NMR STUDY OF RAPID WATER DIFFUSION ACROSS LIPID BILAYERS IN DIPALMITOYL LECITHIN VESICLES.

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SUMMARY: A method for measurement of rapid diffusional exchange between external and internal water in lecithin vesicles is described. Paramagnetic ions were inserted inside DPL vesicles and the NMR relaxation times for water protons were measured as a function of temperature. It was found that water diffusion rate is described by a single activation energy of 15±1 kcal/mole in the temperature range 16 - 35°C and exhibits a maximum at 44°C. The permeability of DPL vesicles to water was calculated to 16-18 x 10^{-4}cm/s at 44°C and 1.7 x 10^{-4}cm/s at 20°C.

INTRODUCTION:

Model membranes, consisting of a lipid bilayer, exhibit high permeability to water. Although water permeability for black lipid films has been the subject of several studies[1,2], there is a lack of methods available for the determination of rapid water diffusion in phospholipid vesicles and similar systems. A method based on the rate of swelling of multilamellar vesicles has been developed by Bangham et al.[3] These experiments yielded water diffusion rates from 0.8 to 18 x 10^{-4}cm/s.

In contrast to the high permeability to water, phospholipid membranes show extreme impermeability to cations. The impermeability of lecithin vesicles to paramagnetic ions has been employed to differentiate the internal and external membrane surfaces[4,5]. Use of paramagnetic ions is well established in biological applications of NMR spectroscopy[6]. Measuring the water exchange time between erythrocytes and plasma has been achieved by pulsed NMR when the plasma was doped with Mn^{2+} [7]. For phospholipid vesicles this method is, however, limited to exchange times longer than about 5 - 10 ms due to risks for osmotic rup-

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tures caused by addition of ions to bulk solution. Interference with other protons in such systems reduces the possibility to apply this method.

In the present study, paramagnetic ions were inserted inside the vesicles by sonicating of phospholipid dispersions in the presence of Mn^{2+} followed by an exchange of Mn^{2+} by Na^+ in the external solution. This method is applicable to even very small samples since the relaxation of bulk water protons is measured.

MATERIALS AND METHODS:

Synthetic dipalmitoyl lecithin (DPL) was a generous gift from Dr Gösta Arvidsson, Lund. All other reagents were reagent grade and used without further purification.

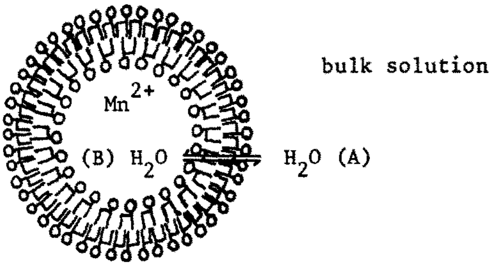
DPL was suspended in water containing added Mn^{2+}. The final concentration of Mn^{2+} varied between 45 - 90 mM. Bilayer vesicles were obtained by sonication of this coarse dispersion on a Branson sonifier (model S 125). During the sonication procedure the temperature of the sample was kept at ca. 45 - 50°C. 20-30 s intervals of sonication were alternated with 30 s resting intervals for about 15 minutes. 3-5 fold excess (in terms of exchanging capacity) of Chelex 100 (BIO--RAD) was added to the resulting translucent solution to exchange Mn^{2+} in the external solution by Na^+. The sample was gently shaked several times and, after 10 minutes, 0.1 - 0.2 ml solution was decanted for NMR measurements. During the whole procedure the temperature was maintained at 45 - 50°C.

The ^1H relaxation time measurements were performed on a Bruker BK-322s spectrometer at 61.00 MHz. The longitudinal relaxation times (T_1) were obtained by $180^\circ - t - 90^\circ$ pulse sequences and the transverse relaxation times (T_2) were measured using the Gill - Meiboom modification of the Carr - Purcell sequence. The oscilloscope recordings of magnetization decays were photographed.

The manganese content in the samples was determined as permanganate[8] on a Perkin - Elmer spectrophotometer, model 124.

