Ethylene glycol causes acyl chain disordering in liquid-crystalline, unsaturated phospholipid model membranes, as measured by ^2H NMR

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Received 18 September 1986; revised version received 10 October 1986

^2H NMR has been used to probe the effects of ethylene glycol at the level of the acyl chains in liposomes prepared from dioleoylphosphatidic acid or dioleoylphosphatidylcholine, labeled with ^2H at the 11-position of both oleic acid chains. Increasing concentrations of ethylene glycol lead to a proportional and substantial decrease in the quadrupolar splittings, measured from the ^2H NMR spectra of both liposomal systems, indicative of acyl chain disordering.

Ethylene glycol *Membrane disordering* *Dehydration* *^2H NMR* *Dioleoylphosphatidylcholine* *Dioleoylphosphatidic acid*

1. INTRODUCTION

In membrane research, EG is widely employed as an anti-freeze in calorimetric measurements involving phase transitions occurring between approximately $-20°C$ and $10°C$ [1,2]. This application of EG relies on its prominent ability to bind and structure water [3,4]. It is generally believed that this water binding capacity also plays an important role in the EG-induced fusion of cells [5,6] and model membranes [7,8] which is another important application of this compound and is especially well-documented for its high molecular mass polymers. The dehydrating effect is thought to allow close intermembrane contact which is a prerequisite for membrane fusion. At present, it is not fully understood whether besides the dehydrating effects of ethylene glycols, membrane stability is decreased thereby promoting fusion [6,9].

Abbreviations: DOPA, dioleoylphosphatidic acid; DOPC, dioleoylphosphatidylcholine; EG, ethylene glycol

In the course of investigations aimed at characterizing the molecular details of the interaction between Ca^{2+} and DOPA embedded in a DOPC matrix, we noticed that EG has substantial effects on the level of the acyl chains. The quadrupolar splittings measured from ^2H NMR spectra of liposomes prepared from DOPA or DOPC, labeled with ^2H at the 11-position of both oleic acid chains, showed a dramatic decrease upon EG addition. It has been shown previously that EG influences the transition temperature and enthalpy of DOPC as measured by differential scanning calorimetry [10]. Arnold et al. [9] reported a decrease of the fluorescence anisotropy of diphenylhexatriene on addition of polyethylene glycol to erythrocyte ghosts. However, interpretation of such studies employing exogenous probe molecules to assess membrane status are severely hampered by the fact that ethylene glycols considerably affect the partition of these compounds [9]. In this study we used ^2H NMR to characterize the effect of EG on the hydrophobic interior of DOPC and DOPA membranes. The ^2H NMR method is a non-perturbing technique which is ideally suited to get quantitative information on

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00145793/86/$3.50

acyl chain order in model membrane systems under fully liquid-crystalline conditions [11,12].

2. EXPERIMENTAL

EG (p.a.) was from Merck (Darmstadt, FRG) and was used without further purification. 1,2-[11,11-di-^{2}H]DOPC was synthesized as described elsewhere [13]. 1,2-[11,11-di-^{2}H]DOPA was synthesized from the ^{2}H-labeled DOPC as described [14]. Both lipids were >99% pure, as demonstrated by HPTLC.

Samples for NMR were prepared by dispersing 34–82 μmol of lipid (dried overnight under high vacuum) in 15 ml of a buffer containing 100 mM NaCl, 25 mM piperazine-*N,N'*-bis[2-ethanesulfonic acid], 40 μM EDTA, pH 7.4. Under these conditions DOPA has a negative charge of -1 [15]. Liposomes were collected by centrifugation (45 min, 40000 × *g*), resuspended in the above buffer to a final volume of 750 μl and transferred into a 10 mm (o.d.) NMR tube. Ethylene glycol was added to the appropriate final concentration directly in the NMR tube, followed by vortex mixing. It was routinely checked that addition of EG did not affect the pH of the suspension.

^{2}H NMR spectra were recorded at 46.1 MHz on a Bruker MSL 300 spectrometer by employing the quadrupolar echo sequence [16]. The ^{2}H 90° pulse was 13 μs, the 90° pulse separation was 50 μs, acquisition of the echo was started 57 μs after the second 90° pulse while a dwell time of 8 μs was used. Recycle delays were 0.2 s and 2500–5000 echos were accumulated for each spectrum.

^{31}P NMR was carried out at 121.49 MHz on the above spectrometer. A single pulse experiment was used with a 90° pulse of 13 μs and a 1 s recycle time. High-power proton decoupling was employed during acquisition.

