X-ray diffraction measurement of the monolayer spontaneous curvature of dioleoylphosphatidylglycerol

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Abstract

Phosphatidylglycerol (PG) is an anionic lipid commonly found in large proportions in the cell membranes of bacteria and plants and, to a lesser extent, in animal cells. PG plays an important role in the regulation and determination of the elastic properties of the membrane. Using small angle X-ray scattering experiments, we obtain that the monolayer spontaneous curvature of dioleoylphosphatidylglycerol (DOPG) is \(-1/150 \pm 0.021\) nm\(^{-1}\) when measured in 150 mM NaCl. When the experiments are carried out in 150 mM NaCl and 20 mM MgCl\(_2\), the value obtained for the monolayer spontaneous curvature is \(-1/8.7 \pm 0.037\) nm\(^{-1}\). These values are of importance in modelling the effects of curvature elastic stress in membrane lipid homeostasis in the bacterium *Acholeplasma laidlawii* [Alley, S.H., Barahona, M., Ces, O., Templer, R.H., in press. Biophysical regulation of lipid biosynthesis in the plasma membrane. *Biophys. J.*] and indicate that divalent cations can play a significant role in altering curvature elastic stress.

1. Introduction

The lipids in the plasma membrane form a bilayer that provides a permeability barrier between the cytoplasm and the environment. Membrane lipids are chemically diverse (Dowhan, 1997a) but are permeability barrier between the cytoplasm and the environment.

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1. Introduction

The lipids in the plasma membrane form a bilayer that provides a permeability barrier between the cytoplasm and the environment. Membrane lipids are chemically diverse (Dowhan, 1997a) but are often classified into two physico-chemical classes: bilayer and non-bilayer lipids. Bilayer lipids will spontaneously assemble into the familiar plasma membrane structure of back-to-back monolayers with flat interfaces. Non-bilayer lipids self-assemble into structures with curved interfaces, such as the inverse hexagonal or H\(_{II}\) phase (Fig. 1). In fact, all lipid monolayers have a tendency to attain an interfacial curvature. Whether or not this is expressed structurally depends on the magnitude of the local forces between lipids, especially in the head-group and hydrocarbon regions. Although bilayer formation is crucial for cell survival, most organisms contain significant amounts of non-bilayer lipids (Goldfine, 1982; Ansell et al., 1973). It has been hypothesised that the cell uses lipid biosynthesis to establish a balance between the bilayer and non-bilayer lipid compositions in order to maintain the mechanical integrity of the bilayer while allowing for dynamical membrane function (Attard et al., 2000; Lindblom et al., 1986; Gruner, 1985). The balance between bilayer and non-bilayer lipids in the plasma membrane, and its relationship with specific cellular processes, has been the subject of extensive research (McMahon and Gallop, 2005).

A quantitative measure of the propensity for interfacial curvature of a lipid component is given by the spontaneous curvature in the Helfrich ansatz (Helfrich, 1973). For a cylindrical interface the monolayer curvature elastic free energy per unit area, \(g\), is:

\[
g = \frac{k_M}{2} (c_1 - J_c)^2
\]

where \(k_M\) is the bending rigidity of the monolayer, \(c_1\) is the spontaneous curvature of the cylindrical surface and \(J_c\) is the monolayer spontaneous curvature. Note that \(c_1 = J_c\) when \(g\) is at a minimum for the cylindrical monolayer. Bilayer lipids have \(J_c\) close to zero and (by convention) non-bilayer lipids that bend towards the water have negative spontaneous curvatures. By varying lipid composition, cells are hypothesised to adjust \(J_c\) to values that ensure the mechanical integrity of membranes in the fluid state whilst also enabling membrane scission and fusion. In this hypothesis, the spontaneous curvatures of lipid species are therefore a critical material parameter for cell survival (Attard et al., 2000; Lindblom et al., 1986; Gruner, 1985).

Phosphatidylglycerol (PG) and its dimer cardiolipin (CL) are two of the anionic lipids found in the cell membranes of many bacteria, including *E. coli* (Dowhan, 1997b) and the cell-wall-less Mycoplasma *Acholeplasma laidlawii* (Andersson et al., 1996), as well as in plants (Hagio et al., 2002) and animals (Postle et al., 2001). Pure PG mixtures have been shown to form bilayers at physiologi-
formed by mixtures of DOPG, dioleoylphosphatidylethanolamine (DOPE) and 9-cis-tricosene. DOPE, with $J_{c, DOPE} = -1/2.85 \text{ nm}^{-1}$, is added to induce the formation of the H$_{II}$ phase since mixtures of 9-cis-tricosene and DOPG do not to form the H$_{II}$ phase. The hydrophobe 9-cis-tricosene is added to minimize the packing frustration and relax the H$_{II}$ phase to the stress-free state. The dimensions of the H$_{II}$ phase saturate in excess water (Fuller et al., 2003), but excess alkene can prevent the water transport necessary to swell the water cylinders of the H$_{II}$ phase (Vacklin et al., 2000; Chen and Rand, 1998). As a result, samples are prepared in excess water with varying alkene composition to determine the dimensions of the H$_{II}$ phase at the stress-free state.

