

Phase-State Dependent Current Fluctuations in Pure Lipid Membranes

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ABSTRACT Current fluctuations in pure lipid membranes have been shown to occur under the influence of transmembrane electric fields (electroporation) as well as a result from structural rearrangements of the lipid bilayer during phase transition (soft perforation). We demonstrate that the ion permeability during lipid phase transition exhibits the same qualitative temperature dependence as the macroscopic heat capacity of a D15PC/DOPC vesicle suspension. Microscopic current fluctuations show distinct characteristics for each individual phase state. Although current fluctuations in the fluid phase show spikelike behavior of short timescales (~ 2 ms) with a narrow amplitude distribution, the current fluctuations during lipid phase transition appear in distinct steps with timescales of ~ 20 ms. We propose a theoretical explanation for the origin of timescales and permeability based on a linear relationship between lipid membrane susceptibilities and relaxation times near the phase transition.

INTRODUCTION

The permeability of cell membranes to ions, proteins, and other transmitters, and the regulation of these processes, is of crucial importance in maintaining basic cell functions. In this context, ion channel proteins have been found to play a major role in the regulation of ionic transport across the membrane (1). In addition to the characteristics of the channel, proteins themselves as an influence of the membrane phase state on channel function have been identified (2,3), indicating the importance of the lipid bilayer for transmembrane currents. Boheim et al., for example, reported on the slowing down of protein induced ion channels during the lipid phase transition (4,5). The first indication of thermodynamically induced changes in permeability was already given by Papahadjopoulos et al. in 1973 (6), who found a maximum leakage of radioactive labeled sodium ions during lipid phase transition.

The application of high electric fields (typically $\sim 10^7$ – 10^8 V/m) presents another mechanism for overcoming the lipid membrane barrier, inducing current fluctuations in the absence of channel forming peptides (electroporation) (7–11).

A third mechanism (in addition to ion channels, pores, and electroporation) to cross the lipid membrane barrier has been elucidated by Antonov et al. (12). They reported that quantized ion-conducting channels appear in unmodified 1,2-distearoyl-*sn*-glycero-3-phosphocholine membranes close to the phase transition and demonstrated more recently, that the conductance of different ions is in accordance with the well-known Hofmeister series (13). Similarly, Kaufmann and Silmann (14) as well as the literature (2,15–18) were able to demonstrate a close relationship between the physical state of the bilayer and ion current fluctuations. This relation-

ship is also the subject of an accompanying article by Blicher et al. (19).

In this article, we study the origin of ion current fluctuations in protein free lipid bilayers by a thorough comparison of timescales and amplitudes of ion current fluctuations with the heat capacity profile of lipid membranes. Although current fluctuations in the fluid phase appear as short (~ 2 ms) spikes, they last for ~ 20 ms as discrete steplike currents in the phase transition regime including long-lasting events of ~ 100 ms. Based on a model recently proposed by Grabitz et al. (20), we suggest that the increased timescales at T_m have their origin in the extended relaxation times generally found during lipid membrane phase transitions and originating from the flat thermodynamic potential and consequent weak restoring forces driving the system back to its equilibrium near T_m .

MATERIALS AND METHODS

Lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, $T_m = -4^\circ\text{C}$) and 1,2 dipentadecanoyl-*sn*-glycero-3-phosphocholine (D15PC, $T_m = 33^\circ\text{C}$) dissolved in chloroform were purchased from Avanti Polar Lipids (Birmingham, AL) and used without further purification. Hexadecane was dissolved in pentane to achieve a final concentration of 2.5% for the prepainting solution.

We performed current measurements using a patch-clamp amplifier (Cornerstone Series) from Dagan (Minneapolis, MN). The temperature was controlled during the experiments with the aid of a standard heat bath (Julabo, Seelbach, Germany). Heat capacity profiles of small unilamellar vesicles were recorded using a VP-DSC calorimeter from Microcal (Avestin, Ottawa, Canada).

