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THE KINETICS OF IONOPHORE X-537A-MEDIATED TRANSPORT OF MANGANESE THROUGH DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES

A ^{1}H-NMR STUDY

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We have studied the kinetics of ionophore X-537A-mediated transport of manganese ions into small unilamellar vesicles formed from dipalmitoylphosphatidylcholine. To follow the transport we used the paramagnetic effect of manganese on the ^{1}H-NMR signal from choline trimethylammonium groups on the inner phospholipid monolayer. The transport of only one manganese ion produces an intravesicular concentration which is high enough (approx. 1 mM) to substantially broaden this signal. The observed signal thus arises predominantly from those vesicles which contain no manganese. Therefore, as manganese is transported into the vesicles the observed signal decreases in intensity, but does not broaden. The initial time-dependence of the intensity of the signal, $S(t)$, can be approximated by the simple first-order rate law: $S(t) = S(0)\exp(-k't)$, where k' is the probability per unit time for the transport of a manganese ion from the external medium to the intravesicular space. From the dependence of k' on the ionophore X-537A concentration we conclude that manganese is transported into the vesicles via both 1 : 1 and 2 : 1 complexes with ionophore X-537A. At low ratios of ionophore X-537A to vesicles transport via the 1 : 1 complex predominates; at high ratios transport via the 2 : 1 complex predominates. From the dependence of k' on manganese concentration we determined that under our conditions the equilibration of ionophore X-537A between vesicles is much faster than the transport of manganese through the vesicles. Lastly, from the dependence of k' on temperature, we conclude that the ionophore X-537A-mediated transport of manganese into the dipalmitoylphosphatidylcholine vesicles is very sensitive to the gel-liquid crystalline phase transition.

Introduction

The carboxylate ionophore X-537A (lasalocid A) has been shown to mediate ion transport across biological and artificial membranes [1–4]. While the interaction of X-537A with metal ions and amines has been extensively studied in homogeneous solutions [6–8] only few such investigations have been performed in membrane systems [2–4] and the mechanism of ion transport mediated by X-537A is still not fully characterized.

NMR spectroscopy can be employed to follow the transport of paramagnetic ions into unilamellar phospholipid vesicles [3,4]. Specifically, the transport of manganese into phosphatidylcholine (PC) vesicles can be measured by following the disappearance of the choline trimethylammonium signal from phosphatidylcholine molecules at the inner interface. Because the NMR technique detects the transport of a single Mn^{2+} ion, the kinetics of Mn^{2+} transport can be characterized without the complications associated with the back diffusion of the ionophore. Also, unlike the conductance measurements in black lipid membranes, which are sensitive only to charged species, this tech-

nique can follow all the transported species independent of their charge.

In this report we have extended our previous studies of X-537A-mediated manganese transport to unilamellar vesicles formed from pure dipalmitoylphosphatidylcholine and have clarified some ambiguities in the interpretation of the NMR data.

Materials and Methods

Synthetic L-α-dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma Chemical Co. (St. Louis, MO) and was used without further purification. Ionophore X537A (Lasalocid A) was generously supplied by Hoffman-LaRoche (Nutley, NJ). Deuterium oxide (99.98%) (Stohler, Rutherford, NJ) was used without further purification. All other chemicals were analytical reagent grade or equivalent.

Sonicated DPPC vesicles were prepared from a 50 mg/ml suspension in 10 mM tris(hydroxymethyl)-aminomethane chloride (Tris chloride) that was deoxygenated by bubbling argon or nitrogen through the solution. The suspension was sonicated for 12 min with a Branson W-350 sonifier. The sonifier was used at power level 5 in the pulsed mode (40% fractional power) and the sonicator chamber was maintained at 50°C throughout the sonification. Sonicated suspensions were centrifuged in a Spinco 50 Ti rotor for 30 min at 45 000 rev./min at 45°C. The zone containing the clear supernatant was removed and kept at 50°C. The final pH of the sample was adjusted to 6.8 ± 0.1.

