A new method of the measurement of the electrically neutral fluxes of cations through lipid bilayer membranes induced by Me^{n+}/nH^+-exchangers

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Electrically neutral ionophores (nigericin, monencin) incorporated into a planar bilayer lipid membrane (BLM) bring about hydrogen ion gradient formation in the unstirred layers of BLM if a metal ion gradient on the membrane is prepared. Under these conditions a diffusion potential of a hydrogen ion is generated after addition of a protonophore. Cation selectivity of nigericin, monencin and A23187 has been studied by means of electrical potential measurements in the presence of a protonophore and Me^{n+}/nH^+-exchangers mentioned above. The data on cation selectivity are in a good agreement with the well known results of the direct measurements of metal ion fluxes. This shows that the effect of generation of the potential on BLM in the presence of a protonophore and a Me^{n+}/nH^+-exchanger can be used for the estimation of electrically neutral ion fluxes through BLM.

Bilayer lipid membrane   *Unstirred layer*   *Nigericin*   *Monencin*   *Ionophore A23187*

1. INTRODUCTION

It has been shown that electrically neutral ionophores (nigericin, monencin) incorporated in a planar lipid bilayer membrane (BLM) bring about hydrogen ion gradient formation in the unstirred layers of BLM if a metal ion gradient on the membrane is prepared [1]. A diffusion potential of hydrogen ion is generated under these conditions after addition of the protonophore [1]. Authors in [2] obtained similar results, observing the generation of the potential on BLM in the presence of monazomycin and A23187 after the formation of calcium gradient on BLM. On the basis of these results we developed a new method of measurement of electrically neutral fluxes through BLM induced by Me^{n+}/nH^+-exchangers. For this purpose

Abbreviations: BLM, bilayer lipid membrane; TTFB, tetrachlorotrifluoromethylbenzimidazole; Mes, morpholinoethane sulfonic acid; Tris, tris-(hydroxymethyl) aminomethane

we investigated the cation selectivity of nigericin, monencin and A23187, and showed that the selectivity of the above exchangers obtained by this method agrees well with the cation selectivity determined by conventional techniques.

2. MATERIALS AND METHODS

Lipid bilayer membranes were formed by the standard technique [3] on a 0.4-mm hole in a Teflon partition. The membrane-forming mixture contained 20 mg egg lecithin and 20 mg cholesterol/ml *n*-decane. The formation of the membrane was controlled visually and also by measurement of its electric capacity [4]. A magnetic stirrer was used for mixing solutions in the cell; the rate of stirring was constant in all experiments. Potentials were measured by means of calomel electrodes connected with solutions by agar–agar bridges filled with 0.01 M KCl. The potential was controlled with a Keithley 301 amplifier connected to a recorder. The measure-

ments were carried out at room temperature. Antibiotics, nigericin, monencin, A23187 and protonophore tetrachlorotrifluoromethylbenzimidazole (TTFB), were added to the solutions on both sides of BLM.

3. RESULTS

Fig.1 shows the magnitude of the potential on BLM detected upon the formation of a metal ion (K^+, Na^+ or Ca^{2+}) gradient as a function of the concentration of a corresponding ionophore: nigericin, monencin and A23187. The magnitude of the potential is practically proportional to the concentration of nigericin and monencin and depends quadratically on the concentration of

