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Proton dissociation from nigericin at the membrane–water interface, the rate-limiting step of K^+/H^+ exchange on the bilayer lipid membrane

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The rate of K^+/H^+ exchange through bilayer lipid membranes (BLM) induced by nigericin was measured by the method of pH gradient offset according to Antonenko, Yu.N. and Yaguzhinsky L.S. [(1990) Biochim. Biophys. Acta 1026, 236–240]. It was shown that under the conditions of high potassium ion concentration the rate of nigericin-mediated K^+/H^+ exchange increased with an increase in the concentrations of such buffer compounds as citric acid and MES. The concentration dependence was different for citrate and MES. The buffer concentration effect was absent at low potassium ion concentrations. Citrate increased the rate of K^+/H^+ exchange being added to the side of BLM where the K^+ concentration was higher and had no effect at the opposite side. At high KCl and citrate concentrations, the rate of K^+/H^+ exchange was about 6 times lower in D_2O when compared to H_2O solutions. It is concluded that under certain experimental conditions the overall rate of the K^+/H^+ exchange induced by nigericin is determined by the rate of proton dissociation from nigericin at the membrane–water interface.

Bilayer lipid membrane; Nigericin; Buffer concentration effect

1. INTRODUCTION

Problems concerning the proton-transfer reactions at the membrane–water interface have been widely discussed in the literature [1–9]. It was shown by the method of rapid kinetics that the rate of proton release from bacteriorhodopsin increased with the increase in buffer concentration [7]. A similar effect of the buffer was shown to be inherent to the dehydration reaction of HCO_3^- catalyzed by carbonic anhydrase [8] and in some model systems [1–3,5].

In another series of studies it was shown that the flux of protons [9,10] and other cations [11] across the gramicidin channel was limited by the diffusion of cations in the zones of the solution near the entrance of the channel. Different buffers differently enhanced the channel conductance, indicating structure specificity. These observations showed that the proton-transfer reaction at the membrane interface can proceed with lower speed than the process of proton translocation across the membrane. Riddell and co-workers [12,13] showed that under certain conditions the rate of nigericin-mediated K^+/K^+ and Na^+/Na^+ exchange was limited by the step of cation dissociation from nigericin at the membrane interface. It is noteworthy that the majority of mathematical models of carrier-mediated ion transport assume that there is an equilibrium of the reactions of cation–carrier interactions at the membrane.

The present work deals with the role of proton-transfer reactions in the nigericin-mediated K^+/H^+ exchange across the planar bilayer lipid membrane (BLM). In particular the effect of such buffers as citrate and MES as well as the effect of D_2O on the rate of K^+/H^+ exchange was studied.

2. MATERIALS AND METHODS

BLM was formed on a Teflon partition, 0.4 mm in diameter, by a conventional method [14]. A membrane-forming solution contained 20 mg phosphatidylcholine from soy beans (azolectin, Sigma) and 10 mg cholesterol (Serva) in 1 ml of *n*-decane. The thinning of the BLM was observed both visually and by measuring its capacity. The main element of the electrical scheme was a Keithley 301 amplifier. The experiments were carried out at room temperature (21–23°C). Ethanol solution of tetrachlorotrifluoromethylbenzimidazole (TTFB) was added at both sides of the BLM. Nigericin was from Calbiochem; the buffers citrate, MES, Tris were from Serva. D_2O contained less than 1.5% H_2O. Other chemicals were from Reachim (USSR).

The hydrogen ion electroneutral flux (J_H) was measured by the method which was described earlier [15]. Briefly, the pH gradient in the unstirred layers was reduced to zero by means of the formation of a gradient of acetic acid across the BLM. Acetate concentrations were converted into J_H according to [15]. The method enabled us to measure J_H in the absence of a pH gradient in the unstirred layers. An example of these kind of measurements is presented in Fig. 2.

3. RESULTS

Fig. 1. shows the effect of the citrate and MES concentration on the nigericin-mediated hydrogen ions flux

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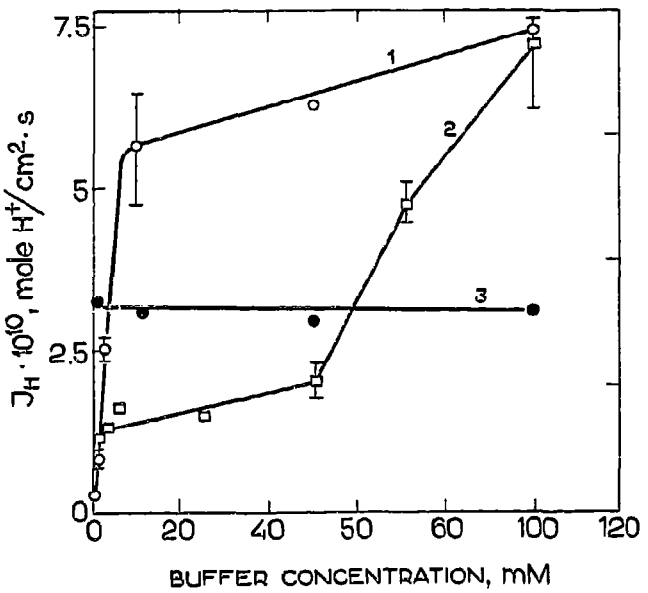

