Biochimica et Biophysica Acta, 1106 (1992) 171–177

BBAMEM 75601

Monensin-mediated transports of H^+, Na^+, K^+ and Li^+ ions across vesicular membranes: T-jump studies

B.S. Prabhananda and Mamata H. Kombrabail

Chemical Physics Group, Tata Institute of Fundamental Research, Bombay (India)

(Received 10 December 1991)

Key words: Monensin; Liposome; Membrane; Ion transport; Temperature jump; Proton transport

Theoretical expression for the rate of decay of ΔpH across vesicular membrane due to carrier-mediated ion transports, $1/\tau$, has been modified taking note of carrier states (such as mon$^-$ and mon-H-M^+) for which the translocation rate constants in the membrane are small. The rates of ΔpH decay due to monensin-mediated H^+ and M^+ transports ($M^+ = Na^+, K^+, Li^+$) observed in our experiments in the pH range 6–8, and [M^+] range 50–250 mM at 25°C have been analysed with the help of this expression. ΔpH across soyabean phospholipid vesicular membranes were created by temperature jump in our experiments. The following could be inferred from our studies. (a) At low pH (~ 6) $1/\tau$ in a medium of Na^+ is greater than that in a medium of K^+. In contrast with this, at higher pH (~ 7.5) $1/\tau$ is greater in a medium of K^+. Such contradictory observations could be understood with the help of our equation and the parameters determined in this work. The relative concentrations of the rate-limiting species (mon-H, mon-K, and mon-Li at Ph ~ 7 in vesicle solutions having Na^+, K^+ and Li^+, respectively) can explain such behaviours. (b) The proton dissociation constant K_H for mon-H in the lipid medium ($pK_H \sim 6.55$) is larger than the reported K_H in methanol. (c) The concentrations of mon$^-$ and mon-H-Na^+ are not negligible under the conditions of our experiments. The latter species cause a [Na^+]-dependent inhibition of ion transports. (d) The relative magnitudes of metal ion dissociation constants K_{HM} (~ 0.05 M) for mon-H-Na^+ and K_M (~ 0.03 M) for mon-Na suggest that the carboxyl group involved in the protonation may not be dominantly involved in the metal ion complexation. (e) The estimates of K_M (~ 0.03 M for Na^+, 0.5 M for K^+ and 2.2 M for Li^+) follow the ionophore selectivity order. (f) The rate constants k_1 and k_2 for the translocations of mon-H and mon-M ($M^+ = Na^+, K^+$ and Li^+) are similar in magnitude (~ $9 \cdot 10^3$ s^{-1}) and are higher than that for nig-H and nig-M (~ $6 \cdot 10^3$ s^{-1}) which can be expected from the relative molecular sizes of the ion carriers.

Introduction

Ion transport across membranes is of fundamental importance in several biological processes. Liposomes can be conveniently used as model membrane systems in the study of ion transport. In liposomes the passive transport of H^+ is accompanied by a compensating flux of charge (such as that due to alkali metal ions, M^+) [1,2]. Such a compensation is needed even in the electroneutral transport of ions so that there is no build up of an electric potential across the membrane. The electroneutral carriers nigericin and monensin are polyalcohol, polyether monocarboxylic acids and can carry both H^+ and M^+ across membranes [3]. In a recent paper we have discussed the mechanism of nigericin-mediated ion transports in detail [4]. Monensin-mediated Na^+ transport has been the subject of study by several groups [5–10]. In the simple mechanism of ion transport by monensin (Fig. 1), H^+ and M^+ are exchanged at the membrane/aqueous medium interfaces [10]. The motivations for the present studies on monensin-mediated H^+ transport and the associated M^+ (= Na^+, K^+ and Li^+) transports are the following: (A) The transport rate-limiting species at a given pH depends on the magnitudes of the apparent proton dissociation constant K_H for mon-H and the metal ion dissociation constant K_M of mon-M [4]. Therefore, to identify the rate limiting species at a given pH it is necessary to know which of the pK_H reported in the literature (~ 6.7 [11], ~ 7.9 [12] or ~ 10.2 [13]) is relevant in lipid membranes. (B) The intrinsic rate constants associated with the translocations of mon-H and mon-M ($M^+ = Na^+, K^+, Li^+$) across membranes (k_1 and k_2, respectively) have not

Correspondence: B.S. Prabhananda, Chemical Physics Group, Tata Institute of Fundamental Research, Bombay 400005, India.

