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HYDRATION OF *ESCHERICHIA COLI* LIPIDS

DEUTERIUM T_1 RELAXATION TIME STUDIES OF PHOSPHATIDYLGLYCEROL, PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE

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The hydration properties of *Escherichia coli* lipids (phosphatidylglycerol, phosphatidylethanolamine) and synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphocholine in $H_2O/^2H_2O$ mixtures (9:1, v/v) were investigated with ^2H-NMR. Comparison of the ^2H_2O spin lattice relaxation time (T_1) as a function of the water content revealed a remarkable quantitative similarity of all three lipid-H_2O systems. Two distinct hydration regions could be discerned in the T_1 relaxation time profile. (1) A minimum of 11–16 water molecules was needed to form a primary hydration shell, characterized by an average relaxation time of $T_1 \approx 90$ ms. (2) Additional water was found to be in exchange with the primary hydration shell. The exchange process could be described in terms of a two-site exchange model, assuming rapid exchange between bulk water with $T_1 = 500$ ms and hydration water with $T_1 = 80$–120 ms. Analysis of the linewidth and the residual quadrupole splitting (at low water content) confirmed the size of the primary hydration layer. However, each lipid-water system exhibited a somewhat different linewidth behavior, and a detailed molecular interpretation appeared to be preposterous.

Introduction

The properties of water in the vicinity of biological interfaces differ significantly from those of free water. Water ordering in the proximity of proteins and membranes has been studied extensively using NMR methods, in particular ^{1}H-NMR (for reviews see Refs. 1–3). The application of ^{2}H-NMR has been relatively rare, even though this method has two significant advantages. (1) Under favorable circumstances, the ordering of the water molecules at the protein or membrane surface may give rise to a quadrupole splitting which provides information on the structure of the hydration layer. (2) The dominant relaxation pathway of ^{2}H-NMR is quadrupolar relaxation, which is a purely intramolecular mechanism. Intermolecular interactions or interactions with paramagnetic ions which complicate the interpretation of ^{1}H-NMR relaxation times have practically no effect on ^{2}H-NMR relaxation times. A quantitative interpretation of the deuterium T_1 relaxation time in terms of an effective molecular correlation time, τ_c, is therefore straightforward.

Deuterium NMR has been applied by several authors to investigate the hydration properties of phospholipid bilayers (mainly lecithin bilayers) [4–9]. The focus of this earlier work was exclusively on the quadrupole splittings of ^2H_2O at various stages of lipid hydration and rather detailed molecular interpretations have been suggested [4–6]. Here we demonstrate that the mea-

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DOPC, dioleoylphosphatidylcholine.

surement of deuterium spin lattice relaxation time (T_1) is an equally convenient and informative tool to probe the structure of the membrane hydration layers. Our work was initiated by the observation that the T_1 relaxation time of ^2H_2O (at approx. 0.02% natural abundance in H_2O) was distinctly shortened in suspensions of *E. coli* cells and membrane preparations [10]. Since the lipids in the *E. coli* membranes were mainly phosphatidylethanolamine (PE; accounting for approx. 80 wt.% of the total membrane lipid) and phosphatidylglycerol (PG; approx. 20 wt.%), measurement of the pure lipid dispersions was thought to shed light on the contribution of the membrane lipids to the relaxation time shortening. Hence, deuterium T_1 relaxation times were obtained for bilayer dispersions of purified *E. coli* PE and PG, measured as a function of water content and temperature. In addition, a synthetic, *cis*-unsaturated phosphatidylcholine (PC), i.e., 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was also measured, since this lipid is similar to natural egg lecithin, the lipid used in most earlier ^2H-NMR studies.

Materials and Methods

^2H-NMR

The ^2H-NMR measurements were made at 46.1 MHz using a Bruker-Spectrospin CXP-300 spectrometer. The return of the magnetization to equilibrium after a 180° pulse was followed by the quadrupole echo sequence [11]. The duration of a 90° pulse was usually 4.8 μs.

Lipids

Phosphatidylethanolamine and phosphatidylglycerol were extracted from *E. coli* cells (strain 131 GP) and purified by column chromatography as described before [10,12]. Synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphocholine was purchased from Avanti, Birmingham, AL, U.S.A. The lipids were dispersed in a mixture of $H_2O/^2H_2O$ (9 : 1, v/v). This isotope ratio was used in order to reduce possible isotope effects on hydrogen bonding but also to ensure sufficient sensitivity for relatively fast T_1 determinations. A well-defined amount of lipid (approx. 80 mg) was weighed in with about the same amount of water. The sample was thoroughly mixed and the T_1 relaxation times were determined at various temperatures. The sample was then partially dehydrated in the desiccator and homogenized again; then the loss of water was determined by weighing and the sample was again used for T_1 measurements. Each set of data was obtained with two different samples.