Fig. 1. Effect of citrate buffer (curves 1,3) and MES buffer (curve 2) on nigericin-mediated electroneutral flux of hydrogen ions (J_H). Measurements were carried out with two concentrations of KCl: 190 mM at one side and 100 mM at the other (curves 1,2) or 30 mM at one side and 10 mM at the other (curve 3). The concentration of choline chloride varied in a way that total concentration of choline chloride and of the buffer was 100 mM. pH 6.5. Nigericin concentration in the membrane-forming solution was 180 μM, TTFB-10 μM.

through BLM at high (curves 1 and 2) and low (curve 3) potassium-ion concentration. At low KCl concentration the flux was independent of the buffer concentration (curve 3). It is worth noting that the BLM voltage measured in the presence of a protonophore decreased considerably with an increase citrate concentration (data not shown). At high KCl concentration, the behavior of the system of the system changed; an increase in the citrate or MES concentration increased the rate of K^+/H^+ exchange 5–7 times (curves 1 and 2). Citrate enhanced the flux in the 1–10 mM range and did not affect the flux at concentrations higher than 20 mM. MES increased the rate of K^+/H^+ exchange at significantly higher concentrations (60–100 mM) without an obvious saturation of the rate even at 100 mM MES. It is noteworthy that these measurements were carried out in the absence of pH gradients on the membrane (see section 2).

It was shown in control experiments in the absence of nigericin that the increase in the buffer concentration (1–50 mM) led to a proportional (within 20% deviation) increase in acetate concentration (added at one side of BLM) needed to generate the BLM potential of definite value. In an other set of control experiments in the presence of nigericin it was shown that the J_H value was independent of the background acetate concentration at both sides of the membrane.

Fig. 2 presents the recording of the BLM potential under the conditions where the rate of K^+/H^+ exchange was measured after the addition of citrate buffer at the *cis* and *trans* side of BLM (*cis* side was the side where 90 mM KCl was added). It was seen that citrate had no effect at the *trans* side while the addition of citrate at the *cis* side increased the flux considerably.

The dependence of J_H on the citrate concentration in H_2O (top curve) and D_2O (bottom curve) solutions is presented in Fig. 3. The use of D_2O instead of H_2O resulted in 5- to 10-fold inhibition of the flux at 10–100 mM citrate.

4. DISCUSSION

Two limiting cases of nigericin-mediated ion transport are theoretically possible: (1) the ion–ionophore translocation through the membrane is a rate limiting step; (2) the ion–ionophore association–dissociation reactions at the surface of the membrane are rate limiting. The kinetic data available in the literature were explained in terms of the dissociation of potassium ions from nigericin being rate-limiting [12,13] as well as the translocation of the ion–ionophore complex being rate limiting [16,17]. In the present work the conditions were found where the rate of K^+/H^+ exchange was changed by factors acting specifically on proton-transfer reactions at the membrane interface. Namely the effect of

