Membrane Bilayer Properties in *Acholeplasma laidlawii* are Sensed and Set by the Lipid-synthesizing Enzymes

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Abstract

In membranes of the small prokaryote *Acholeplasma laidlawii* (i) a constant surface charge density, given by the anionic lipids, (ii) similar phase equilibria, close to a potential bilayer-nonbilayer transition, and (iii) a nearly constant spontaneous curvature, are metabolically maintained for the lipid bilayer under a variety of conditions *in vivo*. This involves extensive changes in the amounts and types of bilayer- and nonbilayer-prone glucolipids synthesized. Analyses of the three first, consecutively acting enzymes in the glucolipid metabolic pathway show that the major metabolic responses of the living cell can be mimicked by the enzymes in reconstituted amphiphile-enzyme aggregates. The enzymes respond to a proper lipid surface charge, certain activator lipids, domain formation, and the spontaneous curvature, and it is concluded that they are the sole sensors and mediators for the maintenance of lipid bilayer packing properties in the cell membrane.

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No Models

We are still a long way from a detailed, structural understanding for the involvement of the many different membrane lipids in various cellular processes. Exept for the metabolic maintenance of a "melted" (liquid-crystalline) state for the hydrocarbon chains of biological lipid bilayers, essentially no physico-chemical models have been proposed to rationalize the typical differences in polar lipid composition, and the related inter-membrane flow, that occur for the many membranes in the eukaryotic cell. The reasons for this are several, e.g. the large number of lipids and synthesis pathways, several genes/enzymes for many individual lipid synthesis reactions, the localization of enzymes to different organelle membranes, plus the technical problems of manipulating and purifying various specific membranes from eukaryotic cells, respectively.

Acholeplasma laidlawii Membrane

A bilayer organization, and the essential character of the liquid-crystalline (LC) state for cell growth, was early shown for the small and simple prokaryote *Acholeplasma laidlawii.* However, this parasitic organism lacks efficient metabolic means to regulate or adjust the gel to LC transition of the lipid bilayer since it can only synthesize saturated (SFA), but not unsaturated (UFA) fatty acyl chains for polar lipid synthesis. Still, these cells can grow with a large variety of bilayer lipid acyl chains, yielding from ~14 to almost ~20 carbons in average chain length (C_n), but with restrictions in the extent of chain unsaturation tolerated in the short and long C_n range, respectively (Wieslander et al., 1995). This causes an allowed span between different membranes for the thickness of the hydrophobic bilayer region, from ~2.3 nm to ~3.1 nm, with little influence of the membrane proteins, according to chain order profiles determined by NMR (Thurmond et al., 1994). However, experimental variation of the C_n and SFA/UFA parameters yield large changes in the amounts of individual polar lipids, as well as between the classes of phospholipids, phosphoglucolipids and glucolipids synthesized.

Similar Charge Density and Phase Equilibria

Extensive analyses of the physico-chemical properties of the individual lipids, and more or less *in vivo*-like mixtures thereof, have revealed a correlation between physiological composition (regulation) and properties of the lipids (summarized in Rilfors et al., 1993; Wieslander and Karlsson, 1997). A constant surface charge density (potential) was maintained by the varying amounts of anionic lipids synthesized as a function of increased chain unsaturation (lipid lateral area). Concomitantly, the amounts of the major nonlamellar-prone (reversed cubic/hexagonal phase) lipid monoglucosyldiacylglycerol (MGlcDAG) decreased, whereas the lamellar-forming diglucosyl derivative (DGlcDAG) increased, see Fig. 1. Based on arguments of "molecular shape" it was proposed that the metabolic balance between MGlcDAG and DGlcDAG aimed at maintaining a certain "bilayer stability" (or instability), i.e. similar phase equilibria, close to a potential lamellar- nonlamellar transition for the membrane lipid bilayer. Evidence for this was gained from studies, where the different but established effects of certain hydrocarbons, alcohols, detergents and steroids on amphiphile phase equilibria, correlated strongly with the metabolic responses (i.e. MGlcDAG/DGlcDAG amounts synthesized) from *A. laidlawii* to the presence of these foreign additives in the membrane bilayer. An elaborate fine-tuning by these regulatory mechanisms was also established. MGlcDAG, although always being made by the cells, will have its phase equilibria progressively shifted towards the lamellar phase by decreasing C_n and increasing chain saturation, respectively (Lindblom et al., 1993). However, at such conditions other, more potent nonbilayer derivatives are successively made or increased in amounts, assisting MGlcDAG in its critical "packing" function. These lipids are the precursor DAG, and variants of the two glucolipids with an extra, third acyl chain, i.e. MAMGlcDAG and MADGlcDAG (*-label in Fig. 1.) (Wieslander et al., 1995; Andersson et al., 1996).

For amphiphiles in general, increased chain unsaturation and decreased C_n, lead to a decreased average chain order (at constant temperature). However, in *A. laidlawii* the metabolic variation of the MGlcDAG amounts as a function of UFA and C_n changes seems to have a stronger impact on chain order, with increased order correlating with increased MGlcDAG amounts (and decreased C_n).

