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Time-resolved protonation dynamics of a black lipid membrane monitored by capacitative currents

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The laser-induced proton pulse (Gutman, M. (1986) Methods Enzymol. 127, 522–538) was used for transient protonation of one side of a black lipid membrane. The charging of the membrane drives an electric (voltage or current) signal selectively representing the fast proton exchange at the membrane/electrolyte interface. The sensitivity of the electric signal to the presence of buffer indicates that proton transfer is measured, not some dyes or membrane photoelectric artifact. The same event can be visualized in an analogous system consisting of a pH indicator adsorbed to neutral detergent-phospholipid mixed micelles. The time-resolved light absorption transient is equivalent to the electrically determined transient charging of the membrane surface. The sensitivity of the current measurement exceeds the spectrophotometric method by 6–8 orders of magnitudes. As little as 10^{-18} mol of H^+ reacting with 0.75 mm^2 of the membrane surface can be monitored in a time-resolved observation. Both types of observed transients were accurately reconstructed by the numerical solution of coupled, non-linear, differential equations describing the system. The rate constants of the various proton transfer reactions were calculated and found to be of diffusion controlled reactions. There is no evidence for any barrier at the interface which either prevents protons from reaching the membrane, or keeps proton on the interface. The electric measurements can be applied for monitoring proton transfer kinetics of complex biomembrane preparations.

Introduction

The proton gradient ($\Delta\mu_{H^+}$) is the driving force for ATP synthesis by H^+-ATPases present in mitochondria, chloroplasts and bacterial membranes [1]. In spite of common interest in proton diffusion in membrane structures, the mechanism is still unknown. Even the basic value of spontaneous proton permeability in phospholipid membranes [2,3] is still under debate. One cause for lack of information is the inadequate time resolution of these studies.

A formalism based on steady-state flux can produce detailed mechanisms like that proposed for the Na^+/H^+ transport [4] but it cannot decide whether the activity is Na^+/H^+ antiport or Na^+/OH^- symport. The solution to such a mechanistic problem is attainable by time-resolved measurements.

The application of time-resolving measurements (voltage jump or charge pulse) of proton

Abbreviations: PS, phosphatidylserine; In, indicator (Bromocresol green); Mes, Mes buffer; ROH, pyranine (8-hydroxypyrene 1,3,6-trisulfonate). All these compounds appear either in their alkaline or acidic states.

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transport via uncoupler [5,6] produce a detailed mechanism. These studies determine the rate of uncoupler protonation, but not the primary step of proton binding to the matrix of phosphoheadgroups covering the membrane.

The reaction of a particle with a specific site on a structure can be accelerated by a surface assisted mechanism [6–8]. A primary collision with any phosphoheadgroup will arrest the proton in a transient covalent bound. Upon dissociation it has a very high probability to be trapped by a nearby protonable group.

According to this scenario, the initial reaction of proton with the membrane matrix is important for all following events. In the present study we investigate the dynamics of the primary proton collision with the membrane and its subsequent release to the bulk. This event is monitored by direct physical measurement, capacitative current in a black lipid membrane charged on one side by protons.

A charged species crossing a membrane produces an electric signal (voltage or current) [9,10] which can be measured accurately with excellent time resolution [11–13]. Fast current measurements are applicable not only for ion transport, but also for conformation changes of membrane proteins [14]. In the past years our laboratories were measuring fast electric transients of planar lipid membranes on one hand and rapid perturbation of acid-base equilibria on the other hand. This research couples the two methodologies for the selective study of proton transfer at a membrane-solution interface.

Selective protonation of one face of a black lipid membrane is achieved by the laser induced proton pulse [15,16]. A light sensitive membrane impermeable dye, 8-hydroxypyrene 1,3,6-trisulfonate (pyranine), is added to one side of the membrane and excited by a short ultraviolet laser pulse. The excited molecule dissociates and the released proton reacts, in diffusion-controlled reaction, with the membranes phosphoheadgroups. The resulting electric imbalance is measured as a current flowing through the external circuit.

The analysis we advocate is a dynamic adaptation of the detailed balance principle. The transient concentration of each reactant, after the light pulse excitation, is described by a differential equation. The equation for a given reactant contains the concentration terms and rate constants of all reactions in which it participates. The simultaneous solution (by numeric integration) of all differential equations provides the rate constants of all reactions involved in the overall, macroscopic observation.

This manuscript demonstrates the potential capacity of such proton-coupled transient current measurements.

