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Proton lateral conduction along a lipid monolayer spread on a physiological subphase

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Abstract

A localized lateral proton pathway is present along the phospholipid polar heads and bound water molecules when the lipids are spread in monolayers at the air/water interface. Conduction can be detected on concentrated buffers as found under physiological conditions if the lateral proton gradient is large enough. The localized movement supports the occurrence of microlocalized proton circuits along a membrane and of lateral proton gradients.

Key words: Lipid monolayer; Proton pathway; Proton conduction; Physiological subphase

1. Introduction

Most energy transducing membrane systems work through proton movements. One key problem is to know if after crossing the membrane through a channel, the translocated proton is free to diffuse into the bulk phase. Very recent results have shown that it is in fact trapped at the interface. This is observed with bacteriorhodopsin [1–3], submitochondrial particles [4] and chloroplasts [5]. It was recently shown on model systems that an energy barrier was present at the interface preventing the free release of protons from the membrane/solution interface [6]. This rigorous study rules out the opposite conclusions of previous studies of a free exchange of protons between the interface and the bulk phase [7–10]. But in these observations, the transmovement of the protons between bulk phase and membrane interface was evaluated through an analysis of fluorescent signals associated to absorbed, i.e., not well localized fluorescent indicators. Their deconvolution used arbitrary choice of many critical parameters. The observation of a time lag before release of proton in the bulk phase observed with biological systems and the occurrence of an energy barrier to proton release in the case of model systems open the question of the possibility for protons to diffuse along the membrane as suggested in theoretical approaches [11–14]. Such a lateral movement was strongly supported by a recent work with alkalophilic bacillus [15]. Preferential lateral proton conduction at the phospholipid/water interface has been observed at a macroscopic scale by using different methodologies: measuring the local concentration changes of protons at the interface level by fluorescence [16–18], measuring surface potential variations [19], measuring changes in surface pressure for acidic lipids [20], or indirectly measuring the increase in surface conductance [21–23] as previously described with multilamellar systems [24–26]. One of the limits of all these approaches was that in all cases experiments were run either on pure water or on a poorly buffered solution. This was far removed from physiological conditions where the bulk pH is strongly buffered. The relevance of these previous observations to biological systems was then questionable. Nevertheless the ionic content of the subphase was shown not to affect the conduction [18].

In the present study, we show that such a lateral movement does take place on physiological-like subphases, i.e., highly concentrated buffers. This study provides tangible support under physiological condi-

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tions to the hypothesis of a local movement of protons along energy transducing membranes.

2. Materials and methods

2.1. Chemicals

Escherichia coli phosphatidylethanolamine (PE) was purchased from Sigma (USA). Synthesis of the pH fluorescent indicator probe, fluorescein phosphatidylethanolamine thiocarbamide (FPE), was described previously [27]. Salts were analytical grade. Ultrapure water, free from surfactant, was prepared with a MilliQ system (Millipore, France).

2.2. Monolayer preparation

Buffered saline solutions were prepared with ultrapure water. Lipids were spread on the aqueous phase from a solution in chloroform/methanol (5:1, v/v) and a 5 min period was observed to allow for solvent evaporation. The film surface pressure was monitored by means of a platinum plate (Prolabo, France) connected to a force transducer of our own construction. The sensitivity of the surface pressure determination was better than 0.2 mN/m. Temperature was 20°C (±0.5°C).

2.3. Fluorescence measurements

An interface fluorimeter constructed in the laboratory was used, in which the front face fluorescence was monitored. The emission from a small illuminated area (about 2 mm in radius) was measured. The trough was milled in plexiglas in order to obtain a low degree of light scattering. Excitation wavelengths were selected by means of optical filters. The fluorescence intensity was measured using a photomultiplier tube (EMI 9558, UK) connected to a data acquisition unit.