To obtain lipid mixtures, lipids dissolved in chloroform were mixed in the desired proportion. Aqueous solution of lipids for calorimetric measurements were obtained as described previously (21). The experiments on the artificial membranes (in this case, black lipid membranes, or BLMs) were performed by using a setup consisting of two Teflon chambers separated by a Teflon foil of 25- μm thickness with a 150- μm diameter hole. The hole was produced by drilling it into the foil. Lipid membranes were obtained by prepainting the Teflon foil with the hexadecane solution and 15 min of waiting until the pentane was evaporated. Subsequently, both

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chambers were filled up to a level sufficiently far below the hole with a 200-mM NaCl solution. Ten microliters of lipid solution was spread on the water surface and after a waiting time of 15 min, the electrolyte level was raised above the hole. Before experiments, the bilayer was characterized by measuring the resistance and the capacity of the membrane.

RESULTS AND DISCUSSION

Theory

In thermodynamics, the different functions of state (enthalpy, energy, etc.), the susceptibilities (heat capacity, compressibilities, etc.) and the response times are coupled via the fluctuation-dissipation theorem (22). For lipid membranes, it has been shown by Grabitz et al. (20) and Seeger et al. (23) that the relaxation timescale τ after a pressure perturbation is proportional to the excess heat capacity Δc_p such that

$$\tau = \frac{T^2}{L} \Delta c_p, \quad (1)$$

where T is the temperature in Kelvin and L is a phenomenological coefficient independent of temperature (see Grabitz et al. (20), and Seeger et al. (23), for details). For 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) multilamellar vesicles (MLV), for instance, $L = 6.6 \times 10^8$ J K/mol and for DMPC large unilamellar vesicle (LUV) it is $L = 15.7 \times 10^8$ J K/mol. This equation implies that relaxation is slow if the system is at the heat capacity maximum, which should be very pronounced in lipid membranes exhibiting a phase transition. For multilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles, this time can be as long as 30 s. According to Onsager (24,25), each fluctuation can be considered as a perturbation of the system away from the entropy maximum. Therefore, fluctuation lifetimes and relaxation times are the same.

According to Einstein (26), fluctuations in the thermodynamic variable x_i are related to the curvature of the entropy potential S by

$$\langle \delta x_i \delta x_j \rangle = -k_B \left(\frac{\partial^2 S}{\partial x_i \partial x_j} \right)^{-1},$$

or for $x_i = x_j$, in the often experimentally more convenient free energy (G) expression

$$\langle \delta x \delta x \rangle = -k_B T \left(\frac{\partial^2 G}{\partial x^2} \right)^{-1},$$

where k_b is the Boltzmann constant and T the temperature.

This can be used to show that heat capacity and fluctuations in enthalpy H are proportional,

$$\langle \delta H^2 \rangle = \langle H^2 \rangle - \langle H \rangle^2 = RT^2 c_p,$$

around the entropy maximum (R is the gas constant). Along the same lines, one can show that the lateral compressibility κ_T is given by

$$\langle \delta A^2 \rangle = ART \kappa_T, \quad (2)$$

i.e., it is proportional to the fluctuations in area A . Heimburg (27) and Ebel et al. (28) have shown that changes in heat, in volume, and in area in lipid transitions are proportional functions. This leads to the relation

$$\Delta \kappa_T^A = \frac{\gamma^2 T}{A} \Delta c_p, \quad (3)$$

meaning that the lateral compressibility changes are proportional to the excess heat capacity (γ is a material constant of ~ 1 m²/J (27)). This further implies that it does not matter whether one considers a perturbation in enthalpy (after a temperature jump) or a perturbation in volume or area. The fluctuations in volume and area have the same lifetimes as those of the heat.

Pore formation in absence of channel-forming proteins occurs because of thermal area fluctuations. According to Nagle and Scott (29), the work to create a pore in the membrane is proportional to its lateral compressibility, which is proportional to the excess heat capacity (Eq. 3). As a consequence, pore formation is facilitated in the melting regime. Membranes are expected to be more permeable at the heat capacity maximum (for details, see (19)). Equating the fluctuation lifetime with the lifetime of pores, this implies that the pore opening times are proportional to the heat capacity. This relation has already been discussed in Seeger et al. (23), and in this article we will demonstrate that this relation leads to reasonable predictions. Both permeability and mean pore open time are at maximum in the chain melting transition. Experimental values for pore lifetimes are close to those estimated from Eq. 1.