Materials and Methods

Experimental system

The observation cell. This cell (made of Teflon) contained two compartments connected by a 1 mm hole. The membrane was formed over this hole by adding 5 μl of the phospholipid dissolved in decane (1.5% (w/v)). The solution of both compartments contained 100 mM of NaCl or choline chloride as conductive electrolyte and 5 μM Mes buffer (pH 6.7) to avoid pH drifting during the experiment. The content of each compartment was mixed by slow rotating stirrers. For more details see Ref. 17.

The laser pulse. $\lambda = 308$ nm, 10 ns full width at half-maximum, 0.47 mJ/pulse at a repetition rate of 0.5 Hz was focused through a quartz window on the black lipid membrane, without illuminating the Teflon septum or the anuluse at the membrane edge.

Electric signal. This signal was picked up by black platinum electrodes shielded from direct illumination. The short-circuit current was amplified 10^7-fold by a fast current-voltage converger and the signal recorded by Biomation 8500 transient recorder and averaged for 256 events.

The time resolution of the system (about 1.5 μs) was determined by the access impedance and the membrane's capacitance. An improved time resolution of about 60 ns was achieved by measuring the charging transient by a high impedance (10^{12} Ω) 20-fold voltage amplifiers.

Membrane stability. The stability exceeded 4 h. Each set of experiments was carried out with the same membrane. Phospholipids were purchased from Avanti, Polar Lipids. Pyranine, laser grade, was a Kodak preparation.

Transient light absorbance measurements. These

measurements were carried out as described elsewhere [15,16]. The time resolution of this method is 20 ns.

Results

Capacitative current signal

A typical current transient of protonation of a phosphatidylserine black lipid membrane is shown in Fig. 1.

The transient initiates with an extremely short, unresolved positive spike, followed by a negative trough. This signal is typical for a system where the dye is present in the back side compartment. Addition of the dye to the front side comprtment reversed the polarity of the signal. When the dye was present on both sides, only a minor membrane artifact was measured, similar to that observed in absence of dye (not shown).

The initial protonation, too fast to be resolved, is represented by the initial positive spite. The major signal is the redissociation of the protons (positive charges) from the dye facing side of the membrane. The rate of dissociation increases to a maximum, then decays with the progression of the reaction until the initial prepulse state is recovered.

Fig. 2A depicts the transient current measured

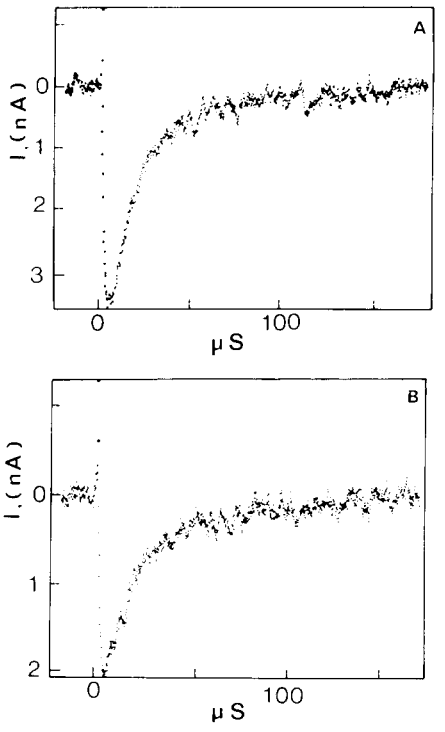

Fig. 1. Capacitance current signal of black lipid membrane due to one side protonation. The membrane made of phosphatidylserine (1.0 mm in diameter) was equilibrated with 100 mM choline chloride and 5 μM Mes buffer (pH = 6.7). On the back side pyranine 100 μM was also present. The proton pulse was generated by a short laser pulse 0.47 mJ, λ = 308 nm, 10 ns full width at half-maximum. The presented signal is an average of 256 pulses at a repetition rate of 0.5 Hz. Negative current corresponds with incremental negative charge on the dye side of the black lipid membrane. (A) The signal was measured under the above conditions. (B) 1 mM Mes buffer (pH 6.7) was added on both sides of the membrane.

