J. Membrane Biol. 35, 303–307 (1977)

Pulse NMR Studies of Water Permeability in Phosphatidylcholine Vesicles Containing General Anesthetics

Howard S. Hochster and J. H. Prestegard

Department of Chemistry, Yale University, Sterling Chemistry Laboratory, New Haven, CT 06520

Received 3 November 1976

Summary. Phosphatidylcholine was extracted from egg yolks and sonicated with *n*-alkyl alcohols to give vesicles. The magnetic environment of the external compartment was then modified by the addition of shift reagent (Co^{2+}). This allowed determination of the average lifetime for exchange of water molecule protons by observing the relaxation rate dependence on the interval between pulses in a Carr-Purcell sequence. For vesicles containing *n*-hexanol as 33% of lipid, the average water molecule lifetime in the internal compartment was found to be substantially less than that for a vesicle without hexanol. The lifetime, 11.3 ± 4 msec, is equivalent to a permeability coefficient of 60 μ/sec at 34 °C.

Introduction and Theoretical Considerations

Suspension of phospholipid in aqueous media results in the spontaneous formation of bilayers. Such a suspension may be sonicated to give closed, spherical vesicles of relatively homogeneous size and volume [9]. Vesicle systems of this nature have been extensively used as models for numerous physical properties of biological membranes [4, 10, 11]. One such property is the permeability of the bilayer to ions or small molecules [6, 12]. Most studies involving membrane permeability have made use of thin-lipid films rather than vesicles. They have led to the characterization of several permeability altering agents. One class which had been found to increase permeability of small molecules is the general anesthetics, such as the long chain organic alcohols [5]. The present research attempts to study the water permeability properties of alcohol-containing vesicles through the observation of the rate of water exchange between two environments of differing magnetic properties. One NMR study of water exchange in lipid vesicles has appeared, relying on differences in the water proton relaxation in the two environments [2]. We will use instead a method dependent on shift differences which allows direct extrapolation of exchange times from the pulse spacing dependence of the spin-spin relaxation.

The well-known Bloch phenomenological equations describe the behavior of a magnetic moment in a static magnetic field with an oscillating field applied perpendicular to it. While the relaxation times, T_1 and T_2, as introduced by Bloch were related to line widths and intensities in the high-resolution spectrum (frequency domain), they may be measured more directly in the time domain by pulsed magnetic resonance techniques [7]. In considering exchange of a magnetic moment between two sites with differing magnetic environments, the Bloch equations may be modified with a dependence on the first order rate constant, k, or its reciprocal, τ. In particular, solving these simultaneous differential equations yields the result that only T_2, the transverse or spin-spin relaxation time, is sensitive to low frequency exchange processes. T_2 may be measured using the Gill-Meiboom modification of the Carr-Purcell method where a 90° pulse is originally applied to the system and then a train of 180° pulses is applied, waiting a given interval, $2t_{cp}$, between each 180° pulse. The spins will rephase at time t_{cp} after each pulse giving a series of spikes whose decay envelope is characterized by T_2.

It is intuitively clear that the observed T_2 will in some way depend on the spacing between pulses in the Carr-Purcell experiment. If the time between pulses is but sufficient for a small number of exchanges to have occurred, the average resonance frequency of a given spin will not be well-defined; these spins will not rephase at $2t_{cp}$ and a contribution to T_2 will result. A solution expressing this dependence on t_{cp} results from the modified Bloch equations for T_2 H1]:

$$\frac{1}{T_2}=\frac{1}{T_2^0}+P_A P_B(\delta\omega)^2 \tau \left(1-\frac{2\tau}{t_{cp}}\operatorname{Tanh}\frac{t_{cp}}{2\tau}\right) \tag{1}$$

where T_2^0 is the relaxation time in the absence of exchange, p_A and p_B are the fractional populations of the two sites, $\delta\omega$ is the difference between resonance frequencies for the sites (radians/sec) and τ is the average lifetime between exchanges (i.e., $1/\tau=p_A/\tau_A+p_B/\tau_B$). The equation, although based on several limiting assumptions, has proven valid over a wide range of experimental situations. It will be applied without modification to the problem at hand.

Materials and Methods

Phosphatidylcholine used in the present study was extracted from egg yolks and purified by the method of Singleton, *et al.* [13]. The egg yolk phosphatidylcholine

(EYPC) was stored at freezer temperature under N_2 until used. Vesicle samples were prepared to give approximately 15% (by weight) phospholipid. If *n*-alkyl alcohols were added, their concentration was 20 or 33% of phospholipid by mole. The EYPC and alcohol, if present, were dispersed in water by mechanical agitation. The dispersion was then sonicated, at approximately 10 °C, using a Branson S125 probe sonicator—with a microtip, at low power, and 70% dury cycle—under N_2 for 0.5 to 1 hr. The transparent sample was then filtered through a millipore membrane and examined.

