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Deuterium NMR studies of the interactions of polyhydroxyl compounds and of glycolipids with lipid model membranes

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The physical properties of bilayers composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) in the presence of four water-soluble polyhydroxyl compounds, trehalose, sorbitol, glycerol, and ethyleneglycol, and three neutral glycolipids – monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and nonhydroxy fattyacyl-cerebrosides (NHFA-Cer) – were investigated using ^{2}H-NMR. All four polyhydroxyl compounds induced small, but comparable concentration-dependent changes in the choline headgroup conformation which were consistent with the presence of a small negative charge being conferred upon the bilayer surface. The latter may be explained by dipolar interactions brought about by changes in the long-range order of the water layer at the membrane surface. Trehalose had a small ordering effect on the hydrophobic interior of the membrane while ethyleneglycol induced a disordering, at both the head group level and in the hydrophobic interior. The presence of high amounts of carbohydrate at the membrane surface was ensured when POPC was mixed with various proportions of one of three glycolipids, MGDG, DGDG and NHFA-Cer. In these cases the conformation of the choline headgroup was only marginally altered when not masked by macroscopic phase changes. The headgroup conformational changes observed in the presence of any of the above-mentioned compounds were modest in comparison to the effects induced by charged substances.

The conformation and physical properties of membrane lipids depend not only on interactions within and between the membrane components but also on interactions with solutes present in the cytoplasmic or water phase. Numerous water-soluble compounds such as metal ions, anaesthetics and certain peptides bind to membrane lipids at the bilayer/water interface as an intrinsic part of their functional mechanism and in doing so influence the properties of the membrane lipids. One further class of substances which has received attention in this regard are the polyhydroxyl compounds. In particular trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), a nonreducing disaccharide, has been suggested to interact with the head group of phosphatidylcholine via hydrogen bonding and it is postulated that this mode of interaction is responsible for the prevention by this disaccharide of the membrane damage normally occurring upon dehydration [1].

Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; NHFA-Cer, nonhydroxy fattyacyl-cerebroside; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; ^{2}H-NMR, deuterium nuclear magnetic resonance.

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Deuterium nuclear magnetic resonance (^{2}H-NMR) of deuterium-labelled lipids provides a sensitive handle on the structural and dynamic properties of lipids in membranes. The technique has been used to gain insights into the conformation of individual lipid headgroups and fatty acyl chains, and to characterize lipid/lipid and lipid/protein interactions, as well as to quantitate and gauge the effects of binding such diverse membrane ligands as soluble metal ions, hydrophobic ions, and local anaesthetics (for a recent review see Ref. 2).

In the present study we have investigated the consequences of the addition of the water soluble polyhydroxyl compounds trehalose, sorbitol, glycerol and ethyleneglycol to model membranes consisting of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) in which deuterons had been located at either the α- or the β-position of the choline headgroup following the nomenclature shown below.

$$\underset{\alpha\quad\beta}{-OCH_2CH_2\overset{+}{N}(CH_3)_3}$$

For two of these polyhydroxyls, trehalose and ethyleneglycol, their effects on the membrane interior were also investigated employing deuterons located at the 9,10-position of the oleoyl fatty acyl chain of POPC. Furthermore, in order to ensure the presence of high carbohydrate concentrations at the lipid/water interface, we have also obtained ^{2}H-NMR spectra of [α-$^2H_2$]POPC mixed with the neutral glycolipids monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), or nonhydroxy fattyacyl-cerebroside (NHFA-Cer).

Fig. 1A shows the ^{2}H-NMR spectrum (obtained using conditions described in detail elsewhere [3]) for pure [α-$^2H_2$]POPC membranes in the presence of 67% (w/v) trehalose. All samples described here were prepared by hydrating dry lipid films with the appropriate buffered solution (150 mM NaCl, 100 mM Hepes (pH 7.5)). The ^{2}H-NMR spectrum is typical of a random dispersion of lipids in a liquid-crystalline bilayer [4]. The quadrupole splitting $\Delta\nu_Q$, which corresponds to the separation in frequency units between the two maxima in the spectrum, equalled

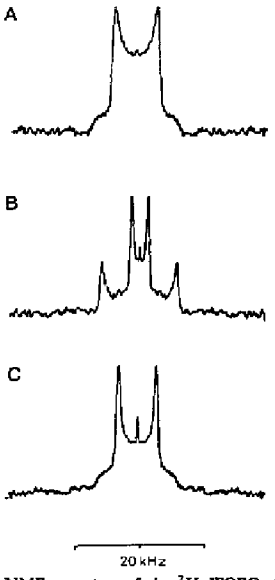

Fig. 1. ^{2}H-NMR spectra of [α-$^2H_2$]POPC (A) and 1-palmitoyl-2-[9′,10′-$^2H_2$]oleoyl-*sn*-glycero-3-phosphocholine (B) both recorded in the presence of 67% (w/v) trehalose. (C) 80 mol% [α-$^2H_2$]POPC plus 20 mol% DGDG. Spectra A and B were measured at 293 K, spectrum C at 298 K (150 mM NaCl, 100 mM Hepes (pH 7.5)).