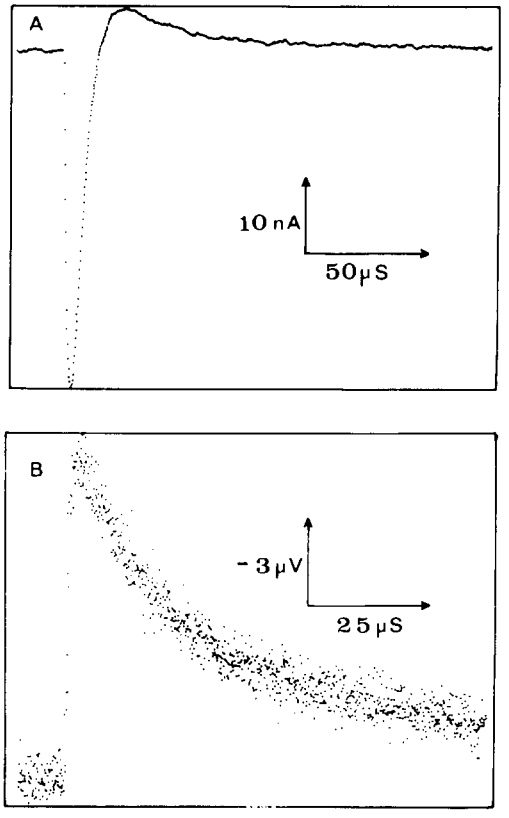

Fig. 2. Capacitance current signal, measured with phosphatidylcholine black lipid membrane equilibrated with pyranine on the back side. The experiment was carried out under the conditions specified for Fig. 1A. (A) Current measurement. (B) Transient voltage measurement. Upward deflection corresponds with build up of negative charge on dye side of black lipid membrane. The signal was amplified by a high resistance (10^{12} Ω) 20-fold voltage amplifier with a response time of 60 ns.

with a phosphatidylcholine membrane. These kinetics are well resolved. The initial negative current transient corresponds with negative charging of the dye side of the membrane, followed by reprotonation. These dynamics are shown as highly resolved voltage transient in Frame B.

The initial negative charging of the dye side is ascribed to the proton dissociation from the pyranine adsorbed on the membrane [18] leaving the anion on the surface. The following recharging is the reprotonation of the surface. The protonation of the phosphatidylcholine phosphoheadgroup is too fast to be resolved; our previous measurements [15,19] demonstrated that such acidic moiety ($pK = 2.25$) will complete its transient protonation cycle in less than 0.2 μs. The measuring system we employed is not fast enough to detect such transients.

In order to focus our attention on the dynamics of proton transfer between bulk and surface, we selected for the following experiments the negative charge membrane phosphatidylserine which does not adsorb the negative charged pyranine [18].

Comparison between current and light absorbance measurements

The fast protonation of the phosphatidylserine surface can be readily visualized by transient light absorption of a surface-bound indicator. For this purpose we used mixed micelles of neutral detergent (Brij-58) and phosphatidylserine. Such micelles adsorb the indicator Bromocresol green [20] which serves as a surface proton probe. The pK value of the the adsorbed indicator ($pK = 5.4$) and the carboxyl of phosphatidylserine ($pK = 4.6$) are close enough so that approx. 1 μs after the proton pulse their dynamics follow the same time course [15,20]. By monitoring the transient protonation of the indicator we visualize the protonation of the surface groups.

Fig. 3 depicts the dynamics of this analogous system. Line A presents the absorption change corresponding with the protonation of the indicator (comparable with accumulation of positive charge on the black lipid membrane). Line B, the first time derivation of the curve in Frame A, corresponds with the current transient shown in Fig. 1.

