Ion Transport through Phospholipid Bilayers Studied by Magnetisation Transfer; Membrane Transport of Lithium Mediated by Monensin

Frank G. Riddell, S. Arumugam, and Brian G. Cox

Department of Chemistry, The University of Stirling, Stirling FK9 4LA, Scotland

The suitability of a magnetisation transfer technique to study ion transport through membranes is demonstrated by studying the monensin mediated exchange of Li^+ ions through phosphatidyl choline membranes in a process shown to be first order in monensin.

The use of ^{23}Na and ^{39}K n.m.r. spectroscopy to study the transport of Na^+ and K^+ ions through the limiting membranes of cells[1] or through model phospholipid bilayers[2—5] has now become an established technique. The methods involve a compartmentalised system of cells (*e.g.* erythrocytes) or of vesicles and employ either a shift reagent[6] or a relaxation agent[7] to contrast the signals from the intracompartmental and extracompartmental metal ions.[7] For slow exchange rates the time course of the signal intensities can be followed. For rapid exchange dynamic n.m.r. line-broadening effects are observed. Magnetisation transfer offers a third option that has so far not been fully investigated for transport studies. Here we show that magnetisation transfer can be employed to measure transport rates in cases where the other techniques are not applicable.

The magnetisation transfer technique for a two site case involves placing a magnetic label at one site by inverting the spin populations (inverted signal), and then following the intensity at the other site as the inverted signal relaxes back. If there is chemical exchange on the timescale of the relaxation process a reduction in intensity of the monitored signal will be observed.[8]

The magnetisation transfer technique is most useful in the region where transfer rates are similar to or somewhat slower than the relaxation rates, *i.e.* in the slow exchange limit just before measurable line broadening begins. Although magnetisation transfer can be used in regions where there are dynamic line broadening effects, the extra acquisition time and data processing required make the method inferior to line broadening measurements in these regions. Thus, in measuring the rates of monensin mediated Na^+ transport we preferred to use line broadening although we confirmed the nature of the process by means of a supplementary magnetisation transfer experiment.[3]

Table 1. Rate constants (k) for transport of Li^+ as a function of the monensin : PC ratio in the lipid bilayer.[a]

$10^3 \times$ Monensin : PC	k (in → out) /s^{-1}
0.0000	0.00
1.1604	0.46
2.3208	0.98
2.3208	0.86
3.4813	1.38
3.4813	1.42
4.6417	1.85

[a] *Conditions*: 75 mM Li^+; vesicles prepared from 27.01×10^{-6} mol egg yolk PC at 313 K; spectra were run at 303 K on a Bruker WP80 spectrometer at 31.14 MHz. Monensin : PC ratios vary between 1 : 862 and 1 : 215.

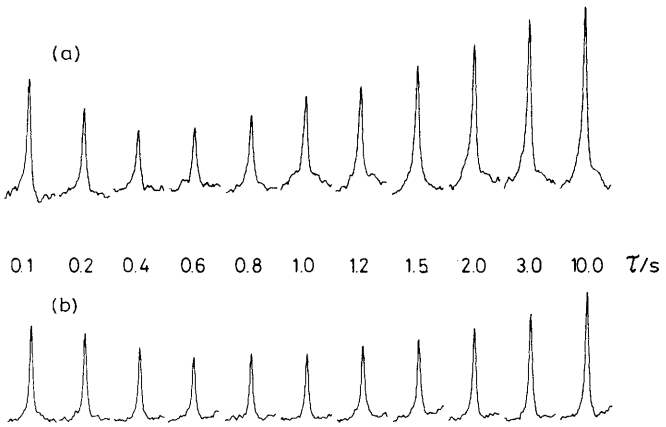

Figure 1. Typical variation of the intensity of the Li^+ (in) peak *vs.* delay time (τ) for 75 mM Li^+ and monensin: PC ratio (a) 1 : 215 (k 1.85 s^{-1}); (b) 1 : 431 (k 0.98 s^{-1}). The reduction in the signal intensity arises from chemical transfer of inverted magnetisation from the Li^+ (out) signal. The recovery arises from normal relaxation processes. The transport rate is deduced from the variation of signal intensity with time.