This is due to the small polar head of MGlcDAG allowing for a denser lateral packing of the lipids with a concomitant increase in chain order, as was shown for different MGlcDAG/DGlcDAG mixtures (Eriksson et al., 1991), similar to other lipid mixtures with contrasting packing properties (e.g. nonbilayer DOPE/bilayer DOPC). Hence, chain order is not kept constant in the *A. laidlawii* bilayer by the lipid metabolism.

Constant Spontaneous Curvature

The presence of MGlcDAG, and the other nonbilayer-prone lipids (see above) with small polar headgroups in relation to their more (laterally) bulky acyl chains, is obviously regulated to maintain certain packing properties as is evident from the similar (temperature) closeness to a bilayer-nonbilayer transition for all *in vivo* lipid mixtures. The imbalance in lateral forces yields a spontaneous curvature for the two monolayers of the bilayer, tending to curl outward (concavely) from each other. This bending force is considered large enough to be able to affect the conformation of proteins. In *A. laidlawii* the radius of the spontaneous curvature (R_0) is actively kept constant by the lipid metabolism as seen for *in vivo* lipid mixtures, where the % UFA chains and C_n in the lipids were varied in a systematic manner, by metabolic adjustments in MGlcDAG (and other nonbilayer lipid) amounts (Österberg et al., 1995). Likewise, the lamellar to nonlamellar phase transition, but not the melting one, occurred at fairly similar temperatures. The R_0 for unsaturated MGlcDAG and DGlcDAG species was 1.7 and 12.3 nm, similar to DOPE (2.1 nm) and DOPC (9.6 nm), respectively (saturated DGlcDAG and PC are bound to have substantially larger R_0). However, R_0 values for the *in vivo* mixtures only varied between 5.8 and 7.3 nm over the series, a remarkable constancy unders these circumstances (Österberg et al., 1995).

Enzymes Sense and Set Bilayer Properties

At the executing level these regulatory mechanisms in *A. laidlawii* must involve a direct sensing of bilayer packing properties by the lipid-synthesizing enzymes, or by specific "sensors" communicating proper signal to activate enzyme genes or to modify activities of the enzymes, respectively. A sensing of indirect effects, like for instance lipid domain formation, must also be considered. The membrane phospholipids and glucolipids are consecutively made in two competing pathways from a common phosphatidic acid (PA) precursor. Purification and reconstitution of the three first enzymes of the glucolipid pathway (Table 1 and enzyme 1, 2 and 3 in Fig. 1) in mixed-micelles and liposome bilayers have revealed that the regulatory mechanisms (cf. above) most likely rely solely on the "sensing" and kinetic properties of the synthesizing enzymes. The synthase enzyme for the major nonbilayer-prone lipid MGlcDAG (DAG-glucosyltransferase = enzyme 2) demands a critical fraction of negatively charged lipids for activity (Karlsson et al., 1994; 1997). This is coupled to a conformational change of the enzyme, as analyzed by proteolytic resistance (Li et al., 1997). Chain length of the DAG substrate, the latter made from PA by a PA-phosphatase (= enzyme 1) (Berg and Wieslander, 1997), but not curvature or phase equilibria, is important for activity. PA and DAG precursors do not accumulate normally. Hence, the MGlcDAG synthase (but not the PA phosphatase) is probably a main site for the lipid surface charge regulation, balancing the two

Table 1. Properties of *A. laidlawii* lipid-synthesizing enzymes.

	PA phosphatase	MGlcDAG synthase	DGlcDAG synthase
In vivo substrates	PA	DAG & UDP-Glc	MGlcDAG & UDP-Glc
In vivo products	DAG & PI	MGlcDAG & UDP	DGlcDAG & UDP
Purification	near homogeneity	homogeneity	near homogeneity
Cofactors	not detected	Mg^{2+}	Mg^{2+}
Membrane assoc.	integral protein	hydrophobic + electrostatic	prob. integral protein
Substrate specificity	low	lipid chain variants	lipid chain variants
Activator	not detected	anionic lipids	PG

pathways, and activation of this enzyme is brougth by all the anionic lipids, see Fig. 1. The following, single glucosylation of MGlcDAG to yield the major bilayer-forming DGlcDAG (MGlcDAG-glucosyltransferase = enzyme 3) only occurs in the presence of substantial amounts of a specific activator lipid (in a cooperative fashion), i.e. phosphatidylglycerol (PG) from the other, phospholipid pathway, see the Fig. 1. (Dahlqvist et al., 1995). *In vivo* these two lipids are coordinately synthesized on an amount basis. Amounts of PG activator needed *in vitro* depend on chain order, bilayer curvature and phase equilibria, i.e. the fractions and nonbilayer propensities of several additives tested. Variations in synthesis rates for both glucosyltransferases *in vitro* are in accordance with, and sufficient for, the recorded *in vivo* changes of MGlcDAG and DGlcDAG amounts needed for the compensatory curvature adjustments due to the inflicted membrane perturbations (Dahlqvist et al., 1995). A domain formation of the activator PG, induced experimentally by an activator-PG/bilayer-matrix chain length mismatch, could superseed the curvature effects for the DGlcDAG synthase and strongly enhance the glucosyltransferase activity at low activator (PG) amounts (Karlsson et al., 1996). Hence, a local enrichment of the activator lipid may be a fast, potential second mechanism for regulating DGlcDAG synthesis (from MGlcDAG), which may override the primary curvature effects. Preliminary data also indicate that certain ions can stimulate this enzyme substantially, yielding a coupling to cell energy metabolism.