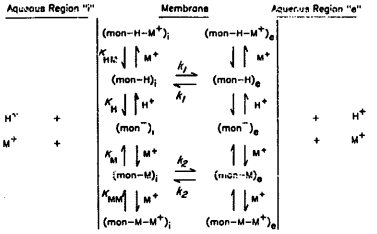

Fig. 1. Suggested mechanism for the monensin-mediated proton transport. Paths of fast equilibria such as mon-M + H$^+$ ⇌ mon-H-M$^+$ ⇌ mon-H + M$^+$ have not been explicitly indicated in the figure.

been determined in previous studies, since the transport data given in the literature are not adequate [5–10]. Also, the possibility that the monensin-mediated Na$^+$ transport rate may be pH dependent has not been recognised in NMR linewidth studies [5–7]. (C) Riddell and Hayer [6] confirmed the observations of Hamilton and Nilsen-Hamilton [9] that at sufficiently high [Na$^+$] the monensin-mediated Na$^+$ transport rate is reduced on increasing [Na$^+$]. A natural explanation for this observation can be found from the mechanism of transport itself (see below). But formation of species such as mon-M-M$^+$ and mon-H-M$^+$ (Fig. 1) could also cause 'inhibition'. We wanted to examine this possibility. (D) If the pK_H is as low as ~6.7 and K_M is not sufficiently small, [mon$^-$] will not be negligible. In the absence of electric fields, the rate constants associated with the transport of charged species such as mon$^-$ or mon-H-M$^+$ across membranes are negligible when compared to those of the neutral species mon-H and mon-M. We wanted to derive the modified equation for ΔpH decay when the concentrations of such species cannot be neglected. (E) The equation for the ΔpH decay derived by us predicts contra-intuitive behaviours: For example, in a vesicle solution containing a mixture of Na$^+$ and K$^+$, on increasing the proportion of [K$^+$], the H$^+$ transport rate increases for small [K$^+$] but decreases for large [K$^+$]. We wanted to see whether such behaviours could be seen in the pH range 6–8 of biological interest.

In the recent past, ionophore mediated fast ion transports across vesicular membranes have been studied using fast reaction techniques such as relaxation studies in NMR [5–7], stopped-flow [14,15] and temperature jump (T-jump) [4,8,16,17]. The reasons for our choice of soyabean phospholipid vesicles (SBPL) to elucidate the mechanism of ion transports and the choice of T-jump to create a proton gradient ΔpH across vesicular membrane are discussed elsewhere [4,8]. (The ΔpH decay is monitored to infer the transport rates of H$^+$ and M$^+$).

Theory

The transport of H$^+$ across a membrane can be treated as a chemical reaction in which a $(H^+)_{in}$ is converted into $(H^+)_{out}$ with an apparent rate constant k_{+H} or vice-versa. When the deviations of the concentrations from the equilibrium values are small, we can adopt the procedures commonly used in 'chemical relaxation' studies [18–20] and write an expression for the ΔpH decay rate, τ^{-1}, if the H$^+$ transport is driven by the H$^+$ concentration gradient (see Eqn. A-18 of Ref. 4).

$$\tau^{-1} = (\ln 10)[1 + b_i V_i/(b_e V_e)]k_{+H}[H^+]_i/b_i \qquad (1)$$

V_i and V_e are the aqueous volumes inside and outside the vesicles. b_i and b_e are the internal and external buffer capacities and have contributions from buffer species in fast proton exchange equilibrium (compared to τ^{-1}) with the aqueous medium inside and outside. They include endogenous contributions from the buffer groups in the inner and outer layers of the vesicular membrane, respectively [4,15]. For SBPL vesicles the internal buffer capacity b_i can be given as follows.

$$b_i = (\ln 10)\left\{\sum_{j=1}^{3} C_j\left[K_j[H^+]_i/(K_j + [H^+]_i)^2\right]\right\} \qquad (2)$$

where C_1 and K_1 are the concentration and acid dissociation constant of the buffer entrapped inside vesicles. The contribution to b_i from the endogeneous buffer group of concentration $[A_j]$ in vesicle solutions, is $[A_j]V_i/(V_i + V_e)V_i$. For 1 mg lipid/ml in SBPL vesicle solutions $V_e/V_i = 2250$ [4]. Making use of Table I of Ref. 4 we get $C_2 = 30$ mM, $K_2 = 10^{-6.9}$ and $C_3 = 45$ mM, $K_3 = 10^{-7.8}$. In our experiments (and in the derivation by Grzesik and Dencher [15]) $V_i b_i/V_e b_e \ll 1$ and hence can be neglected in Eqn. 1.

