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Effect of changes in cation concentration near bilayer lipid membrane on the rate of carrier-mediated cation fluxes and on the carrier apparent selectivity

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A new approach was applied for the measurements of ion transport through bilayer lipid membranes (BLM) induced by electrically neutral cation/H^+ exchangers. This is an improved version of the method of the measurements of the cation/H^+ exchange rate based on recording pH shifts in the unstirred layers near the BLM. Using this approach, the pH gradient in the unstirred layers induced by the cation/H^+ exchanger was reduced by successive addition of the acetate on one side of the BLM until the pH shift reached zero. The difference in acetate concentration across the membrane is a measure of the cation/H^+ exchange rate. In the second part of the work we found that the changes in cation concentration in the unstirred layers under the conditions imposed when measuring cation selectivity (according to Antonenko, Yu.N. and Yaguzhinsky, L.S., Biochim. Biophys. Acta 1988; 938, 125–130) can significantly decrease the apparent value of cation selectivity. It was shown that more accurate results can be obtained if low concentrations of the carrier are used. The values of nigericin cation selectivity for the alkali metals were measured (K^+/Rb^+ 19 ± 1, Rb^+/Na^+ 1.9 ± 0.2, Na^+/Cs^+ 8 ± 0.5, Cs^+/Li^+ 1.8 ± 0.3).

Introduction

The main effect of polyether antibiotics is the induction of electrically silent antiport fluxes of hydrogen and monovalent metal cations across the membranes [1–3]. The mechanism of operation of cation/H^+ exchangers as well as the nature of their ion selectivity have been studied intensitvely during the last years [4–17]. These studies are important for gaining an understanding of the mechanism of action of widely used agents which selectively affect the ion gradients in physiological experiments as well as for the simulation of the complex antiport systems which catalyze cation/H^+ exchange on natural membranes.

It was shown previously that the process of cation/H^+ exchange on the planar bilayer lipid membrane (BLM) induces the formation of a pH gradient on the membrane after the formation of a metal cation gradient [16,17]. This phenomenon was used in our laboratory for the measurement of the rate of cation/H^+ exchange [17], as well as for the estimation of ionophore ion selectivity [13]. The disadvantage of these measurements is the effect of the pH gradient on the rate of cation/H^+ exchange, which may distort the results. Besides, the hydrogen ion and the metal cation are symmetrical in the process of cation/H^+ exchange. Therefore, along with the formation of a pH gradient, a change should take place in the cation concentration gradient formed initially.

In the present work, the new version of the method is elaborated. This new version permits the measurement of the rate of cation/H^+ exchange in the absence of a pH gradient on the membrane. The effect of metal cation concentration changes near the BLM on the rate of cation/H^+ exchange and on the measured ion selectivity were estimated for the nigericin ionophore. A range of nigericin concentrations was found where the effect of the changes in metal cation concentration on the cation/H^+ exchange was negligible. At higher carrier concentrations, the exchange rate is controlled by

Abbreviations: TTFB, tetrachlorotrifluoromethylbenzimidazole, Mes, 2-[*N*-morpholino]ethanesulfonic acid; ψ, the difference in electrical potential between opposite sides of the membrane; J_H, the electrically silent hydrogen ion flux through the membrane; T^-, TH and TK, three forms of nigericin; BLM, bilayer lipid membrane; $S_{Me1/Me2}$, carrier selectivity of a cation Me_1^+ with respect to a cation Me_2^+.

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the diffusion of metal cations and hydrogen ions through the unstirred layers adjacent to the surface of the BLM. Similar studies were performed for the effect of changes in the cation concentration on the carrier cation selectivity parameter ($S_{Me1/Me2}$). We measured the nigericin $S_{Me1/Me2}$ values for the alkali metals and compared them with the corresponding binding constants available in the literature.

Materials and Methods

BLM were formed on a Teflon partition 0.4 mm in diameter, using a conventional method [18]. The membrane-forming solution contained 20 mg phosphatidylcholine from soy beans (Sigma) and 10 mg cholesterol (Calbiochem-Boehringer) in 1 ml of *n*-decane. Thinning of the BLM was observed both visually and by measuring its capacity. The main element of the electrical scheme is a Keithley 301 amplifier. The experiments were carried out at room temperature (21–23° C). The hydrogen ion electroneutral flux (J_H) was measured using a method previously described [17]. This method is based on the formation of local pH gradients in the unstirred layer near the BLM during cation/proton exchange on BLM in a solution with low buffer capacity (1 mM Tris, 1 mM Mes, 100 mM choline chloride, pH 7.0). The pH gradient was determined from the difference of the electrical potentials (ψ) on BLM in the presence of a protonophore in the open circuit mode. The protonophore, tetrachlorotrifluoromethylbenzimidazole and the antibiotic nigericin (Calbiochem) were added at both sides of the BLM. In some experiments the antibiotic was added to the membrane-forming solution of the phospholipid.