Lateral proton diffusion experiments were run with the proton 'window' jump technique using a trough and an experimental procedure previously described [16]. The movement of protons from the injection compartment (1) to the fluorescence observation area (2) was observed by a change in fluorescence emission of the pH-sensitive fluorescent chromophore FPE at the lipid/water interface. The compartment (1) is made acidic by HCl addition. This give a lateral bulk pH gradient with compartment (2). This ΔpH (pH1 − pH2) is a driving force for the movement of protons along the monolayer. This lateral diffusion is monitored by the change in the emission of the FPE probe at 4.1 cm away from the compartment (1). In order to reduce the bulk lateral continuity, barriers were present between (1) and (2) where the thickness of the subphase under the monolayer was reduced to less than 1 mm. This proton lateral diffusion is described by two parameters [16]: T_{H^+}, the time between the acid injection and the beginning of the decrease in fluorescence, and ΔF, the amplitude of this decrease which is representative of the induced steep surface pH gradient (classically about 2 pH units) from the acidic interface towards the bulk [19]. Lateral movement of protons in the bulk phase was very slow as compared to what was taking place at the level of the monolayer [16].

3. Results

3.1. The bulk lateral ΔpH is strongly dependent on the buffer molarity

The driving force for the movement of protons along the monolayer is the lateral ΔpH between the bulk phases of the observation compartment and the injection one. In a previous work, we showed that the magnitude of ΔpH was controlling the diffusion [17]. On a 1 mM phosphate subphase, it was shown that a ΔpH of at least 3 pH units is required to induce a lateral conduction. This ΔpH value was obtained when injecting 150 μl of 3 N HCl. On the other hand, when working on a 20 mM phosphate subphase, no conduction was detected when injecting 150 μl of 3 N HCl [17]. In a control experiment, we presently observed that such an injection induces a ΔpH which is strongly dependent on the buffer strength (i.e., its concentration) (Fig. 1). Nevertheless, it keeps a high value with 20 mM phosphate (3.9 pH units). We checked what was the volume of HCl which was needed to create a given ΔpH for different buffer concentrations (Fig. 2). ΔpHs larger than 4 pH units can be obtained even with concentrated phosphate buffers. It is then clear that, for a given acid volume injection, increasing the buffer

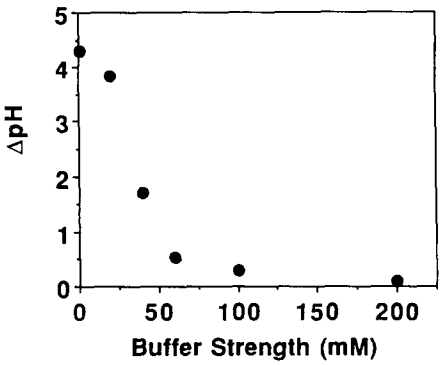

Fig. 1. Change in lateral bulk ΔpH as a function of the buffer concentration. 150 μl of 3 N HCl are injected in the injection compartment (1) inducing an acidification of its content. The subphase buffer is phosphate (initial pH = 6.8). The pH of the injection compartment was measured using a pH electrode.

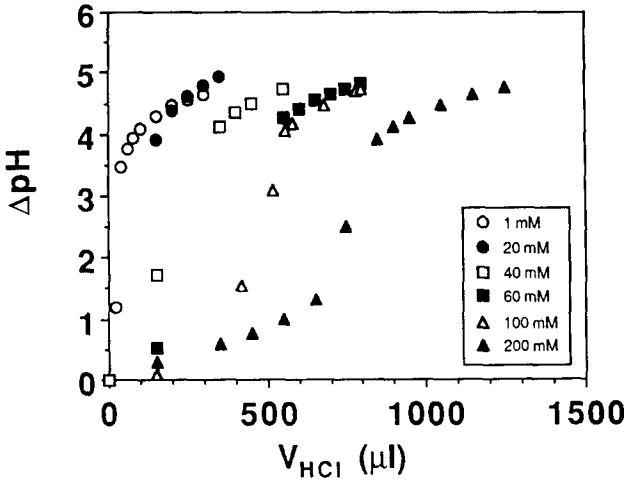

Fig. 2. Change in lateral bulk ΔpH as a function of injected HCl volume. Different volumes of 3 N HCl were injected in the injection compartment (1). The subphase was phosphate (initial pH = 6.8), with varying concentrations as indicated in the inset. The pH of the injection compartment was measured using a pH electrode.

concentration is dramatically decreasing ΔpH, which is controlling the occurrence of the proton lateral conduction.