At zero time the laser pulse discharged protons

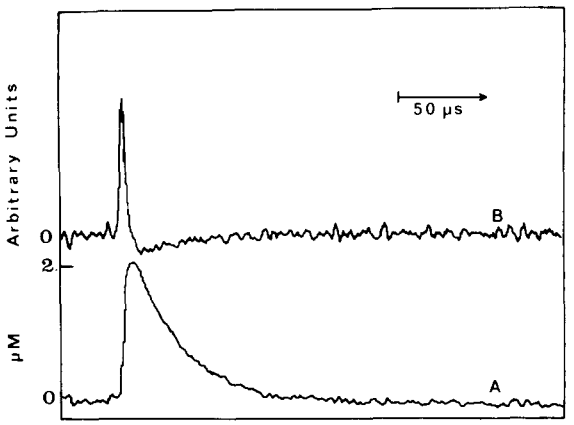

Fig. 3. Transient light absorbance recording of protonation of bromocresol green adsorbed to mixed micelles. The protonation of the indicator was measured at 633 nm where the alkaline form absorbs ($\varepsilon = 33000\ M^{-1} \cdot cm^{-1}$). The experimental system contained 100 mM choline chloride, 100 μM pyranine, 40 mg/ml of Brij-58, 1.5 mM phosphatidylserine (incorporated in the Brij-58 micelles) and 50 μM of Bromocresol green (pH 6.7). The proton pulse was generated by a 10 ns, 0.3 mJ, $\lambda = 337$ nm laser pulse. The optical path length of the monitoring beam was 1.0 cm. The signal is an average of 2048 laser pulses. Line A. Transient absorbance measurement. The relaxation time of the decay is $\tau = 27$ μs, $r = 0.997$. Line B. Time derivative of trace A. The relaxation time of the decay is $\tau = 21$ μs. $r = 0.778$.

from the pyranine into the bulk. These protons react rapidly with the micellar surface groups.

The protonation of the indicator is fast, reaching maximal velocity 1 μs after the pulse. After 3 μs, the indicator's protonation is maximal and the derivative curve crosses the zero line. From now on the indicator is deprotonated. Disregarding the first 2–3 μs, where the current amplifier is too slow to measure the current (Fig. 1), the derivative curve of the deprotonation (line B, Fig. 3) is very similar to the current transient presented in Fig. 1.

The final relaxation times calculated from the curves A and B in Fig. 3 are essentially the same (see legend).

The effect of buffer on the current and absorbance transients

The dynamics of transient protonation is very sensitive to the presence of buffer in the solution [5,6,20,21]. We exploited this effect to vary pro-

gressively the observed dynamics and to collect data for quantitative analysis.

The experiment depicted in Fig. 1A was repeated on the very same membrane with increasing concentration of Mes buffer on both sides of the membrane. As demonstrated in Fig. 1B, the buffer suppressed the amplitude, but had only a minor effect on the relaxation. The same titration was repeated with the indicator-mixed micelle system described in Fig. 3.

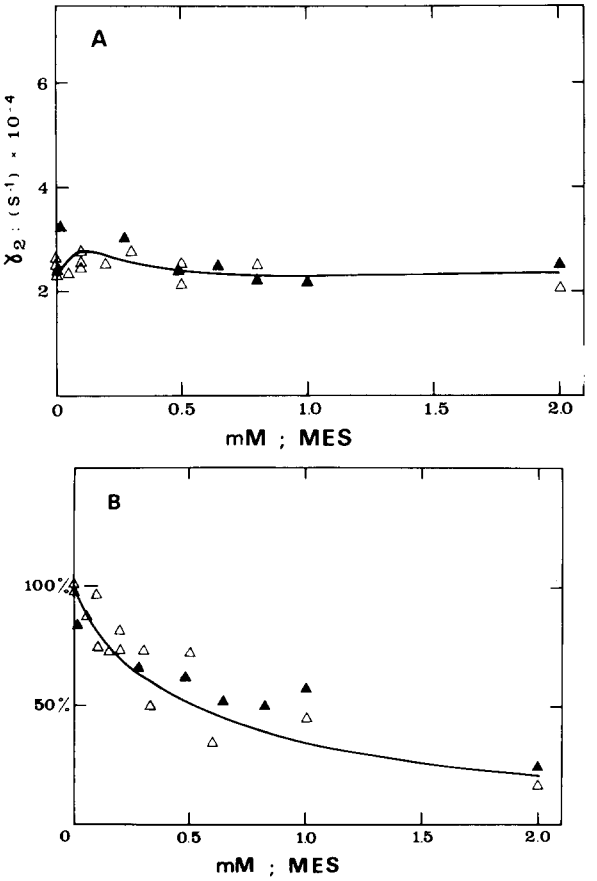

Fig. 4. The effect of buffer on the observed dynamics of current and absorbance measurements. Current measurements were carried out as exemplified in Fig. 1. Absorbance measurements were carried out as in Fig 3A. The relaxation constant γ_2 was calculated from the decay curves. The amplitudes were measured at the point of maximum deflection in the absorbance and the current measurements. (A) The effect of Mes buffer on γ_2. (B) The effect of Mes buffer on the total change ($I_o \cdot \tau$). The value measured in absence of buffer is taken as 100%. The continuous lines are the expected dependence of the two variables on the buffer concentration, according to the rate constant listed in Table I. ▲, current measurements; △, light absorption measurements.

The effect of buffer on the relaxation constants (γ_2) is shown in Fig. 4A. The effect of buffer on the translocated charge (normalized to 100% in the absence of buffer) is shown in Fig. 4B.

