Kinetics of Valinomycin- and Tetranactin-mediated Cation Transport through Liposomal Membrane

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(Received May 28, 1982)

Kinetics of valinomycin- and tetranactin-mediated cation transport were studied. An equation was derived which describes the kinetics of ionophore induced cation leakage from liposomes. The theoretically predicted ion-selectivity of the ionophore-mediated cation transport agreed well with that given by experiments. This supports the assumptions used in the derivation and is consistent with the following characteristics of the ionophores: (1) Ionophore-mediated cation transport is carried out by stoichiometrically forming a ternary complex between ionophore, cation and lipophilic anion. (2) The ion-selectivity of the ionophore is determined primarily by the formation constant of the ionophore cation complex in water. (3) A relative value of the complex formation constant in water can be calculated from both the complex formation constant in methanol, which is estimated more easily than that in water, and the difference between desolvation energy of the cation in water and methanol.

Neutral ionophores, such as valinomycin and tetranactin function as mobile carriers of specific ions through biomembranes or model membranes. Block *et al.* studied the kinetics of valinomycin-induced K^+ leakage from liposomes and determined a partition constant of valinomycin between membrane phase and aqueous phase, and a turnover rate of potassium transport across liposomal membrane.[1] Yamaguchi and Anraku studied the mechanism of SF6847-mediated proton uptake in liposomes and showed that the proton uptake was compensated by valinomycin-induced potassium efflux.[2] Results showed that potassium ion leakage or the exchange transport between proton and potassium ion was induced by ternary complex formation between valinomycin, potassium ion and thiocyanate ion or between valinomycin, potassium ion and SF6847. But in their reports, the ion-selectivity was not discussed.

In the present report, we studied the ion-selective permeation induced by ionophore through liposomal membranes. First, we derived an equation which described the ion selective transport of ionophore through the liposomal membrane. Secondly, we showed experimentally that the equation adequately predicted the ion selectivity of the ionophore. The derivation required two assumptions: (1) It is assumed that ionophore distributes uniformly throughout liposomal membrane. This contrasts with the assumption made by Block *et al.*[1] that ionophore should be localized on the inside and outside membrane surfaces. (2) The association constant for ionophore-cation complexation in water may be expressed in terms of the association constant obtained in methanol and the difference between the desolvation energy of the cation in methanol and water.

Theoretical

In the specific liposomal system under investigation, only the transport of ion through the outer bilayer of the multilamellar liposomes contributes directly to the observed leakage. A model for ionophore-mediated ion transport through membrane is proposed as shown in Fig. 1. In this model it is assumed that only ternary complex (IMX) of ionophore (I), cation (M) and anion (X) can carry ions through membrane, and ionophore is

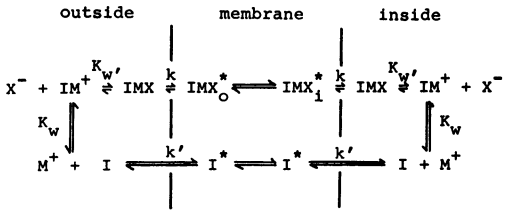

Fig. 1. Model for ionophore-mediated cation leakage from liposomes containing MSCN.

distributed uniformly throughout the membrane phase.

In the water phase inside the bilayer, ionophore (I) and cation (M) form complex (IM) *i.e.* $I+M \overset{K_w}{\rightleftharpoons} IM$. The complex formation constant K_w is expressed by Eq. 1,

$$K_w = \frac{C_{IM}}{C_{IM}C_M}. \quad (1)$$

Complex IM and anion X form ternary complex IMX, $IM+X \overset{K'_w}{\rightleftharpoons} IMX$. K'_w can be expressed by Eq. 2,

$$K'_w = \frac{C_{IM}}{C_{IM}C_X}. \quad (2)$$

Ternary complex IMX is distributed into membrane, $IMX \overset{k}{\rightleftharpoons} IMX^*$, where asterisk (*) indicates the membrane phase. The distribution constant k of the ternary complex is expressed by Eq. 3,

$$k = \frac{C^*_{IMX}}{C_{IMX}}. \quad (3)$$

The distribution constant of ionophore k' is expressed by Eq. 4,

$$k' = \frac{C^*_I}{C_I}. \quad (4)$$

The ternary complex IMX* diffuses through membrane from inside to outside. The flux J of the ternary complex is assumed to be proportional to the concentration gradient of the ternary complex in membrane. We get following equation,

$$J = D\frac{C^*_{IMXi} - C^*_{IMXo}}{d}, \quad (5)$$

where D is the diffusion constant of the ternary complex in methanol and d is the thickness of the membrane.