For the simple mechanism of monensin-mediated H$^+$ transport (Fig. 1), when the translocations of mon-H and mon-M across the membrane are rate limiting and when [M$^+$] is sufficiently large such that the terms involving $\Delta[M^+]$ can be ignored, we can write the rate equations following the procedure given in our earlier paper [4].

$$-d(\Delta[H^+]_i)/dt = a_{11}\Delta[H^+]_i + a_{12}\Delta[mon]_{in}$$
$$-d(\Delta[mon]_{in})/dt = a_{21}\Delta[H^+]_i + a_{22}\Delta[mon]_{in} \qquad (3)$$

where the subscript 'i' refers to the region inside vesicles and the inner layer. $[mon]_{in}$ is the total monensin concentration in the inner layer of the mem-

TABLE I

Parameters which give calculated τ (with the help of Eqns. 1, 2 and 8) in good agreement with those observed at 25°C (Figs. 2 and 3) [a]

[MCl] (μM)	Metal ion M^+	pK_H	$1/K_{HM}$ (M^{-1})	pK_c^*	k_1 ($=k_2$) ($10^3\ s^{-1}$)
50	Na^+	6.55	20	6.35	11.5
			(0)	(6.35)	(11.5)
50	K^+	6.55	~2	7.55	9
50	Li^+	6.55	~0	8.25	9
250	Na^+	6.55	20	5.95	9
			(0)	(6.45)	(4)
250	K^+	6.55	~2	7.35	9
250	Li^+	6.55	~0	8.1	9
50	Na^+	7.9	20	6.3	10
50	K^+	7.9	~2	7.0	2
50	Li^+	7.9	~0	7.05	0.6
250	Na^+	7.9	20	5.9	9
			(0)	(6.25)	(3.5)
250	K^+	7.9	~2	7.0	3
250	Li^+	7.9	~0	7.0	0.6

[a] Solid lines of Figs. 2 and 3 have been obtained using $K_{MM}^* = 0$, $pK_H = 6.55$, and associated parameters which are not within paranthesis in the table.

brane. We have $[mon]_{0c} = [mon]_{0i}$ at equilibrium. For the mechanism of Fig. 1 we have,

$$a_{11} = k_1\{[H^+]/b_i\}[mon]_0(\ln 10)\{K_c/(K_c + [H^+]^2)\}$$
$$\times\{1 + b_iV_i/(b_cV_c)\}$$
$$a_{12} = 2k_1(\ln 10)\{[H^+]/b_i\}\{[H^+]/(K_c + [H^+])\}$$
$$a_{21} = \{[mon]_0/(1 + [M^+]/K_{HM})\}$$
$$\times\{(k_1K_c - k_2K_c^*)/(K_c + [H^+])^2\}\{1 + b_iV_i/(b_cV_c)\}$$
$$a_{22} = \{2/(K_c + [H^+])\}\{1/(1 + [M^+]/K_{HM})\}\{k_1[H^+] + k_2K_c^*\} \quad (4)$$

$$K_c = \{K_H + K_c^*(1 + [M^+]/K_{MM})\}/(1 + [M^+]/K_{HM})$$
$$K_c^* = K_H[M^+]/K_M \quad (5)$$

where K_{HM} and K_{MM} are the dissociation constants of the following two equilibria.

$$mon\text{-}H\text{-}M^+ \rightleftharpoons mon\text{-}H + M^+$$
$$mon\text{-}M\text{-}M^+ \rightleftharpoons mon\text{-}M + M^+ \quad (6)$$

The observed relaxation time τ associated with ΔpH decay is given by the following equation [4].

$$\tau^{-1} = a_{11} - a_{12}a_{21}/a_{22} \quad (7)$$

When Eqns. 4, 5, 7 and Eqn. A-27 of Ref. 4 are used the apparent rate constant k_{+H} of Eqn. 1 takes the following specific form.

$$k_{+H} = 0.95[[Mon]_0/[lip]][k_1k_2K_c^*/(k_1[H^+] + k_2K_c^*)]$$
$$\times[1/(K_H + [H^+])(1 + [M^+]/K_{HM})$$
$$+ K_c^*(1 + [M^+]/K_{MM})] \quad (8)$$

In the above equations k_1 and k_2 are the intrinsic rate constants associated with the translocations of mon-H and mon-M, respectively, across the membrane, $[Mon]_0$ is the monensin concentration in the vesicle solutions expressed with respect to the total volume.

In liposomes, the H^+ flux across the membrane is compensated by a M^+ flux in the opposite direction to maintain electrical neutrality across the membrane. Therefore, if k_{+M} is the apparent rate constant for the transport of M^+ across the membrane, a ΔpH decay experiment itself can be used to estimate k_{+M} using,

$$k_{+H}[H^+] = k_{+M}[M^+] \quad (9)$$

If we have more than one type of M^+ in vesicle solutions and if the metal ion binding equilibria are fast compared to τ^{-1}, in the above equations we must replace M^+/K_M by $\Sigma([M^+]_j/K_{Mj})$ with summation over the metal ion species 'j'. Similar changes have to be made with the terms involving $[M^+]/K_{HM}$ and $[M^+]/K_{MM}$.

