the acyl chain region. This may in turn either hinder the insertion of the denatured protein into the bilayer, or slow down a folding step within the bilayer.

In the above-mentioned examples the activating effect is exerted by lipids forming reversed nonlamellar phases. However, phospholipase A_2 (PLA_2) is an enzyme that is activated when lipid vesicles are transformed to structures similar to the intermediate structures observed in the vesicle-to-micelle transition in detergent–lipid systems [50]. PLA_2 is a soluble enzyme whose activity is markedly enhanced when the substrate is provided in a lipid aggregate [50,51]. The binding of PLA_2 is stronger to bilayers with anionic lipids than to bilayers with zwitterionic lipids [52,53]. However, binding to a membrane is a necessary, but not sufficient, condition for activation of PLA_2. A change in morphology of the lipid bilayer seems to be needed [52], and a recent study reported a direct connection between the enzyme activity and the transformation of intact lipid vesicles to open vesicles, bilayer fragments, and small globular micelles [50]. Interestingly, the lipid synthesizing enzyme PS synthase from *E. coli* is also activated by a vesicle-to-micelle transition, as discussed in Section 4.1. Furthermore, in a study of the regeneration kinetics of bacteriorhodopsin in mixed micelles consisting of a bilayer-forming PC and a micelle-forming PC, it was found that the values of two apparent rate constants increase about 10-fold when the fraction of micelle-forming PC increases from 0.35 to 0.7 [54]. This effect was suggested to be due to the reduced lateral pressure in the acyl chain region caused by the short-chain, micelle-forming PC.

3.3. Membrane fusion

Membrane fusion is a very important phenomenon in all cells. In particular, it is involved in processes of transport in which membranes encapsulate different cell substances—often called membrane traffic. Other examples are the sperm-egg fusion and virus-cell fusion. The molecular mechanisms behind the fusion process have been studied extensively both experimentally and theoretically over the last decade [55,56]. Here we will confine ourselves to the fusion occurring in lipid systems, in particular systems where nonlamellar structures probably are involved in the process [57–60]. The essential step in fusion is a rearrangement of the lipid molecules of two opposed membranes to form a single, continuous membrane. For an understanding of the fusion process both dynamical and structural aspects have to be considered, but here only the latter one will be briefly discussed.

It was early proposed that the reorganization of the membrane structure which occurs during fusion requires that the lipid bilayer is broken up and that other aggregate structures are formed [61]. Lucy and colleagues showed that lysophosphatidylcholine (LPC) can induce fusion between erythrocytes, as well as between erythrocytes and fibroblasts [62], and LPCs are known to form normal micellar solutions at high water contents [63]. Some years later the formation of reversed nonbilayer aggregate structures in erythrocyte phospholipids was found to be closely correlated to the fusion of intact erythrocytes [64,65]. Recently, Siegel developed a modified so called stalk mechanism to describe the lamellar to reversed nonlamellar phases and its implications for membrane fusion [55,56,66,67]. Siegel estimated the energies of hypothetical intermediate structures with respect to planar bilayers, which is an elaboration of the technique of Markin et al. [68]. These extensive theoretical calculations show that similar intermediate structures are involved in membrane fusion processes and in the transitions between lamellar liquid crystalline and H_{II}, or reversed cubic, phases [55,56,66,67]. The results suggest an important new approach by which low levels of particular lipids like lysolipids, or some types of peptides, could significantly enhance the rate of fusion in model membranes and biomembranes.

4. Lipid synthesizing enzymes

The regulation of the membrane lipid composition, occurring in several organisms, implies that the activity of the enzymes synthesizing the lipids (lipid synthases) are adjusted to the prevailing growth conditions of the cells, i.e. some kind of signal(s), reflecting the status of the lipid bilayer, must be transferred from the bilayer to the lipid synthases. The lipid synthases are generally more

or less tightly associated to the lipid bilayer, and one possibility is that the activity of these enzymes is directly influenced by the properties of the lipid bilayer. Another alternative is that the activity of the synthases is regulated by one or more effector molecules that bind to the enzymes. These effector molecules can consist of membrane lipids [69], or of a special protein that in turn senses the status of the lipid bilayer. In this review we will briefly summarize results showing that the activity of lipid synthases are directly affected by the physico-chemical properties of the lipid matrix.

4.1. Phosphatidylserine synthase from E. coli

Two lipid synthases have a central role in the lipid metabolism of *E. coli*: phosphatidylserine (PS) synthase and phosphatidylglycerophosphate (PGP) synthase. PS synthase and PGP synthase catalyze the first step in the metabolic pathways leading to the syntheses of PE, and PG and DPG, respectively. Wild-type cells of *E. coli* keep the balance between the fraction of PE, and the sum of the fractions of PG and DPG, practically constant (see Section 2.2).