Both types of measurements fit the same theoretical curve (see below).

Quantitative analysis of bulk-surface proton transfer

The quantitative analysis of the results is carried out by numerical integration of the non-linear differential equations representing the transient protonation of all reactants present in the reaction system [15,16,21].

The transient currents are somewhat less convenient for analysis due to their time derivative nature. For this reason we started with the analysis of the indicator's protonation, with which we have more practice and experience. As current and absorbance measurement represent two aspects of the same process, we projected that the solution for the absorbance measurements will also provide the description of the current measurements (see below).

The reactions to be considered in the quantitative analysis are defined in Table I. Of these reactions we have already determined the value of all rate constants except the rate of proton exchange between the Mes buffer and the carboxyl groups of phosphatidyl serine (see references in the table). The differential equations pertinent to this case are given in the Appendix to Ref. 12 and in Ref. 16. Using these equations with the known rate constants listed in Table I, we determine by an iteration procedure the rate of proton transfer between protonated phosphatidylserine and Mes buffer.

Within the range given for this rate constant, $k = (2.9 \pm 0.15) \cdot 10^9\ M^{-1} \cdot s^{-1}$, the predicted curve followed the experimental result of both current and transient absorbance measurements (see continuous line in Figs. 4A and 4B). The same rate constant was then used to reconstruct the derivative curve of phosphatidylserine protonation d(PSH)/dt in the presence of 5 μM and 1.0 mM Mes buffer. The time derivatives are shown in Fig. 5, together with the measured current transient of the corresponding experiments. As clearly seen, the predicted functions are superpositioned over the experimental curves.

TABLE I

THE RATE CONSTANTS OF PROTON TRANSFER REACTION ON A PHOSPHOLIPID/WATER INTERFACE

All reactions are written with the thermodynamic favored direction as the forward reaction (k_+). The rate cnstants are given in $M^{-1}\cdot s^{-1}$ or s^{-1} units for second- and first-order reactions, respectively. The reactants are: pyranine (ROH); phosphatidylserine (PS^-); Mes buffer (Mes^-) and the pH indicator, Bromocresol green (In^-). The rate constants in this table produce the continuous lines in Figs. 4 and 5.

Reaction	k_+	k_-	Ref.
1 $RO^- + H^+ \rightleftharpoons ROH$	$1\cdot10^{11}$	$1.3\cdot10^3$	16
2 $In^- + H^+ \rightleftharpoons HIn$	$1\cdot10^{10}$	$4\cdot10^4$	16
3 $PS^- + H^+ \rightleftharpoons PSH$	$1.5\cdot10^{10}$	$3.8\cdot10^5$	16
4 $Mes^- + H^+ \rightleftharpoons MesH$	$1\cdot10^{10}$	$7.9\cdot10^3$	[a]
5 $PSH + In^- \rightleftharpoons HIn + PS^-$	$1\cdot10^{10}$	$1.6\cdot10^9$	16
6 $PSH + RO \rightleftharpoons ROH + PS^-$	$1\cdot10^8$	$5\cdot10^4$	[a]
7 $PSH + Mes^- \rightleftharpoons PS^- + MesH$	$(2.9\pm0.15)\cdot10^9$	$(8\pm0.7)\cdot10^7$	
8 $MesH + RO^- \rightleftharpoons Mes^- + ROH$	$2.5\cdot10^7$	$4\cdot10^5$	[a]
9 $HLn + Mes^- \rightleftharpoons In^- + MesH$	$4\cdot10^4$	$8\cdot10^6$	[a]
10 $HLn + RO^- \rightleftharpoons In^- + ROH$	$1\cdot10^7$	$3.2\cdot10^4$	16

[a] Nachliel, E., unpublished results.