From Eqn. 8 we see that the intrinsic rate constants k_1 and k_2 can be estimated if k_{+H} shows pH dependence. Using Eqns. 5, 8 and 9 we note that k_{+M} should decrease with increase in $[M^+]$ (except for $[H^+] \gg K_c^*$, when [mon-M] is rate-limiting), even when mon-H-M^+ and mon-M-M^+ have negligible concentrations.

Materials and Methods

SBPL vesicles with 2 mM pyranine, and 2–50 mM phosphate or 0.25 mM Aces buffers inside vesicles and 25 mM Aces or 5 mM Aces outside vesicles were prepared from asolectin, by sonication and passing through a Sephadex G-50 column in the aqueous medium having specified concentration of MCl (M^+ = Na^+, K^+ and Li^+), as described previously [4,8]. The pH of buffers were adjusted with HCl or MOH. Optical densities at 700 nm, measured with the help of a Cary 17D spectrophotometer, were used to control vesicle preparations. Microlitre amounts of 1 mM or 3 mM monensin (Sigma) in ethanol were added to vesicle solutions before the *T*-jump experiments. A particular set of experiment was carried out using the same vesicle preparation to avoid scatter of data due to small differences in vesicle preparations. The *T*-jump instru-

ment [21], the transient recorder used to record the fluorescence changes [22] and the identification of pyranine fluorescence changes associated with ΔpH decay [4] have been described elsewhere. At least four ΔpH relaxation traces were used in the determination of each relaxation time. A calibrated exponential generator was used to measure τ as described elsewhere [4].

Results

In SBPL vesicles the endogeneous buffer groups make a substantial contribution to the internal buffer capacity, b_i [4,15]. The temperature dependence of their pK are small when compared to that of Aces buffer (see Table I of Ref. 4). When the buffer trapped inside vesicles is 0.25 mM Aces or 2–50 mM phosphate, the magnitude of the pH jump following a T-jump will be smaller inside vesicles than that in the Aces buffer medium outside vesicles (see eqn. A-9 of Ref. 4). Thus, with a T jump of 1.5°C we could get ΔpH ~ 0.025 across the vesicular membrane in all the vesicle solutions used in our experiments. The ΔpH decay due to H^+ transport across the membrane was monitored from the changes in the fluorescence of pyranine entrapped inside vesicles as described in our previous paper [4]. In the absence of monensin, the ΔpH decay rate τ^{-1} ($< 0.1\ s^{-1}$) was much smaller than that observed with monensin in vesicle solution. Thus, the observed τ^{-1} can be attributed dominantly to the monensin-mediated ΔpH decay. The increase in τ^{-1} with either the increase of $[Mon]_0$ [8] or the decrease of [lip] is similar to that observed in the case of nigericin-mediated ion transports [4] and are consistent with Eqn. 8. The increase in τ with the increase in the phosphate buffer concentration inside vesicles (compare Figs. 2a and 2b) are also consistent with that expected by substituting the endogeneous contributions to the internal buffer capacity reported earlier [4] in Eqns. 1 and 2.

Dependence of τ^{-1} on PH

When Na^+ ions were used as M^+ in the vesicle solutions, the relaxation time τ decreased on decreasing the pH from approx. 8 and tended towards a constant value in the pH range 6–6.5 (Figs. 2a and 2b).