General Validity

Following these studies, analogous mechanism for maintaining similar lamellar-to-nonlamellar phase equilibria have been vizualized in several other, not related bacteria (with no glucolipids), e.g. the Gram-negative *Pseudomonas fluorescens* and *Escherichia coli*, and the Gram-positive *Bacillus megaterium* plus two *Clostridium* species, and are likely to occur in many more by arguments of similarity in lipid composition. For several of these species the regulation takes place at a specific (soluble) enzyme in acyl chain synthesis (elongation), whereas for others it involves changes in polar headgroup composition or even at both these levels. Hence, similar principles operate in various organisms, but with different tools for the metabolic execution. Likewise, the high content of nonbilayer-prone lipids in several eukaryotic organelle membranes (e.g. the endoplastic reticulum, mitochondria and chloroplasts) indicates that in these bilayers a certain spontaneous curvature must indeed be present and actively maintained.

Several integral membrane proteins, and

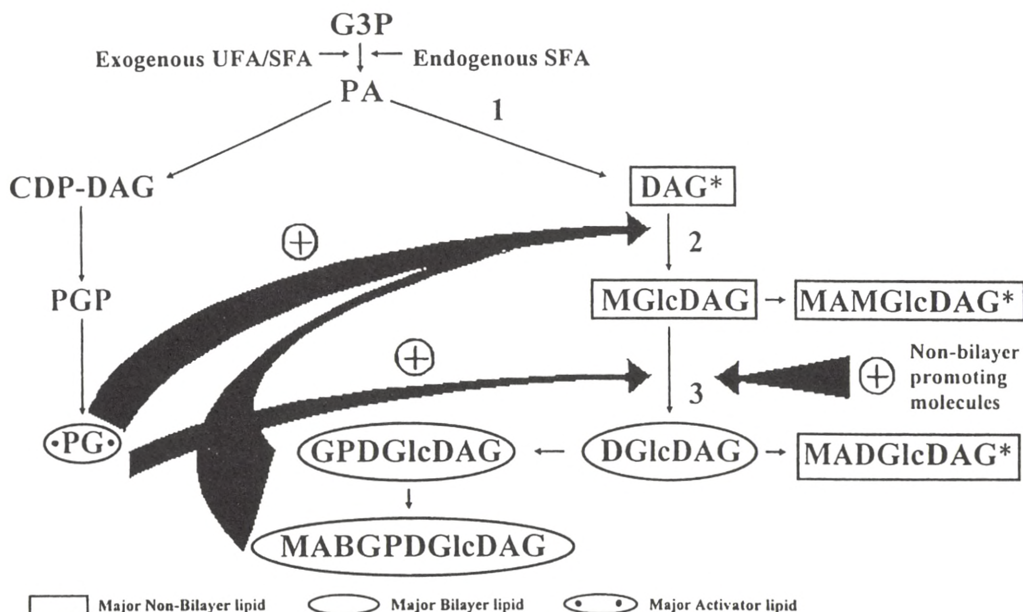

Figure 1. Connected pathways for the biosynthesis of membrane lipids in *Acholeplasma laidlawii* A-EF22. →, enzymatic step; filled, thick arrows indicate (different) enzyme activation by influence (contact?) of anionic lipids or PG (for enzyme 2 and 3, respectively); and influence of curvature and chain order on enzyme 3. Lipids boxed all form nonbilayer/nonlamellar aggregates, but with varying propensities; lipids with an asterisk may accumulate under certain conditions, especially when the phase equilibria of MGlcDAG are shifted from nonlamellar towards lamellar phases by more saturated or shorter acyl chains. Lipids encircled form lamellar phases. The glucolipid pathway is initiated by enzyme 1, a PA-phosphatase producing DAG; enzyme 2 is the MGlcDAG synthase, and enzyme 3 the following DGlcDAG synthase. These three are all very minor, integral membrane proteins ($\approx$ 23, 40 and 41 kDa). Enzyme 2 and 3 are both glucosyltransferases (lipid plus UDP-glucose substrates).

temporarily membrane-associated soluble proteins, have been shown to depend on similar lipid packing properties as the two *A. laidlawii* glucolipid synthases described here, i.e. lipid surface charge and the spontaneous curvature. Interesting examples are the rate-limiting step in the eukaryotic phospholipid synthesis, i.e. CTP:phosphocholine cytidylyltransferase and protein kinase C; both of these have important regulatory functions in the cell.

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