RESULTS AND DISCUSSION:

Water diffusion in the investigated systems is shown in following picture:



Due to the presence of paramagnetic ions inside the vesicles, the NMR relaxation times for internal water protons become very short. The diffusional process can thus be treated as a two-sites chemical exchange between environments A and B. Since the fraction of H_2O inside vesicles is small, the NMR relaxation for all water protons is single exponential process and the ^1H relaxation times, T_1 and T_2, are given by[9]

$$\frac{1}{T_i} = \frac{1}{T_{iA}} + \frac{P_B}{P_A}\,\frac{1}{T_{iB} + \tau_B} \qquad i = 1,2 \qquad (1)$$

where T_{iA} and T_{iB} are the ^1H relaxation times for external and internal water in the absence of exchange; P_A and P_B are the volume fractions of external and internal solutions and τ_B is the mean residence time of H_2O inside the vesicles.

In the absence of Mn^{2+}, the ^1H relaxation times for water in vesicle solutions are long (>1s). Thus, the first term in eq.(1) can be neglected and eq. (1) rewritten as

$$T_i = \frac{P_A}{P_B}\,(T_{iB} + \tau_B) \qquad i = 1,2 \qquad (2)$$

Fig. 1 shows the observed relaxation times for water protons in 7.5 % DPL vesicles as a function of temperature for 3 different concentrations of Mn^{2+}. All the experiments were performed starting from the highest temperature over the period of 2-3 hours.

The experimental T_2 exhibit minima at approximately the phase transition temperature of DPL while the T_1 minima lie at temperatures several degrees lower. Since T_{1B} and T_{2B} are known to decrease with decreasing temperature Fig. 1 indicates that τ_B determines the observed relaxation times at lower temperatures. At higher temperatures T_{1B} and T_{2B} appear to dominate. This conclusion is supported by the fact that the T_1/T_2 ratio decreases with decreasing temperature.

In the temperature range where the relaxation is completely dominated by chemical exchange ($T_1 \approx T_2$), τ_B is calculated directly using eq.(2) with $T_{iB} << \tau_B$. The P_A/P_B ratio is obtained by analysis of Mn^{2+} contents in the samples before and after the treatment with Chelex 100. In order to calculate the exchange time for the whole temperature range we have employed two independent methods:

1) The values of $1/T_{2B}$ should be directly proportional to Mn^{2+} concentration

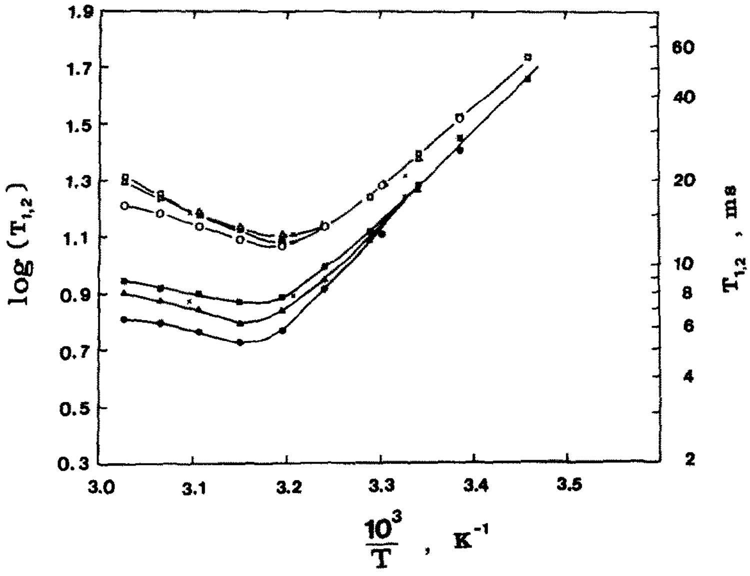


Fig. 1 : The ^1H relaxation times for water protons in 7.5 % DPL vesicles as a function of temperature and Mn^{2+} concentration.
(□) T_1 and (■) T_2 for 0.043 M Mn^{2+} .
(△) T_1 and (▲) T_2 for 0.060 M Mn^{2+} .
(○) T_1 and (●) T_2 for 0.090 M Mn^{2+} .
Several measurements of T_1 and T_2 for vesicles with 0.050 M Mn^{2+} are also included in the figure (×) .

inside the vesicles. This fact was confirmed from observations of the ^1H relaxation times in sonicated DPL dispersions containing Mn^{2+} in both external and internal solutions. Thus, the plots of the observed T_2 (Fig. 1) against the reciprocal Mn^{2+} concentrations give straight lines with the intercept $\tau_B P_A/P_B$. Several values of τ_B calculated in this manner are depicted in Fig. 2.