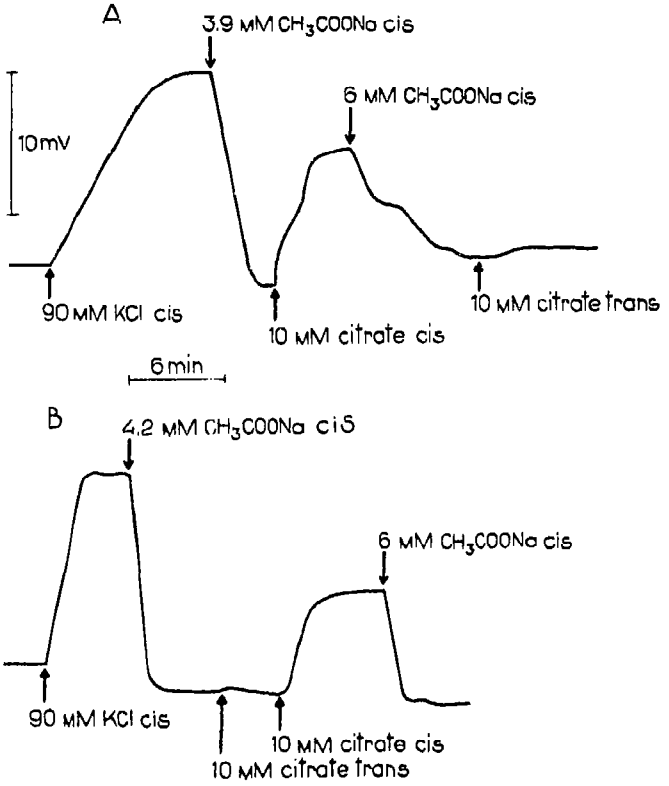

Fig. 2. Effect of citrate buffer added at different sides of the BLM on the J_H induced by nigericin (190 μM in the membrane forming solution). A. The addition of 10 mM citrate at the *cis* and some time later at the *trans* side of the BLM. B. Reversed series of additions. The solution was 1 mM citrate, 100 mM choline chloride, 10 μM TTFB, pH 6.5, and 190 mM KCl at the *cis* and 100 mM at the *trans* side of the membrane.

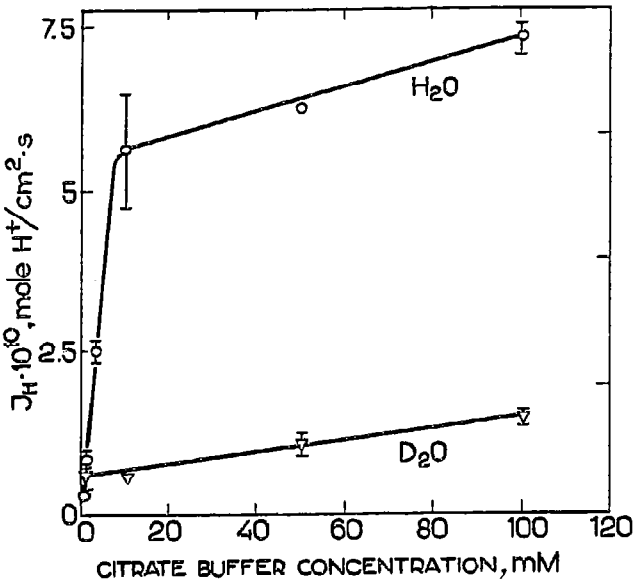

Fig. 3. Effect of D_2O (curve 2) compared to H_2O (curve 1) on the nigericin-mediated J_H at different citrate concentrations. Conditions are the same as in the legend to Fig. 1, curve 1.

buffers (citrate and MES) as well as of D_2O were studied. The results obtained enable us to conclude that the rate-limiting step under our conditions was the proton-transfer reaction with nigericin at the BLM interface. The observation that citrate increased the flux being added at one side only indicates that it is the transfer of a proton from nigericin to the basic form of a buffer molecule that limits the process.

In fact it should be concluded from the analysis of the data presented in Fig. 1 that at low concentrations of citrate and MES the rate of K^+/H^+ exchange is limited by the dissociation of a proton from nigericin and its transfer to H_2O. At 1 mM citrate, the use of D_2O instead of H_2O resulted in only a 30% reduction of the flux. At high citrate concentrations the rate of K^+/H^+ exchange increased in parallel with the increase in isotope effect which attained 10 at 10 mM citrate. Obviously, at high citrate concentrations the limiting step of the process changed, so that the transfer of protons from nigericin to the basic form of citrate became rate-limiting.

In the case of MES buffer the increase in the rate of K^+/H^+ exchange took place at much higher concentrations. Quantitative differences in the effects of these two membrane-insoluble buffer compounds show that there are specific interactions of basic forms of citrate or MES with the acidic form of nigericin at the BLM interface which result in proton transfer from nigericin to a buffer molecule.

The system used for the measurements of K^+/H^+ exchange included the titration by acetic acid, the anion of which exhibits the properties of a strong base and therefore can interact with nigericin. In general, this disadvantage of the system can make the interpretation of the data difficult. Three sets of control experiments showed that acetate had no effect on the properties of the system. 1. Acetate did not increase the buffer capacity of the solutions under our experimental conditions by more than 5%. 2. It was also shown that the results of the measurements did not depend on the background concentration of acetate at both sides of the BLM (see section 3).

As mentioned in the introduction, the study of the conductance of gramicidin channels showed the effect of the buffer structure on the single channel current [9,10]. This effect was similar to ours, however, we studied the ion carrier nigericin, the mechanism of action of which is completely different from that of gramicidin. This similarity indicates that the properties of proton interaction with an ion-transporting system are determined by the properties of the membrane–water interface but not by the properties of a transport system.

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