Fig.2. The magnitude of the potential on BLM as a function of the logarithm of nigericin concentration (in M) at constant gradients of K^+, Rb^+, Na^+, Cs^+, Li^+ (10 mM on one side and 0.3 mM on the other) in the presence of 10 μM TTFB. The solution was: 1 mM Mes; 1 mM Tris, pH 7.0.
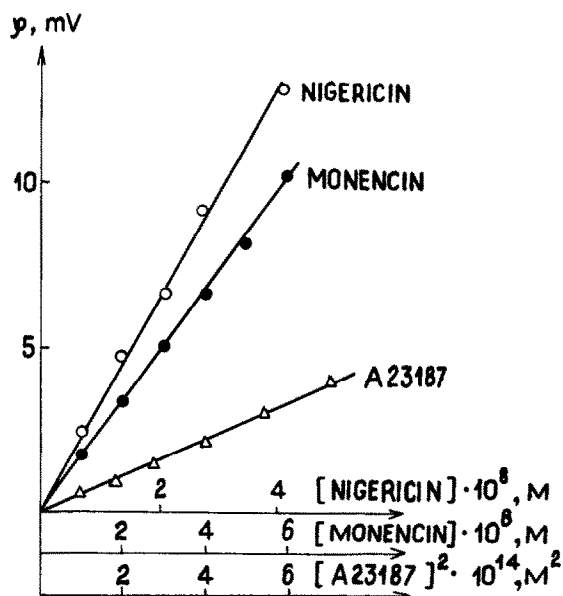
Fig.1. The magnitude of the potential on BLM as a function of the concentration of nigericin, monencin and square concentration of A23187 in the case of KCl, NaCl, and CaCl₂ gradients, respectively (10 mM KCl on one side and 0.3 mM KCl on the other for nigericin and 10 mM NaCl and 10 mM CaCl₂ on one side of the membrane for monencin and A23187, respectively) in the presence of 10 μM TTFB. The potential is positive on that side of the membrane where the concentration of salt is lower. The solution in the experiment with nigericin was: 1 mM Mes; 1 mM Tris, pH 7.0. The solution in the experiment with monencin and A23187 was: 1 mM Mes; 1 mM Tris; 100 mM Cholin.Chlorid, pH 7.0.
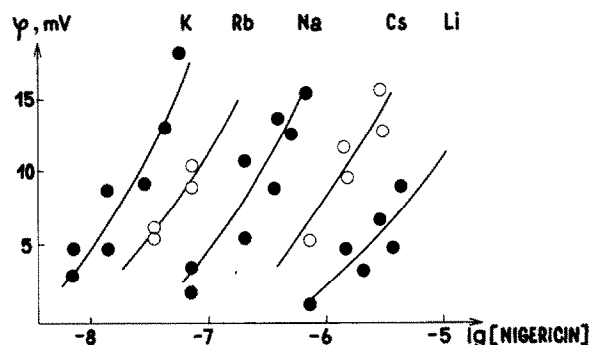
A23187. The magnitudes of potentials, which are shown in fig.1 and further, reach their steady-state values 20 min after the addition of a ionophore. Control experiments have shown that the steady-state values of potentials are constant during at least several hours.

The study of cation selectivity of Me^{n+}/nH^+-exchangers was carried out at constant cation gradients on BLM (10 mM on one side and 0.3 mM on the other). The concentration of antibiotic which gives rise to the potential of 10 mV on BLM was taken as a criterion of cation selectivity of the antibiotic. The following series of
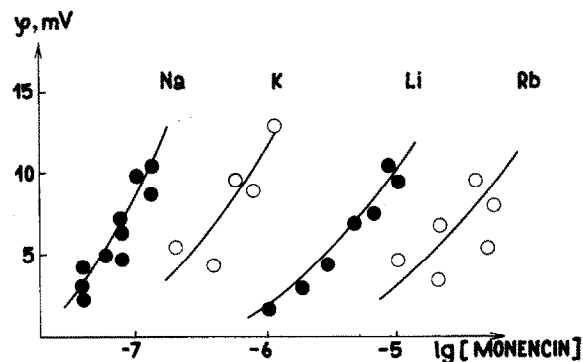
Fig.3. The magnitude of the potential on BLM as a function of the logarithm of monencin concentration (in M) at constant gradients of K^+, Rb^+, Na^+, Li^+ (10 mM on one side and 0.3 mM on the other) in the presence of 10 μM TTFB. The solution was as in fig.2.
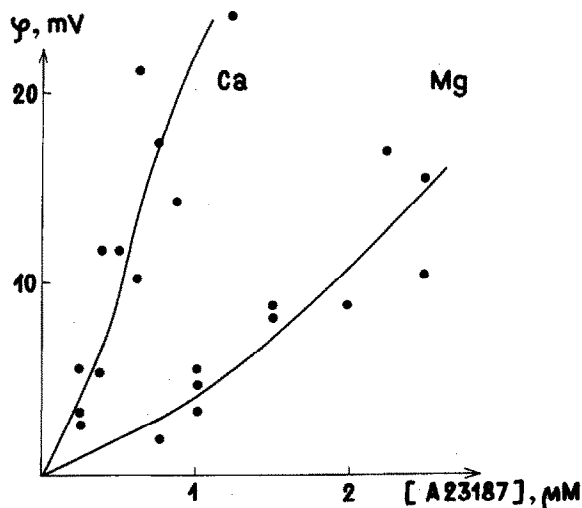
Fig.4. The magnitude of the potential on BLM as a function of the concentration of A23187 at constant gradients of Ca^{2+} and Mg^{2+} (10 mM on one side and 0.3 mM on the other) in the presence of 10 μM TTFB. The solution was as in fig.2.

monovalent cation selectivity was obtained for nigericin: $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$ (fig.2). It has been shown previously that nigericin increases the conductivity of BLM [5,6] and thereby can decrease in principle the real magnitude of the diffusion potential of H^+. However, under our conditions (fig.2) the conductivity in the presence of 10 μM TTFB appeared to be some orders of magnitude higher than the conductivity induced by nigericin.

Similarly a series of cation selectivity of monencin was obtained (fig.3): $Na^+ > K^+ > Li^+ > Rb^+ > Cs^+$. The addition of extremely high concentrations of monencin (10^{-4} M) did not induce generation of the potential on BLM if CsCl gradient is prepared. Fig.4 shows similar data for A23187. It can be shown that the antibiotic has higher selectivity for calcium than for magnesium.