DPPC concentrations were determined by measuring the phosphate concentration of ashed aliquots of the lipid sample. Although all DPPC vesicles were prepared from 50 mg/ml unsonicated suspensions, the final DPPC concentration varied up to ±25% among different preparations. The ratio of ionophore X-537A to vesicles was calculated using the apparent partial specific volume of 0.984 ml/g [8].

A methanolic solution of X-537A (in the acid form) was prepared prior to each experiment and the concentration was determined by the absorbance at 310 nm ($\epsilon_{310} = 4.1 \cdot 10^3\ mM^{-1} \cdot cm^{-1}$). Ionophore addition was performed by evaporation of a known amount of the stock solution, followed by addition of the vesicle suspension. The solution was mixed for several minutes to ensure equilibration of the ionophore with the membranes [4]. Transport of manganese through the vesicle membranes was initiated by the addition of manganese to the vesicle suspensions containing ionophore X-537A.

NMR measurements were performed either on a Bruker WH-360 spectrometer or on a Bruker WH-270 spectrometer. Both spectrometers were equipped with variable temperature accessories which maintained the temperature to within ±1°C.

Fluorescence measurements were carried out on a Perkin-Elmer MPF-44A spectrometer with a micro cell of 2 mm path length. The excitation wave length was 310 nm and the emission wave length was 420 nm. Both slits gave a bandwidth of 5 nm. The fluorescence intensity was calibrated for zero emission with a suspension of vesicles without ionophore X-537A and with $MnCl_2$ in order to eliminate the scattering by the vesicles. $MnCl_2$ was added separately to each sample and was equilibrated for at least 0.5 h before the actual measurement. Egg phosphatidylcholine vesicles were prepared in the same manner as DPPC vesicles except that both sonication and centrifugation were performed at 4°C. In these experiments the ionophore X-537A concentration was 100 μM and $p^2H = 6.8$.

Analysis of data

We assume that the diffusion of manganese through the vesicle can be described by the equation

$$\frac{d\bar{C}_i}{dt} = k_1 C_o - k_{-1} \bar{C}_i \qquad (1)$$

where C_o is the manganese concentration in the extravesicular medium and $\bar{C}_i$ is the average concentration of manganese inside the vesicles. We also assume $k_1 = k_{-1}$, and that C_o is constant. The appropriate boundary conditions are: at $t = 0$, $\bar{C}_i = 0$; at $t = \infty$, $\bar{C}_i = C_o$. The solution to Eqn. 1 which satisfies these boundary conditions is

$$\bar{C}_i(t) = C_o(1 - e^{-kt}) \qquad (2)$$

where $k = k_1 = k_{-1}$.

The transport of a paramagnetic manganese ion into the interior of a vesicle will broaden the NMR signal arising from the polar head groups of the 'inner' phospholipid molecules. With an internal

radius of approx. 74 Å [9], the transport of a single manganese ion produces an internal concentration of approx. 1.0 mM. This concentration is high enough to substantially broaden the ^{1}H-NMR signal from the 'inner' choline trimethylammonium groups. The observed ^{1}H-NMR signal thus arises predominantly from those vesicles that have no manganese ions in the intravesicular space. As manganese ions are transported into the vesicles, the observed signal decreases in intensity, but does not broaden.

The observed intensity of the ^{1}H-NMR signal from the 'inner' choline trimethylammonium groups, $S(t)$, is thus proportional to the probability that a particular vesicle contains no manganese ions, $P(0, t)$. From Appendix I, $P(0, t)$ is given by the following equation:

$$P(0, t) = \exp(-\bar{n}(t)) \tag{3}$$

where $\bar{n}(t)$ is the average number of manganese ion per vesicle at time t. $\bar{n}(t)$ is given by the expression

$$\bar{n}(t) = v\bar{C}_i(t)N_A$$

where N_A is Avagadros number, v is the intravesicular volume and $\bar{C}_i(t)$ is the average manganese concentration in the intravesicular space at time t. $\bar{C}_i(t)$ is given by Eqn. 2. $S(t)$ is thus given by the expression

$$S(t) = S(0) \exp\{-vC_oN_A(1 - e^{-kt})\} \tag{4}$$

where $S(0)$ is the signal intensity at $t = 0$.