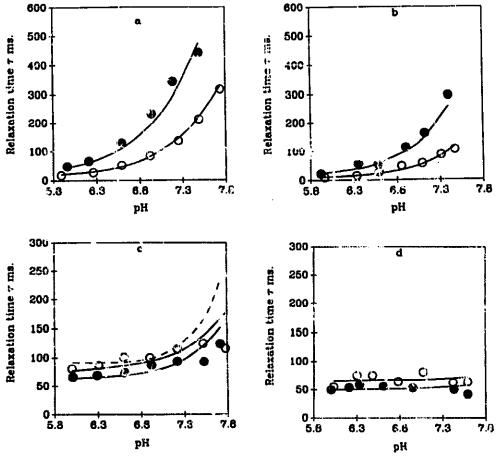

Fig. 2. pH dependence of relaxation time τ associated with the ΔpH decay in SBPL vesicles at 25°C (a) in a medium having $[Na^+] = 50$ mM (○) and 250 mM (●) (50 mM phosphate buffer was inside and 25 mM Aces was outside vesicles, $[Mon]_0 = 1.5\ \mu M$); (b) in a medium having $[Na^+] = 50$ mM (○) and 250 mM (●) (2 mM phosphate was inside and 5 mM Aces was outside vesicles, $[Mon]_0 = 1.5\ \mu M$); (c) in a medium having $[K^+] = 50$ mM (○) and 250 mM (●) (2 mM phosphate was inside and 25 mM Aces was outside, $[Mon]_0 = 1.5\ \mu M$) and; (d) in a medium having $[Li^+] = 50$ mM (○) and 250 mM (●) (0.25 mM Aces was inside and 5 mM Aces was outside vesicles, $[Mon]_0 = 7.5\ \mu M$). [lip] = 5.2 mM in (a)–(d). The solid lines were calculated using $pK_H = 6.55$, Table I, Eqns. 1, 2 and 8. The broken line in (c) was calculated using $pK_H = 7.9$ and the associated parameters in Table I for [KCl] = 50 mM.

In contrast with such data, the pH dependence of τ was small when K^+ ions or Li^+ ions were used (Figs. 2c and 2d). Thus, at $[M^+] = 250$ mM, $\tau^{-1}/[Mon]_0$ (which reflects the monensin-mediated H^+ and M^+ transport rates) followed the order $Na^+ > K^+$ at low pH similar to the ionophore selectivity order [3], but followed the contra-intuitive order $K^+ > Na^+$ at higher pH conditions (~ 7.5) (compare Figs. 2b and 2c).

Dependence of τ on $[M^+]$

In the pH range 6–8 of our study τ increased with increase in $[Na^+]$ but decreased with increase in $[K^+]$ or $[Li^+]$ (Figs. 2a–2d). The pH dependent data (Fig. 3a) obtained in a mixture of Na^+ and Li^+ at 250 mM total ion concentrations show that at higher pH (~ 7.5) τ increases on increasing the proportion of $[Na^+]$. This observation confirms the increase of τ with increase in the concentration of Na^+ ions. Figs. 3b and 3c show that at low pH (~ 6.2) if $[Li^+]$ is decreased from 100 mM with a corresponding increase in $[Na^+]$ or $[K^+]$ (such that the total ion concentration remains 100 mM), τ decreases rapidly at first tending towards a saturating value at higher concentrations of $[Na^+]$ or $[K^+]$. In the experiments with a mixture of K^+ and Na^+ ions at pH 7.4, when $[K^+]$ was decreased from 100 mM with a corresponding increase in $[Na^+]$, τ decreased at first, but increased at higher concentrations of Na^+ (Fig. 3d).

Analysis of data

The metal ion dependent behaviours mentioned above can be explained with the help of Eqns. 1, 2 and 8 by a suitable choice of parameters. However, more than one set of parameters can give the calculated τ in good agreement with the observed values. For example, in Fig. 2c the solid line has been obtained with $pK_H = 6.55$ and the broken line with $pK_H = 7.9$ (along with the associated parameters given in Table I). To select the parameters appropriate to our system we can use the following criteria: (i) The relative magnitudes of K_M (dissociation constants associated with mon-M, $M^+ = Na^+$ and K^+) estimated from our data should be consistent with the ionophore selectivity data. (ii) The intrinsic rate constant k_t associated with the transport of mon-H across the membrane should not depend on the choice or concentration of M^+ (except for small changes due to changes in ionic strength as observed with nigericin [4]).

