IONIC PERMEABILITIES OF MEMBRANES

^{23}Na and ^{7}Li NMR studies of ion transport across the membrane of phosphatidylcholine vesicles

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1. Introduction

Ion transport across membranal barriers plays a fundamental role in cellular activity; the generation of nerve impulses and their transfer to the muscles, the renal activity and activated transport of various metabolites are only a few examples. Thus, studies of the permeability characteristics of membranes are essential for the understanding of many physiological processes. Several methods have been employed to study ion permeability of vesicular membranes. The method mostly used is the tracers technique which employes radioactive isotopes. This method in general is limited to relatively slow transport rates corresponding to lifetimes longer than several seconds. Also its application requires the destruction of the sample. In this communication we present a method in which the transport process can be directly monitored using the nuclear magnetic resonance (NMR) of the transporting species. It makes use of paramagnetic relaxation reagents (PRR) and is similar to the method used [1,2] to study water transport. Here we extend the method by using an anionic PRR for positively charged transporting species for which the usual PRRs are not suitable. Specifically we consider the transport of alkali metal ions (sodium and lithium) through phospholipid membranes of vesicles using ^{23}Na and ^{7}Li NMR and employ $Gd(EDTA)^-$ as a relaxation reagent for cations [3]. The method is non-destructive and can be applied for both very slow kinetics (halflives longer than several minutes) as well as very fast rates (halflives shorter than 10^{-3} s).

2. Materials and methods

Pure egg phosphatidylcholine was purchased from Makor Chemicals Ltd and was used without further purification. The monensin sodium salt was kindly supplied by Eli-Lilly and purified before use by successive recrystallization from methanol. All other chemicals used were of analytical grade.

Vesicles were prepared by sonicating 10% egg phosphatidylcholine at 4°C under nitrogen in $^{2}H_2O$ containing 150 mM salt (either NaCl or LiCl) and 10 mM Tris (hydroxymethyl) amino methane. The vesicle suspension was centrifuged at 40 000 × g for 50 min and the zone containing clear supernatant was removed for subsequent studies. The monensin sodium salt was added prior to each experiment to the clear vesicle suspension and was mixed for ~15 min at room temperature. All experiments were performed within 48 h after the preparation of the vesicles.

The ^{23}Na and ^{7}Li Pulse-FT measurements were performed at 23.81 MHz and 34.98 MHz, respectively, on a Bruker WH-90 spectrometer, with a multinuclear probe, using 10 mm diam. tubes. Proton NMR spectra were obtained on a Bruker WH-270 Pulse-FT spectrometer.

3. Results and discussion

In the upper trace of fig.1 the NMR spectrum of ^{23}Na in a dispersion of egg phosphatidylcholine vesicles is shown. In this preparation about 10% of the sodium

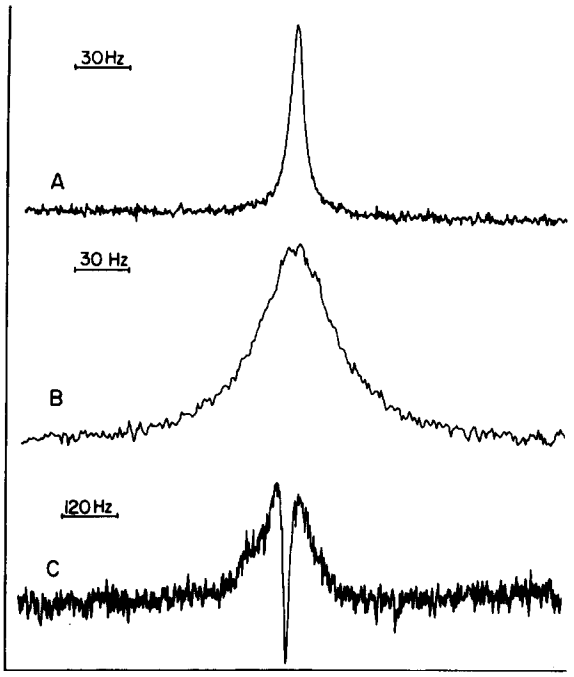

Fig.1. ^{23}Na NMR spectra of: (A) 150 mM NaCl dissolved in phosphatidylcholine vesicle suspension p^{2}H 9.2 at 28°C; (B) the same as (A) after addition of 11.6 mM Gd(EDTA)$^-$; (C) the same as B using $180° - \tau - 90°$ pulse sequence in order to separate the signal due to the ions entrapped in the inner vesicular medium, as explained in the text. 40 transients were accumulated in (A) and (B) and 4800 transients with τ = 4 ms in (C), with a recycle time of 1.1 s.