Results

Rather simple ^2H-NMR spectra were obtained for the system *E. coli* PG-H_2O, exhibiting only a single ^2H_2O resonance with no resolvable quadrupole splitting over the whole concentration range measured (50–2 wt.% H_2O; 25°C). However, the width of the resonance changed considerably with the water content. Fig. 1 shows the variation of the linewidth (measured as the width at half-height, $\Delta\nu_{1/2}$) with the mole fraction of lipid, x_L. Starting from pure water, the linewidth increases linearly with increasing lipid content reaching a plateau value of $\Delta\nu_{1/2} \approx 1.3$ kHz at a hydration level of about 16 H_2O/PG. A further increase to about 2 kHz was observed at very low water contents (approx. 2 H_2O/PG; data not shown).

The recovery of the magnetization after a 180° pulse followed a single exponential. Fig. 2 displays the variation of the relaxation rate, $R_1 = 1/T_1$, with the mole fraction of lipid demonstrating a

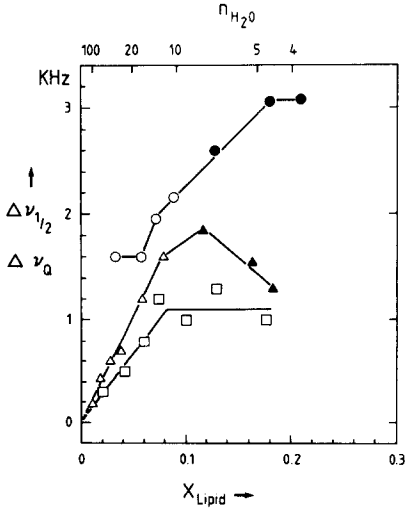

Fig. 1. Variation of the linewidth at half-height ($\Delta\nu_{1/2}$, open symbols) and the quadrupole splitting ($\Delta\nu_Q$, closed symbols) with the mole fraction of lipids. Spectra were recorded at 46 MHz and 25°C. Circles, PE; triangles, PC; squares, PG.

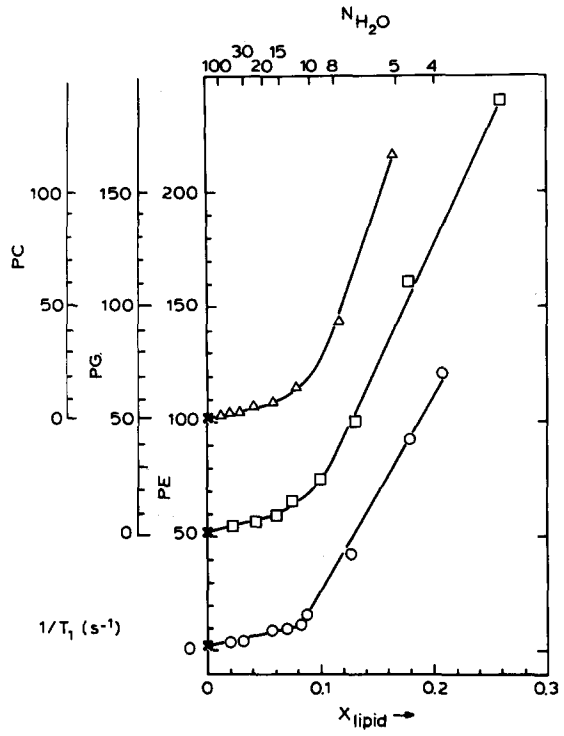

Fig. 2. Variation of the spin lattice relaxation rate $R_1 = (1/T_1)$ with the mole fraction of lipid at 25°C and 46 MHz measuring frequency. The ordinates are staggered to avoid confusion due to overlap of the curves. ○, PE; □, PG; △, PC.

linear dependence of $1/T_1$ on the lipid content from pure water to about 16 H_2O/PG. At this concentration, a distinct change of the slope of the relaxation curve is observed; further dehydration causes a more rapid increase in the relaxation rate (equivalent to a shortening of T_1). At 2 wt.% water, the T_1 relaxation rate is only 5.3 ms, which is already two orders of magnitude shorter than that of pure water (T_1 = 502 ms at 25°C).

The temperature dependence of the T_1 relaxation times of the *E. coli* PG-H_2O system is summarized in Table I. Arrhenius plots of the relaxation times yield straight lines with negative slopes, indicating that the motions determining T_1 fall into the fast correlation-time regime where $\omega_0\tau_c \ll 1$ ($\omega_0 = 2\pi \times 46$ MHz in the present experiments; τ_c is the correlation time for the reorientation rate of the water molecule). The activation energies, E_a as determined from the slopes of the Arrhenius plots are 17.8 kJ/mol for pure water (i.e., H_2O/^2H_2O 9:1) and 27.2 ± 1 kJ/mol for the various *E. coli* PG/H_2O mixtures. Interestingly, the activation energies of the latter are almost independent of the water content.