6.74 kHz at 293 K, somewhat higher than the value of 6.01 kHz measured for [α-$^2H_2$]POPC at the same temperature in the absence of trehalose. Since a single quadrupole splitting was observed, the interaction between trehalose and POPC must be one experienced by all lipids. Any equilibration between 'interacting' and 'non-interacting' lipid molecules must be fast on the ^{2}H-NMR timescale of 10^{-5}–10^{-6} s. Similar line shapes were observed for [α-$^2H_2$]- and [β-$^2H_2$]POPC whether trehalose, sorbitol, glycerol or ethyleneglycol were added and regardless of the actual concentration of the particular polyhydroxyl compound.

Fig. 2 illustrates the concentration dependent changes in $\Delta\nu_Q$ of the α- and the β-segment induced by polyhydroxyl compounds. Deuterium-labeled POPC in aqueous dispersion exhibited a value of $\Delta\nu_Q$ equal to 6.0 kHz for both the α- and the β-segment in agreement with previously re-

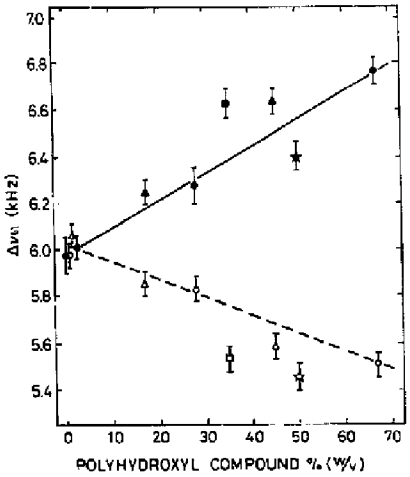

Fig. 2. The dependence of the deuterium quadrupole splitting $\Delta\nu_Q$ in [α-$^2H_2$]POPC membranes (closed symbols) and [β-$^2H_2$]POPC membranes (open symbols) on the concentration of the polyhydroxyl compounds trehalose (circles), sorbitol (squares), glycerol (triangles) and ethyleneglycol (stars) (150 mM NaCl, 100 mM Hepes (pH 7.5) at 293 K). The linear regression is shown for $\Delta\nu_\alpha$ (continuous line) and $\Delta\nu_\beta$ (interrupted line) versus the concentration of trehalose.

ported values [3,5,6]. The addition of either trehalose, sorbitol, glycerol, or ethyleneglycol caused the quadrupole splitting of the α-segment to increase and that of the β-segment to decrease in a manner that was linearly related to weight concentration and only weakly dependent on the type of polyhydroxyl compound added. The decrease in $\Delta\nu_\beta$ was about 0.6-times smaller than the increase in $\Delta\nu_\alpha$ as can be seen in Fig. 3. These counter-directional changes of the quadrupole splitting of the α- and β-segment upon addition of polyhydroxyl compounds resemble the more pronounced changes which occur when electric surface charges in the form of negatively charged amphiphiles [7], aqueous and hydrophobic anions [7,8], or negatively charged phospholipids [6,9,10] are introduced into the lipid bilayer. It would appear that the polyhydroxyl compounds modify the bilayer surface either directly, via hydrogen-bonding interactions with the lipid polar groups, or indirectly, by disrupting the prearranged orientations of the hydrogen-bonding network of water molecules in the surface hydration layer [11,12] such that the phospholipid polar groups sense a slightly more negative surface charge. The precise arrangement of sugar molecules and phospholipid head groups is, however, not known.

In order to determine whether the change in lipid headgroup conformation evident in the presence of polyhydroxyl compounds extended to the hydrophobic interior of the bilayer, we compared the effects of the presence of 67% (w/v) trehalose and 50% (w/v) ethyleneglycol on the ^{2}H-NMR spectrum of [9,10-$^2H_2$]POPC in which the deuterons are located at the 9- and 10-position of the oleic acyl chain, i.e. on the two carbon atoms bridged by the *cis*-double bond. Fig. 1B shows the spectrum of this lipid when dispersed in saline buffer containing 67% (w/v) trehalose at 293 K: two quadrupole splittings are observed, the outer corresponding to the deuteron at the 9-position ($\Delta\nu_Q = 14.2$ kHz) and the inner one to that at the 10-position ($\Delta\nu_Q = 3.1$ kHz). The presence of two quadrupole splittings reflects the tilt of the *cis*-double bond with respect to the bilayer normal which produces different orientations for C-^{2}H vectors of the 9- and 10-positions [13]. In the presence of 67% (w/v) trehalose the quadrupole splittings at the 9- and 10-positions increased by

