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Increase of local hydrogen ion gradient near bilayer lipid membrane under the conditions of catalysis of proton transfer across the interface

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Abstract The measurements of pH profiles in the unstirred layers (USLs) near planar bilayer lipid membranes (BLM) were applied for the evaluation of the hydrogen ion fluxes which were induced by nigericin in the presence of potassium ion gradients. It was shown that at high concentrations of KCl the increase in the concentration of citrate buffer caused an anomalous effect, namely, an increase in the local pH shifts in the USLs. The hydrogen ion flux rose 50 times upon the increase in the citrate concentration from 1 mM to 20 mM. Phosphate stimulated the flux 7 times under these conditions. In agreement with our previous results, at low KCl concentrations, when the process is limited by the K^+-nigericin interaction, an increase in the buffer concentration led to a reduction of the local pH shifts, under these conditions the usual concentration dependence was observed. The data obtained favor the model implying the existence of the kinetic barrier for proton transfer at the membrane-water interface.

Key words: Proton coupling; Bilayer lipid membrane; Unstirred layer; Nigericin; Buffer; Proton exchange

1. Introduction

Proton transfer across membranes attracts much attention as it is relevant to the mechanism of membrane bioenergetics based on the vector proton transfer reactions across membranes [1]. Recently, a series of research works have focused on the mechanism of proton transfer through membrane-water interfaces [2–12]. Specifically, lateral proton conduction along the phospholipid monolayer [3,4,13] as well as proton transfer across planar bilayer lipid membranes (BLM) [5–9,14,15] and related systems [10–12] were studied. Some of these studies as well as two recent works on the patches of purple membranes of *Halobacterium halobium* [16,17] support the existence of a kinetic barrier for proton transfer at the membrane-water interface, while some other works argue against the existence of such a barrier [6,8,9].

The present work deals with the study of the process of proton transfer across planar BLM induced by non-electrogenic cationic carrier nigericin and the action of citrate and phosphate molecules on it. Previously, the effect of buffers on this system was studied by measurements of protonophore-dependent potentials in the absence of local pH gradients in the unstirred layers (USLs) [18]. pH gradients were removed by the addition of sodium acetate on one side of the BLM. It was shown that several buffer compounds (MES, phosphate, citrate) increased the rate of proton transfer across the interface, citrate being the most efficient. In the present study the pH shifts in the USLs were measured directly by means of pH microelectrodes according to [19]. A goal of the present work was to study the catalyzing effect of citrate in the presence of local pH changes in the USLs. Conditions were found where the flux stimulating effect of citrate was so high that it caused a increase in the pH change in the USLs. In other words, the catalyzing action of citrate predominated under these conditions over the usual buffer effect which led to the diminishing of local pH gradients in the USLs.

2. Materials and methods

The BLMs were formed by a conventional method [20] on a hole, 0.4 mm in diameter, in the center of a PTFE diaphragm dividing a chamber into two compartments. The membrane forming solutions, unless otherwise stated, contained 20 mg azolectin from soybeans (phosphatidylcholine type II-S, Sigma) in 1 ml of *n*-decane (Merck). The bathing solutions were agitated with magnetic bars at the same velocity in all experiments.

Nigericin-mediated hydrogen ion flux brought about pH gradients within the USLs near BLM which were measured by means of a glass-insulated tip-sensitive antimony microelectrode according to [19] with small modifications. The sensor was driven perpendicular to the surface of the BLM through the open space in the rear part of the cell. The process of BLM formation and pH microelectrode movements were observed through the transparent window in the front side of the cell. A smooth approach of the microelectrode to the membrane is carried out using a hydraulic system attached to the reversible drive. Voltages were recorded by microprocessor ionalyzer/901 (Orion Research) connected to a computer through an interface card.

