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Combination of the electrogenic ionophores, valinomycin and CCCP, can lead to non-electrogenic K^+/H^+ exchange on bilayer lipid membranes

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Abstract

The method of pH shift measuring by means of a pH microelectrode was applied to measure hydrogen ion fluxes across a planar bilayer lipid membrane (BLM) in the presence of the potassium ion ionophore, valinomycin, and a protonophore, carbonylcyanide *m*-chlorophenylhydrazone (CCCP), under conditions of the voltage clamp. The voltage dependence of the flux was determined to be in the range of ± 150 mV under the conditions of both symmetrical KCl as well as a KCl gradient across the BLM. Surprisingly, at a clamped zero voltage on BLM a significant hydrogen ion flux was observed in the presence of a KCL gradient and both valinomycin and CCCP. This finding was interpreted as a result of induction of non-electrogenic K^+/H^+ exchange in the presence of valinomycin and CCCP, presumably through the formation of electrically neutral complexes of these two ionophores and K^+ (H^+) ions: valinomycin–K^+–$CCCP^-$ and/or possibly valinomycin–$CCCP^-$–H^+.

Key words: Bilayer lipid membrane; Unstirred layer; K^+/H^+) exchange; Valinomycin; Protonophore CCCP; pH microelectrode

1. Introduction

Ionophores, and protonophores in particular, are widely used to study the mechanisms of ion transport through natural membranes and reconstituted systems. The most frequently used ionophores are the potassium ion electrogenic ionophore, valinomycin, and the proton electrogenic ionophore (protonophore), carbonylcyanide *m*-chlorophenylhydrazone (CCCP); often these ion carriers are added simultaneously (see for example [1]). The individual mechanisms of CCCP-mediated proton transport and valinomycin-mediated potassium transport are well studied (for reviews see [2,3]).

Simultaneous addition of valinomycin and CCCP leads to the induction of K^+/H^+ exchange via the formation of an electrical potential across the membrane. This electrical coupling of fluxes can be opposed to the coupling of other types which can be induced by ionophores of the nigericin family and can proceed without a potential across the membrane (non-electrogenic K^+/H^+ exchange [4]). It is shown in the present work that the combined action of valinomycin and CCCP can lead to the induction of non-electrogenic K^+/H^+ exchange across a bilayer lipid membrane (BLM). This phenomenon can be explained by the existence of complexes of valinomycin and CCCP, the formation of which was demonstrated earlier in several laboratories [1,8].

2. Materials and methods

BLM was formed on a 0.9 mm hole in a Teflon partition, by a conventional method [5]. A membrane-forming solution contained 20 mg phosphatidylcholine from soy beans (type IV-S; Sigma) and 10 mg cholesterol (Serva) in 1 ml of *n*-decane. The thinning of the BLM was observed visually. The experiments were carried out at room temperature (21–23°C). The protonophore, CCCP (Serva), the ionophore, valinomycin (Serva), and gramicidin (Sigma) were added to both sides of the BLM and the solutions were stirred carefully. Measurements were carried out in the absence of stirring at least 5 min after the additions and/or voltage changes. The bathing solution was 1 mM MES, 1 mM Tris, 100 mM choline chloride, pH 7.0. The measurements of pH shifts near the BLM were carried out by means of a pH microelectrode: the system was described in detail in our previous work [6,7]. The microelectrode speed was 5 μm/s. The sensitivity of microelectrode was 50 mV per pH unit. pH shifts were induced by imposing either an electrical potential or potassium ion gradients across the BLM.

We used a three electrode system for the simultaneous measurement of pH shifts and the BLM potential clamp. The pH microelectrode and a common reference electrode (connected to a signal ground) were placed in the rear part of the cell whereas the second reference electrode was in the front part. Voltages were held constant (clamped) by the voltage source with a low output resistance (G6-15; Russian Federation). Membrane voltages indicated in figures represent the potentials of the front compartment with respect to the zero potential of the rear compartment. The pH shifts in an unstirred layer were expressed as the difference between the local near-membrane pH and the bulk pH in the rear compartment. In order to impose a KCl gradient, KCl was added to the front compartment.

3. Results and discussion

Fig. 1 (curve 4) shows the pH profile near the BLM induced by the addition of valinomycin and CCCP in the presence of a KCl gradient across the BLM. The BLM

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Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; MES, 2-[*N*-morpholino]ethanesulfonic acid; BLM, bilayer lipid membrane.

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voltage was zero. As seen from Fig. 1, curve 4, when the electrode approached the membrane (up to the arrow), the pH microelectrode voltage started to change. However, at a certain distance the electrode movement brought about a steep change in pH electrode potential of the opposite sign. We showed [6,7] that this phase was due to the interaction of the electrode with the membrane and was not associated with a pH change near the membrane. At the moment marked by an arrow, the electrode was withdrawn. Valinomycin or CCCP alone were ineffective in inducing pH shifts (curves 1 and 2). Curve 3 shows that if the channel former, gramicidin, is substituted for valinomycin, at the concentration which induces a similar BLM current, the pH shifts do not arise.