DOPG and DOPE were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and 9-cis-tricosene was obtained from Sigma–Aldrich, UK. All chemicals were stated to be >97% pure. Mixtures of different DOPG/DOPE mole fraction were co-dissolved in cyclohexane to give stock solutions. A stock solution of 9-cis-tricosene in cyclohexane was prepared. Volumes of DOPG/DOPE and 9-cis-tricosene stock solutions were added and weighed to give DOPG/DOPE/9-cis-tricosene samples with the appropriate alkene wt%. The mixtures were lyophilized to remove cyclohexane before being transferred to a glass capillary. Buffer was then added to the capillary in excess. Two buffers were prepared and adjusted with osmotic conditions (Dowhan, 1997b). PG can constitute up to 25% of the plasma membrane of E. coli, and similar percentages in A. laidlawii. It is therefore important to provide an experimental measurement of the spontaneous curvature of PG in order to model its role in the regulation of the mechanical properties of the membrane in bacteria (Alley et al., 2008).

Here, we estimate $J_{c, DOPG}$, the monolayer spontaneous curvature of dioleoylphosphatidylglycerol (DOPG), through small angle X-ray scattering experiments. To do this, the hydrophobe 9-cis-tricosene is added to DOPG mixtures so that DOPG monolayers achieve their spontaneous cylindrical curvature (Fig. 1). This method, first established by Rand et al. (Leikin et al., 1996; Rand et al., 1990) and extended in our laboratory (Vacklin et al., 2000), works on the principle that the hydrophobe reduces the free energy of chain packing in the H$_{II}$ phase to an almost stress-free state (Duesing et al., 1997; Kirk et al., 1984). The use of a long alkene, instead of an n-alkane (Chen and Rand, 1998), reduces the unwanted interdigitation with lipid chains (Vacklin et al., 2000). The spontaneous curvature is measured at the interface where bending and stretching modes are decoupled, i.e., the pivotal plane (Kozlov and Winterhalter, 1991). This plane always exists for small deformations near the spontaneous state (Leikin et al., 1996), and has been found in lipid mixtures (Zimmerberg and Kozlov, 2006; Vacklin et al., 2000; Leikin et al., 1996), lipid–fatty acid mixtures (Templer et al., 1998) and in lipid–cholesterol mixtures (Chen and Rand, 1997). In our method, we do not use gravimetric hydration measurements to determine the location of the pivotal plane but rather assume that the position of the pivotal plane lies at a constant 0.9 nm from the lipid chain terminus (Fig. 1). This assumption is discussed in Section 3. We carry out experiments to obtain $J_{c, DOPG}$ in two aqueous solutions, 150 mM NaCl and 150 mM NaCl with 20 mM MgCl$_2$, in order to study the effect on $J_{c}$ of cations that are typically present in the bacterial systems we have been modelling.

2. Materials and methods

2.1. Sample preparation

The monolayer spontaneous curvature of DOPG is determined from the dimensions of the inverse hexagonal (H$_{II}$) phase (Fig. 2)
HCl to give a pH of 7.4 at 40 °C. The first buffer consisted of 50 mM Tris and 150 mM NaCl. The second buffer consisted of 50 mM Tris, 150 mM NaCl and 20 mM MgCl₂. This MgCl₂ concentration ensures that the samples have a molar ratio of approximately one Mg²⁺ to every 10 anionic lipids, as measured in A. laidlawii (Niemi et al., 1997).