Modification of the magnetic environment outside the vesicles was achieved by the addition of shift reagent. 50 mM Co^{2+} gave the most desirable effect of inducing a large chemical shift difference in the water peak, 0.23 ppm, with minimal broadening in the high resolution spectrum (i.e., the ratio $\delta\omega$: T_2^0 is large).

T_2 measurements were performed with a Bruker Minispec P-20 pulse spectrometer thermostated at 34 °C. Pulse trains were taken for appropriate t_{cp} values, recorded with a Tektronix 5031 storage oscilloscope, and peak heights were measured. These data, which were single exponential in nature, were subjected to linear regression analysis, giving the best exponential decay constant, T_2.

Results and Discussion

$1/T_2$ data are presented in Fig. 1 for 50 mM aqueous $CoCl_2$ solution and vesicles containing *n*-hexanol (33% of phospholipid by mole). The

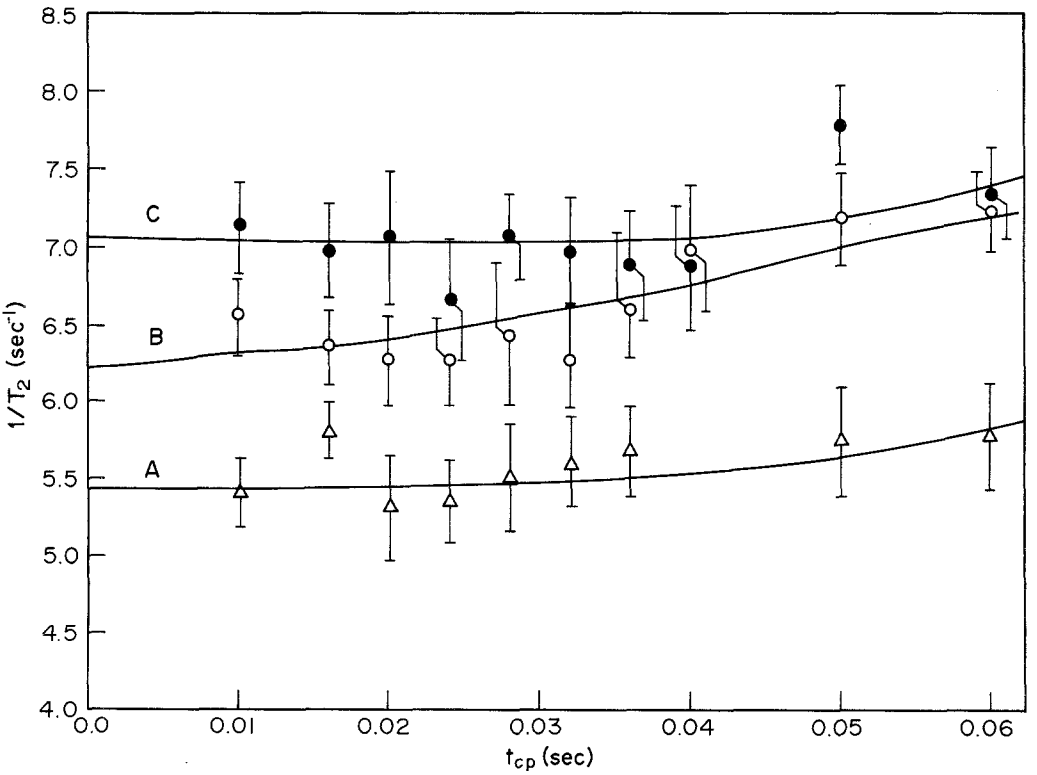

Fig. 1

upper limit for t_{cp} of 60 msec is set by the self-diffusion of water in the inhomogeneous magnetic field. In the "doped" water solution (curve *A*) one would not expect to see a t_{cp} dependence and the rise at long pulse intervals very likely reflects this diffusion process. A similar experiment was carried out with an EYPC vesicle sample comprised of 15% phospholipid by weight (no anesthetic) yielding a total water volume of 9.6% in the internal compartment. $1/T_2^0$ increased as compared to the H_2O curve, but no greater variation in $1/T_2$ with t_{cp} was observed. This overall increase in $1/T_2^0$ is most probably due to binding of Co^{2+} to the vesicle surface.