Permeability changes during lipid membrane phase transition

To study the temperature dependence of lipid bilayers, a BLM was prepared at 40°C well above the phase transition of the lipid mixture used (5:95 DOPC/D15PC). Before the BLM experiments, the heat capacity of the lipid mixture has been measured (*inset* of Fig. 1), revealing a melting transition at $T_m \approx 30^\circ\text{C}$. To assure bilayer formation, the electric capacitance of the membrane film has been monitored. The bilayer was then left to equilibrate for 15 min. After film formation, the stability of the bilayer was tested by a stepwise increase of the clamp voltage until significant current fluctuation occurred (i.e., threshold voltage). Subsequently, the applied voltage V_M was decreased until the current fluctuations disappeared, leaving the system close to the conducting state. After 10 min waiting for equilibration, the temperature was lowered at a rate of 0.2°C/min at constant clamp voltage. Fig. 1 shows the overall transmembrane conductivity (averaged over 60 s) at $V_M = 400$ mV as a function of temperature. At $T = 36^\circ\text{C}$ the transmembrane conductivity begins to rapidly rise to reach a maximum at $T \approx 31.5^\circ\text{C}$. Clearly, ion permeability and heat capacity follow the same qualitative temperature dependence with a full-width at half height

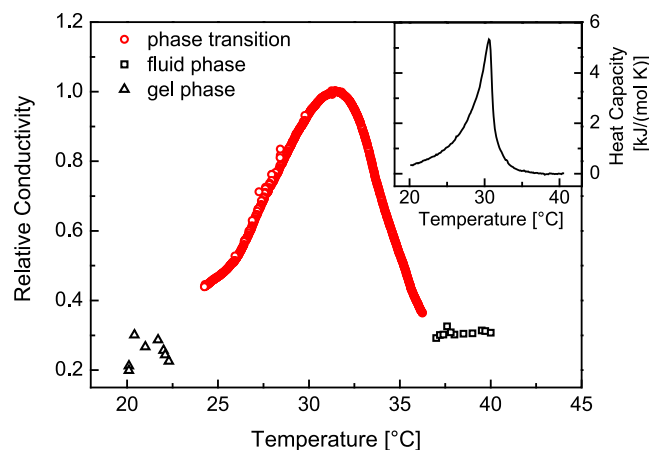


FIGURE 1 Relative conductance of a D15PC/DOPC (95:5) mixture as a function of temperature at $V_M = 400$ mV. A maximum is observed between 35°C and 25°C . The current trace clearly correlates with the heat capacity profile (see *inset*). The maximum corresponds to a conductivity of 2×10^{-7} S/cm². Conductivity measurements outside the transition region in the fluid (*squares*) and gel phase (*triangles*) have been performed on individual BLMs and are further described in the [Supporting Material](#).

of 3–6 K. The slight shift of $I_{\text{tot}}(T)$ toward higher temperatures is probably due to the different sample preparations. After sonification, small unilamellar vesicles are believed to be under some tension, whereas BLMs are rather relaxed. Tension, however, tends to increase the membrane area, thereby supporting a lowering in T_m . Finally, uncontrollable lipid accumulation around the septum might be an additional source of changes in T_m . We were able to observe the same correlation between heat capacity and ion permeability for D15PC/DMPC mixtures (see [Supporting Material](#)). However, the introduction of DOPC in our lipid mixtures significantly increased the stability of the planar lipid membrane. This can also be seen in [Fig. 2 b](#), where the bilayer resists membrane potentials as high as 1000 mV.

One possible origin of the increase in overall permeability is the increasing number of lattice defects at the gel/fluid phase boundary. This was suggested by several theoretical findings (30) and is supported by the work of Papahadjopoulos et al. (6), who found a maximum leakage of radioactive labeled sodium ions during lipid phase transition. An alternative approach to explain the increased permeability in the phase transition region was given by Kaufmann et al. (31) as well as by Nagel and Scott (29) and Blicher et al. (19), who address the changes in lateral compressibility κ_T and their relation to the fluctuations in area $\langle \partial A^2 \rangle$ (Eq. 2). Considering such area fluctuations as a source of defects excellently agrees with the correlation between heat capacity and conductivity.

