NMR SPIN–SPIN SPLITTINGS IN LIPID MEMBRANES

Headgroup conformation in phosphatidylglycerol bilayers

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1. Introduction

It is now accepted that a major part of the lipid in biological membranes exists in a bilayer form with the hydrocarbon tails of the phospholipid molecules directed in towards the centre of the membrane and only the polar head-groups of the molecules exposed to the aqueous phase [1]. That it was possible to obtain a high resolution proton NMR spectrum from sonicated dispersions of phospholipid bilayers was first demonstrated [2]. However, because of linewidth limitations in these 2×10^6 mol. wt vesicles, it has not until now been possible to resolve the spin–spin splittings used in the conformational analysis of small organic molecules. Here we report the observation of spin–spin splittings in sonicated bilayer dispersions of dipalmitoyl phosphatidylglycerol (DPPG) and their use in determining the headgroup conformation of the phospholipid molecules. Phosphatidylglycerol is a negatively charged phospholipid found at high abundance in the plasma membranes of micro-organisms and the chloroplast membranes of plants, and to a smaller extent in higher organisms. The conformation of the phospholipid headgroup can be expected to affect such functionally important factors as membrane fluidity, surface binding and specific lipid binding to integral proteins.

2. Materials and methods

DPPG was prepared as the sodium salt by trans-phosphatidylation of L-dipalmitoyl phosphatidylcholine (Fluka, Bucks) by phospholipase D (Boehringer, Mannheim) in the presence of excess glycerol [3,6]. The product was purified by silica-gel chromatography and co-chromatographed with egg phosphatidylglycerol (Lipid Products, Surrey) as a single spot on thin-layer chromatograms (solvent system: $CHCl_3$:CH_3OH:NH_4OH(25% v/v) 65:15:1). Specific optical rotation was $[\alpha]_D^{24} + 6.3°$, as would be expected for a racemic mixture of D- and L-isomers in the glycerol headgroup [3].

Lipid dispersions (50 mM) were prepared by sonication in 0.1 M KCl/D_2O, p*D* 9.0 at a temperature above the ordered–fluid bilayer phase transition (41°C). Thin layer chromatography revealed no evidence for lipid degradation after sonication or during collection of the NMR spectra. 270 MHz ^1H NMR spectra were recorded on a Bruker WH-270 spectrometer operating in the fourier transform mode. Convolution difference spectra were obtained as described [4]. Chemical shifts in D_2O were referred to internal sodium-3-(trimethylsilyl)-d_4-propionate and in $CDCl_3$:CD_3OD (2:1) to internal tetramethyl silane. Spectra were simulated using the Bruker ITERCAL programme which is based on the LAOCN3 algorithm. Convolution difference spectra were simulated by subtracting the same simulated spectra with different line broadenings.

3. Results and discussion

The phospholipid headgroup region of the 270 MHz proton NMR spectra of DPPG is given in fig.1. Assignments were made on the basis of relative intensities, chemical shifts, multiplicities, ^{31}P- and

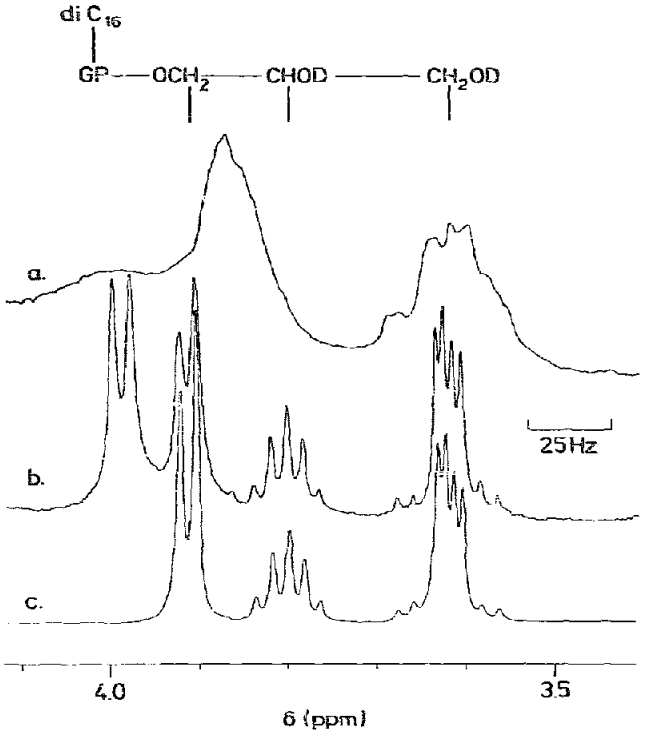

Fig.1. Polar headgroup region of the ^{31}P-decoupled 270 MHz proton NMR spectrum of DPPG: (a) sonicated dispersions in 0.1 M KCl/D_2O, p*D* 9.0 at 54°C; (b) solution in $CDCl_3$–CD_3OD (2:1) at 20°C; (c) simulation of (b) using the iterated parameters given in the text. The unlabelled resonance at 4.0 ppm in (a) and (b) arises from the $-CH_2OP$ group of the glycerol backbone.

homonuclear-spin decoupling. The spectra in $CDCl_3$–CD_3OD are reasonably well resolved and can be analysed to yield the vicinal H–H coupling constants required for conformational analysis. Iteration on the CH_2OD line positions of the CH_2OD–CHOD multiplet yield the following values:

J_{AB} = 11.6 Hz, J_{AX} = 5.9 Hz, J_{BX} = 4.3 Hz and δ_{AB} = 0.03 ppm. (rms = 0.07 Hz).