2) Use is made of the fact that the T_{1B}/T_{2B} ratio is approximately temperature independent for the water protons in vesicle solutions containing Mn^{2+} in both external and internal solutions. With $T_{1B}/T_{2B} = C$, the experimental T_1/T_2 ratio is given by

$$\frac{T_1}{T_2} = \frac{C\,T_{2B} + \tau_B}{T_{2B} + \tau_B} \qquad (3)$$

where C is constant. Combining eq.(3) with eq.(2) for T_2, τ_B can be calculated at every temperature if C is known. The value of C is 10 for aqueous Mn^{2+}. Lower values of C were found for vesicles with Mn^{2+} in both external and internal solutions, presumably due to binding of Mn^{2+} to phospholipid bilayers. τ_B calculated using the latter values of C are shown in Fig. 2. The full line is an average of all three measurements.

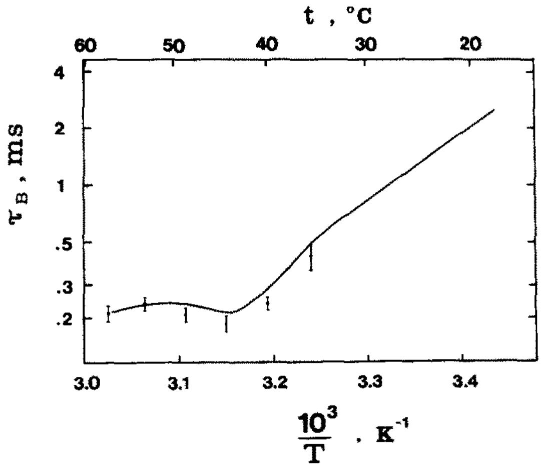


Fig. 2 : The temperature dependence of the exchange time, τ_B, calculated using method 1 ($\updownarrow$) and method 2 (the full line) described in the text. The values of C employed in method 2 were 2.7 for 0.043 M Mn^{2+}, 3.3 for 0.060 M Mn^{2+} and 4.1 for 0,090 M Mn^{2+}.

Fig. 2 reveals an interesting temperature dependence of the calculated exchange time. At temperatures close to the transition temperature τ_B exhibits a slight minimum, then increases with temperature and at about 50°C decreases again. A similar phenomenon, although more pronounced, has recently been observed by Papahadjopoulos et al.[10] for the permeability of DPL to Na^+. The activation energy of 15 ± 1 kcal/mole is calculated for the temperature range 16 - 35°C.

The mean value of 0.07 was found for the P_B/P_A ratio in the investigated systems. Assuming spherical vesicles with thickness of the bilayer 46Å this value corresponds to vesicles with ca. 400 Å in diameter and indicates the presence of vesicle fractions I and II[11]. Thus, our data give the average permeability of DPL vesicles to water $16\text{-}18 \times 10^{-4}$ cm/s at 44°C and 1.7×10^{-4} cm/s at 20°C.

It is necessary to briefly outline some of the factors capable to influence our measurements. Mn^{2+} can leak out from the vesicles (this phenomenon could be observed as an increase in the T_1/T_2 ratio with time during the experiments). Membrane fusion and formation of multilamellar vesicles have been observed for DPL vesicles at temperatures above 40°C [12]. Binding of Mn^{2+} to lecithin molecules could render the observed H_2O exchange rates not directly comparable to those of "non-doped" solutions. Finally, some aggregation of vesicles at temperatures below the phase transition of DPL has been reported[13].

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