Discussion

Evaluation of capacitative current vs. transient absorption measurements

The pH jump is a synchronized proton release from hydroxy aromatic compounds triggered by a brief laser pulse. The experiments we carried out recorded the very same perturbation by two methods; the spectrophotometric monitoring of an indicator and the transient current of capacitor

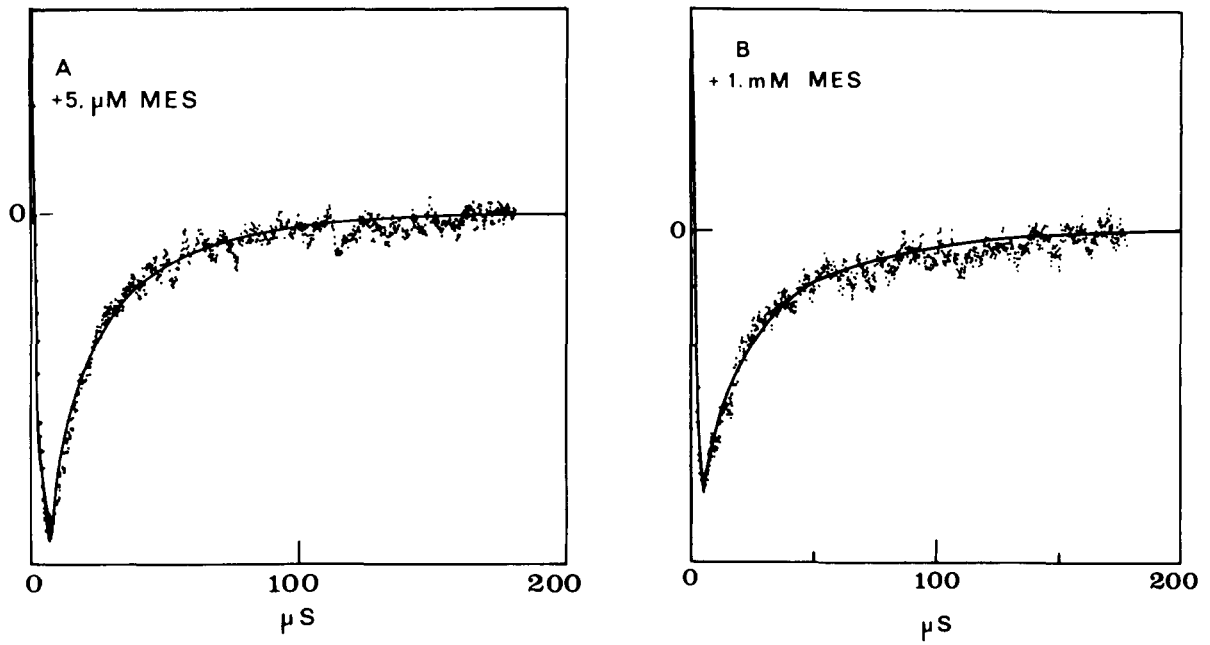

Fig. 5. Reconstruction of the current signals by numerical integration of the differential equation for transient protonation of phosphatidylserine. The rate constants given in Table I, omitting those where indicator is a reactant (i.e., lines 2, 5, 9, 10) were used to generate the function d(PSH)/dt. This function is superimposed over the transient current signal taken from Fig. 1A and Fig. 1B for lines A and B, respectively.

charging. We shall evaluate these methods and their relative advantages.

A typical experiment like that shown in Fig. 1 records a current I_o of approx. 3 nA with decaying time constant of $\tau \approx 30$ μs. The total number of detected charges ($Q = I_o \cdot \tau$) is about $0.9 \cdot 10^{-13}$ C or $2 \cdot 10^{-18}$ mol.

In the transient absorbance measurement (Fig. 3) the incremental protonation of indicator was $\Delta c = 2.3$ μM (the lower level of detection is approx. 0.1 μM) in an observation volume of $V \approx 50$ μl. The measured transient corresponds with $\Delta c \cdot V \approx 1 \cdot 10^{-10}$ mol.

Apparently the electric signal picks up a perturbation (or a transient charging) which is 10^{-8} times smaller than the spectrophotometric one. Yet we know that the perturbation in both cases (estimated from the pulse energy, pyranine concentration and quantum yield at 308 nm and 337 nm) are about equal in both measurements.

The main cause for the apparent unequal recorded amplitues is the difference of distribution and quantity of the proton detecting material.

The electric measurements monitor the charging of a single layer of phospholipids 1.0 mm in diameter. The amount of phosphoheadgroup available for protonation is (using 70 Å^2 per molecule) $1.6 \cdot 10^{-12}$ mol.

The optical probing consists of micellar solution containing 1.5 mM of phosphatidylserine. The volume of the observation space (that illuminated both by the excitation pulse and the monitoring beam) is approx. 50 μl. The amount of phosphatidylserine participating in the optical probed reaction is $7 \cdot 10^{-8}$ mol. Thus the smaller smount of detecting material increases the sensitivity of the electric measurement by a factor of 10^5.

