Density Fluctuations in Lipid Monolayers and Their Possible Relevance to the Formation of Conductive Defects in Bilayers

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Received December 2, 1994. In Final Form: June 16, 1995[⊗]

It is known that bilayer membranes formed with a variety of phospholipids behave similarly to corresponding monolayers at lateral pressures in the range of 30–50 dyn/cm. Under this context, we analyze the thermodynamic fluctuations in the lateral density of phospholipid molecules in a monolayer, distinguishing between global and local fluctuations. For local fluctuations, the problem of finding the correlation area is solved by dividing the space into equal *domains* of areas $A_0 = 2000A$, where A_0 is the minimum area in which there is correlation between molecules in A and those in A_0. Then, the probability of a defect being produced in area A is $w(0) = \exp(-A/a)$, where a is the available area per molecule. From this, we calculate the conductance (G_{BLM}) of a hypothetical bilayer membrane as the product $G_{BLM} = P_{open}G_{defect}\Omega$, where the three terms are, respectively, the probability of bilayer spanning defect, the conductance of the defect, and the number of membrane domains experiencing a defect. We found that G values are of the order of experimental ones when $r = 9-11$ Å, what compares to the Nagle and Scott proposed value for bilayer holes. The relaxation time of defects was also estimated from Einstein's formula and using the coefficient of lipid lateral diffusion reported by Thompson and Huang, giving a time of the order of 20×10^{-7}, too short for experimental detection.

I. Introduction

Lipid bilayers membranes (BLMs) have been employed for the last few decades as models for many properties of cell plasma membrane. BLMs are essentially impermeable to small ions such as Na, K, etc., due to an extremely unfavorable partition coefficient lipid/water of about 10^{-30}.[1] As such, electrical resistances of unmodified bilayers are typically above the 10^8 Ω·cm² mark.[11] In order for lipid bilayers to conduct small ions, special permeation pathways are usually required. These structures have been described either as channels or as carriers.[1]

Despite of the above requirements, it has been extensively reported that bare bilayers conduct, albeit poorly, electrical current. Under strict conditions, which include proximity to the phase transition, intense applied electric fields, extreme degrees of curvature, and other bare lipid bilayers either may conduct electrical current in the form of discrete conductance steps[2] or simply may display macroscopic conductance.[3,4] Such conductivity has been generally explained using the concept of lipidic pores[5] or, in the case of near the critical temperature, T_c, defects of lipid domains produced by fluctuations in the density of phospholipid molecules.[6,8–10,15] Under normal conditions, at low voltages and far from critical regions, the conductivity of the bare bilayers has been explained by defects in the membrane matrix.[11] Also, application of strong and pulsed electrical voltages leads to another phenomenon, electroporation.[12] The "spontaneous" formation of pores may have a physiological role as in exocytosis and associated membrane fusion.[5]

The well-known enhancement of the ionic permeability near the main transition temperature has been described in terms of thermodynamic density fluctuations[8] or in terms of the static lateral compressibility of the bilayer.[7,13]

Since studies in lipid monolayers have yielded most of the present information regarding the interplay of lateral forces and the degree of packing of constituent phospholipid molecules, we have, in this study, resorted extensively to the use of monolayer-derived parameters.

Phospholipid monolayers are of special interest because they resemble half of the lipid bilayer. However, in order to transfer the information obtained by monolayer experiments to the bilayer system, it is important to establish those experimental conditions where the bilayer and monolayer are similar in their degree of packing of phospholipid molecules and the corresponding intermolecular interactions.

Comparison of monolayers and bilayers suggests that, as the temperature is changed, the thermodynamic state

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[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1995.

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0743-7463/95/2411-3943$09.00/0

of the bilayers may come close to the critical point in the monolayers.[7] The proximity of the transition state in bilayers to the critical point in monolayers suggests that lateral density fluctuations may be responsible for the increased permeability in bilayers. These fluctuations are associated with high lateral compressibility, as measured, for example, in lipid monolayers.[7]

It is generally agreed that, for a variety of phospholipids, the behavior of the bilayer system is similar to that of the respective monolayer at a lateral pressure in the range of 30−50 dyn/cm, because at this pressure the absolute area and the area change in both systems are the same.[14,15]

As suggested by many techniques, the lateral mobility of the molecules in the lattice is high in the liquid-crystalline state.[16] Fluctuations in the number of phospholipids molecules occupying a given area of membrane are then expected to occur as a consequence of the thermal energy of the system.