The ^{2}H-NMR spectra of the *E. coli* PE-H_2O and the PC-H_2O system are more complex, which is consistent with earlier observations on related PE and PC membranes [4–9]. Fig. 3 displays a series of ^{2}H-NMR spectra of the system *E. coli* PE-H_2O recorded at various lipid-to-water ratios. At the highest water content, only a single sharp resonance can be detected. However, at a mole fraction x_{H_2O} = 0.967 (corresponding to 30 H_2O/PE) a second, broad component appears and grows at the expense of the sharp signal. At x_{H_2O} = 0.91 (11 H_2O/PE) only the broad iso-

TABLE I

DEUTERIUM T_1 RELAXATION TIMES OF THE SYSTEM *E. COLI* PHOSPHATIDYLGLYCEROL-H_2O AS A FUNCTION OF WATER CONTENT AND TEMPERATURE

Mixture H_2O/^2H_2O; 9/1, v/v

Mole fraction H_2O	T_1 relaxation time (ms)				E_a (kJ/mol)
	5°C	15°C	25°C	40°C	
1	291	386	502	698	17.8
0.979	76	121	181	304	28.1
0.958	54	86	143	195	26.5
0.94			104	144	
0.926	25	41	62	97	27.4
0.899			39	50	
0.871	9.3	11.7	20	33	26.7
0.823			9	13.5	
0.742			5	7.2	

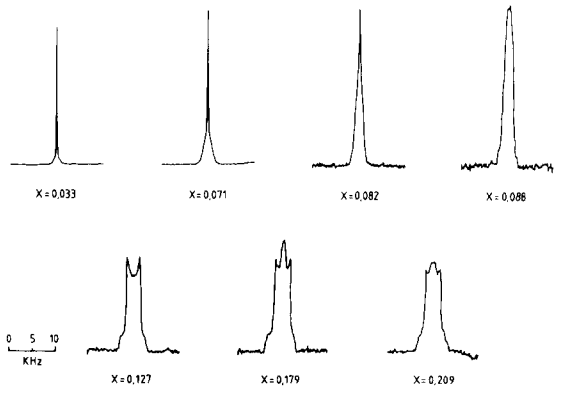

Fig. 3. Variation of the ^{2}H-NMR spectra of the *E. coli* phosphatidylethanolamine-$H_2O/^2H_2O$ system with the mole fraction of lipid at 25°C.

tropic signal remains which upon further dehydration transforms into a typical quadrupole doublet, indicating a restricted and axially symmetric motion of water. The quadrupole splitting, $\Delta\nu_Q$, of the powder-pattern is 2.6–3.1 kHz, which is much less than the maximum value of about 165 kHz of immobilized water molecules in ice (calculated with a static quadrupole coupling constant of $(e^2qQ/h) = 220$ kHz [13]).

Qualitatively similar behavior was obtained for bilayers composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine ($18:1_c$-, $18:1_c$-PC) at 25°C. A sharp isotropic ^{2}H-NMR signal, observable at high water contents ($x_{H_2O} = 0.982$), was gradually replaced by a broader component (in the range of $0.982 \leqslant x_{H_2O} \leqslant 0.962$) which was finally transformed into a typical quadrupole splitting at $x_{H_2O} = 0.884$. The variation of the linewidth $\Delta\nu_{1/2}$ and – at lower water contents – the quadrupole splitting, $\Delta\nu_Q$, of both lipids are shown in Fig. 1. Inspection of Fig. 1 reveals that the anisotropy parameters, $\Delta\nu_{1/2}$ and $\Delta\nu_Q$, remain quite small for all three systems investigated. However, further generalizations are not possible, since each membrane possesses its own set of spectral parameters.

In contrast, the relaxation behavior of all three systems investigated is surprisingly similar, as evidenced by Fig. 2, which provides a comparison of the relaxation rates as a function of the mole fraction of lipid. When the spectra exhibited two isotropic resonances, the T_1 relaxation times of both signals were evaluated separately. Only the relaxation rate of the broad component was included in Fig. 2. On the scale of Fig. 2, the differences between the two relaxation rates were almost negligible in most cases. At high water contents ($0 \leqslant x_L < 0.08$), the three relaxation curves are practically superimposable and their ordinates are displaced for the sake of clarity. All curves reveal a break point at a hydration level between 16 and 11 H_2O per phospholipid. Beyond the break point, a rapid increase in the relaxation rates is observed.

Discussion

The similarity of the T_1 relaxation time curves (Fig. 2) suggests some common features of lipid hydration independent of the chemical nature of the three polar groups involved. The two branches of the relaxation time curves apparently correspond to two different hydration processes.