ions are intravesicular while the rest is dissolved in the bulk solvent. The single line observed in the spectrum thus consists of a superposition of both the signals of the inner and outer sodium ions. In order to monitor the transport between the inner and outer compartments we must be able to record at least one of the signals separately, preferably the weaker signal of the intravesicular sodium ions. A discrimination between the 2 signals can be achieved by using (paramagnetic) shift of relaxation reagents. However, the common reagents Mn^{2+}, Gd^{3+} and Pr^{3+} were found to have no effect on the sodium (as well as on the other alkali) ions' resonance. This is not surprising in view of the fact that these reagents are positively charged and will not bind to the alkali cations. On the other hand, the ethylenediamine tetraacetate (EDTA) complex of gadolinium (III), which is negatively charged, was found to be quite effective in broadening the alkali ion resonances. The trace in fig.1B corresponds to the same solution used to record the upper trace but to which 11.6 mM Gd(EDTA)$^-$ was added. Although the relaxing effect on the sodium resonance is quite remarkable, no discrimination between the signals due to inner and outer sodium is apparent. There may be several reasons for that:

(i) The Gd(EDTA)$^-$ complex diffuses through the membrane and thus affects both compartments.
(ii) There is very fast transport of sodium across the membrane causing the relaxation of the inner and outer sodium to average.
(iii) The inner signal is too weak to observe.

We shall show shortly that (i) and (ii) may be ruled out and we are left with the problem of separating the weak signal from the strong superimposed signal. A possible, but undesirable way to do this is to use very high concentrations of Gd(EDTA)$^-$ so as to completely wash out the outer signal. Instead we used the following modification of the Fourier transform method: Rather than Fourier transforming the free induction decay (FID) signal following single pulses we used a pulse sequence of $180°$–τ–$90°$ and Fourier transformed the FID signal following the 90° pulses. The interval τ was chosen so that $\tau \simeq T_1^{ex} \ln 2$ where T_1^{ex} is the longitudinal relaxation time of the sodium ions in the bulk solution containing the Gd(EDTA)$^-$. This procedure essentially abolishes the outer signal but since T_1^{in} of the inner sodium, which is not affected by the Gd(EDTA)$^-$, is longer than T_1^{ex}, the inner signal is only slightly reduced (although its phase is inverted).

An example of spectra obtained using this procedure is shown in fig.1C. The sharp, inverted signal corresponds to the inner sodium ions. The extracellular ions are responsible for the weak, broad, uninverted signal. This spectrum clearly demonstrates that there is no diffusion of Gd(EDTA)$^-$ into the vesicles and that transport of sodium through the membrane is slow compared to the nuclear relaxation time. This conclusion is in agreement with previous measurements of ionic self diffusion rates across phosphatylcholine vesicles [4].

The technique described above can be used in 2 ways to study cation transport in and out of the vesicles. If the transport is slow enough (life times of several minutes), the change in intensity of the

inner signal can be monitored in real time following a change in the ion concentration of the intravesicular bulk solution. The second way applies when the transport rate is of the order of the nuclear relaxation rates. In this case the relaxation times T_1 and T_2 of the inner signal are given by [5]:

$$\frac{1}{T^{in}_{1,2}} = \frac{1}{T^{in}_{1,2}(0)} + \frac{1}{\tau} \quad (1)$$

where the index (0) refers to the relaxation rates in the absence of transport, and τ is the mean life time of the ions inside the vesicles. It is related to the permeability P of the vesicular membrane by [6]:

$$P = \frac{V}{A}\frac{1}{\tau} = \frac{R}{3}\frac{1}{\tau} \quad (2)$$

where V, A and R are, respectively, the internal volume, surface area, and radius, of the vesicles.