Unless otherwise indicated, the NMR experiments were performed at 25°C.

3. RESULTS

Fig.1 shows a series of ^{2}H NMR spectra recorded from suspensions of DOPC and DOPA, labeled with two deuterons at the 11-position of both acyl chains, as a function of the percentage of EG in the buffer. The ^{2}H NMR spectra consist of two contributions. First, deuterons present at natural abundance in the aqueous buffer give rise to the sharp isotropic signal in the center of the spectra. Secondly, the deuterons in the oleic acid chains of DOPC and DOPA give rise to axially symmetric powder patterns [11,12] with reasonably sharp perpendicular and somewhat more diffuse parallel edges. The residual quadrupolar splittings ($\Delta\nu_Q$), as measured from the frequency separation of the doublet centered symmetrically around the zero-position, are 5.6 and 5.0 kHz for DOPC and DOPA, respectively, in the absence of EG. In both liposomal systems, increasing amounts of EG cause a gradual decrease in $\Delta\nu_Q$ (fig.1). Moreover,

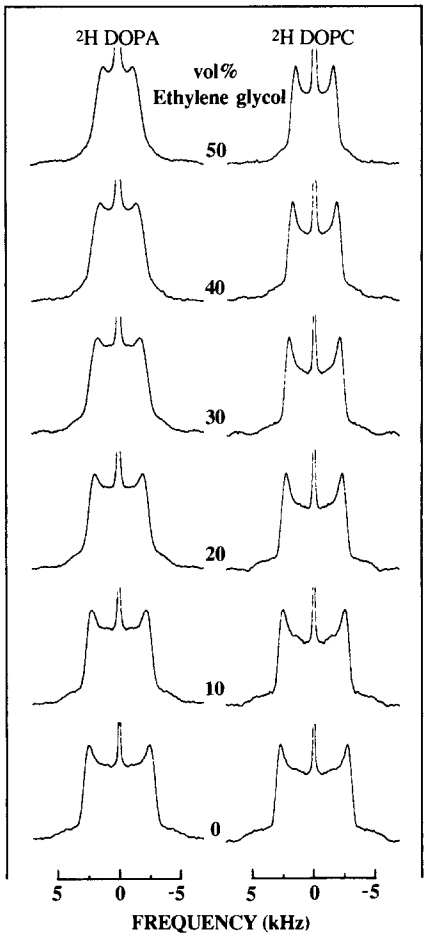

Fig.1. Dependence of the ^{2}H NMR spectra of 1,2-[11,11-di-^{2}H]DOPC and -[DOPA] on the volume percentage of ethylene glycol in the buffer. Lipid concentration was 67 mM in both cases. For further details see section 2.

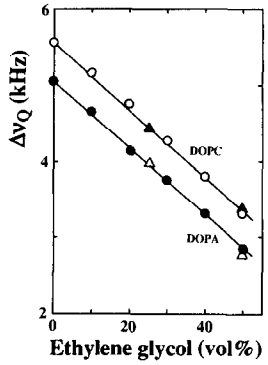

Fig.2. Dependence of the residual quadrupolar splitting measured from ^2H NMR spectra of 1,2-[11,11-di-^2H]DOPC (○, ▲) and -DOPA (●, △) on the volume percentage of ethylene glycol in the buffer. Data were obtained from the series shown partly in fig.1. Lipid concentrations were 45 mM (△), 67 mM (○,●) and 110 mM (▲). Further details in text.

the reduction in $\Delta\nu_Q$ as a function of the percentage of EG present is linear, identical for DOPC and DOPA, and independent of the lipid concentration (fig.2). It should be noted that the spectra shown were obtained immediately after EG addition. Upon prolonged incubation, no further changes took place. Furthermore, the EG affects all acyl chains in the entire population to the same extent since the ^2H spectra consist of one single component.

^{31}P NMR spectra of DOPC and DOPA were recorded as a function of EG concentration at 25°C (not shown). It appeared that the residual ^{31}P chemical shift anisotropy which is a measure for the local order in the phosphate region of the polar head group remained essentially unchanged up to 50 vol% EG at values of 44 and 37 ppm for DOPC and DOPA, respectively.