The analysis of the data using Eqn. 4 may be considerably simplified by noting that in the region where $kt \ll 1$ the equation simplifies to

$$S(t) = S(0) \exp\{-vC_oN_Akt\} \tag{5}$$

Eqn. 5 may then be used to analyse the data for the initial time period. The validity of Eqn. 5 for the analysis of the data may be confirmed by the linearity of a semilogarithmic plot of the intensity of the NMR signal as a function of time.

Using Eqn. 5, the slope of the semilogarithmic plot of the height of the 'inner' choline signal versus time gives the product vC_oN_Ak, and not simply the intrinsic rate constant, k. We define an observed rate constant, k', where

$$k' = vC_oN_Ak \tag{6}$$

Eqn. 5 may then be rewritten as

$$S(t) = S(0) \exp(-k't) \tag{7}$$

The observed rate constant, k', is simply the probability per unit time for the transport of a manganese ion from the extravesicular to the intravesicular space. The intrinsic rate constant, k, can be easily calculated from k' by using Eqn. 6, the known vesicular volume, v, and the initial external manganese concentration, C_o. In most of the experimental data presented here (i.e., Figs. 3 and 5) the external manganese concentration was 1 mM. With the assumed internal vesicle radius of 74 Å [9], the product vC_oN_A is calculated to be approx. 1.0. For this data, k and k' will be essentially identical.

Results

Typical ^{1}H-NMR signals from the trimethylammonium groups on the DPPC molecules on the outer and inner monolayers of the bilayer membranes are partially resolved under these experimental conditions, as previously noticed [10]. The addition of 1 mM manganous chloride to the external medium broadens the ^{1}H-NMR signal from the 'outer' choline trimethylammonium groups by at least an order of magnitude (see trace B, Fig. 1), leaving essentially only the signal from the 'inner' choline trimethylammonium groups. The apparent outside/inside ratio is approx. 2.0, in agreement with previous results [11]. In the absence of ionophore X-537A the signal from the 'inner' choline trimethylammonium groups is unaffected up to 24 h after the manganese addition to the external medium. This indicates that in the absence of X-537A the DPPC vesicles are impermeable to manganese. However, in the presence of ionophore X-537A, the intensity of the ^{1}H-NMR signal from the 'inner' choline trimethylammonium groups slowly decreases after the addition of manganese to the external medium (see Fig. 2). When the narrow signal from the 'inner' choline trimethylammonium groups has almost disappeared, the broad signal from the 'outer' choline trimethylammonium groups can be observed (last spectra, Fig. 2). This broad signal also includes the signal for 'inner' choline trimethyl

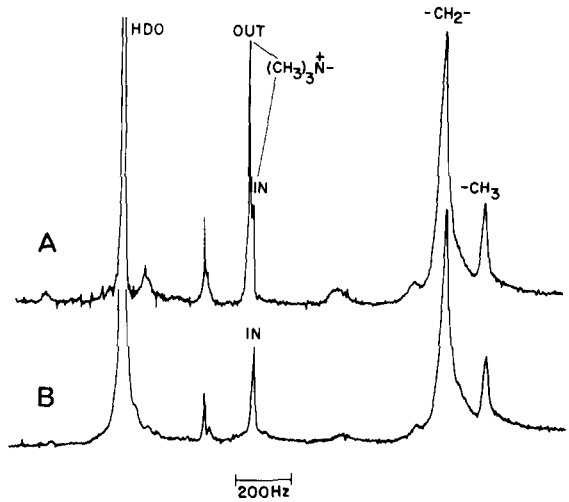

Fig. 1. 360 MHz ^{1}H-NMR spectra of sonicated DPPC vesicles (50 mg/ml) at 48°C. Vesicles were prepared in ^2H_2O containing 6.7 mM Tris chloride, $p^2H = 6.8$. Each spectrum is the Fourier transform of one transient. The assignments are [17]: CH_3-, terminal methyl group of hydrocarbon chains; -CH_2-, methylene groups of hydrocarbon chains; $(CH_3)_3N^+$-choline trimethylammonium groups; HDO, residual water in solvent. The spectrum shown in trace B is taken after the addition of 1 mM manganese chloride to the external medium. All other conditions are the same as for the spectrum shown in trace A. 'IN' and 'OUT' refer to the choline trimethylammonium groups on the inside and outside faces of the bilayer vesicles, respectively.