3.2. Lateral proton conduction is present along monolayers spread on concentrated buffers

Experiments were run by taking into account the dependence of ΔpH on the buffer concentration described in Figs. 2 and 3. In order to get a ΔpH larger than 4 pH units, larger volumes of 3 N HCl were injected in highly concentrated phosphate buffers. As described previously [16], it was checked that the diffusion in the subphase was always very slow when no film was spread at the air/water interface (data not shown). Then the diffusion along the film (phosphatidylethanolamine at a surface pressure of 20 mN/m) was

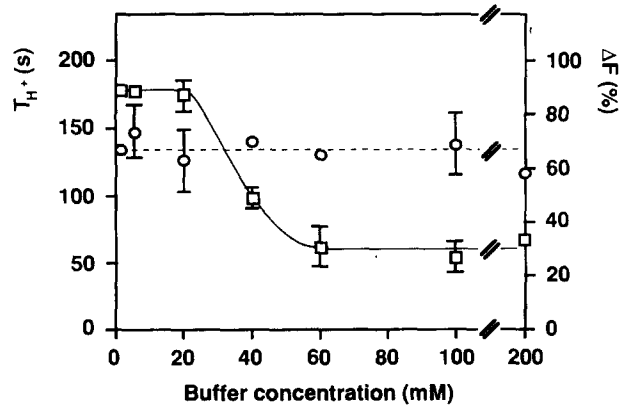

Fig. 3. Changes in the conduction parameters as a function of the buffer concentration. The subphase was phosphate (pH 6.8). PE monolayer was spread at 20 mN/m. (○) T_{H^+}; (□) ΔF.

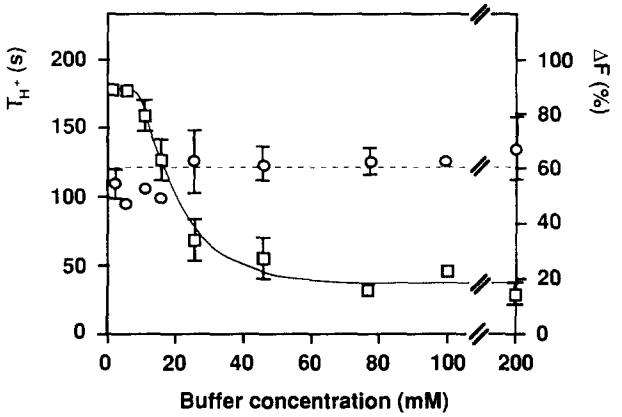

Fig. 4. Changes in the conduction parameters as a function of the buffer concentration. The subphase was TES (pH 6.8). PE monolayer was spread at 20 mN/m. (○) T_{H^+}; (□) ΔF.

assayed through the FPE fluorescence change kinetics. Conduction was always present whatever the phosphate concentration (up to 200 mM) (Fig. 3). T_{H^+} values were not affected but the ΔF values were of smaller amplitude on concentrated buffer. A decrease was observed between 20 and 60 mM; then a plateau was present up to 200 mM.

3.3. The nature of the buffer does not affect the occurrence of conduction on concentrated buffers

Up to now, all experiments dealing with proton lateral conduction along a monolayer were run in our group mostly on phosphate buffer subphase [16–20,27,28]. Cytoplasmic content in cells is buffered by more complex systems than this simple inorganic buffer. A buffer which is known to interact with membranes, 2-{[tris(hydroxymethyl)methyl]amino}-1-ethane-sulfonic acid (TES), was then used. As in the case of phosphate buffer, if we used acid injection conditions such as to get a ΔpH larger than 4 pH units, conduction was observed along a PE monolayer (20 mN/m), for all TES concentrations we used (i.e., up to 200 mM) (Fig. 4). T_{H^+} was not affected, as in the case of phosphate buffer. ΔF was observed to be decreased for lower values of the TES concentration than for the phosphate one, and a lower plateau value was observed at high buffer concentrations.