Subscripts i and o denote inside and outside surfaces of the membrane, respectively. When the distribution equiliblium of IMX between aqueous and membrane phases is achieved quickly, that is, the transmembrane diffusion of the ternary complex is the rate-limiting process of ion transport, $C^*_{MIXo} \ll C_{IMX_i}$ in the initial stage after addition of ionophore, since $C_{IMXo}=0$ in the initial stage. Then the leakage L $(=JS)$ is expressed by Eq. 6,

$$L = JS = D\frac{S}{d}C^*_{IMX_i} = D\frac{S}{d}kC_{IMX}, \tag{6}$$

where S is the surface area of the membrane. From Eqs. 1, 2, 3, and 6, we get

$$L = D\frac{S}{d}kK'_wK_wC_IC_MC_X. \tag{7}$$

If we define b and m as the liposomal volumes of water and membrane phases, and assume the concentration of cation M in extra liposomal water can be regarded as zero in the initial stage after the addition of ionophore, then the total amount of cation a_{MT} and anion a_{XT} are represented by Eqs. 8 and 9.

$$a_{MT} = b(C_M+C_{IM}+C_{IMX}) + m(C^*_M+C^*_{IM}+C^*_{IMX}) \tag{8}$$

$$a_{XT} = b(C_X+C_{IMX}) + m(C^*_X+C^*_{IMX}) \tag{9}$$

In the present case, $C_M \gg C_I$, Eqs. 8 and 9 can be reduced to Eqs. 8′ and 9′,

$$a_{MT} = bC_M, \tag{8'}$$

$$a_{XT} = bC_X. \tag{9'}$$

For uni-valent electrolyte

$$a_T = a_{MT} = a_{XT} = bC_M = bC_X, \tag{10}$$

where a_T is the amount of the salt contained in liposomes before addition of ionophore. When w is the total volume of water (*i.e.* in and out of liposomes), total amount of ionophore a_T is represented as follows;

$$a_T = w(C_I+C_{IM}+C_{IMX})+m(C^*_I+C^*_{IM}+C^*_{IMX}). \tag{11}$$

Since K'_w can be regarded as far less than K_w[1] and ion concentration within the membrane regarded as negligible. Eq. 11 is reduced to Eq. 11′,

$$a_I = w(C_I+C_{IM})+mC^*_I. \tag{11'}$$

From Eqs. 1, 4, and 11′, we get following equation.

$$a_I = w\left(C_I+K_wC_I\cdot\frac{a_T}{b}\right)+mk'C_I \tag{12}$$

From Eqs. 7, 10, and 12, we get following equation.

$$L = D\frac{S}{d}kK'_wK_w\frac{a_I}{w+mk'+\dfrac{wa_T}{b}K_w}\left(\frac{a_T}{b}\right)^2 \tag{13}$$

S, d, m, w, and k' are invariant with cation species. k, D, and K'_w can be regarded as invariant with cation species, because in ionophore-univalent cation complexes cations are enveloped by ionophore and the complexes are similar in shape and size with each other.[3-6] Therefore setting $D\frac{S}{d}kK'_w=A$=constant and $w=mK'=B$=constant, we get

$$L = AK_w\frac{a_I}{B+K_ww\cdot\dfrac{a_T}{b}}\left(\frac{a_T}{b}\right)^2. \tag{14}$$

When the amount of lipid is constant, C_{IT} can be defined as the total amount of ionophore per mg of lipid, and C_T as the concentration of metal thiocyanate in water phase in liposome before addition of ionophore, that is $C_T=\frac{a_T}{b}$, Eq. 14 can be represented as Eq. 15 where A' is A per amount of lipid.

$$L = A'K_w\frac{C_{IT}C_T^2}{B+K_wwC_T} \tag{15}$$

The relation between leakage L and association constant K_w, under the condition where the concentration of ionophore and metal thiocyanate are constant, as defined by Eq. 15, is shown in Fig. 2. When $K_w \ll \frac{B}{wC_T}=\frac{w+mk'}{wC_T}$, Eq. 15 can be reduced to Eq. 16.

$$L = \frac{A'}{B}K_wC_{IT}C_T^2 \tag{16}$$

In this case, leakage L is proportional to K_w, C_{IT}, and C_T^2. According to Eq. 16, the leakage of ion from liposome is proportional to the complex formation constant between ionophore and the cation in water which agrees with the electrical study of Eisenman *et al.*[7-10] When $K \gg \frac{B}{wC_T}=\frac{w+mk'}{wC_T}$, Eq. 15 can be reduced to Eq. 17.

$$L = \frac{A'}{w}C_{IT}C_T \tag{17}$$

In this case, L is proportional to the concentrations of ionophore and metal thiocyanate and has no relation to the association constant.