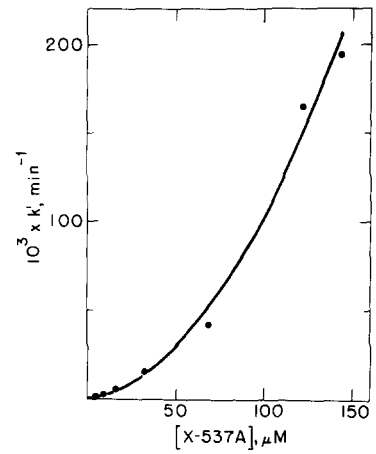

Fig. 3. X-537A dependence of the probability per unit time for manganese transport, k', in DPPC vesicles at 44°C. The DPPC concentrations was $6 \cdot 10^{-2}$ M. Other conditions were the same as in Fig. 1B.

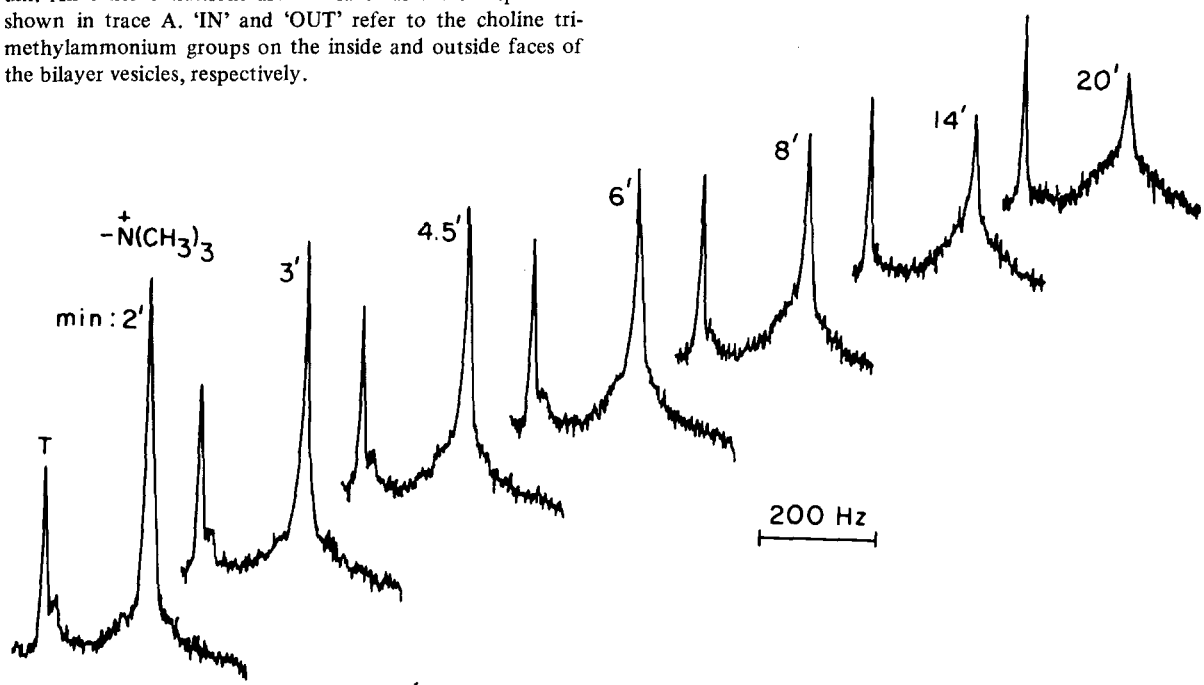

Fig. 2. Time dependence of the 360 MHz ^{1}H-NMR signal from the inner choline trimethylammonium groups of sonicated DPPC vesicles after the addition of 1 mM manganese chloride to the external medium. Conditions are the same as for Fig. 1B, except for the presence of 71 μM X-537A. The peak labelled 'T' is assigned to Tris. The numbers shown on the left of the choline trimethylammonium peak are the times (in minutes) after the addition of manganese chloride to the solution.

