Coupling of proton source and sink via H^+-migration along the membrane surface as revealed by double patch-clamp experiments

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Abstract Long-range proton transfer along the surface of black lipid bilayers was observed between two integral membrane channels (gramicidins), one operating as a proton source, the other as a sink, by patch-clamp technique. In contrast, potassium ions were shown to equilibrate with the aqueous bulk phase before being consumed. Both channels opened and closed simultaneously only if the charge between them was carried by protons. In this case an anomalous high conductance between two patched membrane fragments was measured, each of them containing one single gramicidin channel. The coupled state disappeared when the distance between these two channels was increased above the critical value. The latter was shown to increase with the channel lifetime. Our results support the idea of the 'localized' proton coupling, in which protons that have been pumped across membranes migrate along the membrane surface to reach another membrane protein that utilizes the established pH gradient.

Key words: Gramicidin; Double patch-clamp; Proton lateral conductance; Planar bilayer lipid membrane

1. Introduction

Transmembrane proton gradients play a vital role in membrane-linked energy transduction [1]. Between pumping proteins, protons are believed to pass through the bulk phase. Alternatively, lateral proton diffusion along membrane surfaces can be more efficient [2–5] provided that proton transfer from the interface into the bulk is hindered by a kinetic barrier [6–8]. This postulate is questioned, however [9,10]. Moreover, evidence for extremely rapid equilibration of surface protons with the aqueous phase was obtained in experiments with the protonophore S-13 and the alpha-toxin channel. In that case, the collision of buffer molecules with adsorbed anions or the hydrolysis of water could account for the fast transfer of protons to the membrane surface either in the absence or the presence of fixed charges and Stern layers [10,11].

This question is dealt with in the present work with the help of a new approach, namely double patch clamp of planar bilayer lipid membranes (BLM). Two different fragments of a single planar BLM are patched simultaneously. A voltage clamp between both pipettes induces a transmembrane ion flux as well as an ion flux between both membrane fragments. The latter goes either along the membrane/water interface or across the aqueous bulk. The data obtained support the existence of a kinetic barrier for the proton transfer from the surface of the BLM to the bulk phase.

2. Materials and methods

BLMs were formed by a conventional method [12] of 20 mg diphytanoyl phosphatidylcholine (DPhPC, Avanti Polar Lipids) per ml *n*-decane (Merck). The membranes (1.6 mm in diameter) were spread across a circular hole in a diaphragm separating two aqueous phases of a PTFE chamber. The pipettes were made of glass capillaries on a Narishige PP-83 puller. Their tips had a diameter of about 5–10 μm. At the *cis* and *trans* sides of the BLM, respectively, two patch pipettes and a reference electrode were inserted into the solution. As described earlier [13], the pipettes were moved perpendicular to the surface of the BLM by a hydraulic microdrive manipulator (Narishige). The touching of the membrane was observed with the help of a microscope that also allowed us to measure the distance between the pipettes. Pipettes which formed stable gigaohm seals upon the BLM touching were selected. Across the electrically isolated membrane fragments inside the pipettes [14,15] the current was monitored with an amplifier (Model 428, Keithley Instruments) connected to an x-t recorder. A special switch enabled us to measure the current between (i) the pipettes, (ii) one pipette and the reference, (iii) the other pipette and the reference. Gramicidin A (Fluka) was added at both sides of the BLM from an ethanolic stock solution to a final concentration of 2–5 nM.

3. Results

The conductance of bilayer lipid membranes doped with gramicidin was approximately 10^{-7} Ohm^{-1}. A patch pipette was attached to the BLM and the current across single channels was monitored (Fig. 1A). The membrane fragment inside the pipette was electrically isolated from the rest of the membrane [14,15]. Potassium ions were not added, so that the conducting ions were protons (pH 1.5) as confirmed by zero current measurements upon the formation of a pH gradient. Conductance and corresponding average duration time of the predominant transitions were 120 pS and 0.6 s, respectively. After a second pipette was attached at a distance of 500 μm, the current between two membrane fragments was measured at the same voltage of 100 mV (Fig. 1C). It was expected to be smaller by a factor of two, because under these conditions the electrical equivalent circuit consisted of two resistors in series, both of them representing one gramicidin channel in one membrane patch (compare Fig. 2). Indeed, irregular channel fluctuations were monitored with a predominant current amplitude of 45 pS and a duration time of 0.8 s (Fig. 1C). However, the current measured in the double patch configuration depended considerably on the distance λ between the pipettes. At $\lambda = 70$ μm, regular channel activity was observed. The conductance of 125 pS was very close to the one in single patch