The sensitivity of current measurement is not fully exploited due to the fact that the surface carboxyls are partially neutralized by counter ions. It is a well-known phenomenon that at high packing density the effective degree of dissociation is smaller than in the dispersed state. Binding of SDS to neutral micelles (Brij-58) suppresses the dissociation of the SO_3-Na^+ group to approx. 30% [22]. On pure SDS micelles this value is even smaller, 15% [23]. Similar counterion neutralization of serine's carboxyls [24] suppress the sensitivity of current measurements; a proton replacing a counterion will cause no electric transient, yet it is readily detected by a pH indicator.

Quantitative analysis of the dynamics

We observed the same bulk-surface proton transfer by two independent monitoring systems, thus the results should be analysed by the same procedure. This analysis was specially designed for reversible proton transfer in multicomponent systems [16,17]. The observed reaction is described as a sum of simultaneously occurring elementary reactions, each step is reversible and characterized by appropriate rate constants (see Table I). A detailed balance dynamics is employed to write a set of coupled, non linear differential equations ocrresponding with the transient concentration of each reactant (see appendix to Refs. 15, 16 and 21). The numerical solution of the coupled differential equation reproduces the dynamics of the observed component (protonation of indicator or first time derivative of protonated phosphatidylserine ($dPSH/dt$)). As seen in Fig. 5 this procedure accurately reproduces the individual current recordings. The same rate constants also describe the effect of varying buffer concentrations on the macroscopic characteristics (amplitude and γ_2) of the spectrophotometric and the electric signals (see Fig. 4).

The accurate reconstruction of the observed current transient demonstrate that through chemical formalism we have analyzed an event more commonly treated by analogue circuit analysis [14]. We have to stress that the analogue circuit analysis is an essential step in understanding the observation. Through this formalism one can determine when (or to what extent) the measured signal is free from distortion imposed by the capacitance and resistance of both measured and measuring circuits. Within the 'safe limits' of the observation period, where the signal is properly resolved, the numerical solution can be applied for determination of the rate constants of the fundamental reactions.

Dynamics of bulk-surface H^+ transfer

The advantage of the analysis we advocate is demonstrated in Fig. 6. These tracings are the reconstruction of the transient protonation of

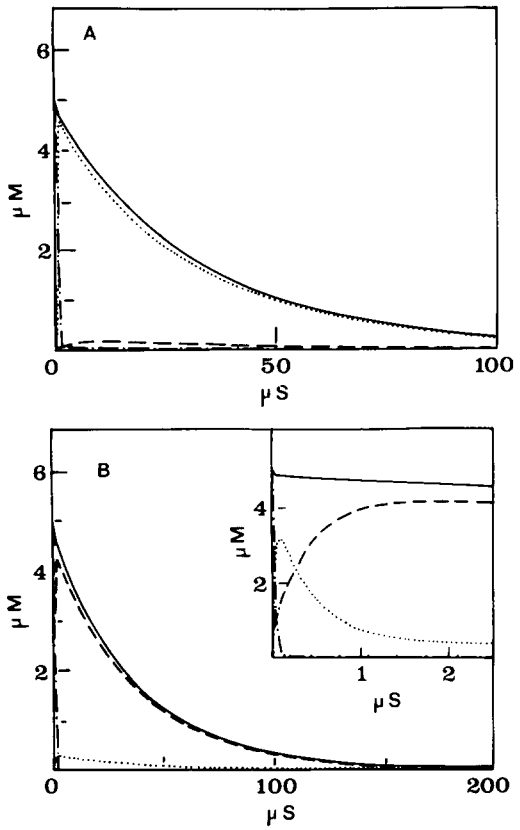

Fig. 6. Reconstruction of the transient protonation of phosphatidylserine black lipid membrane. The curves are the numerical solution of the equation represented the dynamics of the pyranine (———), phosphatidylserine (· · · · · ·), Mes buffer (– – –) and free protons (·–·–·). The derivative curve of phosphatidylserine are those shown in Figs. 5A and 5B, respectively. (A) The time course of the events in presence of 5 μM Mes. (B) The time course of the events in presence of 1 mM Mes. The insert is an expansion of the first 2 μs of the reaction.

phosphatidylserine black lipid membrane. (The corresponding current signals of these lines are those given in Fig. 5). At time zero, a certain fraction of the proton emitter dissociated to RO^- and H^+. After a very brief period (approx. 200 ns) we find most of the protons on the membrane. Only a few reacted with RO^- and free protons are practically absent. This rapid proton transfer from bulk to the surface negates the existence of a putative energy barrier which is 'abolished by strong stirring which provides protons with kinetic energy and reappears as the stirring is suppressed' [25].