groups of vesicles that contain at least one manganese ion. The semilogarithmic plots of the height of the narrow 'inner' choline trimethylammonium NMR signal were found to be linear down to at least 50% of the initial height, and this part of the data was analyzed according to Eqn. 7 (see Analysis of data) to calculate the apparent rate constant, k'.

The dependence of k' on the X-537A concentration is shown in Fig. 3. The data could not be simulated by either a linear or a quadratic function, but could be fit to a theoretical curve of the form

$$k' = \alpha[X] + \beta[X]^2 \quad (8)$$

where $[X]$ is the X-537A concentration. The best fit to the data is given by the values

$\alpha = 2.1 \cdot 10^{-4}\ \mu M^{-1} \cdot min^{-1}$ and $\beta = 9 \cdot 10^{-6}\ \mu M^{-2} \cdot min^{-1}$

and is shown by the solid line in Fig. 3. In these experiments the vesicle concentration was calculated to be 23 ± 1 μM, assuming 3 000 phospholipid molecules per vesicle. The ratio of X-537A to vesicles ranged from much less than one to much greater than one (see Table I).

In the data given in Fig. 3 the manganese concentration was kept constant at 2 mM. Fig. 4 shows the effect of varying the manganese concentration for two samples that had a ratio of X-537A to vesicles of much less than on (0.32) and much greater than one (3.9), respectively. For the low ratio of ionophore X-537A to vesicles the dependence of k' on the manganese concentration is linear over the concentration range studied (1 to 10 mM). For the sample with the high ratio of X-537A to vesicles the dependence of k' on the manganese is apparently not linear. However, as discussed below, the fluorescence results indicate that the apparent curvature in Fig. 4B cannot be due to saturation of the X-537A molecules with manganese. The curvature probably results from inaccuracies in the measurement of k' for the very fast transport rates.

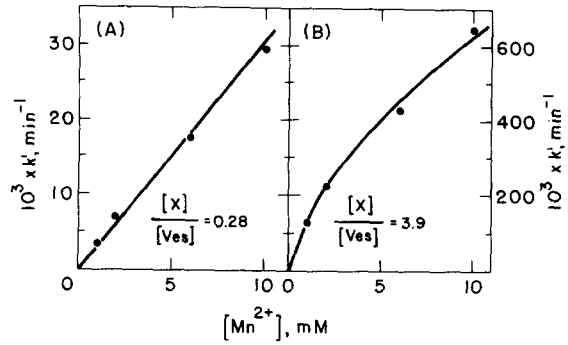

Fig. 4. Manganese dependence of the probability per unit time for manganese transport, k', at 44°C. The DPPC concentration was $4.8 \cdot 10^{-2}$ M. The X-537A concentration was 4.8 μM in A and 67 μM in B. Other conditions were the same as in Fig. 1B.

TABLE I

X-537A DEPENDENCE OF THE PROBABILITY PER UNIT TIME FOR MANGANESE TRANSPORT, k', IN SONICATED DPPC VESICLES AT 44°C

The last two columns are the values of k' calculated from Eqn. 8 assuming $\alpha = 2.1 \cdot 10^{-4}\ \mu M^{-1} \cdot min^{-1}$ and $\beta = 9.0 \cdot 10^{-6}\ \mu M^{-2} \cdot min^{-1}$. [X] is the X-537A concentration.