4. Discussion

The present results show that if a large enough ΔpH is generated, lateral diffusion of protons takes place along lipid monolayers spread on concentrated buffers. Such subphases are close to physiological conditions. Our observation that the nature of the buffer, either phosphate or TES, is playing only a secondary

role in the process strongly supports the conclusion that such lateral transport is physically possible inside a cell if the membrane interface organization is locally structured as found with lipid monolayers. It was proposed that conduction takes place along a bidimensionnal network of hydrogen bonds organized by the membrane [17,18,22]. Local destruction of such a network by the presence of amphiphiles prevented the lateral conduction [29]. These previous observations and the present one give tangible support to the possible occurrence of a local lateral proton transfer along the membrane in energy transducing systems. This is the basic hypothesis of the mosaic coupling model [13,14].

The recent characterization of an energy barrier preventing a free diffusion of protons from the interface to the bulk supports our observation of lateral diffusion [6]. This barrier was not taken into account in a previous description [30]. In our case, the increase in interfacial proton concentration is created locally (in contact with subphase (1)) and a lateral gradient is created. This is the driving force for the lateral movement which takes place preferentially along the interface, the perpendicular movement being prevented by this energy barrier. Leakage is of course present. This is shown by the need of a higher ΔpH to detect the conduction on 20 mM phosphate (ΔpH larger than 4 pH units) than on 1 mM phosphate (ΔpH larger than only 3 pH units) [17]. This is further supported by the decrease in ΔF with an increase in the buffer concentration (Figs. 3 and 4). Such a decrease cannot be explained by the small shift in pK of the pH indicator due to the change in ionic content [27]. It just shows that less protons are reaching the observation area, i.e., more protons are lost by the leakage during the lateral movement. But as a plateau level in ΔF is observed (Figs. 3 and 4), this means that a limited extent of leakage is present on concentrated buffers and does not prevent the lateral diffusion under physiological conditions.

Previous mathematical computations of diffusion process at the interface [17] showed that increasing the buffer concentration while ΔpH remained constant, should not affect the efficiency of the conductance along the interface but should increase the trapping efficiency: a strong buffer is a very efficient proton sink. With these assumptions, the mathematical simulations indicated that T_{H^+} should not be affected but that ΔF should decrease [17]. This is what we experimentally observed in the present work.

Buffers act as carriers which clear the proton from the site of conduction [7]. Their action is then dependent on their affinity for proton, their local concentration and their diffusion. Clearance requires that protonated buffer molecules move away from the interfacial layer. The saturating effect in the magnitude of proton transfer with the increase in buffer concentration can be associated to a plateau value and to the limited value of its diffusion coefficient. As the experiments are run with flat monolayers, the unstirred layer is not negligible [31]. The Brownian motion is slow close to the interface and the buffer-mediated clearance of proton reaches a saturating level. This reduced exchange between the bulk and the interface was taken into account in our previous experiments where we showed that stirring was needed to absorb protons to the interface in the injection compartment [17]. One should notice that such a limited exchange across the unstirred layer is mostly important with monolayers but is negligible in the case of higly curved vesicles [31].

The local concentration of buffer molecule in the Gouy-Chapman layer is known to be only slightly dependent on the bulk concentration when this last one is longer than 10 mM [32]. It is then not surprising that the lateral diffusion is observed when ΔpH is large enough.

A final conclusion is that our observation supports the semi-localized model of proton transfer. Nevertheless, since a membrane is a more complicated assembly than a lipid bilayer, proteins have to be taken into account in the lateral communication along membranes and may hinder the proton movement [33].