2.2. X-ray diffraction measurements

The hexagonal repeat spacing of the HII phase, dhex, in Fig. 1 of DOPE/DOPG/9-cis-tricosenoate mixtures was measured using small-angle X-ray diffraction, X-rays from a Kristalloflex 760 X-ray generator (Siemens AG, Germany) were supplied to a NanoSTAR SAXS system (Bruker AXS GmbH, Germany). The X-rays were focused with crossed Gobel mirrors to a 250 μm point focus. The samples were placed in a HR-PH2 sample chamber (Anton Paar GmbH, Austria) under vacuum and heated to 40 ± 0.5 °C by a servo-controlled thermoelectric heater. X-ray diffraction patterns were measured using a HI-STAR 2D X-ray detector (Bruker AXS GmbH, Germany). Experiments were controlled with the Bruker SAXS software package and diffraction patterns were analyzed with the AXcess software package developed by Andrew Heron at Imperial College London. X-ray measurements were calibrated with silver behenate (d₀₀₁ = 58.38 Å). The HII phase gives rise to Bragg peaks in the ratio 1:√3:2:√7:√9. In all samples, a minimum of three peaks was used to determine the hexagonal repeat spacing, dhex.

2.3. Structure analysis

\( J_{s,DOPG} \) is calculated from the dimensions of the HII phase with minimal packing frustration using:

\[
J_s = \frac{-1}{R_m - 0.9} \left( \text{nm}^{-1} \right) = \frac{2}{a - 1.8} \left( \text{nm}^{-1} \right)
\]

where \( a \) is the lattice parameter, and \( R_m \) is the interaxial radius of the monolayer, as defined in Fig. 1.

3. Results

We obtain \( c_m = -1/R_m \), the cylindrical curvature measured at the lipid chain termini, for DOPE/DOPG/alkene mixtures of varying alkene wt% at fixed proportions of DOPE/DOPG. Fig. 2A and B show that \( c_m \) increases with 9-cis-tricosenoate wt% and saturates above 10 wt% in both buffers. We use a least-squares optimization to fit the data for each DOPE/DOPG mixture to a three-parameter piecewise linear saturating function (Vacklin et al., 2000; Chen and Rand, 1998):

\[
c_m = \begin{cases} 
 p_1 \phi_{alkene} + p_2, & \text{if } \phi_{alkene} < p_3 \\
 p_1 p_3 + p_2, & \text{if } \phi_{alkene} \geq p_3 
\end{cases}
\]

Here \( p_1, p_2, \) and \( p_3 \) are parameters, and \( \phi_{alkene} \) is the alkene wt%. Some of the samples show variation in \( c_m \) at large alkene wt%. These variations result from excess alkene that coats the sample and creates a barrier to the absorption of water into the HII phase.

The \( c_m \) values of pure DOPE/alkene mixtures in 150 mM NaCl saturate at a value of −1/3.73 nm⁻¹. \( J_s,DOPE \) in water has been measured to be −1/2.85 nm⁻¹ (Leikin et al., 1996), which helps us locate the pivotal plane at 0.9 nm from the ends of the lipid chain in the direction of the lipid headgroup. We have used this offset distance to calculate \( J_s \) for every DOPE/DOPG mixture reported here. Fig. 3 plots \( J_s \) for DOPE/DOPG mixtures as a function of DOPG molar fraction. The extrapolated values at 100% DOPG are: \( J_s,DOPE = -1/150 ± 0.021 \) nm⁻¹ (in 150 mM NaCl) and \( J_s,DOPE = -1/8.7 ± 0.037 \) nm⁻¹ (in 150 mM NaCl and 20 mM MgCl₂). Mg²⁺ leads to a more negative monolayer spontaneous curvature, significantly closer to the boundary at which non-bilayer phases begin to form. Apart from the screening of electrostatic repulsion between neighbouring anionic lipids by divalent cations (Fragata et al., 1997) they are also able to form bridges between two mono- valent anionic lipids, an effect observed with phosphatidylserine (PS) lipid (Seddon, 1990). Our measurement of \( J_s,DOPE \) in the presence of Mg²⁺ assumes that the addition of divalent cations affects the negatively charged DOPG but does not affect the neutral lipid DOPE. This assumption is supported by a previous study (Kooijman et al., 2005), which showed that the addition of 25 mM Ca²⁺ had a negligible effect on the spontaneous curvature of DOPE.