Current characteristics strongly depend on lipid phase state

To unravel the origin of the transient conductivity behavior, we prepared BLMs from a DOPC/D15PC (5:95) mixture

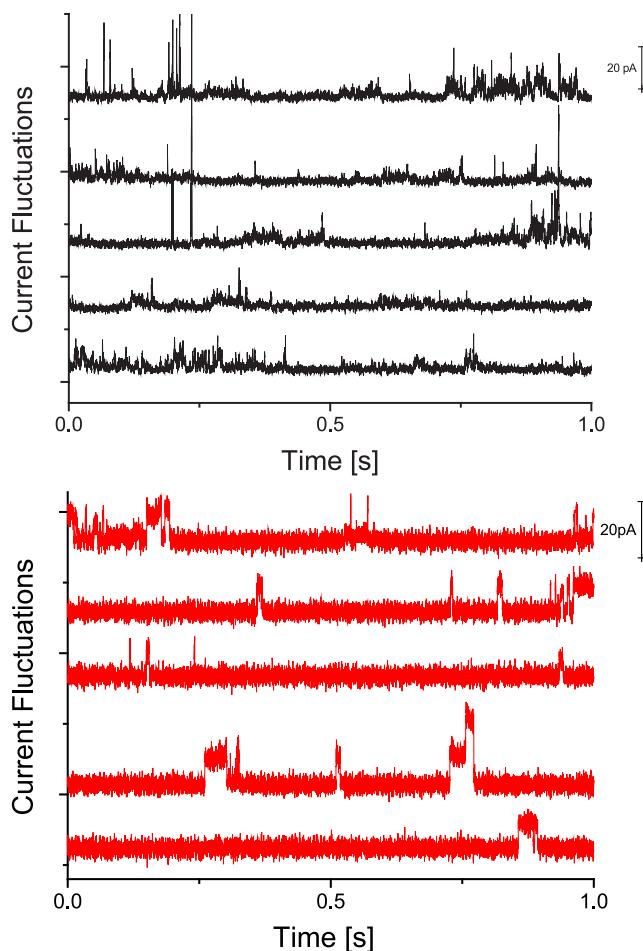


FIGURE 2 Typical current traces of a D15PC/DOPC (95:5) (*top panel*) in the fluid phase (33°C , $V_M = 100 \sim \text{mV}$) and (*bottom panel*) in the phase transition regime (31.5°C , $V_M = 1000$ mV). In the fluid phase, spikelike current fluctuations on short timescales are observed. In the phase transition regime, however, quantized fluctuations appear at longer timescales. Note that the typical timescales are strongly increased as compared with the top panel.

both in the fluid phase (40°C) and in the phase transition region (27°C). After a stepwise increase in V_M until values were reached at which current fluctuations were observed, the voltage V_M was again slightly reduced, resulting in stable current fluctuations over several minutes.

In the fluid phase ([Fig. 2 a](#), $T = 33^\circ\text{C}$, $V_M = 100$ mV), spikelike events were observed and did not change their characteristics at even higher temperatures. The current fluctuations in the phase transition regime ([Fig. 2 b](#), $T = 31.5^\circ\text{C}$, $V_M = 1000$ mV), however, appear rather steplike and quantized, with longer opening times. Again, the bilayer exhibited very high stability (the relation between applied voltage and initial resistance will be addressed in a separate article). For the DMPC/D15PC mixture, for example, strong current fluctuations were found at ~ 500 mV and below (see [Supporting Material](#)). An analysis of the current amplitudes results in a rather broad distribution with two small maxima at ~ 12 pS and 24 pS in the fluid state ([Fig. 3 a](#)). In the phase transition