4. DISCUSSION

For the axially symmetric ^2H NMR powder patterns observed here, the residual quadrupolar splitting $\Delta\nu_Q$ can be directly related to the orientational order parameter of the C-^2H bond ($S_{C\text{-}2H}$) through the equation [11,12]:

$$\Delta\nu_Q = \frac{3}{4} \times \frac{e^2 q_Q}{h} \times S_{C\text{-}2H},$$

where $(e^2q_Q)/h$ is the static quadrupolar coupling constant which amounts to 170 kHz for parafinic C-^2H bonds [17]. From the above equation, it follows that the absolute value of $S_{C\text{-}2H}$ decreases from 0.044 to 0.026 and from 0.039 to 0.022 for DOPC and DOPA, respectively, when increasing the amount of EG from 0 to 50 vol%. Since

$$S_{C\text{-}2H} = \frac{1}{2} < 3\cos^2\theta_{C\text{-}2H} - 1 >,$$

where $<3\cos^2\theta_{C\text{-}2H} - 1>$ denotes the time-average of the angle $\theta_{C\text{-}2H}$ between the bilayer normal and the C-^2H bond vector, the above data indicate that either the angle $\theta_{C\text{-}2H}$ is affected by EG or the fluctuations in this angle greatly increase in amplitude upon EG addition. It also cannot be excluded that a combination of both effects is responsible for the decrease in $S_{C\text{-}2H}$. The observations clearly demonstrate that EG significantly perturbs the acyl chain organization in the liquid-crystalline lipid systems studied here and that this perturbation is identical for the neutral DOPC and negatively-charged DOPA liposomes.

It is well known that ethylene glycols cause concentration-dependent changes in the temperature and energy content of the gel to liquid-crystalline phase transition of aqueous dispersions of lipids [10,19]. Thus, we have recently reported [20] that the transition temperature of DOPC increases from −20°C in the absence to −14°C in the presence of 50% EG. By contrast, the transition temperature of DOPA was found to decrease from −8 to −11°C upon addition of 50% EG. These shifts in transition temperature might in principle lead to changes in $\Delta\nu_Q$. However, since it was measured (not shown) that in 50% EG $\Delta\nu_Q$ increases by 0.7 and 0.5 kHz per 10°C reduction in temperature for DOPA and DOPC, respectively, the EG-induced changes in phase transition temperature are too small to account for the massive reduction in $\Delta\nu_Q$ found at 25°C (fig.2).

Polar headgroup dehydration by EG presents another potentially important factor to interpret the present observations. By measuring ^2H NMR spectra as a function of water content, Chupin et al. [13] have recently shown that decreased hydration of the polar head group of 1,2-[11,11-di-^2H]DOPC results in increased quadrupolar splittings. Since EG has the opposite effect, a pure dehydrating action of EG cannot explain our data. Similar conclusions were recently reached in

studies concerning the fusogenic properties of polyethylene glycols [7,21].

Instead of decreasing the molecular area of the polar headgroups by partially extracting bound water [9], it rather appears that EG addition leads to an increase in the effective size of this moiety, as was also suggested from monolayer experiments [22]. This would lead to less dense packing of the acyl chains, as reflected in the present data. Solvation of the polar headgroups by the hydrated EG is most probably responsible for this increase in size. It should be noted that interaction of EG at the level of the headgroup is not accompanied by changes in the local order of the phosphate group, as evidenced by ^{31}P NMR, in agreement with earlier findings [9,23]. Apart from indirectly affecting acyl chain order by increasing the effective size of the polar headgroups, it may well be that partial penetration of EG into the hydrocarbon region occurs, thereby more directly affecting acyl chain organization.

The finding that the EG-induced effects on $\Delta\nu_Q$ are independent of the lipid concentration within the range tested here is of interest in relation to the findings of Boni et al. [7]. These authors reported that also the threshold concentration of polyethylene glycol for aggregation and fusion of small unilamellar vesicles of egg phosphatidylcholine is independent of lipid concentration.

The data on membrane destabilization presented here are of relevance for explaining the capacity of ethylene glycols: (i) to cause aggregation and fusion of model and biological membranes; and (ii) to cause a large increase in permeability towards ions in liposomes and cellular systems [4,24]. The outcome of our experiments also implies that great caution should be taken in the use of EG in membrane research since it dramatically affects acyl chain packing and is far from being an inert antifreezing agent.

ACKNOWLEDGEMENTS

The authors wish to gratefully acknowledge Mr Johan Timmermans for synthesizing the ^{2}H-labeled DOPC. This research was supported by the Dutch Cancer Foundation (to K.N.) and the Netherlands Foundation of Biophysics (to E.B.S.).

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