The formation of pH shifts in the presence of valinomycin and CCCP under the conditions of a KCl gradient and zero BLM voltage (Fig. 1) can be explained by the induction of non-electrogenic K^+/H^+ exchange. In fact the coupling of hydrogen and potassium ions via the electrical potential should be ruled out under voltage-clamp conditions.

Control experiments showed that the membrane resistance in the presence of valinomycin was 3 MΩ, for CCCP it was 50 MΩ, and for both ionophores it was 1.8 ΩM. The resistance of the system without the membrane (with agar bridges) was 10 kΩ, thus showing that under our experimental conditions the applied voltage drops across the membrane rather than in the buffer solutions.

Fig. 2 shows the dependence of the maximum pH shifts, A_{max} (see Fig. 1), on the BLM voltage in the absence (curves 1–3) and in the presence (curve 4) of a KCl gradient across the membrane. It can be seen that the stimulating effect of valinomycin on the CCCP-mediated proton flux is observed across the whole range of voltages applied (curves 1–3). It can also be seen, in accordance with Fig. 1 (curve 4), that at zero voltage a significant pH shift is observed under the conditions of a KCl gradient across the BLM, confirming that there is a transmembrane proton flux across the membrane. Obviously the driving force for the flux is the KCl gradient since under symmetrical KCl conditions there was no hydrogen ion flux (Fig. 2, curve 3). It is interesting to note that the flux reaches a zero value at BLM voltages considerably higher than +60 mV (about + 80 mV) under the conditions of a 6 mM:60 mM KCl gradient across the membrane (Fig. 2, curve 2). This observation supports the idea of electrically neutral exchange of potassium ions for protons since at + 60 mV the electrochemical gradient of potassium ions is zero. The inset presents the dependence of A_{max} on the KCl gradient across the BLM under the conditions of zero applied voltage.

Fig. 3 shows the effect of CCCP concentration on the pH shifts at different concentrations of valinomycin under the conditions of a KCl gradient and zero BLM voltage. It can be seen that valinomycin increases the amplitude of the dose-dependent curve. In addition, the increase in valinomycin concentration raises the saturat-

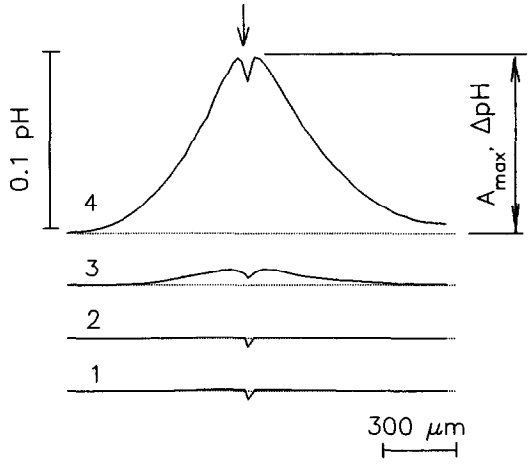

Fig. 1. The pH profile near the BLM measured upon bringing the electrode close to it (left side before the arrow) and taking the electrode away, from it (after the arrow) in the presence of 0.3 μM valinomycin (curve 1), 17 μM CCCP (curve 2) and both ionophores at indicated concentrations (curve 4) under the conditions of zero BLM applied voltage and a 6:60 mM KCl gradient. Curve 3 shows the pH profile under the same conditions as for curve 4 except that valinomycin was replaced by the channel former, gramicidin (2 nM), which induced a similar BLM current. Arrows under the figure indicate the directions of the movement of the electrode.