The ion-selectivity of the ionophore can be evaluated indirectly. It is difficult to estimate the complex formation constant in water (K_w) directly by experimental measurement, because of very low solubility of ionophore in water and small K_w. However, it is only necessary to estimate relative value of K_w in evaluating ion-selectivity and not the absolute value. As discussed in the preceding study,[18] the free energy of complex formation, ΔG^0, is defined as a sum of the conformational energy difference of ionophore between complex form and uncomplex form (ΔU), the desolvation energy of cation with complex formation (ΔG^0_M), and the other factor (I), which includes the solvation effect and the change in entropy of the ionophore upon complex formation and can be regarded as independent of ion

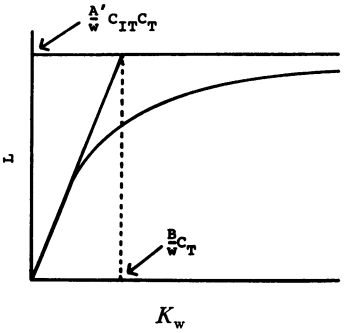

Fig. 2. Scheme of L(leakage) and K_w (complex formation constant) relation under the condition of constant concentration of ionophore and ion.

species.

Then in water,

$$-RT\ln K_w = \Delta G_w^\circ = \Delta U + \Delta G_{Mw}^\circ + I_w \quad (18)$$

and in methanol,

$$-RT\ln K_m = \Delta G_m^\circ = \Delta U + \Delta G_{Mm}^\circ + I_m. \quad (19)$$

By Eqs. 18 and 19, we get

$$K_w = K_m \exp\frac{\Delta G_{Mm}^\circ - \Delta C_{Mw}^\circ}{RT} \exp\frac{I_m - I_w}{RT}. \quad (20)$$

From Eqs. 16 and 20, we get

$$L = \frac{A'}{B} C_{IT} C_T^2 \exp\frac{I_m - I_w}{RT} \cdot K_m \exp\frac{\Delta G_{Mm} - \Delta G_{Mw}}{RT}. \quad (21)$$

Since the $\exp\frac{I_m - I_w}{RT}$ can be regarded as independent of ion species, in the case of C_{IT}=constant and C_T= constant, Eq. 21 is reduced to Eq. 22 with $\frac{A}{B} C_{IT} C_T^2 \exp\frac{I_m - I_w}{RT}$=constant=$E$.

$$L = EK_m \exp\frac{\Delta G_{Mm}^\circ - \Delta G_{Mw}^\circ}{RT}. \quad (22)$$

The complex formation constant between ionophore and cation in methanol (K_m) and desolvation energy of cation in methanol (ΔG_{Mm}^0) and in water (ΔG_{Mw}^0) has already been determined.[12-14]

Experimental

Materials. Valinomycin was obtained from Calbiochem Co. Ltd. Tetranactin was gifted from Chugai Pharmaceutical Co. Ltd. Egg lecithin was a purified substance given by Asahi Kasei Co. Ltd. Phosphatidic acid was obtained from Sigma Co. Ltd. The other reagents were special grade of Wako Pure Chemical Industries Ltd.

Methods. Multilayer liposomes were prepared as follows: The inner surface of a 50 ml round-bottomed flask was coated with 10 mg of egg lecithin containing 2% of phosphatidic acid by evaporation of solvent. Then, 5 ml of a 150 mM KSCN/10 mM Tris solution which was adjusted to pH 7.0 by adding H_2SO_4 was poured into the lecithin coated flask and gently shaken until all lipid was removed from the flask. The liposome suspension was centrifuged for 20 min at 40000 g at 4 °C. The liposome preparation was washed with isotonic 150 mM $MgSO_4$/10 mM Tris/H_2SO_4 solution followed by centrifugation at 40000 g for 20 min at 4 °C. The obtained liposome pellet was dispersed in 10 ml of isotonic 150 mM $MgSO_4$/10 mM Tris/H_2SO_4 solution (pH 7.0). Ionophore, dissolved in methanol, was added with a microsyringe (20 μl) to the liposome-dispersed solution. The ion leakage was measured by an ion-meter (Orion Research) with appropriate ion-selective electrodes.