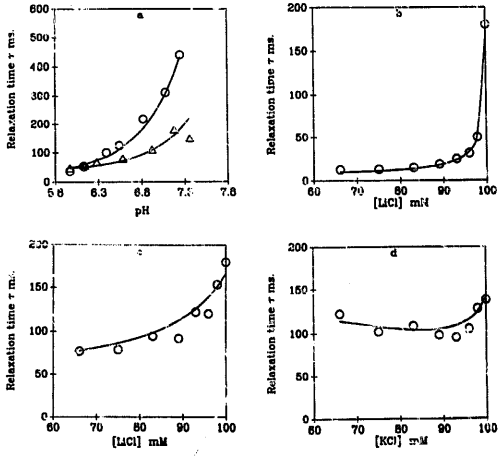

Fig. 3. Variation of τ (a) with pH in a medium having $[Na^+] = 250$ mM (○) and $[Na^+] (= 50$ mM$) + [Li^+] (= 200$ mM$)$ (Δ) (2 mM phosphate inside and 5 mM Aces outside vesicles, $[Mon]_0 = 0.7$ μM, $[lip] = 5.2$ mM); (b) with $[Li^+]$ keeping $[Li^+] + [Na^+] = 100$ mM (pH ~ 6.2, 0.25 mM Aces inside and 5 mM Aces outside vesicles, $[Mon]_0 = 3$ μM, $[lip] = 5.4$ mM; (c) with $[Li^+]$ keeping $[Li^+] + [K^+] = 100$ mM (pH ~ 6.2, 0.25 mM Aces inside and 5 mM Aces outside vesicles, $[Mon]_0 = 3$ μM, $[lip] = 5.4$ mM; (d) with $[K^+]$ keeping $[K^+] + [Na^+] = 100$ mM (pH ~ 7.4, 0.25 mM Aces inside and 25 mM Aces outside vesicles, $[Mon]_0 = 1.0$ μM, $[lip] = 5.2$ mM) at 25°C. The solid lines were calculated using $pK_H = 6.55$ and the associated parameters (Table I) in Eqns. 1, 2 and 8.

For example, with $pK_H = 7.9$ and $k_1 = k_2$ if the calculated τ should agree with the observed values the estimates of k_1 have to be decreased by large factors when M^+ is changed from Na^+ to K^+ or Li^+ (Table I). Also, for this choice of pK_H the relative magnitudes of K_M (estimated from pK_c^* using Eqn. 5 and Table I) are not consistent with those expected from the relative ionophore selectivity. Similar observations can be made for the estimates obtained by fitting the data of Fig. 2 to Eqns. 1 and 8, if k_1 is assumed to have a value largely different from that of k_2 and/or pK_H is chosen to be 10.2. Furthermore, if the probability of formation of the complex mon-H-M is taken to be negligible in the medium containing Na^+ (i.e. $1/K_{HM} \sim 0$), we find that k_1 and K_c^* have to decrease with increase in $[Na^+]$ to explain the data of Figs. 2a and 2b (see Table I). All these are unreasonable in view of the above mentioned criteria and Eqn. 5.

The parameters given in Table I corresponding to $pK_H = 6.55$ (and not enclosed within parenthesis) do explain the data of Fig. 2 and satisfy the above mentioned two criteria. Further support for their validity comes from the observation that the data obtained in a medium containing a mixture of metal ions (Fig. 3) can also be explained using these parameters. However, in these cases we have to sum over the metal ions for the terms involving K_M and K_{HM} as described in the 'Theory' section. The solid lines of Figs. 2 and 3 have been obtained using these parameters.

Discussion

The life-times τ_M ($= 1/k_{+M}$) of the metal ion inside vesicles obtained from the NMR linewidths [6,7,23] can be estimated from the ΔpH decay data also (see Eqn. 9). Thus, the apparent rate constant for the metal ion transport 'k' defined by Riddell and co-workers [6,7,23] is $(k_{+M}\,[\text{lip}]/[\text{Mon}]_0)$. Using Table I, Eqns. 8 and 9, we get 'k' in SBPL vesicles at 25°C to be $\sim 8 \cdot 10^3\ s^{-1}$ for unit value of $[\text{lip}]/[\text{Mon}]_0$ when $[Na^+] = 100$ mM and pH = 7. When we take note of the temperature and lipid constituent dependences of k_{+M}, the above estimate can be considered to be in good agreement with the NMR estimates [7] ($1.2 \cdot 10^4\ s^{-1}$ at 30°C in egg PC vesicles), if the NMR experiments had been carried out at pH $\sim$ 7.

However, our interpretation of the transport data differs substantially from that of Riddell and co-workers [7,23]. (a) The NMR linewidth data have been analysed using a transport mechanism which ignored the compensating flux due to monensin/nigericin-mediated H^+ transport [7,23]. But, Sandeaux et al. [10] have demonstrated the need to include the H^+ transport also when discussing monensin-mediated Na^+ transport. Also, the pK_H values reported in the literature [12] suggest substantial [mon-H] and [nig-H] in the pH region of interest and hence they cannot be ignored. The mechanism used by us is similar to that of Sandeaux et al. [10]. (b) Riddell et al. [7,23] interpret their results assuming $k_{diff} \gg k_d$ (k_{diff} is the rate constant for the diffusion of mon-M across the membranes which is k_2 in our notation; k_d is the rate constant for mon-M dissociation). Thus, in their interpretation k_2 is not obtained. However, our observations on the decrease of τ^{-1} with increase in buffer concentrations and $[Na^+]$ (Figs. 2a and 2b) can be used to say (from arguments similar to those used in the case of nigericin [4]), that equilibria associated with the binding and dissociation of H^+ or Na^+ to monensin are not the rate-limiting steps of ion transports (which cause ΔpH to decay). Thus, in our interpretation k_1 and k_2 are obtained. (c) Riddell et al. have argued that if the diffusion controlled translocation of mon-M is assumed to be rate limiting, k_2 for mon-Na becomes 3 times less than that for mon-K (in their analysis of data) and this is unreasonable since the Na^+ and K^+ complexes of monensin have similar volumes and shapes [7]. On the otherhand, our analysis of ΔpH decay data (in which translocations of mon-M and mon-H across membrane are rate-limiting) does yield similar magnitudes for the k_2 estimates ($= k_1 \sim 9 \cdot 10^3\ s^{-1}$) for the three ions Na^+, K^+ and Li^+ as required by Riddell et al. [7].