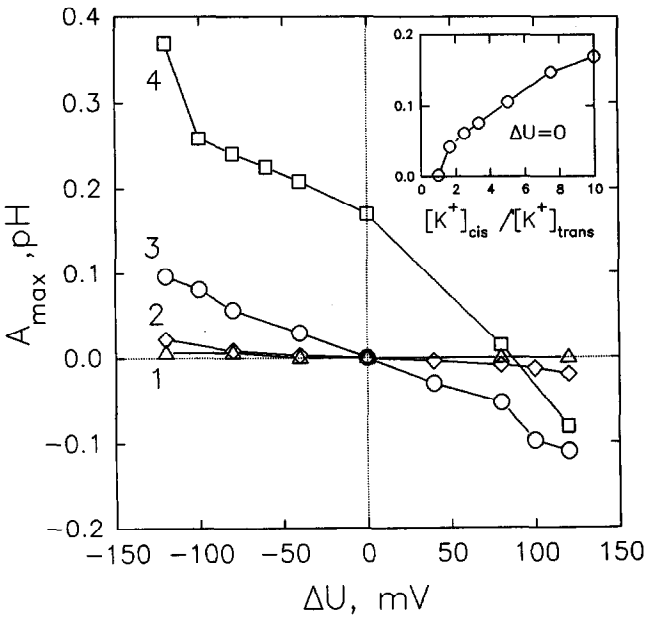

Fig. 2. Voltage dependence of the pH shifts near the surface of the BLM under the conditions of symmetrical 6 mM KCl (curves 1–3) and a 6:60 mM KCl gradient (curve 4), respectively. The concentrations of ionophores were as follows: 0.3 μM valinomycin (curve 1), 17 μM CCCP (curve 2), and 0.3 μM valinomycin and 17 μM CCCP (curves 3 and 4). Inset: the dependence of A_{max} on the KCl gradient across the BLM under the conditions of zero applied voltage.

ing concentrations of CCCP. It can also be seen that the saturating CCCP concentrations significantly exceeded the added valinomycin concentrations.

As was shown in different laboratories [1,8], valinomycin and CCCP can form stable complexes with each other. Fig. 4 shows a scheme of non-electrogenic K^+/H^+ exchange based on the formation of the complexes. Electrically neutral translocation of potassium ions proceeds via the formation of the complex, VK^+A^-, i.e. valinomycin–K^+–$CCCP^-$, while for the translocation of hydrogen ions the electrically neutral form of CCCP, i.e. $CCCP^-H^+$, can be used. The dependence of the saturating CCCP concentrations (Fig. 3) on the concentration of valinomycin favors the idea that hydrogen ion flux proceeds via CCCP–valinomycin complexes. It is worth noting that the formation of functionally active complexes of different protonophores has also been observed [9,10].

As can be seen from Fig. 2, the electrogenic component of the CCCP-mediated hydrogen ion flux is stimulated by the addition of valinomycin as well. This result is valid across a whole range of applied voltages and therefore can not be attributed to an effect of valinomycin on the membrane potential as it has been interpreted routinely in work on liposomes (see for example [8]). As far as we know, this phenomenon has been observed for the first time and can be explained by several reasons: (i) the formation of complexes between CCCP and valinomycin leading to an increased electrogenic proton conductance across the BLM; (ii) the modification of the membrane by positively charged valinomycin–K^+ complexes facilitating the limiting stage of the CCCP-mediated proton transport (presumably the translocation of $CCCP^-$).

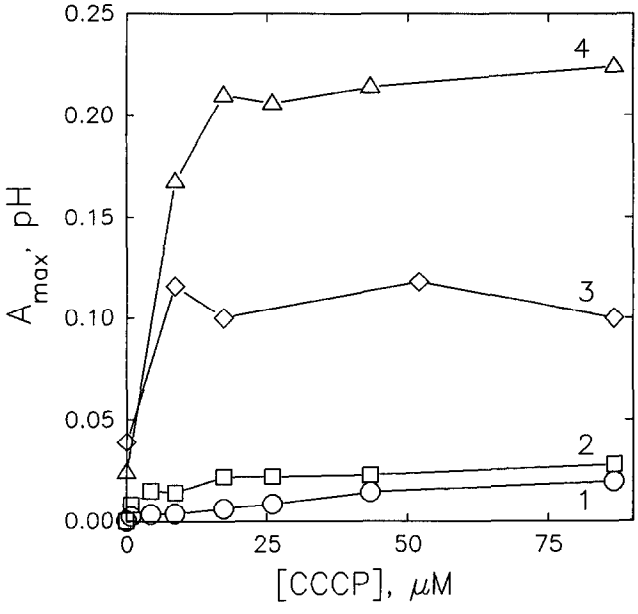

Fig. 3. The effect of CCCP concentration on the pH changes near the surface of the BLM under the conditions of zero BLM applied voltage and a 6:60 mM KCl gradient. Valinomycin concentrations were 0 nM (curve 1), 16 nM (curve 2), 95 nM (curve 3) and 310 nM (curve 4).

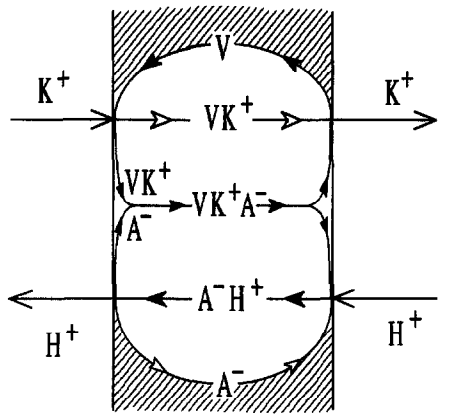

Fig. 4. Scheme of ion movements across the membrane in the presence of valinomycin (V) and CCCP (A). The putative complexes of the ionophores perform the electrically silent K^+/H^+ exchange across the membrane. The transmembrane movement of the $CCCP^-$ anion (A^-) under an imposed electrical field is responsible for the electrogenic proton flux.

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