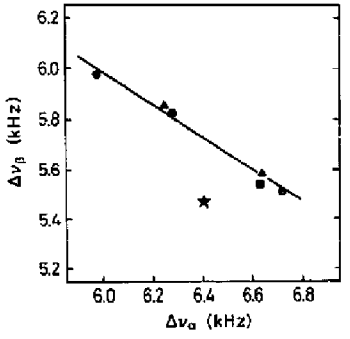

Fig. 3. The interdependence of $\Delta\nu_\beta$ and $\Delta\nu_\alpha$ (linear regression coefficient $r = -0.990$; slope $m = -0.64$) for probes with different concentrations of trehalose (circles), glycerol (triangles), and sorbitol (square). The data point recorded in the presence of 50% (w/v) ethyleneglycol (star) does not fit on this line because a general disordering effect is superimposed on the dipolar effect of ethyleneglycol on the POPC bilayer (see text for details).

0.7 ± 0.2 kHz and 0.5 ± 0.1 kHz, respectively, relatively to the values measured in saline buffer. In the presence of 50% (w/v) ethyleneglycol the C-9 and C-10 quadrupole splittings decreased by 0.6 ± 0.2 kHz and 0.8 ± 0.2 kHz, respectively. The increase in $\Delta\nu_Q$ caused by trehalose and the decrease in $\Delta\nu_Q$ caused by ethyleneglycol were independent of temperature, at least in the range of 10–30°C. A much more pronounced disordering effect of ethyleneglycol has been reported by Nicolay et al. [14] for 1,2-dioleoyl-*sn*-glycero-3-phosphocholine membranes. The disordering effect of ethyleneglycol is reflected also in the lipid headgroup region where it is superimposed upon the changes arising from electric surface effects such that the quadrupole splittings of both [α-$^2H_2$]- and [β-$^2H_2$]POPC are somewhat lower in the presence of ethyleneglycol than in the presence of trehalose (Figs. 2 and 3). Since trehalose and ethyleneglycol differ markedly in size and in the number of hydroxyl groups it is reasonable to attribute their differential effects on the hydrocarbon chain ordering to their ability to penetrate the lipid bilayer. Ethyleneglycol appears to be more hydrophobic with a concomitantly larger tendency to disrupt the parallel packing of the fatty acyl chains.

In relation to the effects of metal ions or negatively charged lipids [5,6,9,10,15] on the choline headgroup conformation, the polyhydroxyl induced changes in $\Delta\nu_Q$ are modest. Moreover, the change in $\Delta\nu_Q$ depends not on the specific structure of the polyhydroxyl compound but rather on the weight of hydroxyl groups present. This indicates that, in the presence of excess water, these compounds have a limited and nonspecific interaction with the lipid headgroups. Since hydrogen bonding will mediate these interactions, under conditions of dehydration the presence of polyhydroxyls could provide alternatives to water-lipid bonding [16,17]. Presumably under such conditions the detailed structure of the H-bond donor would then heavily influence its effectiveness in stabilizing a viable membrane (e.g. trehalose [1]).

As an alternate means of obtaining high sugar concentrations at the membrane surface and investigating their effects on lipid headgroup conformation, we mixed [α-$^2H_2$]POPC with various proportions of different glycolipids. In keeping with their established macroscopic phase behavior [18–20] mixtures of [α-$^2H_2$]- POPC/DGDG, 1 : 1 or 4 : 1 retained an overall bilayer configuration and provided ^{2}H-NMR spectra such as that shown in Fig. 1C. In these cases $\Delta\nu_Q$ was either unchanged (4 : 1) or decreased by 0.5 kHz (1 : 1) relative to the value measured for pure [α-$^2H_2$]POPC. The two monoglycosyl glycolipids, MGDG (1 : 1) and NHFA-Cer (1 : 1), induced the appearance of narrowed spectral components indicative of the presence of nonbilayer phases (spectra not shown) again in accordance with their established macroscopic phase preferences [18–24]. However, when MGDG or NHFA-Cer were present at 20 mol% the ^{2}H-NMR spectra remained typical of an overall bilayer organization. In these cases $\Delta\nu_Q$ was observed to decrease marginally relative to pure [α-$^2H_2$]POPC. Thus the influence of these glycolipids on the choline headgroup conformation is small when not masked by macroscopic phase changes.

In conclusion, the addition of low molecular weight sugars and glycols to aqueous dispersions of phosphatidylcholine bilayers imparts a unique conformational change to the choline headgroup which increases linearly with the weight concentration of the added polyhydroxyl compound. The conformational change is similar in nature to that observed upon the addition of negatively charged anions and negatively charged lipids to POPC membranes. Hence the structuring effect of the polyhydroxyl compounds on water appears to create a more negative electric dipolar field in the vicinity of the phosphocholine head group. In contrast, the presence of relatively high carbohydrate concentration in the plane of the membrane as ensured by the addition of neutral glycolipid has practically no effect on the choline head group conformation. This argues against specific headgroup interactions between the specific glycolipids employed and phosphocholine head groups, at least at temperatures above that of the liquid-crystalline phase transition.

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