Results and Discussion

Fig. 1 shows the scheme of ion fluxes through membrane (M) and unstirred layers (UL) induced by the

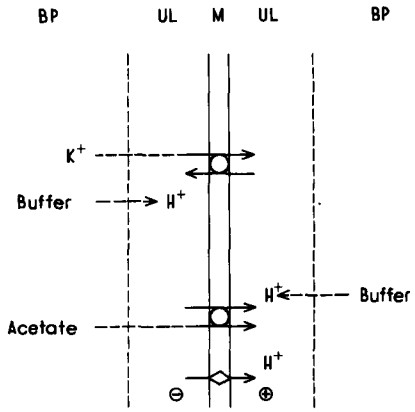

Fig. 1. The scheme of ion fluxes through the membrane (M) and the unstirred layers (UL) induced by the cation (K^+)/H^+ exchanger and in the presence of acetate. BP, bulk phases.

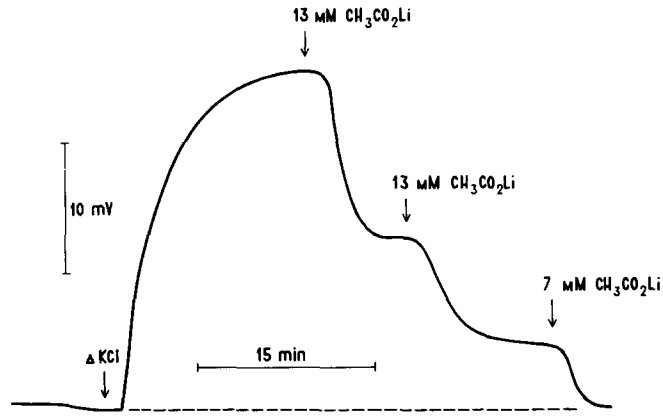

Fig. 2. An example of the measurement of the electroneutral flux of hydrogen ions through the BLM induced by nigericin (200 μM in the membrane-forming solution). KCl, addition of 30 mM and 10 mM KCl at the opposite sides of the membrane. Lithium acetate was added at the side of BLM with the higher KCl concentration.

cation (K^+)/H^+ exchanger. The fluxes induce the formation of a pH gradient when the cation concentrations differ on the two sides on the membrane. The pH gradient on the membrane should decrease the cation/H^+ exchange rate. Therefore, the measurements of the exchange rate should be carried out at low ion fluxes which cause small shifts in the pH near the membrane and therefore have a lesser effect on the ion fluxes.

Using the modified method of cation/H^+ exchange measurements – namely the reduction of pH gradient by means of the addition of acetate at one side of the membrane, one avoids these complications entirely. Acetic acid can easily penetrate through the membrane and due to proton transfer reactions on the surfaces of the membrane, acetate causes an electroneutral hydrogen ion flux through the membrane [19–22]. Schematically, this process is depicted in Fig. 1. When the acetate-induced J_H^1 flux and the cation/H^+ exchange-induced hydrogen ion flux (J_H^2) are oppositely directed and produce a zero total hydrogen ion flux, $J_H^t = J_H^1 + J_H^2 = 0$, the value of J_H^1 can be calculated from the acetate concentration. Experimentally, the $J_H^t = 0$ point corresponds with the moment of the zero pH gradient on the membrane. This method enables measurement of the cation/H^+ exchange rate in the absence of a pH gradient on the BLM.

Fig. 2 shows an example of the J_H measurement at zero pH gradient by means of acetate titration. We used lithium acetate in these experiments, since lithium ions had no effect on the nigericin-induced membrane potential under these conditions. In contrast to our previous approach, the present experiments show that the value of the acetate concentration necessary to attain zero potential is independent of the buffer concentration (data not shown).