We now demonstrate the application of this latter method to the study of the ionophore monensin mediated transport of sodium and lithium ions across the vesicular membrane [7]. The effect of adding monensin to the vesicular preparation on the linewidth of the inner signal of sodium is shown in the 3 traces in fig.2. There is a conspicuous increase in the linewidth upon increasing the ionophore concentration which we attribute to enhancement of the transport rate across the membrane. Using eq. (1) and eq. (2) for R = 60 Å (based on the ratio between the integral areas of the inner and outer choline methyl protons [8]), the ionophore-mediated permeabilities could be determined. Sodium permeabilities were found to be 62 nm/s, 82 nm/s, 126 nm/s and 158 nm/s for 15 μM, 22.5 μM, 30 μM and 37.5 μM monensin, respectively, and lithium permeabilities were 12 nm/s and 33 nm/s for 400 μM and 800 μM monensin, respectively. Thus the permeabilities extrapolated to 1 μM monensin are: for Na^+ 4.0 ± 0.4 nm/s; for Li^+ 0.035 ± 0.005 nm/s. These results show that within the concentration range studied the sodium transport rate increases fairly linearly with the ionophore concentration, indicating that the dominant transporting species is a 1:1 complex of the sodium ionophore. The actual transport mechanism by an ion carrier can be discussed in terms of the following steps:

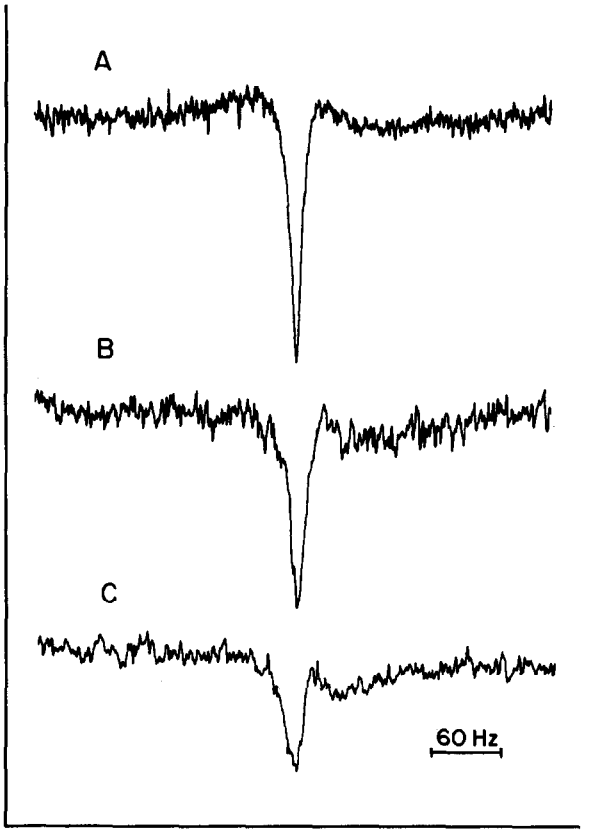

Fig.2. Sodium signal obtained as in fig.1C of ions entrapped inside the vesicles at various monensin concentrations at 28°C. The vesicle suspension was the same in fig.1. Monensin concentrations: (A) 0 μM; (B) 15 μM; (C) 37.5 μM. (A) 7200 transients; (B,C) 4500 transients.

1. Formation of a carrier-ion complex at the interface of the membrane;
2. Diffusion of the complex through the bilayer;
3. Dissociation of the ion from the carrier at the other interface of the membrane;
4. Back diffusion of the carrier through the bilayer.