Results and Discussion

When valinomycin was added to the liposomes containing KSCN, the K^+ leakage as well as the SCN^- leakage increased maximally immediately following the addition of the ionophore (Figs. 3-a, b), but when valinomycin was added to the liposomes containing KCL, the increase in K^+ leakage was far smaller than those containing KSCN (Fig. 3-c). These observations suggest that the permeation of K^+ from the liposomes induced by valinomycin is facilitated by lipophilic

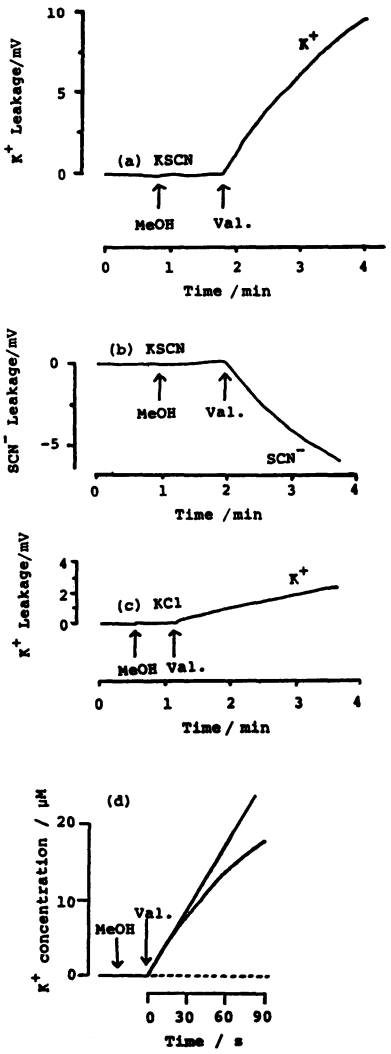

Fig. 3. Recorder trace of the valinomycin-induced ion leakage and the time course of K^+ concentration in medium.
(a) and (b) show K^+ and SCN^- leakages from liposomes containing 150 mM of KSCN. (c) shows K^+ leakage from liposomes containing 150 mM of KCl. (d) shows the time course of K^+ concentration in medium dispersing liposomes containing 150 mM of KSCN.

anions, such as SCN^-. Equation 16 suggests that cation leakage is proportional to the concentration of ionophore, C_{IT}, when C_T and K_w are constant or to the square of the concentration of metal thiocyanate in the liposomes, C_T^2, when C_{IT} and K_w are constant. In Fig. 4, the initial rate of K^+ leakage was plotted against the concentration of valinomycin at constant KSCN concentration. The plot showed a good linear relation. In the Fig. 5, the initial rate of K^+ leakage was plotted against the logarithm of the KSCN concentration at constant valinomycin. The plot showed a linear relation with a slope of near 2. From these observations, Eq. 16 has been found primarily to hold in our experimental condition. Accordingly the assumption, $K_w \ll B/wC_t$, is practically satisfied. In addition, the fact that the slope of the line in Fig. 5 was near 2 supports

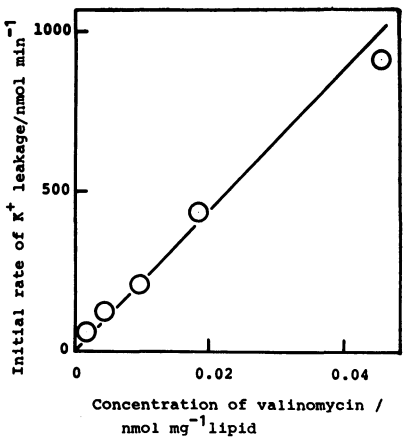

Fig. 4. Initial rate of the K^+ leakage against concentration of valinomycin at constant concentration of KSCN (150 mM) in liposomes.

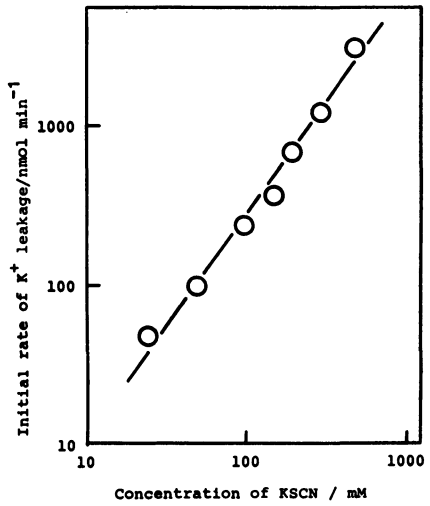

Fig. 5. Initial rate of the K^+ leakage against logarithmic scale of concentration of KSCN in liposomes at constant concentration of valinomycin (0.018 nmol/mg of lipid).