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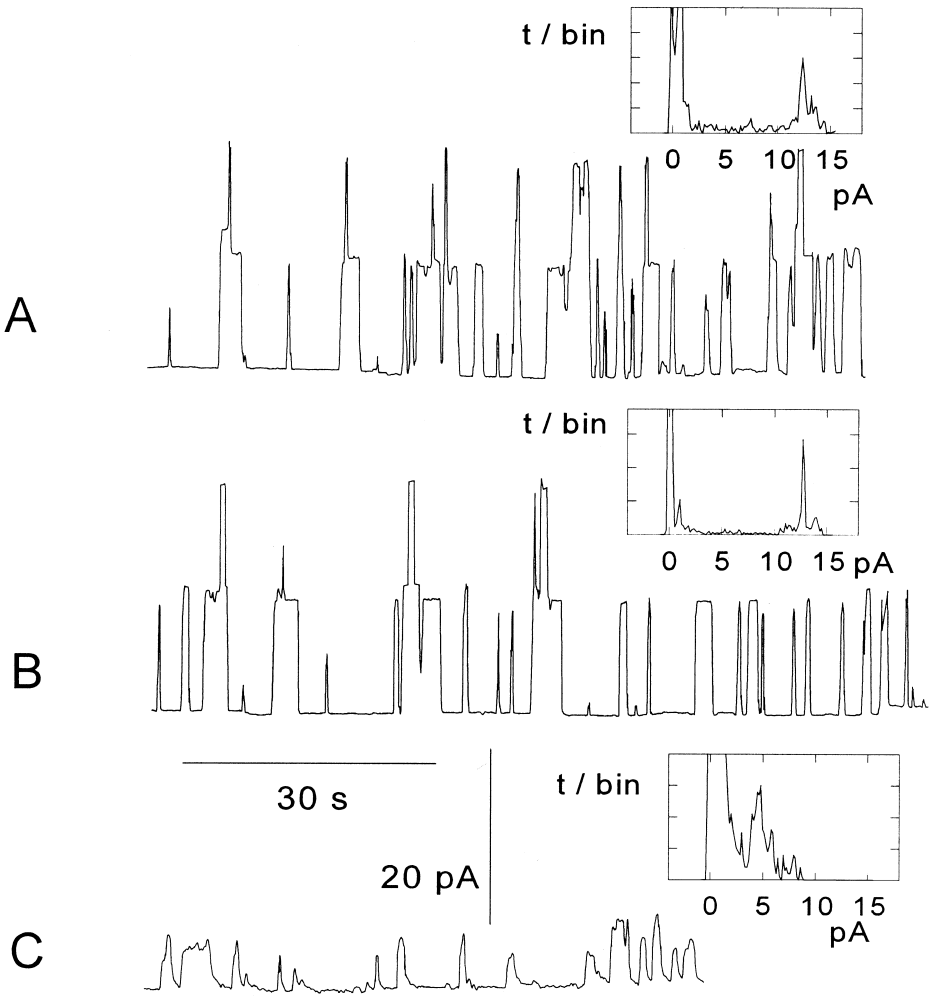

Fig. 1. Traces of current through gramicidin channels and corresponding amplitude histograms recorded from single patch (curve A), double patch with 70 μm distance between the pipettes (curve B) and double patch with 500 μm distance between the pipettes (curve C). Voltage was clamped at +100 mV. Planar BLM was patched in a solution (inside and outside the pipettes) of 100 mM choline chloride, pH 1.5 by HCl.

experiments (Fig. 1B). Moreover, the channels in both membrane fragments seemed to open and close concurrently. The average duration time when both channels were found in the open state simultaneously was 0.7 s.