A first picture of how the activity of PS synthase is affected by the interaction with lipid aggregates of different structures and compositions has been obtained [18]. PS synthase is a so-called amphitropic enzyme, i.e. an enzyme that shifts between an inactive cytosolic form and an active membrane-bound form. PS synthase exhibits practically no activity after reconstitution with lipid vesicles. Addition of the nonionic detergent octylglucoside in concentrations well above its CMC value increases the enzyme activity about 20–1000-fold, the degree of activation depending on the lipid composition of the proteoliposomes. A gradual transformation of the lipid vesicles to micelles brings about an activation of the enzyme that is proportional to the degree of micellization. It remains to be elucidated how this mode of activation is related to the actual functioning of the enzyme in the living cell. The enzyme activity increases about 10-fold when the fraction of PG is increased 7-fold in the mixed micelles. The highest activities of PS synthase are exhibited with the anionic lipids synthesized by *E. coli*.

The interaction of PS synthase with lipid bilayers of various compositions has been studied with coupled plasmon-waveguide resonance (CPWR) spectroscopy [17]. PS synthase exhibits a biphasic interaction with the bilayers: the first phase (at low protein concentrations) is dominated by hydrophobic interactions, and the enzyme causes a local decrease of the ordering of the lipid molecules; the second phase (at high protein concentrations) is predominantly controlled by electrostatic interactions, and results in a cooperative binding of the enzyme to the membrane surface. Addition of anionic lipids to a PC bilayer causes a 5–15-fold decrease in the protein concentration at which the first binding phase occurs.

Taken together, the results show that PS synthase seems to be one of the lipid synthases that is directly involved in maintaining the polar head group composition in *E. coli* at a nearly constant value. A model for regulating the activity of PS synthase has been suggested [70,71]. The essential feature of the model is that PS synthase molecules are supposed to be more tightly bound to the membrane when the fraction of anionic lipids increases. As the enzymatic reaction is carried out when the enzyme is bound to the membrane, the synthesis of PE (proceeding via PS) will increase under these conditions, and the response balances the elevated levels of anionic lipids. The experimental data support, and extend, the model, (i) the enzyme binds more strongly to a lipid bilayer when the fraction of anionic lipids increases [17]; and (ii) besides being modulated by the fraction of anionic phospholipids in lipid aggregates, the PS synthase activity is influenced by the chemical structure of the polar head group of the anionic phospholipid; DPG has a more pronounced activating effect than PG [18].

4.2. CTP: phosphocholine cytidylyltransferase

PC is a major membrane lipid in most eukaryotic cells and a precursor to other membrane lipids. Therefore, the regulation of the activity of CTP, phosphocholine cytidylyltransferase (CCT) is very important for membrane biogenesis. CCT is localized in the nucleus of eukaryotic cells and can interconvert between an inactive form in the

nucleosol and an active membrane-bound form [72]. CCT is thus another example of an amphitropic enzyme. The activity of CCT is increased by anionic phospholipids and by neutral lipids like diacylglycerol [73]. An amphipathic α-helical peptide of CCT binds to anionic lipid vesicles [74], and the activating effect of anionic lipids is attributed to an electrostatic interaction between these lipids and basic amino acid residues in the amphipathic helix [73,75].

Cornell and colleagues suggested that diacylglycerols may give a looser packing in the polar head group region of the bilayer, and activate CCT by facilitating the intercalation of the amphipathic α-helical peptide into the bilayer [74,76]. Recently, it was shown that the activity of CCT is modulated by the stored curvature elastic stress in the monolayers of a lipid membrane [77]. The underlying idea of the experiments is that incorporation into a bilayer of a detergent, or a lipid forming reversed nonlamellar phases, will decrease and increase the stored curvature elastic stress in the monolayers, respectively. It is assumed that the partial submersion of the amphipathic binding domain of CCT into a monolayer allows the release of some of the stored curvature elastic stress. Attard et al. [77] convincingly showed that the activity of CCT increases monotonically when the stored curvature elastic stress (or the spontaneous curvature) in the monolayers increases. In contrast, the enzyme activity decreases significantly by incorporation of small fractions of detergent molecules into the bilayer, i.e. when the spontaneous curvature of the monolayers is decreased. The results show that a purely physical feed-back signal can play a key role in the regulation of membrane lipid synthesis. Finally, it can be pointed out that the membrane binding of CCT involves both electrostatic and hydrophobic interactions, which is similar to the *E. coli* PS synthase.

4.3. Glucolipid synthases from A. laidlawii

MGlcDAG and DGlcDAG are the dominating glucolipids in *A. laidlawii* strain A under most growth conditions. The activity of the purified enzymes MGlcDAG synthase and DGlcDAG synthase, catalyzing the synthesis of these lipids, has been investigated in mixed micelles consisting of the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and membrane lipids or other amphiphilic molecules [78–80].