An ideal model system on which to demonstrate the usefulness of magnetisation transfer in studies of ion transport through membranes is the monensin mediated transport of Li^+ ions. Although ^7Li is a quadrupolar nucleus its relaxation times in aqueous solution are sufficiently long for this technique. Furthermore, the rate of exchange mediated by monensin is insufficient to generate substantial amounts of dynamic line broadening. Studies of Li^+ transport through membranes are of great importance because of the widespread biochemical effects of lithium and in particular the use of Li^+ in the treatment of manic depressive illness.[9]

Vesicles were prepared by the dialytic detergent removal technique.[3,4] Typically, egg yolk phosphatidyl choline (PC; 27 μmol) was dissolved in aqueous LiCl solution (1.5 ml) containing n-octyl glucopyranoside (*ca.* 405 μmol). Three dialyses against aqueous LiCl produced a suspension of large detergent-free unilamellar vesicles with the same concentration of Li^+ inside and out. The internal volume was *ca.* 11.5% of the total volume. A final dialysis introduced a small amount (0.5—5 mM) of lithium linear tripolyphosphate (LiPPPi) into the external medium. The Li^+ : PPPi ratio in the external medium was 25 : 1 with the ionic balance made up by chloride ions.

Sufficient $DyCl_3$ (typically, 1—8 μl, 80 mM) was then added to the vesicle suspension to generate a *ca.* 25 Hz chemical shift difference (d Hz). The pulse sequence 90°–t_1–90°–τ–90°–FID was then employed with the transmitter set d Hz to high frequency of the Li^+ (in) signal and $2d$ Hz to high frequency of the Li^+ (out) signal and with $t_1 = 1/(2d)$. The first two pulses of this sequence specifically invert the Li^+ (out) magnetisation. The variable delay then allows chemical exchange and relaxation to occur. This simple three pulse sequence can be used for a two site system in preference to the DANTE sequence[10] which is required where there is a larger number of sites.

The relaxation times (T_1) at each site were determined separately before addition of any ionophore and were *ca.* 12 s (in) and 0.7 s (out). The Li^+ (out) signal was relaxed by a quadrupolar interaction with the shift reagent. The amount of shift reagent used was consistent with generating a sufficiently large chemical shift difference whilst retaining a sufficiently long relaxation time for the Li^+ (out) signal.

The equations describing the behaviour of two exchanging sites in a magnetisation transfer experiment have been derived by Morris and Freeman.[10] These equations were used in a least-squares program that allowed best fit values for the exchange rate to be calculated. Exchange rates varied between 0.2 and 5.0 s^{-1}. These values are an order of magnitude lower than those measured for Na^+ transport by dynamic line broadening techniques. Typical intensity *vs.* time measurements for the Li^+ (in) peak are shown in Figure 1.

As in the case of Na^+, the rate of exchange is observed to be first order in monensin suggesting that one monensin molecule transports one lithium ion (Table 1). This was substantiated by a Li^+ ion concentration dependence study.[11] The derived rate constants for the formation and dissociation of the Li^+–monensin complex are considerably smaller than those for the Na^+ case, although the stability constant for the complex in the membrane is only slightly lower.[11]

We thank the S.E.R.C. for support of this work.

Received, 16th July, 1986; Com. 1034

References

1 For a review, see J. Grandjean and P. Laszlo, *Life Sci. Adv., Ser. D*, in the press; J. A. Balschi, V. P. Cirillo, and C. S. Springer, Jr., *Biophys. J.*, 1982, **38**, 323; T. Ogino, J. A. Den Hollander, and R. G. Shulman, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 5185; R. K. Gupta, P. Gupta, and R. D. Moore, *Ann. Rev. Biophys. Bioeng.*, 1984, **13**, 221; E. Fernandez, J. Grandjean, and P. Laszlo, *Eur. J. Biochem.*, 1987, **167**, 353.
2 H. Degani and G. A. Elgavish, *FEBS Lett.*, 1978, **90**, 357.
3 F. G. Riddell and M. K. Hayer, *Biochim. Biophys. Acta*, 1985, **817**, 313.
4 F. G. Riddell, S. Arumugam, P. J. Brophy, B. G. Cox, M. C. H. Payne, and T. E. Southon, *J. Am. Chem. Soc.*, in the press.
5 M. M. Pike, S. R. Simon, J. A. Balschi, and C. S. Springer, Jr., *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 810.
6 M. K. Hayer and F. G. Riddell, *Inorg. Chim. Acta*, 1984, **92**, L37.
7 F. G. Riddell and T. E. Southon, *Inorg. Chim. Acta*, 1987, **136**, 133.
8 For a more detailed discussion, see J. Sandstrom 'Dynamic N.M.R. Sepctroscopy,' Academic Press, London, 1982, ch. 4.
9 N. J. Birch, 'Metal Ions in Biological Systems,' Vol. 14, ed. H. Sigel, Marcel Dekker, New York, 1982, 257.
10 G. A. Morris and R. Greeman, *J. Magn. Reson.*, 1978, **29**, 433.
11 F. G. Riddell, S. Arumugam, and B. G. Cox, unpublished results.