At intermediate distances between the pipettes, sometimes conversion between the coupled (Fig. 1B) and the uncoupled (Fig. 1C) states of the channels was found. At least in some of the studies, the electrical isolation of both patches was confirmed by resistance measurements before and after the experiments. Artifacts due to patch damage during the experiments are therefore excluded.

Experiments were carried out at different temperatures to investigate the relation between the channel lifetime and the maximal distance λ_{max} at which the anomalous high conductance can be observed (Figs. 3 and 4). The distance between both pipettes was increased stepwise until the coupled status of both pipettes was lost. From $\lambda_{max} = 200 \pm 50$ μm and the channel duration time τ of 0.3 s the ratio λ_{max}/τ was calculated to be 0.07 cm/s at 21°C and pH 2.5. After lowering the temperature to 13°C, λ_{max} and τ increased to 650 ± 100 μm and 0.9 s respectively, whereas their ratio remained 0.07 cm/s. Fig. 3 shows typical double patch current recordings at two distances between pipettes (150 μm and 650 μm). At 21°C the average channel duration time for single pipette recording was 0.3 s, while that at 13°C was 1.0 s.

Although still under debate, the rapid movement along the interface is a feature that is unique for protons. Nothing comparable to proton wires was ever discussed for metal cations. To test this hypothesis, the experiments were repeated substituting potassium ions for protons (100 mM KCl, pH 4.5). In fact, coupled channels were not found even at very small distances (30 μm) between the pipettes (Fig. 5). Transitions of current in the double patch configuration were always irregular in size and their amplitude was considerably lower than in the case of single pipette recording. The average duration time of well pronounced current transitions was 0.6 s, while the channel duration time was 0.8 s for a single pipette recording.

4. Discussion

In the present work, the functioning of two gramicidin channels, one operating as a source, the other as a sink, was studied. The energy profile of proton movement across

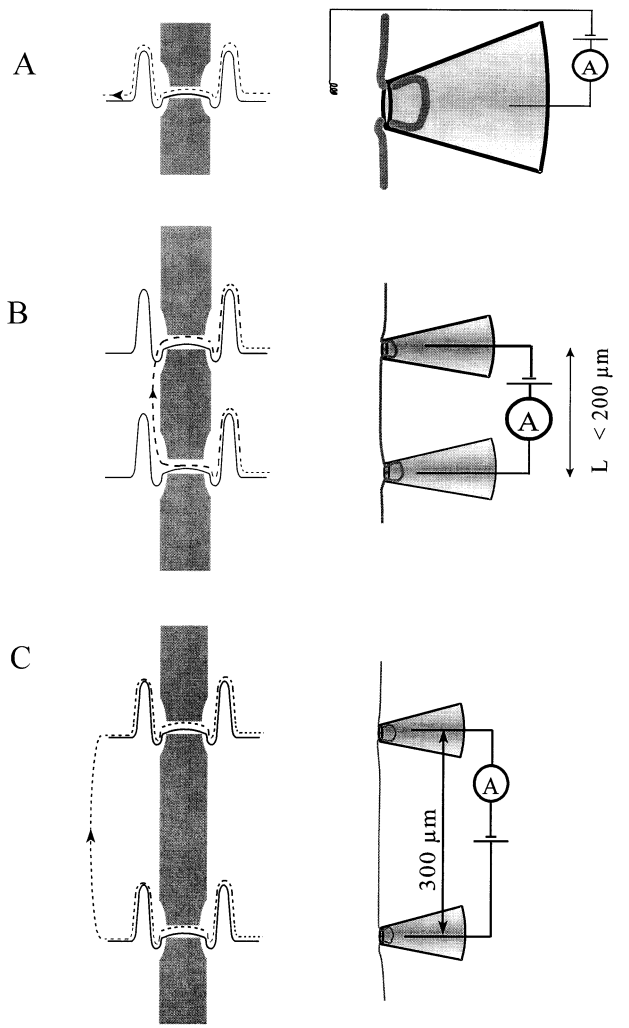

Fig. 2. Schemes of patch configurations (right side) and energy profiles (left side, solid lines) for possible proton movements (dashed lines) through single gramicidin channel (A) and two gramicidin channels (B,C) under double patch conditions at different distances between the pipettes.