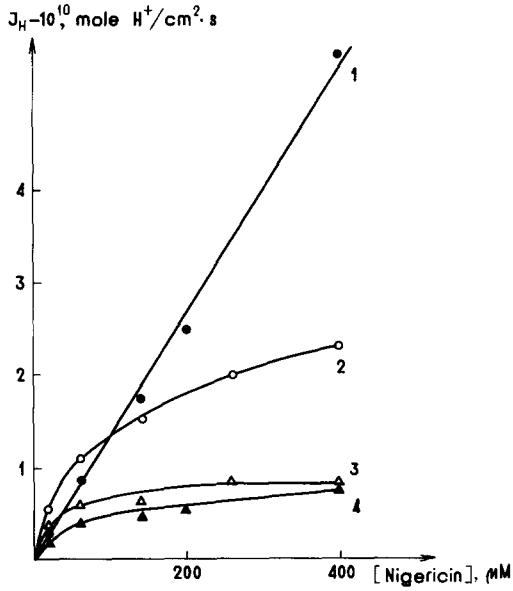

Fig. 3. Effect of the nigericin concentration in the membrane-forming solution on the hydrogen ion flux through the membrane measured from the BLM potential in the presence of a protonophore (curves 3, 4) and from the acetate titration as shown in Fig. 2 (curves 1, 2) under the conditions 3 mM: 1 mM KCl gradient (curves 2, 3) and 180 mM: 60 mM (curves 1, 4).

Fig. 3 shows the effect of nigericin concentration on the J_H flux. The measurements were carried out according to the previous technique (curves 3 and 4) with the new modification (curves 1 and 2). The J_H flux 10^{-11} mol H^+/cm^2 per s corresponds to the potential 2.2 mV under these conditions. This calibration was carried out according to Ref. 17. In this work we use a considerably higher nigericin concentration than in a previous study [23], where the linear potential versus concentration dependence was observed. It is seen from Fig. 3 (curves 3 and 4) that this dependence reaches saturation at [nigericin] > 100 μM. This should be attributed primarily to the inhibition of the J_H flux by the formation of a pH gradient on BLM which reaches 65% of the potassium gradient value. It may be expected that the J_H dependence measured by the new technique, which excludes the formation of a pH gradient, would be linear in the entire range of nigericin concentrations. This holds true for a high potassium ion concentration (180 mM: 60 mM, curve 1). However, at a low concentration of potassium ions (3 mM: 1 mM) significant deviation from linear dependence is observed (Fig. 3, curve 2). This result can be explained by the changes in potassium ion concentration at the surfaces of the membrane due to the process of K^+/H^+ exchange. These changes in the surface $[K^+]$ are not significant if the higher potassium concentration is used (Fig. 3, curve 1).

In our previous paper [13], changes in metal cation concentration near the membrane in the process of cation/H^+ exchange were not taken into account when measurement of the ionophore cation selectivity ($S_{Me1/Me2}$) was carried out. According to the method applied, after the potential on the BLM had attained a steady-state value in the presence of a nonelectrogenic ionophore and a protonophore, given the existence of a concentration gradient of the cation $\Delta[Me_1^+]$, a reverse gradient of the other cation $\Delta[Me_2^+]$ was formed and increased until the BLM potential became equal to zero. Under these conditions, the ratio of transmembrane gradients ($\Delta[Me_1^+]/\Delta[Me_2^+]$) was shown to be a good measure of cation selectivity of an ionophore ($S_{Me1/Me2}$). The process of Me_1^+/Me_2^+ exchange proceeding under the conditions of zero J_H flux can change the surface concentration of cations, especially of the cation whose concentration is lower. The concentration profiles of two cations in the unstirred layers under the conditions imposed during measurements of ionophore selectivity are presented in the Fig. 4. The dashed lines show the cation distribution at a low rate of ionophore operation (low ionophore concentration) and the solid lines show those at a high rate. The value of Δ (cation concentration change in the unstirred layer) is the same for both cations, since the flux of a cation in one direction is equal to the flux of the other cation in the opposite direction. The increase in the exchange rate should lead to the decrease in the measured $S_{Me1/Me2}$ according to the equation

$$[Na^+]/[K^+] = ([Na^+]_{lr} + \Delta)/([K^+]_{lr} + \Delta) \quad (1)$$

where lr refers to low (exchange) rate, the value of Δ is proportional to exchange rate. The following experiments confirm the above considerations.

Fig. 5 shows the effect of nigericin concentration on the $S_{K/Na}$. It is seen that at low carrier concentrations, the parameter is independent of nigericin concentration and amounts to 32. At higher concentrations (more than 10^{-7} M), $S_{K/Na}$ decreased as expected.