Comparing the results and parameters given in Table I with those obtained for the nigericin mediated transports [4], we note the following: (i) The pK_H ($= 6.55$) of monensin determined by us in the SBPL medium is close to that reported in 66% dimethylformamide-water mixture [11] (The higher value of pK_H ($= 10.2$) observed in methanol could be due to the protic nature of the solvent [13].) Thus, we can expect $[\text{mon}^-]$ to be non-negligible in the pH range of our interest, unlike the situation with nigericin. (ii) Our analysis of the data suggests that at the metal ion concentrations used in our experiments $[\text{mon-H-Na}^+]$ could be substantial. Therefore, part of the contribution to the reduction in ion transport rates on increasing $[Na^+]$ could be attributed to the 'inhibition' due to the increase in $[\text{mon-H-Na}^+]$. (iii) The mon-M dissociation constants, K_M (~ 0.052 M for Na^+, 0.5 M for K^+ and 2.2 M for Li^+ at 50 mM ion concentration estimated using Eqn. 5 and pK_c^* (Table I)) follow the expected ionophore selectivity order [3,24,25]. A similar comment had been made for nig-M also [4]. (iv) Similar to the conclusion of our work on nigericin [4], we can say that when $K_c^* \gg [H^+]$, τ^{-1} is proportional to [mon-H] and when $K_c^* \ll [H^+]$ it is proportional to [mon-M]. Thus, as the pH is increased the dominant species limiting the rate changes from mon-M to mon-H. Using Table I we can say that at pH $\sim$ 7, translocation of mon-H across the membrane dominantly limits the ion transport rates when the metal ion in the medium is Na^+. In contrast with this, at this pH the transport of mon-K and

mon-Li dominantly limit the rates when the metal ion in the medium is K^+ and Li^+, respectively. (v) The pK_c^* (and hence the concentrations of the dominant rate-limiting species) at pH ~ 7.5 are such that we can have higher ion transport rates (and hence higher ΔpH decay rate) in a medium of K^+ rather than in a medium of Na^+ (at 250 mM ion concentration) although the ionophore selectivity order for monensin is $Na^+ > K^+$. Such contra-intuitive behaviour has been noted with nigericin also [4]. (vi) In a medium containing a mixture of Na^+ and K^+ at pH ~ 7.4, when the proportion of K^+ is small, the transport of mon-H can be rate-limiting. Increasing the concentration of the relatively weakly binding K^+ with a corresponding decrease in $[Na^+]$ would then increase [mon-H] with an associated increase in τ^{-1}. However, when $[K^+]$ becomes sufficiently large it is the transport of [mon-Na] which will be rate-limiting, in which case τ^{-1} will decrease with an increase in $[K^+]$ and a corresponding decrease in $[Na^+]$. Such a behaviour is predicted by the equations given above and is observed in experiments (Fig. 3d). (vii) Species with negligible translocation rate constants in membranes (in the absence of electric fields) such as mon$^-$ and mon-H-M^+ require us to include additional terms to the expression for the ΔpH decay derived in our earlier work [4]. (Contribution from the concerted transport of mon-H-M^+ and mon-M-M^+ is negligible since [mon-M-M^+] is negligible. The stabilisation of charged species in the polar interface region is an additional factor making the translocation rates of the charged species small.) (viii) Our data show that $1/K_{MM} \sim 0$ indicating that the binding of a second metal ion to mon-M is negligible. However, $1/K_{HM}$ ($\sim 20\ M^{-1}$) associated with the formation of mon-H-M^+ is comparable to $1/K_M$ ($\sim 30\ M^{-1}$) associated with the formation of mon-M. This observation might imply that the protonation state of the carboxyl group in monensin does not affect the stability of mon-M much and that the bindings of M^+ and H^+ to monensin are not competitive. This conclusion has the support of structural data [26,27]. A similar situation does not exist with nigericin. (ix) K_c^* (corresponding to $pK_H = 6.55$) given in Table 1 increases with $[M^+]$. The observed deviation from the expected linear dependence (Eqn. 5) is similar to that observed with nigericin [4] and can be attributed to the decrease in the activity coefficients on increasing the ionic strength at high ion concentrations. (x) The observation $k_1 \approx k_2$ for monensin is similar to that made in the case of nigericin. k_1 estimated for mon-H ($\sim 9 \cdot 10^3\ s^{-1}$) is higher than that for nig-H ($\sim 6 \cdot 10^3\ s^{-1}$). This small difference is understandable when we take note of the small differences in their structures and the relative molecular weights (670 and 725, respectively) which are reflected in their molecular volumes and hence in the values of k_1. The translocation rate constants of mon-H and mon-M in SBPL vesicles are smaller than that of neutral valinomycin ($\sim 4 \cdot 10^4\ s^{-1}$ in phosphatidylinositol bilayer) [12], probably because of their electric dipole moment which can increase the transport barrier and the differences in the constitution of lipid.