gramicidin channels is determined by two identical energy barriers at the entrance and at the exit of the channel as proposed in [16]. There is no significant difference in the total height of energy barriers in the case of a single gramicidin channel and in the case of two coupled gramicidin channels because the conductance measured is nearly identical. In the double patch configuration, the protons have to overcome only the energy barrier at the mouth of the first channel and the energy barrier at the exit of the second channel. After travelling through the first channel the protons are not released into the bulk phase because migration along the membrane surface is energetically favorable. They move directly into the second channel, avoiding the kinetically unfavorable transfer across the mouth of the second channel (compare the scheme in Fig. 2).

It is obvious that two gramicidin channels in two pipettes do not function independently. In fact, open state lifetimes in double and single patch measurements are very close (Fig. 1). Besides, the open probability of the channels in the coupled state (0.25, Fig. 1A) differs only slightly from the probability (0.31, Fig. 1B) of single channels. The reasons for this phenomenon are unclear now. It can be speculated that the change of the proton route (from bulk phase-bulk phase to bulk phase-membrane surface) may affect the kinetic properties of gramicidin channels. In this connection it is interesting to note that the single channel lifetime of gramicidin increases upon the increase in H^+ concentration at low pHs [17].

The system of two simultaneously working proton channels with 'cycling' hydrogen ions is a good model for studying the mechanism of coupling between respiratory proton pumps and ATPases. Our data support the idea of 'local' proton coupling, which was a subject of debate during the last decades [1,2,18–20].

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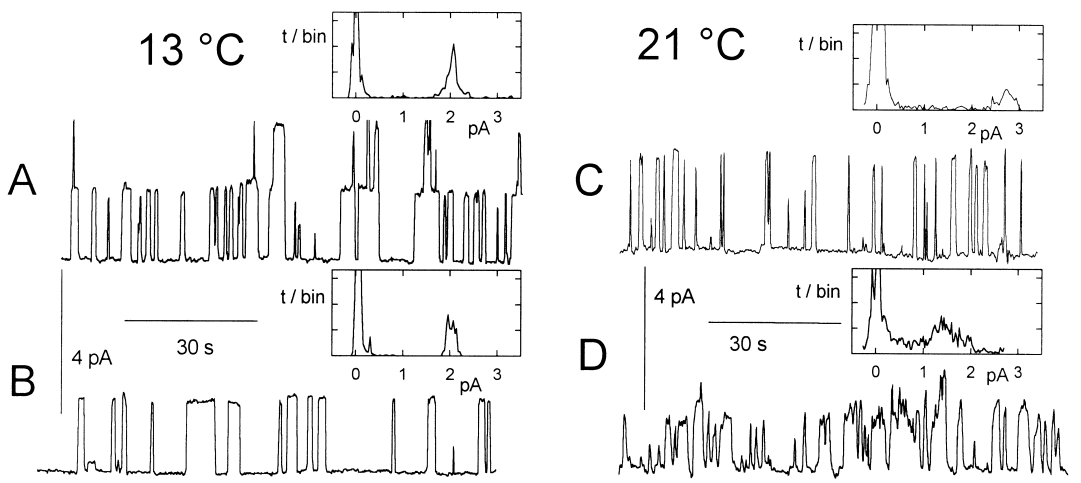

Fig. 3. Traces of current through gramicidin channels and corresponding amplitude histograms recorded from a double patch at 150 µm (A,C) and 650 µm distance between the pipettes (B,D). Voltage was clamped at +100 mV. Planar BLM was patched in a solution of 100 mM choline chloride, pH 2.5 by HCl (inside and outside the pipettes) at temperatures of 21°C (C,D) and 13°C (A,B).