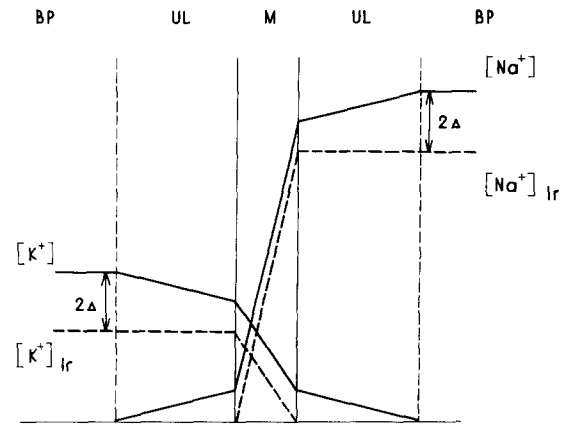

Fig. 4. The profiles of cation concentrations in the unstirred layers (UL), membrane (M) and bulk phases (BP) under the conditions of measurement of ionophore cation selectivity. Dashed lines, the concentration profiles at a low exchange rate; solid lines, the concentration profiles at a high rate.

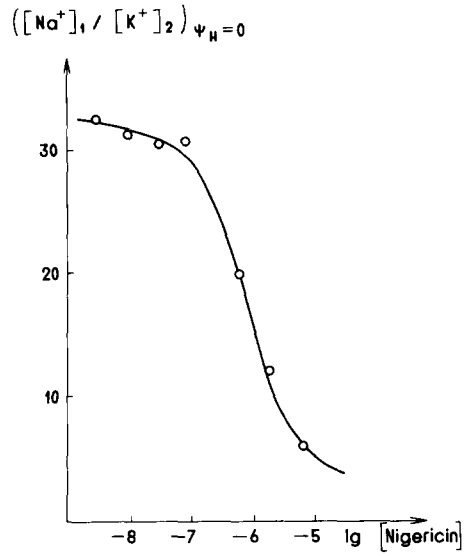

Fig. 5. Effect of the nigericin concentration on the ratio of sodium and potassium concentrations under the conditions of zero hydrogen ion flux. The sodium concentration was 40 mM at one side of the membrane.

In our previous paper [13], the measurement of nigericin $S_{K/Na}$ was carried out at different carrier concentrations and the average $S_{K/Na}$ obtained was 25. The new estimation, $S_{K/Na} = 32$, obtained at a low nigericin concentration is thought to be more accurate.

Eqn. 1 predicts that the increase of the cation concentration at a low exchange rate ($[Na^+]_{lr}$ and $[K^+]_{lr}$) should diminish the effect of Δ on $S_{K/Na}$. We measured the dependence of $S_{K/Na}$ on the sodium (potassium) ion concentration in the range 1–200 mM NaCl under high nigericin concentration conditions (3 mM). A small increase in the $S_{K/Na}$ value was found, from 7 to 11 (data not shown). This result may be explained by the exchange rate increasing with increasing the cation concentration and, therefore, the concomitant increase in the Δ value in Eqn. 1.

It is important to measure the nigericin cation selectivity for all alkali cations rather than for potassium and sodium only. Two kinds of cation combinations seem appropriate: (i) all cation selectivity parameters may be applied to potassium ion; (ii) cation selectivity parameters may be measured in couples according to the nigericin cation selectivity sequence. The measurements were carried out under the same conditions as those described in the legend to Fig. 5. The following values of $S_{Me1/Me2}$ were obtained: K^+/Rb^+, 19 ± 1; Rb^+/Na^+, 1.9 ± 0.2; Na^+/Cs^+, 8 ± 0.5; Cs^+/Li^+, 1.8 ± 0.3. The value of $S_{K/Na} = 32$ is similar to the result of multiplying the values of $S_{K/Rb}$ and $S_{Rb/Na} = 19 \cdot 1.9 = 36$. The measured value of $S_{Rb/Cs} = 16$ is similar to the result of multiplying the values of $S_{Na/Cs}$ and $S_{Rb/Na} = 1.9 \cdot 8 = 15$. However, the measurement of Li^+/K^+ selectivity requires the addition of 3 mM nigericin for the induction of a detectable J_H flux. According to Fig. 5, the exchange rate under these conditions should change the potassium ion concentration near BLM considerably. This is the reason that the measured value of $S_{K/Li} = 150$ is less than the multiplied values of $S_{K/Rb} \cdot S_{Rb/Na} \cdot S_{Na/Cs} \cdot S_{Cs/Li} = 580$. These results show that the couple cation selectivity measurements, according to the carrier sequence, have some advantages over the other method of measuring all selectivity parameters with respect to one cation.