There are two points concerning the assumption that the pivotal plane lies 0.9 nm above the bilayer mid-plane, i.e. \( R_m - R_0 = 0.9 \) nm. Firstly, the distance between \( R_m \) and \( R_0 \) is assumed to be uniform. This is motivated by evidence that the hydrocarbon thickness of the lipid monolayers is not very sensitive to the HII phase dimensions, and has been measured to be within 1 ± 0.05 nm for different lipid compositions and changes in \( d_{hex} \) between 5 and 6 nm (Leikin et al., 1996). It is also important to note that while \( d_{hex} \), and consequently \( J_s \), can be very sensitive to the salt concentration, the water concentration, and the lipid composition, the hydrophobic thickness and the position of the pivotal plane above the bilayer mid-plane are much less sensitive to these effects. The pivotal plane lies within the hydrophobic core of the lipid monolayer (Vacklin et al., 2000), which allows for an assumed uniform distance between \( R_m \) and \( R_0 \) in the HII phase. It is possible to show that \( J_s \) is not very sensitive to this distance. If the pivotal plane lies within the bounds, \( R_m - R_0 = 0.9 ± 0.2 \) nm, the extrapolation for \( J_s \) in Fig. 3 leads to the ranges −1/54 nm⁻¹ ≤ \( J_s,DOPE \) ≤ +1/123 nm⁻¹ (in 150 mM NaCl), and −1/8.5 nm⁻¹ ≤ \( J_s,DOPE \) ≤ −1/9.0 nm⁻¹ (in 150 mM NaCl and 20 mM MgCl₂).

Previous experiments provide some general bounds on the expected value of \( J_s,DOPE \). NMR measurements of the anionic lipids PG, CL, phosphatidic acid (PA) and PS suggest that as the temperature is increased, PA is the first to form inverse nonlamellar phases, followed successively by CL, PG, and PS (Lewis and McElhaney, 2000). Furthermore, the \( J_s \) value of DOPA has been measured to be −1/4.6 nm⁻¹ (Kooijman et al., 2005), and the \( J_s \)
value of DOPS has been measured to be $+1/14.4\,\text{nm}^{-1}$ (Fuller et al., 2003). Since the formation of the $H_3$ phase is favoured by more negative $J_s$ values, these NMR and SAXS measurements suggest that the $J_s$ value of DOPG is bounded by the sequence: $J_s,\text{DOPA} = -1/4.6 \,\text{nm}^{-1} < J_s,\text{CL} < J_s,\text{DOPC} < J_s,\text{DOPS} = +1/14.4 \,\text{nm}^{-1}$.

In addition, it has been suggested that only lipids that have $|J_s| > 1/6 \,\text{nm}^{-1}$ form lipid bilayers (May and Ben-Shaul, 1999). Since DOPG forms a bilayer at physiological conditions, this implies that $-1/6 \,\text{nm}^{-1} < J_s,\text{DOPC} < 1/6 \,\text{nm}^{-1}$. Our measurements and subsequent calculation of $J_s,\text{DOPC}$ appear consistent with these limits.

4. Discussion

Although the formation of a bilayer phase is important for cell survival, the plasma membranes of all organisms contain lipids that form non-bilayer phases at physiological conditions. Moreover, there is no strong chemical specificity for the non-bilayer lipid species. For instance, although PE is the main non-bilayer lipid in E. coli, a mutant that cannot synthesize PE, uses PG and CL as non-bilayer lipids (Rietveld et al., 1994). Our results show that DOPG is only likely to be a non-bilayer lipid when the concentration of Mg$^{2+}$ is high. This is consistent with experiments that show that the growth of this particular E. coli mutant is over 10 times higher in the presence of 50 mM of Mg$^{2+}$ and Ca$^{2+}$ than at 5 mM concentrations of these divalent cations (Rietveld et al., 1993).

In related experiments, the $J_s$ of the anionic lipid DOPA showed less marked changes on addition of divalent cations: $J_s,\text{DOPA} = -1/4.55 \,\text{nm}^{-1}$ in 150 mM NaCl and $J_s,\text{DOPA} = -1/4.35 \,\text{nm}^{-1}$ in 150 mM NaCl and 25 mM CaCl$_2$ (Kooijman et al., 2005). This smaller dependence can probably be ascribed to the different chemistry of the lipid head groups (Murzyn et al., 2005), as there are only small differences between Ca$^{2+}$ and Mg$^{2+}$ regarding hydrated radii and exchange rates of water molecules in their first hydration shells (Israelachvili, 1991). This point would deserve further experimental consideration.

The measured $J_s,\text{DOPC}$ is of interest for a recently developed model of cellular lipid biosynthesis in A. laidlawii (Alley et al.), in which the $J_s$ of all membrane lipids are used to understand how the lipid biosynthetic network controls the average monolayer spontaneous curvature of the membrane. PG can constitute up to 35% of the A. laidlawii plasma membrane (Andersson et al., 1998) and $J_s,\text{DOPC}$ is therefore an important parameter. Although not yet considered in our biophysical modelling, the present work hints at the possibility that the control of the concentration of divalent cations could also be an important factor in the regulation of the average $J_s$ of the membrane.

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References