Comparison of Figs. 6A and 6B demonstrates the effect of buffer on the distribution of protons between surface and bulk. In the presence of 1 mM Mes, the surface charging becomes a two-phasic event. Initially the phosphatidylserine molecules are rapidly protonated, trapping approx. 40% of the discharged protons (see insert to Fig. 6B, expanding the events during the first two microseconds). Yet the dwell time of protons on the surface is very short and within 1 μs most of them are lost to the buffer.

This fast buffer-facilitated proton transfer is in accord with our previous measurements [26], and with Kasianowicz et al. [5,6] and Junge and McLaughlin [27]. presently there is ample evidence to conclude that under physiological conditions proton flux is mostly mediated by buffer diffusion and not by free H^+.

The study we presented demonstrates that surface-bulk ion exchange can be measured in the time-resolved domain. Such measurements are essential tools for understanding the mechanism of basic cross membranal ion motion in biomembranes.

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References

1 Mitchel, P. (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation, Glynn Research Ltd.
2 Gutknecht, J. (1984) J. Membr. Biol. 82, 105–112
3 Grzesiek, S. and Dencher, N.A. (1986) Biophys. J. 50, 265–276
4 Aronson, P.S. (1985) Annu. Rev. Physiol. 47, 545–560
5 Kasianowicz, J., Benz, R. and McLaughlin, S. (1984) J. Membr. Biol. 82, 179–190
6 Kasianowicz, J., Benz, R. and McLaughlin, S. (1987) J. Membr. Biol. 95, 79–83
7 Adam, G. and Delbruck, M. (1968) in Structural Chemistry and Molecular Biology (Rich, A. and Davidson, N., eds.), pp. 198–215, W.H. Freeman, San Francisco
8 Berg, O.G. and Von Hippel, P.H. (1985) Annu. Rev. Biophys. Chem. 14, 131–160

9 Lauger, P., Benz, R., Stark, G., Bamberg, E., Jordan, P.C., Fahr, A. and Brock, W. (1981) Q. Rev. Biophys. 14, 513–598
10 Ruf, H. and Grell, E. (1981) in Membrane spectroscopy (Grell, E., ed.), pp. 333–376, Springer-Verlag, Berlin
11 Huebner, J.S. (1979) Photochem. Photobiol. 30, 233–242
12 Huebner, J.S. (1980) Biophys. J. 31, 279–283
13 Trissl, H.W. and Graber, P. (1980) Bioelectrochem. Bioenerget. 7, 167–186
14 Fahr, A., Lauger, P. and Bamberg, E. (1981) J. Membr. Biol. 60, 51–62
15 Gutman, M. (1984) Methods Biochem. Anal. 30, 1–103
16 Gutman, M. (1986) Methods Enzymol. 127, 522–538
17 Bamberg, E., Apell, H.-J., Dencher, N.A., Sperling, W., Stiven, H. and Lauger, P. (1979) Biophys. Struct. Mech. 5, 277–292
18 Clement, N.R. and Gould, J.M. (1981) Biochemistry 20, 1534–1538
19 Gutman, M., Nachliel, E. and Fishman, M. (1986) in Ion Interactions in Energy Transfer Biomembranes (Papageorgiou, C.G., Barber, J. and Papa, S., eds.), pp. 93–103, Plenum Press, New York
20 Nachliel, E. and Gutman, M. (1984) Eur. J. Biochem. 143, 83–88
21 Gutman, M., Nachliel, E. and Gershon, E. (1985) Biochemistry 24, 2937–2941
22 Gutman, M., Huppert, D., Pines, E. and Nachliel, E. (1981) Biochim. Biophys. Acta 642, 15–26
23 Feinstein,.M. and Rosano, L. (1967) J. Colloid Chem. 34, 73–79
24 Winiski, A.P., McLaughlin, A.C., McDaniel, R.V., Eisenberg, M. and McLaughlin, S. (1986) Biochemistry 25, 8206–8214
25 Prats, M., Tocanne, J.F. and Teissie, J. (1985) Eur. J. Biochem. 149, 663–668
26 Gutman, M. and Nachliel, E. (1985) Biochemistry 24, 2941–2946
27 Junge, W. and McLaughlin, S. (1987) Biochim. Biophys. Acta 890, 1–5