Effects of the sugar headgroup of a glycolipid on the phase behavior of phospholipid model membranes in the dry state

Antoaneta V. Popova2 and Dirk K. Hincha1

Max-Planck-Institut für Molekulare Pflanzenphysiologie, D-14424 Potsdam, Germany

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Glycolipids are important components of almost all biological membranes. They possess unique properties that have only been incompletely characterized so far. The plant glycolipid digalactosyldiacylglycerol (DGDG) strongly influences the physical behavior of phospholipid model membranes in both the dry and hydrated state. It was, however, unclear whether the strong effect of DGDG on the gel to liquid-crystalline phase transition temperature (Tm) in dry phosphatidylcholine (PC) bilayers is mainly due to the high degree of unsaturation of the DGDG fatty acyl chains or to interactions between the DGDG and PC headgroups. Also, no information on the relative effectiveness of membrane bound and free sugars on membrane phase behavior was available. We have used Fourier-transform infrared spectroscopy (FTIR) to investigate the phase properties and H-bonding patterns in dry membranes made from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) containing one saturated and one mono-unsaturated (16:0/18:1) fatty acid and different fractions of DGDG or 1,2-dilinolenoyl-sn-glycero-3-phosphatidylcholine (DLPC) (18:3/18:3). This was compared to the effects of galactose (Gal) and digalactose (diGal). All additives depressed Tm of the dry membranes, but DGDG was much more effective than DLPC or Gal. diGal had a similar effect as DGDG, pointing to the sugar headgroup as the component with the strongest influence on membrane phase behavior. A combination of DLPC and diGal, which should theoretically be equivalent to DGDG, was much more effective than the galactolipid. H-bonding interactions with the P=O group of PC were also stronger for free diGal than for DGDG, indicating that the free sugar may be structurally more flexible to adopt an optimal conformation for interactions with the PC headgroup.

Key words: desiccation/glycolipid/membranes/phase transition/phospholipid

Introduction

Different forms of glycolipids, such as glycosylated glycerolipids, sterols, ceramides, or sphingolipids, are present in virtually all biological membranes. However, our knowledge of their physicochemical properties and their physiological roles and effects are still far behind our knowledge about most common phospholipids, such as phosphatidylcholine (PC) or phosphatidylethanolamine. Because of the sugar headgroups, the intermolecular and interbilayer interactions of glycolipids are distinct from those of nonglycosylated lipids. For example, sugar headgroups of glycosphingolipids have been suggested to stabilize lipid rafts in membranes (Simons and Ikonen, 1997), and membrane stacking in the myelin sheath around nerve cells is mediated by interactions between glycosylceramides and cerebrosides sulfate (Koshy and Boggs, 1996; Koshy et al., 1999; Kulkarni et al., 1999; Boggs et al., 2000). Photosynthetic membranes of plants and cyanobacteria contain a high proportion of galactolipids, such as the nonbilayer lipid monogalactosyldiacylglycerol (MGDG) and the bilayer lipid digalactosyldiacylglycerol (DGDG) (Webb and Green, 1991; Lee, 2000), which contribute to thylakoid aggregation and stacking (Webb et al., 1988; Menikh and Fraga, 1993; Hincha, 2003).

The physiological role of DGDG has been investigated with knock-out mutants in the biosynthetic pathway, which showed that this lipid is essential for plant growth, thylakoid function, and protein import into chloroplasts (see Dörmann and Benning, 2002 for review). It has recently been shown that under conditions of phosphate starvation, plants reduce the amount of phospholipids, which are a major phosphate sink in plant cells, in favor of DGDG. Under these conditions, DGDG is also found in extraplastidal membranes and can account for up to 70% of the total plasma membrane lipids (Andersson et al., 2003). Effects on function and stability of the plasma membrane under such conditions have not been reported to date.

It has, however, been shown that up to 50% DGDG in egg phosphatidylcholine (EPC) membranes have no significant influence on the stability of model membranes during freezing, hyperosmotic stress (Hincha, 2003), or air drying (Hincha et al., 1998). DGDG is a bilayer-forming lipid (Webb and Green, 1991), and both membranes composed of 50% EPC and 50% DGDG, and membranes made from pure DGDG show an extremely low gel to liquid-crystalline phase transition temperature (Tm below −20°C) in the dry state (Popova and Hincha, 2003). For pure DGDG in the fully hydrated state, a Tm of −50°C has been reported (Shipley et al., 1973). The high degree of unsaturation of DGDG, with mainly 18:3 fatty acids (Quinn and Williams, 1983; Klaus et al., 2002), may be a reason for this low Tm.