X-537A (μM)	X-537A/vesicle	k' (s^{-1}) ($\times 10^3$)	α [X] (s^{-1}) ($\times 10^3$)	β $[X]^2$ (s^{-1}) ($\times 10^3$)
4.4	0.15	1.1	0.92	0.17
8.6	0.29	2.6	1.81	0.66
15.9	0.53	5.7	3.33	2.26
32.1	1.1	15.6	6.74	9.27
68.5	2.3	42.1	14.4	42.2
121	4.0	165	25.4	131
143	4.8	195	30.0	184

The observed fluorescence of a solution of sonicated egg phosphatidylcholine vesicles in the presence of X-537A is tabulated as a function of manganese concentration in Table II. Assuming that the quantum yield of free X-537A is much larger than the quantum yield of X-537A complexed to manganese [6], the decrement in the observed fluorescence may be attributed to the formation of X-537A complexes. At the low manganese concentration (up to 100 mM) the observed decrement in fluorescence is proportional to the manganese concentration. Extrapolating this linear region to low manganese concentrations, we calculate that at 1 mM manganese approx. 0.2% of the total X-537A is bound to manganese.

Fig. 5 shows the temperature dependence of k' in

TABLE II

FLUORESCENCE INTENSITY OF X-537A IN DISPERSIONS OF EGG PHOSPHATIDYLCHOLINE VESICLES CONTAINING VARYING AMOUNTS OF $MnCl_2$ (38.5°C)

$MnCl_2$ (mM)	Fluorescence intensity (arbitrary units)
0	50.0
7	49.0
50	44.5
100	40.0
250	33.0
500	30.5
1 000	21.0

the temperature range from 33 to 55°C. Between 35 and 39°C, which is the region of the gel-liquid crystalline phase transition for sonicated DPPC vesicles [13, 14], k' increased by approximately two orders of magnitude. Below 35°C the activation energy is approx. 50 kcal/mol, whereas above 39°C it is approx. 9 kcal/mol.

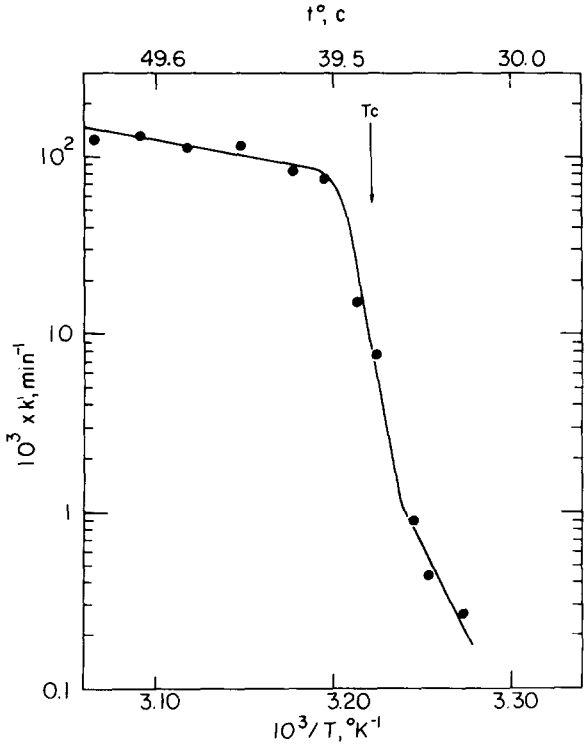

Fig. 5. Temperature dependence of the probability per unit time for manganese transport, k', in sonicated DPPC vesicles. The X-537A concentration was 71 μM. Other conditions were the same as in Fig. 1B.

Discussion

The presence of one manganese ion in the intravesicular space is enough to substantially broaden the ^{1}H-NMR signal from the choline trimethylammonium groups on the inner surface of phosphatidylcholine vesicles. This 'all-or-nothing' effect means that the observed NMR signal from the 'inner' trimethylammonium groups arises only from those vesicles that have no manganese in the intravesicular space. This explains the apparent paradox that, in the presence of ionophore X-537A and manganese in the external medium, the ^{1}H-NMR for the 'inner' trimethylammonium groups does not broaden as a function of time. Instead, the observed intensity of the signal decreases as a function of time. It also explains the previous finding that, if phosphatidylcholine vesicles are sonicated in the presence of increasing manganese concentrations, the ^{1}H-NMR signal from the 'inner' choline trimethylammonium groups also does not broaden as a function of manganese concentration. Instead the observed intensity of the signal decreases as a function of manganese concentration [4].