References

[1] Heberle, J. and Dencher, N.A. (1990) FEBS Lett. 277, 277–280.
[2] Heberle, J. and Dencher, N.A. (1992) Proc. Natl. Acad. Sci. USA 89, 5996–6000.
[3] Heberle, J., Oesterhelt, D. and Dencher, N.A. (1993) EMBO J. 12, 3721–3727.
[4] Gutman, M., Kotlyar, A.B., Borokov, N. and Nachliel, E. (1993) Biochemistry 32, 2942–2946.
[5] Renganathan, R.S., Pan, R.S., Ewy, R.G., Theg, S.H., Allnutt, F.C.T. and Dilley, R.A. (1991) Biochim. Biophys. Acta 1059, 16–27.
[6] Antonenko, Y.N., Koubasnjuk, O.N. and Yaguzhinsky, L.S. (1993) Biochim. Biophys. Acta 1150, 45–50.
[7] Gutman, M. and Nachliel, E. (1990) Biochim. Biophys. Acta 1015, 391–141.
[8] Rochel, S, Nachliel, E., Huppert, D. and Gutman, M. (1990) J. Membr. Biol. 118, 225–232.
[9] Gutman, M., Nachliel, E. and Moshiach, S. (1989) Biochemistry 28, 2936–2940.
[10] Gutman, M., Nachliel, E. and Kiryati, S. (1992) Biophys. J. 63, 281–290.
[11] Williams, R.J.P. (1961) J. Theor. Biol. 1, 1–17.
[12] Kell, D.B. (1979) Biochim. Biophys. Acta 549, 55–99.
[13] Westerhoff, H.V., Melandri, B.A., Venturolli, G., Azzone, G.F. and Kell, D.B. (1984) FEBS Lett. 165, 1–5.
[14] Westerhoff, H.V., Melandri, B.A., Venturolli, G., Azzone, G.F. and Kell, D.B. (1984) Biochim. Biophys. Acta 768, 257–292.
[15] Guffanti, A.A. and Krulwich, T.A. (1992) J. Biol. Chem. 267, 9580–9588.
[16] Teissié, J., Prats, M., Soucaille, P. and Tocanne, J.F. (1985) Proc. Natl. Acad. Sci. USA 82, 3217–3221.
[17] Prats, M., Tocanne, J.F. and Teissié J. (1985) Eur. J. Biochem. 149, 663–668.

[18] Prats, M., Tocanne, J.F. and Teissié, J. (1987) Eur. J. Biochem. 162, 379–385.
[19] Prats, M., Teissié, J. and Tocanne, J.F. (1986) Nature 322, 756–758.
[20] Prats, M., Tocanne, J.F. and Teissié, J. (1987) J. Membr. Biol. 99, 225–227.
[21] Sakurai, I. and Kawamura, Y. (1987) Biochim. Biophys. Acta 904, 405–409.
[22] Morgan, H., Taylor, D.M. and Oliveira, O.N. (1988) Chem. Phys. Lett. 150, 311–314.
[23] Sakurai, I. and Kawamura, Y. (1989) Biochim. Biophys. Acta 985, 347–350.
[24] Leslie, R.B., Chapman, D. and Hart, C.J. (1967) Biochim. Biophys. Acta 135, 797–811.
[25] Jendrasiak, G.L. and Mendible, J.C. (1976) Biochim. Biophys. Acta 424, 133–148.
[26] Jendrasiak, G.L. and Mendible, J.C. (1976) Biochim. Biophys. Acta 424, 149–158.
[27] Soucaille, P., Prats, M., Tocanne, J.F. and Teissié, J. (1988) Biochim. Biophys. Acta 939, 289–294.
[28] Gabriel, B. and Teissié, J. (1991) J. Am. Chem. Soc. 113, 8818–8821.
[29] Gabriel, B., Prats, M. and Teissié, J. (1991) Biochemistry, 30, 9359–9364.
[30] Junge, W. and McLaughlin, S (1987) Biochim. Biophys. Acta 890, 1–5.
[31] Mierle, G. (1985) Biochim. Biophys. Acta 812, 827–834.
[32] Tocanne, J.F. and Teissié, J. (1990) Biochim. Biophys. Acta 1031, 111–142.
[33] Fergusson, S.J. (1985) Biochim. Biophys. Acta 811, 47–95.