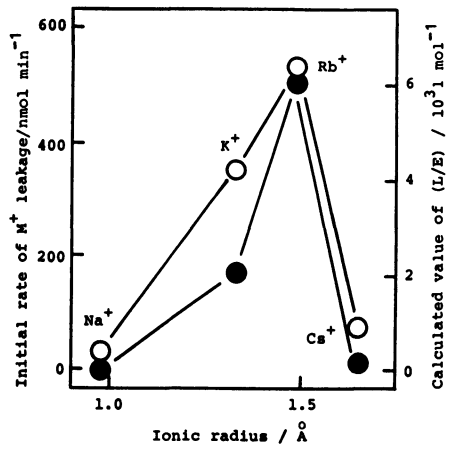

Fig. 6. Initial rate of M^+ leakage and calculated value (L/E) plots against ionic radius for valinomycin.
○: The values obtained from direct experimental measurements. ●: The values summarized in Table 1.

Table 1. Association constant of valinomycin[a] and tetranactin[b] with metal cations in methanol and calculated values, L/E[c]

	$K_m/10^3$ l mol^{-1}	$(L/E)/10^2$ l mol^{-1}
Valinomycin		
Na^+	0.005	0.0003
K^+	80	20
Rb^+	180	61
Cs^+	2.6	2.1
Tetranactin		
Na^+	0.5	0.034
K^+	20	4.9
Rb^+	10	3.4
Cs^+	3	2.4

a) Nishino, Izumiya, *Membrane*, **5**, 338 (1980).
b) M.Ueno *et al.*, *Yakugaku Zasshi*, **97**, 46 (1977).
c) $=K_m\frac{\Delta G^\circ_{Mm}-\Delta G^\circ_{Mw}}{RT}$, see context regarding to Eq. 22.

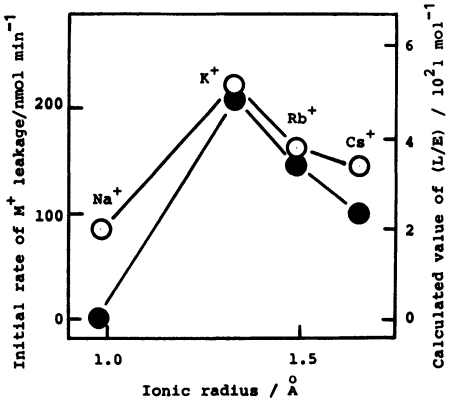

Fig. 7. Initial rate of M^+ leakage and calculated value (L/E) plots against ionic radius for tetranactin.
○: The values obtained from direct experimental measurements. ●: The values summarized in Table 1.

the assumption that valinomycin functions only as a mobile carrier by forming stoichiometrically a ternary complex with K^+ and SCN^-, as described originally by Block *et al.*[1] That the above slope was slightly smaller than 2 may be attributed the <1 activity coefficient of KSCN at 150 mM.

Equation 22 suggests that cation leakage induced by ionophore is proportional to the product of the association constant between the cation and the ionophore in methanol and the exponent of the desolvation energy difference between methanol and water at constant ionophore concentration and constant metal thiocyanate concentration. The association constant in methanol, K_m, and desolvation energy difference, $\Delta G^0_{Mm}-\Delta G^0_{Mw}$, have been reported.[12-14] In Table 1, K_m and $K_m\frac{\Delta G^0_{Mm}-\Delta G^0_{Mw}}{RT}(=L/E)$ were summerized. The experimental values of initial rates of several cations and the calculated values, $K_m\frac{\Delta G^0_{Mm}-\Delta G^0_{Mw}}{RT}$, were plotted against the ionic radii of the cation in Fig. 6 for valinomycin as ionophore and in Fig. 7 for tetranactin. The ion-selectivity of the ionophores given from direct

experimental measurements agreed well with those predicted from Eq. 22. The term, $\Delta G^0_{Mm} - \Delta G^0_{Mw}$, in Eq. 22 ensures the effects of solvation energy of ions on the ion selectivity of the ionophores. The fact that the permeation of Cs^+ or Rb^+ was relatively large as compared with that predicted from the K_m alone shows that the contribution energy of the cation to ion-selectivity of the ionophore is different in methanol and in water. Thus the experimental results justified the validity of application of Eq. 22 to the elucidation of ion selectivity of ionophore-mediated ion transport through liposomal membrane.

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