Equal monensin induced permeability of sodium and lithium would be observed if steps 2 or 4 were rate determining. The much higher value obtained for sodium either indicates that the complex association–dissociation processes (steps 1 and 3) determine the overall rate of transport or reflects the difference in the binding constants for these 2 ions. Both possibilities are in accordance with the significantly higher

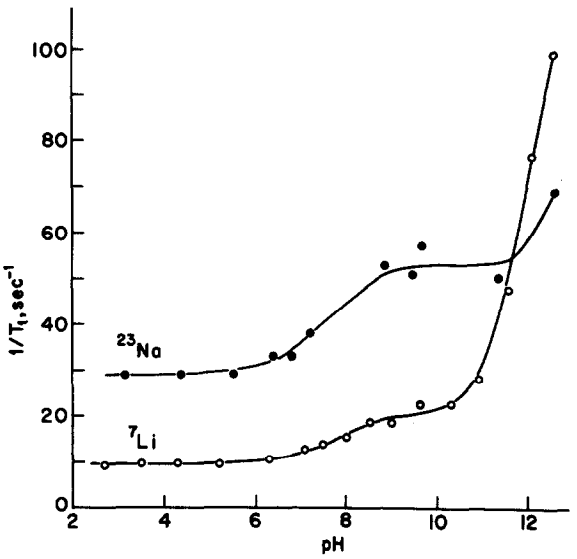

Fig.3. The pH dependence of the longitudinal relaxation rates of aqueous solutions (50% ^2H_2O) containing 0.53 M NaCl and 0.56 M LiCl in the presence of 11.6 mM Gd(EDTA)$^-$ at 28°C. T_1 values were measured by applying 180°–τ–90° pulse sequence with varying τ values. For both nuclei a single transient was sufficient.

affinity of monensin towards sodium relative to that of lithium [9].

Two comments concerning the use of Gd(EDTA)$^-$ in such studies are in order.

(1) We must rule out the possibility that Gd(EDTA)$^-$ is itself transported by monensin into the vesicles thus causing the broadening of the inner signal. This was easily confirmed using the proton NMR signals of the vesicular phosphatidylcholine. Addition of Gd(EDTA)$^-$ caused line broadening of the methyl choline protons of the outer membranal surface, but did not affect the linewidth of the corresponding protons on the inner surface in the presence of 80 μM monensin even after allowing the preparation to stand for several hours.

(2) The effect of Gd(EDTA)$^-$ upon the nuclear relaxation rates of sodium and lithium ions in aqueous solutions which will be discussed [10]. At constant pH, temperature and ion concentration, the relaxation enhancement was found to be linear with the Gd(EDTA)$^-$ concentration. The Gd(EDTA)$^-$-enhanced relaxation rates of ^{23}Na and ^{7}Li was found to be pH dependent. The pH profiles shown in fig.3 reveal an appreciable enhancement of the relaxation rates with increasing pH indicating the existence of 2 titrations around pH 8 and pH 12. Therefore the pH of the studied systems should be kept under control.

Acknowledgements

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References

[1] Conlon, T. and Outhred, R. (1972) Biochim. Biophys. Acta 288, 351–361.
[2] Shporer, M. and Civan, M. M. (1975) Biochim. Biophys. Acta 385, 81–87.
[3] Elgavish G. A. and Reuben, J. (1977) J. Am. Chem. Soc. 99, 1762–1765.
[4] Hauser, H., Oldani, D. and Phillips, M. C. (1973) Biochemistry 12, 4507–4517.
[5] Haran, N. and Shporer, M. (1976) Biochim. Biophys. Acta 426, 638–646.
[6] Schwartz, T. L. (1971) in: Biophysics and Physiology of Excitable Membranes (Adelman, W. J. jr, ed) p. 98, Van Nostrand Reinhold Company, NY.
[7] Agtarap, A., Chamberlin, J. W., Pinkerton, M. and Steinrauf, L. (1967) J. Am. Chem. Soc. 89, 5737–5738.
[8] Huang, C., Sipe, J. P., Chow, S. T. and Martin, R. B. (1974) Proc. Natl. Acad. Sci. USA 71, 359–362.
[9] Gertenbach, G. A. and Popov, A. I. (1975) J. Am. Chem. Soc. 97, 4738–4744.
[10] Elgavish, G. A. and Reuben, J. (1978) in preparation.