MGlcDAG synthase is activated by the anionic lipids PG and PS, and a higher fraction of PG is needed to reach a certain enzyme activity when the cationic lipid sphingosine is present in the mixed micelles [78]. These results indicate that the activity is enhanced by negative charges on the aggregate surface. However, $MgCl_2$ is present in the assay system, and the molar ratio Mg^{2+} to anionic lipids is 2:1 or higher. The negative charges are probably efficiently quenched by the divalent ions [78], and it is, therefore, uncertain how crucial the negative surface charges are for the activity of MGlcDAG synthase. Moreover, the enzyme is inactive when PG constitutes less than 30–40 mol% of the membrane lipids in the mixed micelles, and the fraction of PG very seldom reaches above 30 mol% in *A. laidlawii* membranes [9,81]. In contrast to DGlcDAG synthase, MGlcDAG synthase is not activated by water-soluble phosphate-containing molecules [80].

The MGlcDAG synthase is claimed to maintain the balance between two competing pathways for membrane lipid synthesis [78]. One pathway leads to PG, and the other to MGlcDAG, DGlcDAG, and most probably to two phosphoglucolipids (see Section 2.1). The presence of the phosphoglucolipids in the glucolipid pathway complicates the situation, and it may thus be reasonable that the surface charge density of the membrane lipids is not tightly regulated in *A. laidlawii* [82].

DGlcDAG synthase is activated by PG, PS, DPG, and phosphatidic acid [79,80]. It is somewhat unclear also for this enzyme if it is activated by the negative charges on the aggregate surface, since $MgCl_2$ is present in the assay system, and no decrease in enzyme activity is obtained when sphingosine is present in the mixed micelles. DGlcDAG synthase is not activated until PG constitutes more than 20–25 mol% of the membrane lipids in the mixed micelles [80], and the PG fraction occurring in *A. laidlawii* membranes may, therefore, be insufficient for activation under sev-

eral growth conditions [9,81]. However, this synthase is activated manyfold by water-soluble molecules like orthophosphate, pyrophosphate, ATP, fructose 1,6-bisphosphate, and double-stranded DNA, at least in the presence of 20–25 mol% PG [80].

DGlcDAG synthase is activated 3–5-fold by three amphiphiles forming, or promoting the formation of, nonlamellar phases, namely dioleoyl-PE, 1,3-dioleoylglycerol, and cholestenone [79]. The authors, therefore, suggest that the ability of *A. laidlawii* cells to regulate the ratio between lipids forming lamellar and nonlamellar phases resides partly or fully in the enzyme DGlcDAG synthase. Two objections can be made to this suggestion: (i) the two minor nonlamellar-forming lipids in *A. laidlawii*, MAMGlcDAG and MADGlcDAG, do not activate the enzyme [79], and the enzymes synthesizing these lipids have not been studied; and (ii) a model system consisting of mixed micelles may not be the most suitable to study the effect of nonlamellar-forming lipids on the structure and activity of membrane-associated proteins (see also [49]). The form as well as the size of the micelles may be altered by the presence of the nonlamellar-forming lipids, and this alteration per se can affect the activity of an enzyme (see Sections 3.2 and 4.1). Despite these objections, a very good resemblance was observed between in vivo and in vitro studies when the two glucolipid synthases were reconstituted together in mixed micelles. By increasing the fraction of cholestenone in the micelles, the syntheses of MGlcDAG and DGlcDAG was decreased and increased, respectively [49], and this response mimics the adjustment of the lipid composition occurring in living cells [83].

5. Concluding remarks

It has been shown for the two prokaryotic organisms *A. laidlawii* and *E. coli* that the cells maintain a particular balance between lamellar-forming and nonlamellar-forming membrane lipids by adjusting the polar head group or the acyl chain composition to the prevailing growth conditions. The adjustment performed by *E. coli* cells also results in that the gel–liquid crystalline phase transition is completed below the growth temperature. *E. coli* cells are in this way able to keep their membrane lipids in a 'window' between a lamellar gel phase and reversed nonlamellar phases. Studies of the regulation of the membrane lipid composition in organisms from several kingdoms indicate that this is a general feature of living cells. It is well documented by now that the physico-chemical properties of the lipid matrix can be a direct feed-back signal on the activity of lipid synthesizing enzymes. The activity of such enzymes can be modulated by the fractions of anionic lipids and nonlamellar-forming lipids present in micelles or bilayers. Numerous investigations also show that nonlamellar-forming lipids are important for membrane structures and membrane-associated processes, short-lived nonbilayer structures are probably formed in the processes of fusion and fission of lipid bilayers; long-lived bilayer structures having a small radius of curvature occur in several types of biological membranes (e.g. smooth endoplasmic reticulum, prolamellar bodies, and septum formation in cell division); and the activity of membrane-associated proteins can be modulated by adding detergents and nonlamellar-forming lipids to bilayers.

The present review highlights the involvement of nonlamellar-forming lipids in several aspects of membrane functioning. Evidence is also continuously accumulating that many membrane lipids play important roles as signaling molecules in cells [84]. It may, therefore, be timely to introduce a new area of research that we humbly would like to designate functional lipidomics in analogy with the established fields of functional genomics and functional proteomics.

Acknowledgements

This work was supported by the Swedish Natural Science Research Council and the Knut and Alice Wallenberg Foundation.

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