Under our conditions the time dependence of the observed intensity of the ^{1}H-NMR signal for the 'inner' choline trimethylammonium groups may be analytically described by Eqn. 7, where the observed first-order rate constant for transport through the membrane, k', is directly related to the intrinsic first-order rate constant, k, by Eqn. 6. The X-537A dependence of k' (see Fig. 3) contains both a linear and a quadratic term. At low X-537A/vesicle ratios the linear term dominates k' while at high X-537A/vesicle ratios the quadratic derm dominates k'. The simplest interpretation is that at high X-537A/vesicle ratios manganese transport occurs predominantly via the X-537A dimer complex, but at low X-537A/vesicle ratios occur predominantly via the X-537A monomer complex.

At the high X-537A/vesicle ratios k', may be approximately written as

$$k' \simeq \frac{N_2}{\tau_2}$$

where N_2 is the number of $(X\text{-}537A)_2$ manganese

complexes per vesicle and τ_2 is the intrinsic lifetime for the transport of manganese through the membrane via this complex. From the fluorescence titrations (see Fig. 5) we can estimate that, for egg phosphatidylcholine in the presence of 1 mM manganese and high X-537A/vesicle ratios, approx. 0.2% of the X-537A molecules would be complexed to manganese. Assuming that the same is true for DPPC, we can estimate an upper limit on the value of τ_2 of approx. 3 s (at 44°C) from the data in the last two rows of Table I.

At the low X-537A/vesicle ratios, most of the vesicles will not contain an X-537A molecule. k' will then be a function of the lifetime for transport through the membrane, τ_t, and the lifetime of X-537A on a vesicle, τ_m. From Appendix II, k' is given by the equation

$$k' = \frac{\beta}{\tau_m + \tau_t}$$

where β is the ratio of ionophore X-537A to vesicles. If $\tau_m \ll \tau_t$, then $k' \sim \beta/\tau_t$, and k' is determined by the effective lifetime for the transport of the complex through the membrane. If $\tau_t \ll \tau_m$, then $k' \sim \beta/\tau_m$, and k' is determined by the lifetime of the X-537A molecule on the vesicle. These two cases may be distinguished by the manganese dependence of k'. τ_m would be expected to be independent of the manganese concentration while $1/\tau_t$ would be expected to be directly proportional to the manganese concentration (if only a small fraction of X-537A were complexed to manganese). The observed result (i.e., Fig. 4A) shows that k' is directly proportional to the manganese concentration. We conclude that $\tau_m \ll \tau_t$ and that the rate-determining step under these conditions is the transport of the complex through the membrane. This result is consistent with the observation that, at low X-537A/vesicle ratios, all of the 'inner' choline trimethylammonium groups decrease in intensity at the same rate on the addition of manganese (data not shown). These results also imply that only a small fraction of the X-537A is complexed to manganese under these conditions, which is similar to the results of the fluorescence experiments at high X-X-537A/vesicle ratios.

At the high X-537A/vesicle ratios the rate determining step for the transport process could be the formation of the X-537A-manganese complex or the rate of transport of the complex through the membrane. Some evidence for the latter case comes from the fact that k' changes by approximately two orders of magnitude in the region of the gel to liquid-crystalline phase transition (see Fig. 6). However, this effect could also be due to a large decrease in the partition coefficient below the phase transition.

Appendix I

The probability distribution for the number of manganese ions inside a vesicle

We define V as the total number of vesicles in the sample and N as the total number of manganese ions inside the vesicles. We also define $\bar{n}$ as the average number of manganese ions per vesicle, which is then simply given as

$$\bar{n} = N/V$$

The problem of calculating the statistical distribution of manganese ions per vesicle is analogous to the familiar problem of throwing N marbles into V boxes, and asking for the distribution of marbles in the boxes. This distribution is the familiar binomial distribution [14] and the probability of finding n paramagnetic cations inside a vesicle, $P(n)$, is given by the equation