References

1 Henderson, P.J.F., McGivan, J.D. and Chappell, J.B. (1969) Biochem. J. 111, 521–535.
2 Hinkle, P.C. (1973) Fed. Proc. 32, 1988–1992.
3 Pressman, B.C. (1976) Annu. Rev. Biochem. 45, 501–530.
4 Prabhananda, B.S. and Ugrankar, M.M. (1991) Biochim. Biophys. Acta 1070, 481–491.
5 Degani, H. and Elgavish, G.A. (1978) FEBS Lett. 90, 357–360.
6 Riddell, F.G. and Bayer, M.K. (1985) Biochim. Biophys. Acta 817, 313–317.
7 Riddell, F.G., Armugam, S. and Cox, B.G. (1988) Biochim. Biophys. Acta 944, 279–284.
8 Krishnamoorthy, G. (1986) Biochemistry 25, 6666–6670.
9 Hamilton, R.T. and Nilsen-Hamilton, M. (1980) Biochem. Biophys. Res. Commun. 95, 140–147.
10 Sandeaux, R., Sandeaux, J., Gavach, G. and Brun, B. (1982) Biochim. Biophys. Acta 684, 127–132.
11 Agtarap, A., Chamberlin, J.W. Pinkerton, M. and Steinrauf, L. (1967) J. Am. Chem. Soc. 89, 5737–5739.
12 Jain, M.K. and Wagner, R.C. (1980) Introduction to Biological Membranes, 205 pp., John Wiley, New York.
13 Gertenbach, P.V. and Popov, A.I. (1975) J. Am. Chem. Soc. 97, 4738–4744.
14 Clement, N.R. and Gould, J.M. (1981) Biochemistry 20, 1534–1548.
15 Grzesiek, S. and Dencher, N.A. (1986) Biophys. J. 50, 265–276.
16 Ruf, H. (1980) in Hydrogen Ion Transport in Ephitalia (Schulz, I., Sachs, G., Forte, J.G. and Ullrich, K.J., eds.), pp. 11–18, Elsevier, Amsterdam.
17 Ahmed, I. and Krishnamoorthy, G. (1990) Biochim. Biophys. Acta 1024, 298–306.
18 Eigen, M. and DeMayer, L. (1963) in Tech. Org. Chem., Vol VIII, (Weissberger, A., ed.), pp. 895–1054, Interscience, New York.
19 Bernasconi, G.H. (1976) Relaxation Kinetics, 288 pp., Academic Press, New York.
20 Czerlinsky, G.H. (1966) Chemical Relaxation, 314 pp., Marcel and Dekker, New York.
21 Prabhananda, B.S. (1977) in Proceedings of the Symposium on Fast Reactions at Trivandrum, India, pp. 317–343, Department of Atomic Energy of India, Bombay.
22 Braganza, O.P. and Prabhananda, B.S. (1991) in Magnetic Resonance Current Trends (Khetrapal, C.L. and Govil, G., eds.), pp. 393–405, Narosa Publishing, New Delhi.
23 Riddell, F.G., Armugam, S., Brophy, P.J., Cox, B.G. Payne, M.C.H. and Southon, T.E. (1988) J. Am. Chem. Soc. 110, 734–738.
24 Antonenko, Y.N. and Yaguzhinsky, L.V. (1988) Biochim. Biophys. Acta 938, 125–130.
25 Lutz, W.K., Wipf, H.K. and Simon, W. (1970) Helv. Chim. Acta 53, 1741–1746.
26 Lutz, W.K., Winkler, F.K. and Dunitz, J.D. (1971) Helv. Chim. Acta 54, 1103–1108.
27 Steinrauf, L.K., Czerwinski, E.W. and Pinkerton, M. (1971) Biochem. Biophys. Res. Commun. 45, 1279–1283.