(1) In an initial step, 11–16 water molecules become bound to the lipid polar region (inner or primary hydration shell [6]). With a surface area of 60–70 Å^2 per lipid molecule and an average projected surface area of 7–9.6 Å^2 for water, this number corresponds to approximately one to two layers of water. This is also in agreement with neutron diffraction studies which have shown that water may penetrate up to the level of the glycerol backbone [14]. Previous estimates of the inner hydration shell based on an analysis of the quadrupole splittings have yielded values of approx. 12–14 H_2O/lipid for phosphatidylcholine and phosphatidylethanolamine [6,7]. Within the primary hydration shell, the individual water molecules will occupy different sites with different T_1 relaxation times. However, due to rapid exchange of protons or water molecules (rapid on the T_1 time-scale) only an average T_1 relaxation time is experimentally accessible. The initial hydration is accompanied by a continuous rearrangement and readjustment of the lipid polar groups and the water molecules at the water surface as is evidenced by the continuous changes of the T_1 relaxation time.

(2) Once the primary hydration shell is filled, addition of further water produces much smaller effects on T_1. The addditional water may be trapped between the lipid lamellae (interstitial water) or may exist simply as bulk water, alterna-

tives which are not distinguishable in the present T_1 experiments. However, the strictly linear dependence of $(1/T_1)$ on the mole fraction of lipid suggests that the additional water molecules (or at least the protons) are in exchange with the inner hydration shell. For small lipid contents ($0 \leqslant x_L < 0.08$) the observed relaxation time $T_{1,obs}$ can be described in terms of a two-site exchange model.

$$\frac{1}{T_{1obs}} = \frac{nx_L}{1-x_L}\left(\frac{1}{T_{1L}} - \frac{1}{T_{1,H_2O}}\right) + \frac{1}{T_{1,H_2O}} \quad (1)$$

where $T_{1,H_2O} = 502$ ms is the relaxation time of pure water, n the number of water molecules in the primary hydration shell and T_{1L} their average relaxation time. A good approximation for $x_L \ll 1$ is

$$\frac{1}{T_{1obs}} \approx x_L\left\{n\left(\frac{1}{T_{1L}} - \frac{1}{T_{1,H_2O}}\right)\right\} + \frac{1}{T_{1,H_2O}} \quad (2)$$

indicating a linear dependence of $(1/T_{1,obs})$ on the lipid mole fraction, in agreement with Fig. 2. The break points of the figure further demonstrate that n varies between 11–16, yielding T_{1L} values between 80 and 110 ms. The validity of the above equations implies that all water molecules (protons) of the primary hydration shell participate in the exchange process.

Using the standard expression for the quadrupolar relaxation time (e.g. Ref. 15, p. 314), it is possible to calculate an effective correlation time, τ_c, for the rotation of water molecules. For pure water (containing 10 vol.% ^2H_2O) one finds $T_1 = 502$ ms (at 25°C) and $\tau_c = 2.8$ ps, consistent with literature values of 2–3.5 ps [1,16].

Likewise, the primary hydration shell is characterized by $T_1 = 90$ ms and $\tau_c = 16$ ps. Finally, at very low water contents, e.g., $n_{H_2O}/n_{Lip} = 5$, the T_1 relaxation time is 10 ms, yielding $\tau_c = 140$ ps. Hence even at the lowest water content measured, the rotation of the water molecules remains very rapid and essentially isotropic, explaining the almost complete averaging of the static quadrupole splitting of 220 kHz to a residual value of less than 2 kHz.

We may now compare the ^2H_2O T_1 relaxation time of pure lipid dispersions with that of *E. coli* membrane suspensions. The wet pellet of *E. coli* membranes as used in the ^{2}H-NMR studies normally contains 70 wt.% water. At 25°C, the T_1 relaxation time of ^2H_2O (at natural abundance) amounts to approx. 120 ms [10], which is about 4-times shorter than the relaxation time of pure H_2O. Phospholipid accounts for at most 50 wt.% of the membrane dry weight, the remainder being proteins and carbohydrates. The lipid mole fraction, x_L, in *E. coli* membrane suspensions referred to total water content therefore does not exceed $5 \cdot 10^{-3}$, which, in connection with Eqn. 1, predicts a relaxation time, T_1, of 380 ms. This is much longer than the experimental value and allows the conclusion that the major contribution to the relaxation time originates from the non-lipid components in the *E. coli* membrane. For water in immobilized protein systems, distinct reductions in the T_1 relaxation times have been observed [17], but the experimental conditions are not comparable to those of *E. coli* cell suspensions. Thus, it remains to be seen in future work to what extent membrane-bound proteins or the lipopolysaccharide (dominant in the outer *E. coli* membrane) are responsible for the short T_1 relaxation time of this system.

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