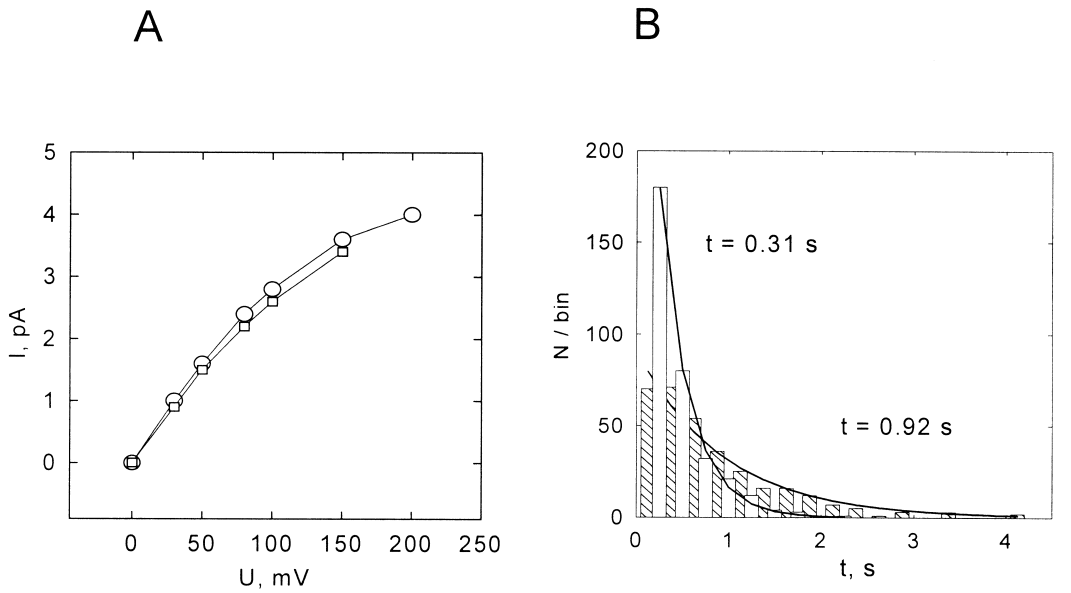

Fig. 4. A: Voltage dependence of the gramicidin channel amplitudes for single (squares) and double (circles) patch conditions. B: The duration distribution for gramicidin channels under double patch clamp conditions at 150 μm (13 and 21°C) was fitted by a single exponential (spline lines). Solutions: 100 mM choline chloride, pH 2.5 with HCl, 100 mV.

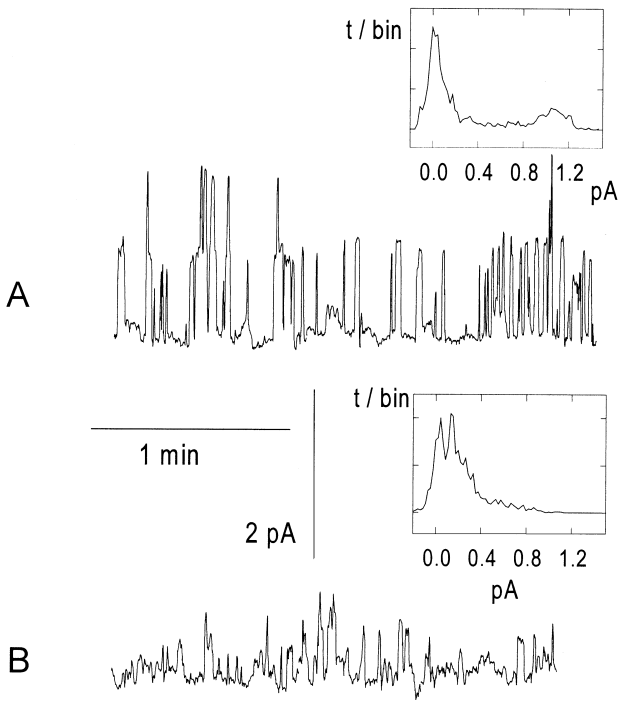

Fig. 5. Traces of current through gramicidin channels and corresponding amplitude histograms recorded from single patch (curve A), double patch with 50 μm distance between the pipettes (curve B). Voltage was clamped at +100 mV. Planar BLM was patched in a solution (inside and outside the pipettes) of 100 mM KCl, pH 4.5 by HCl.

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