It is, however, well documented that soluble sugars can depress the Tm of phospholipid membranes in the dry state. It is, therefore, also possible that the low Tm of mixed EPC/DGDG membranes is mainly due to effects of the sugar

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Results and discussion

The physical state of membrane lipids can be investigated by FTIR. A change in the position of the symmetric CH$_2$ stretching vibration at around 2850 cm$^{-1}$ in FTIR spectra is usually equated with a corresponding change in the motional freedom of the fatty acyl chains, for example, during phase transitions from the gel to the liquid–crystalline state (see Lewis and McElhaney, 1998 for a review). Figure 1 shows melting curves for dry liposomes constructed from series of FTIR spectra taken at different temperatures. From these curves, $T_m$ was determined as the midpoint of the phase transition. It is obvious from the curves in Figure 1 and from the $T_m$ values shown in Table I that the addition of DGDG, Gal, and DLPC resulted in a reduction in the phase transition temperature.

The effect on $T_m$ was most pronounced for DGDG. At the same time, the inclusion of increasing amounts of the galactolipid also increased the motional freedom of the fatty acyl chains in the gel state, and to a lesser degree in the liquid–crystalline state, as indicated by a shift of the CH$_2$ peak position to higher wave numbers at a given temperature (Figure 1). At 30% DGDG in the POPC membranes, no clear phase transition could be determined anymore. This is consistent with our earlier reports (Popova and Hincha, 2003, 2004) that membranes consisting of 50% EPC and 50% DGDG, or membranes made from pure DGDG also showed no phase transition in the dry state. This was also true for hydrated membranes, which were not used in the present investigation. The upfield shift in the CH$_2$ vibration in the gel and liquid–crystalline state was also observed in hydrated membranes containing 50% or 100% DGDG (Popova and Hincha, 2003).

The higher degree of motional freedom of fatty acyl chains in liposomes containing DGDG could be due to the high degree of unsaturation of the fatty acids in DGDG (mainly 18:3; Quinn and Williams, 1983; Klaus et al., 2002), but also to interactions between the headgroups (Popova and Hincha, 2003). We tested this by either drying POPC
membranes in the presence of free Gal at concentrations corresponding to the amount of Gal present in DGDG headgroups of membranes containing 10%, 20%, or 30% DGDG, or by including different fractions of the 18:3/18:3 phospholipid DLPC in POPC membranes. These quantitative comparisons implicate that the free sugars are distributed in the samples in the same way as the membrane-bound sugars and are not confined to sugar crystals. The FTIR spectra of crystalline and noncrystalline (H-bonded to membranes or vitrified) sugars are easily distinguishable (Wolkers et al., 1998). We did not find any evidence for crystalline sugars in any of our samples (data not shown) and, therefore, conclude that no significant fraction of the free sugars crystallized during drying.

Figure 1 shows that the addition of Gal decreased $T_m$, as would be expected for a small sugar (Crowe et al., 1988). It should be mentioned that the mass ratio of sugar to lipid was extremely low in these samples, that is, 0.04, 0.08, and 0.12 for samples corresponding to 10%, 20%, and 30% DGDG, respectively. Therefore, it is not surprising that the shift in $T_m$ was small. Also, the upfield shift of the CH$_2$ vibration in the gel and liquid–crystalline states was much smaller than in the corresponding samples containing DGDG instead of the free sugar. A similar response was observed when instead of a free sugar an unsaturated phospholipid (DLPC) was added to the membranes. In this case, however, the increase in the CH$_2$ peak position with increasing fractions of DLPC was more pronounced in the liquid–crystalline state.

It has been shown that both soluble sugars and the sugar in the DGDG headgroup interact with dry phospholipids through H-bonding of sugar OH groups with the P=O moiety of phospholipids (Oliver et al., 1998, 2002; Popova and Hincha, 2003, 2004). We, therefore, investigated the ability of DGDG and Gal for this interaction with POPC. Figure 2 shows the dependence of the position of the asymmetric P=O stretching vibration (Table I) on the presence of the different additives. A downfield shift of this band in FTIR spectra indicates H-bonding interactions (Lewis and McElhaney, 1998). It can be seen that both Gal and DGDG led to similar shifts in the P=O band of POPC and that the magnitude of the shift was a linear function of the fraction of DGDG or Gal in the samples. Because of the extremely low sugar/lipid ratios (see above), the downfield shifts of the P=O vibrations were small compared to the shifts reported, for example, for sucrose or trehalose (Oliver et al., 2002; Hincha and Hagemann, 2004) at 20- to 100-fold higher sugar/lipid ratios.

As expected, the addition of DLPC, which has the same headgroup as POPC, induced no shift, except at 30% DLPC. In this case, however, the FTIR spectra showed a band at 1650 cm$^{-1}$ (data not shown), a clear indication of the presence of water that had H-bonded to the P=O groups and led to this shift. It was not possible to remove this water from the samples by longer pretreatment at higher temperatures under vacuum even for extended times (data not shown).