For the *n*-hexanol containing vesicles, in 33% alcohol concentration, a more pronounced rise in $1/T_2$ as pulse spacing increased toward 60 msec was noted (curve *B*). Similar behavior was noted for vesicles containing 20% hexanol by mole ratio. This suggests a variation in relaxation rate due to exchange of water between the inner and outer compartments, which was not observable in the pure phosphatidyl choline sample. That this is the result of exchange between differing magnetic environments is confirmed by the fact that resonication, allowing Co^{2+} to enter the vesicle, obviated the t_{cp} dependence (curve *C*). The further increase in $1/T_2^0$ is probably the result of increased availability of binding sites for Co^{2+} (on the interior surface of the vesicle membrane).

Eq. (1) can be fit to the data in curve *B* allowing the weighted average of the water lifetimes to be determined. $\delta\omega$ was determined independently as described above. Gel permeation chromatography [8] for the 33% hexanol vesicles yielded a most probable diameter of 425 Å, from which a total internal water volume of 20% can be estimated. Thus, $p_A p_B$ of Eq. (1) equals 0.16. Curve *B* shows the best fit for Eq. (1) using these parameters and τ as an independent variable. τ is found to be 30 msec (± 10 msec). *n*-butanol vesicles showed the same qualitative behavior as *n*-hexanol, though τ was somewhat larger due to the smaller oil/water partition coefficient. *n*-decanol did not produce a sufficient increase in water permeability to allow measurement. This is consistent with an expected decrease in effectiveness in altering permeability with increasing chain length of the anesthetic [5].

In summary we can say that hexanol increases water permeability of vesicles to the point that an average liefetime may be measured by pulsed NMR techniques relying on its pulse spacing dependence. The measured τ value yields an average water molecule lifetime *inside* a 33% hexanol-lecithin vesicle of 11.3 ± 4 msec or a permeability coefficient of 60 μ/sec at 34 °C. This number compares favorably to permeability coefficients for

biological cells, reported to be 0.4–400 μ/sec at 20–25 °C [14], and to permeability coefficients for liposomes, in the range of 17–104 μ/sec at 25 °C [3]. Both of the above quantities were determined by osmotic swelling techniques. The NMR measurements here are particularly appealing because they are determined at equilibrium conditions for water exchange.

We would like to acknowledge the support of the National Institutes of Health through research grant RO1-GM19035.

References

1. Allerhand, A., Gutowsky, H.S. 1964. Spin echo NMR studies of chemical exchange. *J. Chem. Phys.* **41**:2115
2. Andrasko, J., Forsen, S. 1974. NMR study of rapid water diffusion across lipid bilayers in dipalmitoyl lecithin vesicles. *Biochem. Biophys. Res. Commun.* **60**:813
3. Bangham, A.D., DeGier, J., Greville, G.O. 1967. Osmotic properties and water permeability of phospholipid crystals. *Chem. Phys. Lipids* **1**:225
4. Bangham, A.D., Hill, M.W., Miller, N. 1974. Preparation and use of Liposomes as models of biological membranes. *Methods Membr. Biol.* **1**:1
5. Bangham, A.D., Standish, N.M., Miller, N. 1965. Cation permeability of phospholipid membranes: Effect of narcotics. *Nature (London)* **208**:1295
6. Cass, A., Finkelstein, A. 1967. Water permeability of thin lipid membranes. *J. Gen. Physiol* **50**:1765
7. Farrar, T.C., Becker, E.D. 1971. Pulse and Fourier Transform NMR. Academic Press, New York
8. Gent, M.P.N., Prestegard, J.H. 1974. Cholesterol-phosphatidylcholine interactions in vesicle systems. *Biochemistry* **13**:4027
9. Huang, C. 1969. Studies on phosphatidylcholine vesicles. Formation and physical characteristics. *Biochemistry* **8**:344
10. Levine, Y.K. 1972. Physical studies of membrane structure. *Prog. Biophys. Mol. Biol.* **24**:3
11. Papahadjapoulos, D., Kimelberg, H.K. 1973. Phospholipid vesicles as models for biological membranes. *Prog. Surf. Sci.* **4**(II):141
12. Reeves, J.P., Dowben, R.M. 1970. Water permeability of phospholipid vesicles. *J. Membrane Biol.* **3**:123
13. Singleton, W.S., Gray, M.S., Brown, M.L., White, J.L. 1965. Chromatographically homogeneous lecithin from egg phospholipids. *J. Am. Oil. Chem. Soc.* **42**:53
14. Thompson, T.E., Henn, F.A. 1970. Experimental phospholipid model membranes. *In:* Membranes of Mitochondria and Chloroplasts. E. Racker, editor. pp. 1–52. Van Nostrand and Reinhold, New York