4. DISCUSSION

It has been shown that the magnitude of an electrically neutral flux of sodium through BLM is proportional to the concentration of monencin [7]. Elsewhere it was shown that an electrically neutral flux of calcium depends quadratically on the concentration of A23187 [2]. One can see from fig.1

that the relationship between the potential on BLM and concentration of antibiotics is consistent with the literature. We have found no data in the literature concerning the dependence of potassium flux on the concentration of nigericin. Fig.1 shows that it is proportional to the concentration of nigericin.

We compared a series of cation selectivity of nigericin established in [8,9] with a series of cation selectivity obtained in our experiments. The method of BLM potential measurements proved to give the same series of cation selectivity as direct ion flux measurements: $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$. A series of cation selectivity of monencin coincided with a series of cation selectivity obtained in [8]: $Na^+ > K^+ > Li^+ > Rb^+ > Cs^+$. It should be noted that a series of cation selectivity of monencin varies [8-11]. The comparison of the results of these studies makes it possible to write the following incomplete series of cation selectivity of monencin which is in agreement with the data of all authors and our own measurements: $Na^+ > K^+ > Li^+, Rb^+, Cs^+$. The determination of the fluxes of calcium and magnesium in the presence of A23187 revealed that the antibiotic has selectivity for calcium over magnesium [12]. Our method leads to the same ratio of specificity (fig.4).

The agreement between the data obtained by the method of the BLM potential measurements and the literature indicates that the values of the potentials on BLM under our conditions are proportional to the values of the electroneutral fluxes of ions. This allows us to propose the measurement of the potentials on BLM in the presence of a couple Me^{n+}/nH^+-exchanger–protonophore as a method for the determination of the relative cation fluxes induced by Me^{n+}/nH^+-exchangers.

Our results obtained in [13] make it possible to determine not only the relative values of electroneutral fluxes induced by Me^{n+}/nH^+-exchangers, but to estimate the absolute values of ion fluxes as well. Since equal fluxes of undissociated forms of acids RCOOH by definition cause the generation of equal potentials on BLM, irrespective of the kind of RCOOH, the dependence of the BLM potential, induced by the gradient of CH_3COONa, on the acetate flux through BLM (fig.5), J, can be used for the estimation of the values of the fluxes for Me^{n+}/nH^+-exchangers (this conclusion is correct if the gradient pH in the unstirred layers equal-

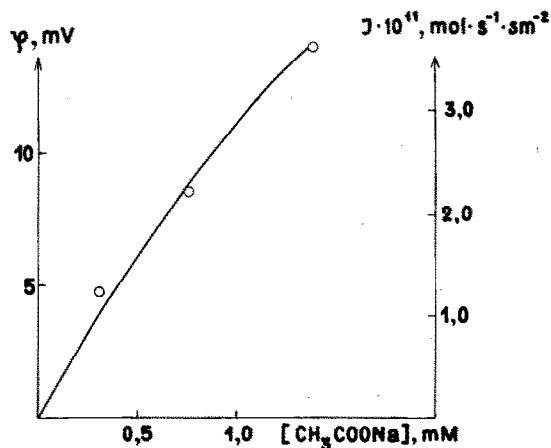Fig.5. The magnitude of the potential on BLM as a function of the gradient of the concentration of sodium acetate in the presence of $10 \mu M$ TTFB. Sodium acetate was added to one side of the membrane; the potential is positive on that side of the membrane where the salt was added. The solution was: 1 mM Mes; 1 mM Tris; 100 mM KCl, pH 7.0.

ly affects the transmembrane fluxes of CH_3COOH and Me^{n+}/nH^+-exchangers. This assumption is valid at low pH gradients). The value of J was calculated as follows [13]:

$$1/J = 1/P^{UL}_{CH_3COO^-}[CH_3COO^-] +$$

$$1/P^{M}_{CH_3COOH}[CH_3COOH]$$

where:
$P^{UL}_{CH_3COO^-}$ is the permeability of the unstirred layers for CH_3COO^- (1×10^{-3} cm/s [13]);
$P^{M}_{CH_3COOH}$ is the permeability of the membrane for CH_3COOH (3.6×10^{-3} cm/s [13]).

The determination of electroneutral ion fluxes through BLM by means of the measurement of the potential in the presence of a protonophore has a number of advantages: (i) experimental procedure is the same for all Me^{n+}/nH^+-exchangers and ca-

tions; (ii) the rapidity of the flux measurement; (iii) the high sensitivity of the method. Owing to these properties the new method can be useful for the search and study of small amounts of unstable natural Me^{n+}/nH^+-exchangers.

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