An analysis of the correlation between P=O peak position and $T_m$ shows that both are linearly correlated for Gal and DGDG containing samples (Figure 3). As expected, there is no correlation between the wavelength of the P=O vibration and $T_m$ for samples containing DLPC. However, the slopes of the two curves obtained from samples containing Gal or DGDG are different, indicating that DGDG depresses $T_m$ more strongly than Gal for the same amount.

<table>
<thead>
<tr>
<th>Sample composition</th>
<th>$T_m$ ($^\circ$C)</th>
<th>P=O peak position (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% POPC</td>
<td>76</td>
<td>1262.2</td>
</tr>
<tr>
<td>90% POPC/10% DGDG</td>
<td>75</td>
<td>1260.6</td>
</tr>
<tr>
<td>85% POPC/15% DGDG</td>
<td>58</td>
<td>1257.5</td>
</tr>
<tr>
<td>80% POPC/20% DGDG</td>
<td>52</td>
<td>1255.1</td>
</tr>
<tr>
<td>75% POPC/25% DGDG</td>
<td>40</td>
<td>1253.7</td>
</tr>
<tr>
<td>70% POPC/30% DGDG</td>
<td>Not detectable</td>
<td>1249.9</td>
</tr>
<tr>
<td>100% POPC + Gal (=10% DGDG)</td>
<td>68</td>
<td>1259.4</td>
</tr>
<tr>
<td>100% POPC + Gal (=20% DGDG)</td>
<td>56</td>
<td>1254.8</td>
</tr>
<tr>
<td>100% POPC + Gal (=30% DGDG)</td>
<td>50</td>
<td>1253.0</td>
</tr>
<tr>
<td>90% POPC/10% DLPC</td>
<td>75</td>
<td>1261.7</td>
</tr>
<tr>
<td>80% POPC/20% DLPC</td>
<td>65</td>
<td>1261.7</td>
</tr>
<tr>
<td>70% POPC/30% DLPC</td>
<td>58</td>
<td>1257.9</td>
</tr>
</tbody>
</table>

Table I. Phase transition temperatures ($T_m$) and peak positions of the asymmetric P=O stretching vibration at 90°C of dry liposomes of different compositions (compare Figures 1 and 2).
of H-bonding interactions. This could be due to effects of the unsaturation of the DGDG fatty acyl chains and/or to the fact that the DGDG headgroup is a disaccharide and not a monosaccharide. It has been shown for many soluble sugars that disaccharides are generally more effective in depressing $T_m$ in dry membranes than monosaccharides (Crowe et al., 1988).

This can also be seen when the effect of Gal is compared to that of diGal (Figure 4; Table II). 20% DGDG in POPC resulted in the same $T_m$ as the addition of the corresponding amount of diGal to POPC liposomes, whereas the same amount of Gal was less effective. This would argue against a strong role for lipid unsaturation and in favor of a dominant influence of the sugar headgroup in determining the phase behavior of mixed phospholipid/glycolipid membranes. However, when membranes containing 20% DLPC were dried in the presence of Gal or diGal, the effect of the sugars on $T_m$ was increased dramatically over that seen in pure POPC membranes. The position of the P=O asymmetric stretching vibration was very similar in all membrane preparations containing either glycolipid or free sugar (Figure 5; Table II). There was no correlation between P=O peak position and $T_m$ for these samples (data not shown), indicating that the differences observed under these conditions are not due to differential H-bonding to the P=O group. It should be mentioned at this point that in the diGal used in our experiments, the two Gal molecules are connected by a $\beta_1$–4 linkage, whereas in DGDG the headgroup contains a $\beta_1$–6 linkage (Quinn and Williams, 1983). This difference may have effects on sugar–membrane interactions (Hincha, 1990), but unfortunately, the $\beta_1$–6 linked diGal was not commercially available.

Also, the C=O stretching band in FTIR spectra in the dry membranes was not influenced by the presence of DGDG, Gal, or diGal (data not shown), indicating that neither the lipid-bound nor the free galactoses are able to penetrate deeply enough into the membrane to interact with the C=O groups, but are rather completely bonded to the P=O groups. This is in agreement with previous observations for membranes containing 50% DGDG and 50% EPC (Popova and Hincha, 2003) and for pure EPC
membranes dried in the presence of different sugars (Hincha and Hagemann, 2004). This indicates that the observed lack of correlation between P=O peak position and T_m (Figure 5) is not due to an alternative H-bonding to the C=O groups in the lipids.