3. Results

Fig. 1 shows pH profiles in the USLs near BLM induced by the difference in KCl concentration (190 mM and 100 mM, *cis* and *trans* sides, respectively) in the presence of 0.9 mM nigericin in the membrane-forming solution. Profiles were measured at 1 mM (curves 1, 2) and 10 mM (curves 3, 4) citrate; at the *cis* (curves 1, 3) and *trans* (curves 2, 4) sides of the BLM. The profiles measured at 10 mM citrate have higher amplitudes compared to 1 mM buffer. This result shows that the action of citrate on the transmembrane flux predominates over the usual action of the buffer, namely the decrease in the local pH shifts in the USLs.

It is worth noting that the effect of citrate depended on the lipid composition in our experiments. In the case of diphytanoyl phosphatidylcholine the pH shifts in the USLs were almost independent of the citrate concentration and their amplitudes were on average 0.063 pH at each side. Accordingly,

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Abbreviations: TTFB, a protonophore, tetrachlorotrifluoromethylbenzimidazole; BLM, bilayer lipid membrane; USL, unstirred layer near the BLM

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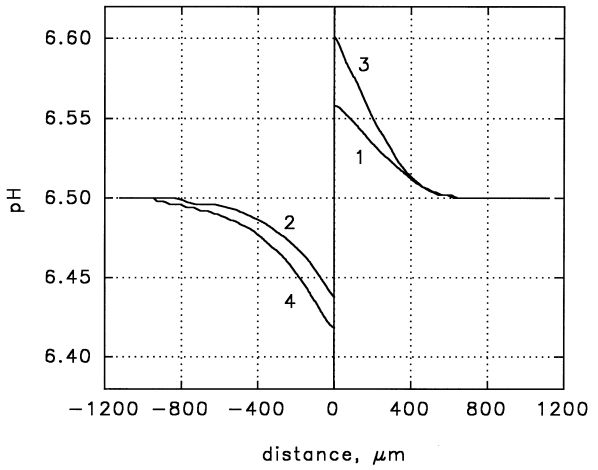

Fig. 1. pH profiles in the *cis* unstirred layer (left, 190 mM KCl) side and *trans* (right, 100 mM KCl) unstirred layer near the BLM. Citrate concentration was 1 mM (curves 1, 2) and 10 mM (curves 3, 4). The solution was: 1 (10) mM citrate, 100 mM choline chloride, pH 6.5. Nigericin concentration in the membrane-forming solution was 0.9 mM.

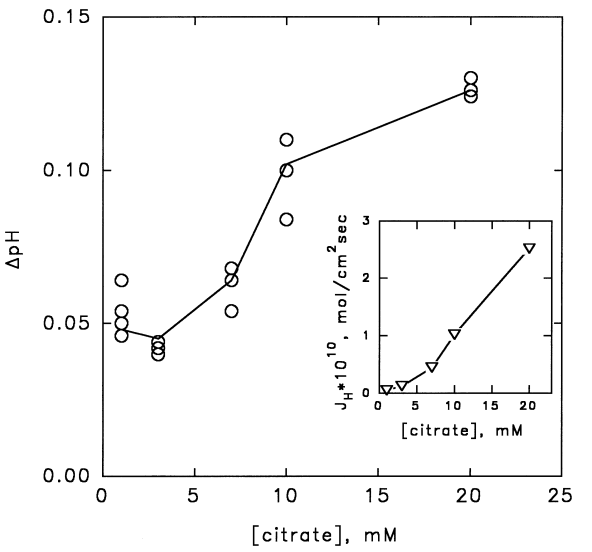

Fig. 2. The effect of citrate concentration on the amplitude of the *trans* pH profile near the BLM. The conditions were as in Fig. 1. Inset: H^+ fluxes were plotted instead of the amplitudes of pH profiles.

the average flux stimulation was 9.5-fold upon increasing the citrate concentration from 1 to 10 mM.