The $-CH_2OP$ protons are accidentally equivalent with a doublet splitting of 1/2 ($J_{AX} + J_{BX}$) = 4.8 Hz.

The linewidths in the aqueous bilayer spectra are considerably broader, but the multiplet structure of the $-CH_2OD$ resonance can be partially resolved, and identified as the AB part of the expected ABX partial spectrum. Resolution can be enhanced considerably using convolution difference (c.d.) spectroscopy [4]

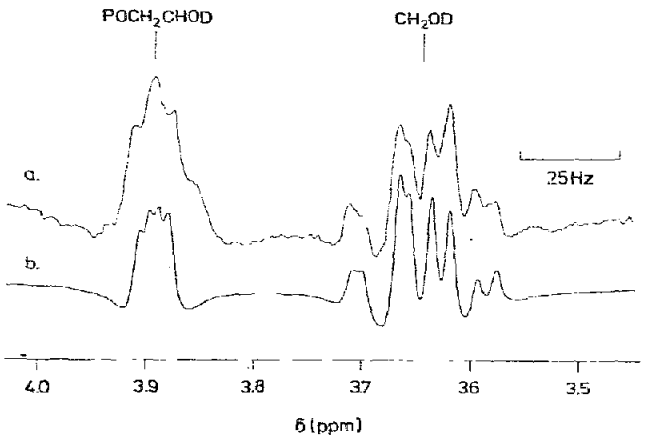

Fig.2. Convolution difference spectrum: (a) aqueous DPPG (c.f. fig.1a); (b) simulation of $-CH_2OD$ multiplet using the iterated parameters given in the text. Intensities cannot be compared in the simulated c.d. spectrum.

as shown in fig.2, from which it is possible to measure all the line positions of the $-CH_2OD$ multiplet and analyse them to yield the required vicinal H–H couplings. The values obtained by iterating on the $-CH_2OD$ line positions are:

J_{AB} = 11.7 Hz, J_{AX} = 5.2 Hz, J_{BX} = 2.4 Hz, δ_{AB} = 0.06 ppm (rms = 0.33 Hz).

There is considerable redundancy in analysing the $-CH_2OD$ resonances, thus the good fit obtained provides an internal check on the reliability of the measured line positions. An additional check was made by using the same parameters to simulate the AB spectrum obtained when the –CHOD proton was decoupled.

The observed vicinal coupling constants may be used to obtain information about the conformation of the DPPG headgroups. The expressions for the motionally-averaged vicinal coupling constants in terms of the fractional populations of the three staggered conformations about the C–C bonds in the glycerol fragment (see fig.3) are given by [5]:

$$J_{AX}\,(\mathrm{Hz}) = 5.8\,p_{\mathrm{I}} + 11.5\,p_{\mathrm{II}} + 0.6\,p_{\mathrm{III}} \qquad (1)$$

$$J_{BX}\,(\mathrm{Hz}) = 11.7\,p_{\mathrm{I}} + 2.7\,p_{\mathrm{II}} + 2.7\,p_{\mathrm{III}} \qquad (2)$$

where $p_{\mathrm{I}} + p_{\mathrm{II}} + p_{\mathrm{III}} = 1$, and the numerical constants in the equations represent the coupling constants of

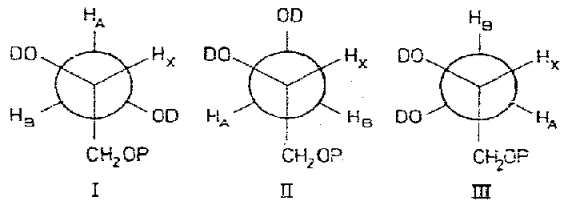

Fig.3. Minimum-energy conformations for rotation about the terminal C–C bond in the glycerol headgroup of DPPG.

the individual rotamers. Since we cannot distinguish between H_A and H_B there are two possible sets of solutions corresponding to the interchange of J_{AX} and J_{BX}. For DPPG bilayers we obtain:

$p_I = 0.0$ (0.28), $p_{II} = 0.42$ (0.03) and $p_{III} = 0.58$ (0.69), and for DPPG in $CDCl_3$–CD_3OD:

$p_I = 0.18$ (0.36), $p_{II} = 0.40$ (0.17) and $p_{III} = 0.42$ (0.47).

Thus there are conformational interconversions in the polar headgroups of DPPG membranes. Conformational differences are detected between D_2O dispersion and $CDCl_3$–CD_3OD solution, corresponding to the intermolecular restrictions imposed on forming the bilayer membrane. Particularly notable is the high proportion (70–100%) of the conformations in which the hydroxyl groups are gauche to each other. Examination of molecular models indicates that these conformations could be favourable for forming intermolecular hydrogen bonds within the bilayers surface. It is hoped that these methods may be applied to detect functional conformational changes in the lipid headgroups, e.g., on ligand binding.

References

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