Conclusions

Our data indicate that the glycolipid headgroup plays a much stronger role in the depression of T_m in dry membranes than lipid unsaturation. In addition, the difference between the effects of Gal and diGal suggests that the diGal headgroup is especially effective in this regard. Unfortunately, corresponding experiments with the monogalactolipid MGDG are not feasible, because it is a nonbilayer lipid and would, therefore, have additional effects that confound any interpretation (Lee, 2000). The higher effectiveness of diGal in depressing T_m cannot be explained by stronger H-bonding to the phospholipid headgroups, as the downfield shift of the P=O vibration was consistently smaller for diGal than for Gal. This suggests that the monosaccharide can penetrate into the membranes more effectively to establish H-bonds, but that the disaccharide was more effective in increasing lipid spacing, most likely due to its larger size. This argument is supported by the fact that the downfield shift of the P=O vibration in the presence of free sugar was more pronounced in membranes containing 20% DLPC than in membranes containing only POPC. The higher degree of unsaturation in the fatty acyl chains of DLPC leads to a larger spacing already in the absence of sugars (Nagle and Tristram-Nagle, 2000; Rog et al., 2004) and allows the sugars easier access. Surprisingly, adding a soluble sugar to DLPC-containing membranes had a much larger effect on T_m (and to a lesser extent the P=O peak position) than adding DGDG, although the effects on the chemical composition of the system should be comparable. The main difference between the two situations is that in DGDG the sugar is anchored to the membrane surface. Therefore, it should be structurally much less flexible and less able to assume an optimum configuration for H-bonding and to act as a spacer and depress T_m. In a physiological context, these data indicate that differences in the fraction of glycolipid in a membrane should have much stronger effects on lipid phase properties and dynamics than changes in fatty acid unsaturation that have so far been a main focus of research into the environmental modulation of membrane composition and properties.

Materials and Methods

Materials

POPC and DLPC were obtained from Avanti Polar Lipids (Alabaster, AL). The chloroplast glycolipid DGDG was purchased from Lipid Products (Redhill, Surrey, UK). Gal and diGal (4-O-β-D-galactopyranosyl-D-galactopyranose) were obtained from Sigma.

Liposome preparation

Liposomes were composed of different weight fractions of POPC and DGDG or DLPC. The lipids (10 mg) were dried from chloroform under a stream of N_2 and stored overnight under vacuum to remove traces of solvent. The lipids were hydrated in 200 µL of distilled water or solutions containing Gal or diGal corresponding to the sugar content of liposome suspensions containing different fractions of DGDG. Unilamellar liposomes were prepared from the hydrated lipids using a hand-held extruder (LiposoFast, Avestin, Ottawa, Canada; MacDonald et al., 1991) with two layers of 100 nm pore filters. Liposomes were extruded in the presence of Gal or diGal, so that the sugars were distributed on both sides of the membranes.

FTIR spectroscopy

Liposome samples (50 µL) were spread on CaF_2 windows and dried at 0% relative humidity in desiccators at 28°C for 24 h in the dark. A window with a dry sample was then fixed in the vacuum chamber of a cuvette holder connected to a temperature control unit (Specac Eurotherm, Worthington, UK; see Hincha et al., 2002 for details). Temperature was monitored by a fine thermocouple fixed on the surface of the window, next to the sample. The sample was incubated at 30°C for 30 min under vacuum to remove residual moisture the sample had taken up during handling. The effectiveness of this procedure was verified by the absence of a water band in the FTIR spectra at 1650 cm⁻¹. Only in the case of liposomes containing 30% or more DLPC, it was impossible to completely remove the water. This was also
evident from a shift in the position of the asymmetric phosphate stretching vibration to lower wave numbers (compare Results and discussion).

The samples were cooled to ~30°C and after a 10 min equilibration, the temperature was increased at a constant rate of 1°C/min. Spectra were recorded every minute with a PerkinElmer GX 2000 FTIR spectrometer. After normalization of absorbance using the interactive abex routine, the peak frequencies of the CH\textsubscript{2} symmetric stretching vibration at ~2850 cm\textsuperscript{-1} were determined by the automatic peak identification routine. The gel to liquid-crystalline lipid phase transition temperature (T\textsubscript{m}) was estimated as the midpoint of the lipid melting curve. The phosphate asymmetric

\[ \beta \]-D-galactopyranosyl-D-galactopyranose); DLPC, 1,2-dilinolenoyl-sn-glycero-3-phosphatidylcholine; EPC, egg phosphatidylcholine; FTIR, Fourier-transform infrared; Gal, galactose (4-0-β-D-galactopyranosyl-D-galactopyranose); MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; T\textsubscript{m} gel to liquid-crystalline phase transition temperature.

Abbreviations

DGDG, digalactosyldiacylglycerol; diGal, digalactose (4-0-β-D-galactopyranosyl-D-galactopyranose); DLPC, 1,2-dilinolenoyl-sn-glycero-3-phosphatidylcholine; EPC, egg phosphatidylcholine; FTIR, Fourier-transform infrared; Gal, galactose; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; T\textsubscript{m} gel to liquid-crystalline phase transition temperature.

References