The dependence of the amplitude of the pH shift on the citrate buffer concentration is shown in Fig. 2. The effect has S-type concentration dependence, rising with the increase in the citrate concentration. The values of transmembrane hydrogen ion fluxes were calculated from the pH profiles according to [21] and are presented in the inset in Fig. 2. The rise of the citrate concentration from 1 mM to 20 mM led to a 50-fold increase in the flux. The stimulating effect of phosphate was 7-fold under these conditions and the values of pH shifts were lower at high phosphate concentrations than at low concentrations.

At low KCl concentrations, the increase in the concentration of the citrate buffer led to a decrease in the pH shifts. Fig. 3 illustrates the pH profiles at the *trans* side of BLM at 10 mM (*cis*) and 1 mM (*trans*) KCl.

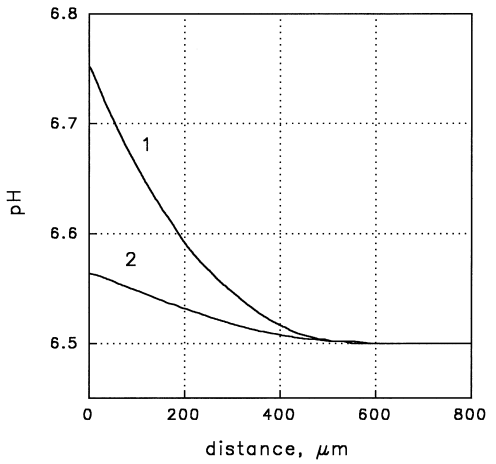

Fig. 3. pH profiles in the *trans* unstirred layer near the BLM measured at low KCl concentration (10 mM *cis* and 1 mM *trans*). Citrate concentration was 1 mM (curve 1) and 10 mM (curve 2). The solution was: 1 (10) mM citrate, 100 mM choline chloride, pH 6.5. Nigericin concentration in the membrane-forming solution was 0.04 mM.

4. Discussion

As was mentioned above, previously the study of the catalytic effect of buffers on nigericin-mediated fluxes required the presence of a gradient of lithium acetate to diminish the change of the hydrogen ion flux due to the alteration of pH shifts in the USLs upon increasing the buffer concentration [18]. In the present work conditions were found where citrate increases the pH gradient in the USLs (and the transmembrane pH gradient as well). Thus, the effect of citrate on the stimulation of the transmembrane hydrogen ion flux cannot be attributed to the decrease of the pH gradient on the BLM as can be assumed in the case of low KCl concentrations. The difference in the effect of citrate at high and low KCl concentrations (Fig. 3) can be explained by the change of the rate-limiting step of the total process of the K^+/H^+ exchange. Namely, at low KCl concentrations the reaction of potassium ions with the acid form of nigericin becomes the rate-limiting step while at high KCl concentrations proton transfer reactions determine the total transport process.

Considering the action of citrate on the nigericin-mediated hydrogen ion flux (Fig. 1), two different cases should be taken into account. (i) Citrate molecules interact directly with nigericin, accelerate the proton transfer and consequently the rate of nigericin turnover. (ii) There is an additional stage of proton transfer from nigericin to surface proton-binding sites of phospholipids [5,22]. Citrate catalyzes the proton transfer from these binding sites. This is possible in the case of the existence of a kinetic barrier for the proton transfer at the membrane-water interface.

The second case is supported by the following observations. (i) As was shown in our previous work [5], nigericin induces the formation of a difference in the boundary potentials on bilayers containing anionic phospholipids which implies the appearance of a transmembrane gradient of hydrogen ions

closely associated with the negatively charged membrane surface. (ii) The value of the stimulating effect of the buffer also depends on the phospholipid composition.

Thus, the present work introduced a new experimental approach showing the large flux-stimulating effect of citrate on nigericin-mediated proton transport. The system studied supports the existence of a kinetic barrier for proton transfer at the membrane surface which can be reduced upon increasing the citrate concentration. This barrier controls the transmembrane hydrogen ion flux not only in the absence of a pH gradient on the BLM, but in the presence of the gradient as well.

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