$$P(n) = \binom{N}{n}\left(\frac{1}{V}\right)^n \left(1 - \frac{1}{V}\right)^{N-n} \tag{9}$$

where $\binom{N}{n}$ is the binominal coefficient, defined as

$$\binom{N}{n} = n!\, N!/(N-n)!$$

Under our experimental conditions $N \sim V \sim 10^{16}$, so we can assume that $N \to \infty$ and $V \to \infty$ with the condition

$$\lim_{\substack{N\to\infty \\ V\to\infty}} (N/V) = \bar{n}$$

Under these conditions the binomial distribution may be very accurately approximated by the Poisson distribution [15]

$$P(n) = \frac{e^{-\bar{n}}\,\bar{n}^{-n}}{n!} \tag{10}$$

If $\bar{n}$ is a function of time, then $P(n)$ is still given by the same expression (Eqn. 10), although $\bar{n}$ will now be replaced by $\bar{n}(t)$. In the experiments presented in this manuscripts, $\bar{n}(t)$ is given by the expression

$$\bar{n}(t) = N_A v \bar{C}_i(t)$$

where N_A is Avogadro's number, $\bar{C}_i(t)$ is the average concentration of manganese in the vesicles, and v is the volume per vesicle. The time dependence of $\bar{C}(t)$ is given by Eqn. 2. To analyse the time dependence of the probability that the vesicles have no manganese ion in the intravesicular space, we use Eqn. 10 with the special case that $n = 0$, i.e.,

$$P(0, t) = e^{-\bar{n}(t)} \tag{11}$$

We note that Eqn. 11 take into account not only those vesicles that have not had any manganese ions transported into the intravesicular space, but also those vesicles that have had one (or more) manganese ions transported in, but subsequently transported back out again by time t.

Appendix II

Calculation of the probability per unit time for transport of manganese for a low ratio of X-537A to vesicles

We assume that the ratio of X-537A to vesicles is very small and ignore the very small population of vesicles that contain two or more X-537A molecules. We define τ_m as the lifetime of X-537A on the vesicle and τ_t as the lifetime for the transport of manganese through the vesicle via the X-537A complex. For those vesicles that contain one X-537A molecule, p is defined as the probability that manganese will be transported into the vesicle before the X-537A dissociates from the vesicle. p is then given by the expression

$$p = \frac{1/\tau_T}{1/\tau_T + 1/\tau_M} \tag{16}$$

We defined $\pi_n(t)$ as the probability that a molecule of X-537A has 'jumped' between n vesicles in a time t. $\pi_n(t)$ is given by the Poisson distribution [16]

$$\pi_n(t) = \frac{e^{-t/\tau_M}(t/\tau_M)^n}{n!} \tag{17}$$

Since the ratio of X-537A to vesicles is much less than one, we consider only those vesicles that initially did not contain an X-537A molecule. The observed transport of manganese via the X-537A complex will then require that an X-537A molecule dissociate and 'jump' to a new vesicle and that this new vesicle transports manganese through the membrane. $N(t)$ is defined as the number of manganese ions that have been transported at time, t. $N(t)$ is given by the expression

$$N(t) = r \sum_{n=1}^{\infty} np\pi_n(t) \tag{18}$$

$$= rpe^{-t/\tau_M} \sum_{n=1}^{\infty} \left(\frac{n(t/\tau_M)^n}{n!} \right)$$

which, after a few straightforward algebraic steps, gives

$$N(t) = rpt/\tau_m \tag{19}$$

where r is the number of vesicles that have one X-537A molecule. Dividing both sides on Eqn. 19 by V, we obtain

$$\bar{n}(t) = \frac{N(t)}{V} = \beta pt/\tau_M \tag{20}$$

where $\bar{n}(t)$ is the average number of manganese ions per vesicle (as a function of time), and β is defined as the ratio of X-537A to vesicles. The probability per unit time for the transport of manganese through the vesicle, k', is given by the expression

$$k' = \frac{d\bar{n}(t)}{dt} = \beta p \; 1/\tau_M = \frac{\beta}{\tau_M + \tau_T} \tag{21}$$

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