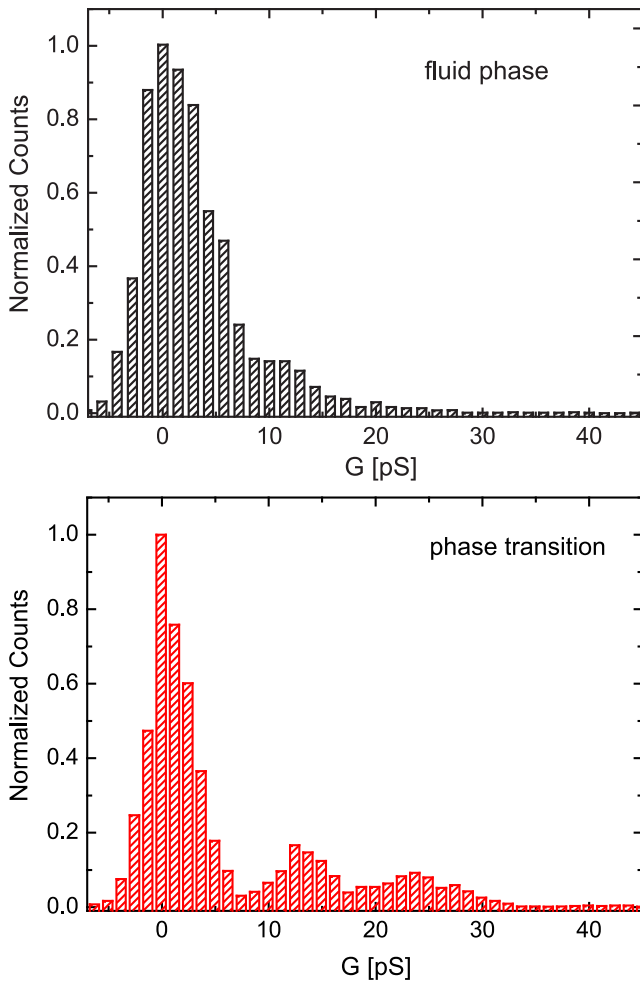


FIGURE 3 Conductivity histogram of current fluctuations in (*top panel*) the fluid phase ($T = 33^\circ\text{C}$, $V_M = 100\text{ mV}$) and (*bottom panel*) the phase transition regime ($T = 31.5^\circ\text{C}$, $V_M = 1000\text{ mV}$). The peak at $G = 0\text{ pS}$ corresponds to the baseline. In the fluid phase, no significant peaks appear. In the phase transition regime, distinct maxima around $G = 12\text{ pS}$ and $G = 24\text{ pS}$ appear, with some additional substructure around $G = 20$ and $G = 27\text{ pS}$. Data were collected from three individual bilayers by analyzing a 200 s long trace. The observed maxima agree in all measurements within a range of 20%.

regime, these maxima become very pronounced (Fig. 3 *b*). In addition, the gel state exhibited some current fluctuations, which, however either slowly disappeared when waiting for extended times or led to membrane rupture (see [Supporting Material](#)). Distinct quantized current fluctuations in lipid membranes have been reported before (2,12–18,29), ranging between $\sim 1\text{ pA}$ and $\sim 1\text{ nA}$ close to the phase transition temperature. However, so far, no clear relation among current amplitudes, distribution, and fluctuations for the different thermodynamic states of the lipid membrane has been given.

The existence of current fluctuations in both states suggests that the nature of the underlying mechanism for these current fluctuations is the same, but the specific characteristics seem to be determined by the physical properties of the membrane. In agreement with our results, we believe, that fluctuations in area (δA^2) are the origin of lattice defect

formation and hence permeating ions. The origin of the step-like shape of the current fluctuations remains unclear, yet is probably related to the physical properties of the liquid crystal. In principle, and based on our experimental results, one could model the pore size necessary for an ion to pass the membrane. For a one-step fluctuation (Fig. 2 *b*), this suggests a single pore diameter of $\sim 1\text{ nm}$, using a cylindrical pore approximation assuming free instead of surface-bound water within the pore.