Fig. 5 shows that for the measurement of a true value of ionophore cation selectivity ($S_{Me1/Me2}$), one should plot the dependence of apparent $S_{Me1/Me2}$ on the ionophore concentration and use the result of extrapolation to zero concentration. An experimentally different method of measuring $S_{Me1/Me2}$ may be proposed, which uses the ionophore property of slow incorporation into the membrane. The time of nigericin incorporation is much higher than the time needed to measure the J_H flux. So the real nigericin concentration in the BLM is small after its addition. According to the new approach, one should obtain the definite ratio of the concentrations of two cations which results in zero J_H flux after the addition of an ionophore. The J_H flux is measured routinely by the electrical potential recording in the presence of a protonophore.

Fig. 6 shows the time course of potential recording after nigericin addition under conditions of 40 mM NaCl at one side of BLM and different concentrations of KCl at the opposite side in different experiments. It is seen that at $[K^+] > 1.17$ mM and $[K^+] < 1$ mM the BLM potential has opposite signs. At $[K^+] = 1.1$ mM the potential remains almost unchanged after nigericin addition. These experiments give the range of $S_{K/Na}$ estimation from 34 to 40. It is noteworthy that at

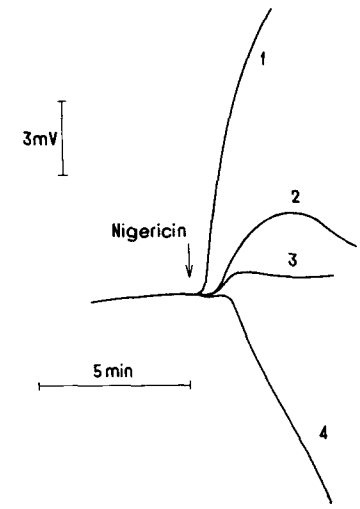

Fig. 6. Kinetics of potential generation on the membrane in the presence of a protonophore after the nigericin addition (0.7 μM) under the conditions of 40 mM NaCl at one side of BLM and 1.33 mM (curve 1), 1.17 mM (2), 1.1 mM (3), 1.0 mM (4) KCl.

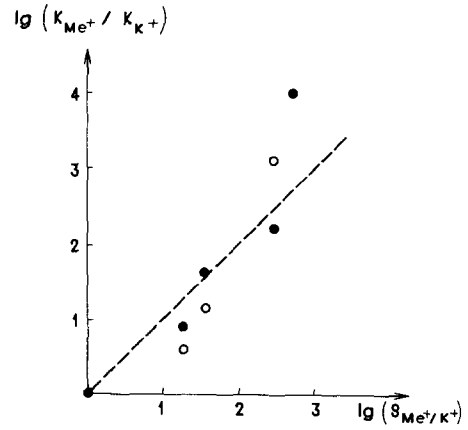

Fig. 7. The correlation of the logarithm of nigericin cation selectivity, measured on the BLM with the logarithm of cation binding constants in methanol [25] (open circles) and in the two-phase system [26] (filled circles). All magnitudes are given in relation to potassium ions.

$[K^+] > 1.1$ mM, the initially observed plus sign of the potential at the sodium side becomes negative some time later (data not shown). This result agrees well with the previously observed decrease in the measured $S_{K/Na}$ at a higher nigericin concentration (Fig. 4).

It is interesting to compare the data of nigericin cation selectivity measured on BLM with the data on the cation binding constants with the carrier in methanol [24,25] and in the two phase system [26]. It is seen from Fig. 7 that although there is a qualitative correlation between $S_{K/Me}$ and K_{Me}/K_K, the quantitative correlation is poor. This result is somewhat unexpected, since the nigericin cation selectivity is traditionally attributed to the difference in cation binding constants [1–3]. It may be proposed that the deviation from the correlation is due to the difference between the methanol and membrane interface cation binding constants. On the other hand, the contribution of the translocation rate constants of cation-nigericin complexes in $S_{K/Me}$ cannot be excluded. In general, the cation selectivity of the ionophore should also be determined by the association/dissociation rate constants of the cations with the ionophore. It can be proposed that for at least some cations the equilibrium of these reactions does not take place and these constants may contribute to the experimentally measured parameter of cation selectivity.

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