Timescales of current fluctuation increase during phase transition

To unravel the relationship between thermodynamic behavior and the observed fluctuations, we analyzed the typical timescales of the opening times. Peaks were identified and characterized with a threshold criterion on the same set of data used to describe the conductance characteristics above (Fig. 3). In Fig. 4, we show the resulting data for the fluid phase (*circles*) and the phase transition (*triangles*). The logarithmic plot already reveals that the opening times are significantly increased within the phase transition regime. In the fluid phase, we found a mean average opening time of $\sim 3\text{ ms}$ (exponential fit). The behavior alters significantly when the phase transition region is entered. The mean average timescales shift by almost an order of magnitude to $\sim 20\text{ ms}$, including long-lasting states of up to $\sim 100\text{ ms}$. We also observed a broad variety of events including short events in the transition region and long fluctuations in the fluid state during measurements over extended periods of times. This is also reproduced by the broad distributions

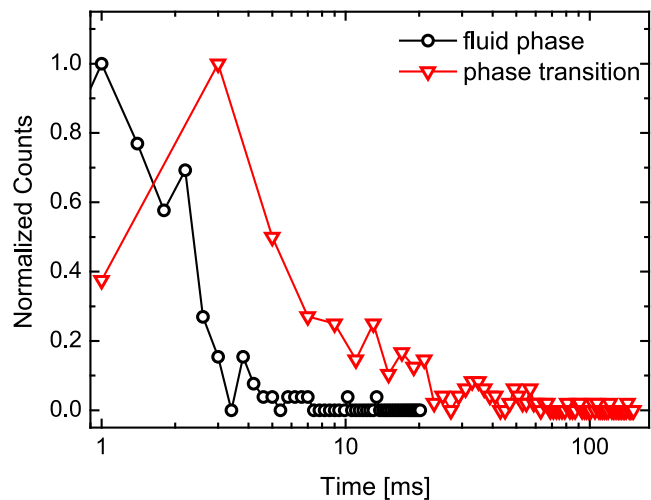


FIGURE 4 Logarithmic plot of the timescales of the current fluctuation in the fluid phase (*triangles*) and the phase transition regime (*circles*). The transmembrane currents have been measured under constant membrane potential V_m . The timescales of the fluid phase follow an exponential decay, whereas those in the transition can only be approximated by a double-exponential decay. The average lifetimes τ center around $\tau = 3\text{ ms}$ in the fluid and $\tau = 20\text{ ms}$ in the phase transition. This is in good agreement with our theoretical prediction.

shown in Fig. 3, and reflects uncontrollable variations in the lipid membrane structure and dynamics like, for example, the pool of lipids accumulating around the septum or the degree of asymmetry. However, the tendency toward increased timescales during lipid phase transitions is obvious and reproducible in every experiment. Importantly, these enhanced timescales are in line with the increasing relaxation times of the membrane in this regime (20) and originate most likely from the small restoring forces of the flat thermodynamic potential around T_m . Experimentally, increased relaxation times during the main phase transition of artificial membranes have been reported before by Tsong (32) as well as by Blume and Hillmann (33). Since the relaxation times reflect the reorganization of lipids in the membrane, one would expect that pore nucleation, pore opening, and closing, respectively, follow the same time evolution as lipid relaxation.

Taking the phenomenological coefficient L for DMPC MLV (as given above) and numbers for c_p from Fig. 1 (at $\sim 31^\circ\text{C}$), Eq. 1 yields lifetimes of 165 ms, whereas using the L from DMPC LUV yields lifetimes of 73 ms. Considering the transition half-width of only 2°C , this is very close to the experimentally found average lifetime of 20 ms (Fig. 4).

CONCLUSION

The behavior of current fluctuations in an unmodified phospholipid membrane under a constant voltage was found to be strongly sensitive to the phase state. Whereas fluctuations in the fluid phase reveal an unordered pattern on short timescales, in the phase-transition region, quantized fluctuations on much longer timescales occur. Using the fluctuation dissipation theorem and the linear relationship between heat capacity and area compressibility, we were able to predict the correct timescales and identify weak restoring forces of the flat thermodynamic potential as the origin of the extended timescales during lipid phase transition. Therefore, this article demonstrates the thermodynamic potential of lipid membranes to be the physical origin of lipid membrane current fluctuations.

SUPPORTING MATERIAL

Five figures are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(09\)00663-8](http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)00663-8).

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