The main purpose of the present work is to examine the possibility that thermodynamic fluctuations of membrane lateral density may result in spontaneous and transient rarefactions in membrane density important enough as to be relevant to ion permeation.

II. Theory

Our rationale is based on the calculation of the probability of occurrence of a fluctuational density rarefaction in the BLM, important enough as to create a transient pathway for ion movement.

We first consider that there is some finite probability that, due to a fluctuational density change, a complete absence of molecules (henceforth called a DEFECT) of area A occurs in one of the two monolayers that constitute the BLM. Even though a complete void of molecules may never occur above a given value of A, it is nevertheless possible to define an equivalent void for any rarefation in the lateral density of phospholipid molecules.

We then define a minimum area A_0, encompassing A, where the behavior of molecules in A_0 influences those in A. The area A_0 is determined considering a value A_0^{max} beyond which the Poisson distribution for the probability $w(0) = f(A_0)$ becomes independent of A_0. From the above reasoning, it is possible to calculate the probability $w(0)$ of a defect of area A comprising a mean number of molecules $\bar{N}$ (eq I-9). In this way, the membrane is partitioned into *domains* in which there is the same probability of defect occurrence.

It is well-known that a system in thermodynamic equilibrium performs fluctuations in the number of its particles $\bar{N}$. The dispersion $\sigma_{\bar{N}} = \sqrt{\overline{\Delta N^2}}$ of this fluctuation is given by (see, for instance, ref 17a)

$$\sigma_{\bar{N}} = \sqrt{\overline{\Delta N^2}} = \sqrt{-\frac{\bar{N}^2 kT}{V^2\left(\frac{\partial P}{\partial V}\right)_T}} \qquad \text{(II-1)}$$

where k is the Boltzmann constant, T is the absolute temperature, P is the pressure, and V is the volume. This dispersion corresponds to a probability distribution in the particle number given by

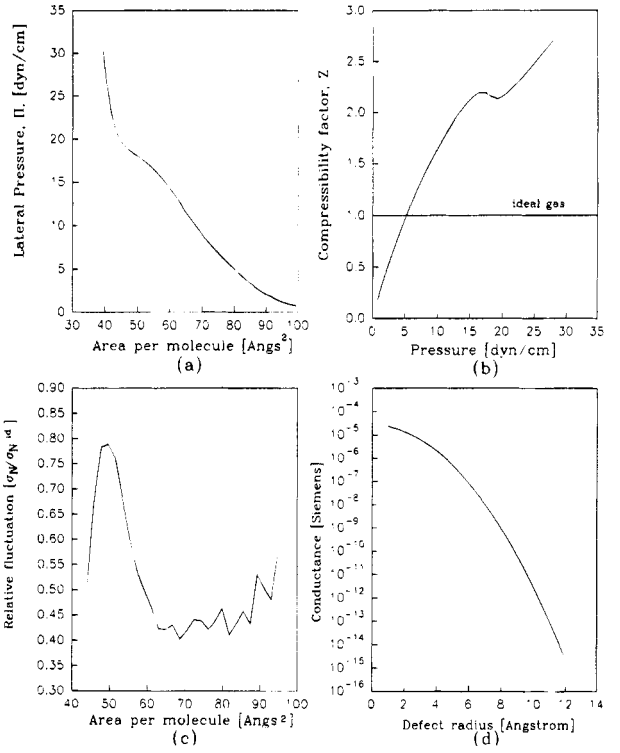

Figure 1. (a) Phase diagram of the model lipid dimyristoylmethylphosphatidic acid, temperature 20 °C, pH = 5.0. Data extracted from ref 18. (b) Ditto. Compressibility factor. (c) Ditto. Relative particle number fluctuations $\sigma_{\bar{N}}/\sigma_{\bar{N}}^{ideal}$. (d) Fluctuational conductance for a 1-cm² membrane in 1 M KCl solution (logarithmic scale).

$$w(N) = \frac{1}{\sqrt{2\pi}\sigma_{\bar{N}}} \exp\left[-\frac{1}{2}\left(\frac{N-\bar{N}}{\sigma_{\bar{N}}}\right)^2\right] \qquad \text{(II-2)}$$

In the case of an ideal gas $P = \bar{N}kT/V$, eq II-1 gives

$$\sigma_{\bar{N}}^{ideal} = \sqrt{\bar{N}} \qquad \text{(II-3)}$$

For a bidimensional system with a given available area per molecules a, eqs II-1 and II-3 give

$$\sigma_R = \frac{\sigma_{\bar{N}}}{\sigma_{\bar{N}}^{ideal}} = \sqrt{-\frac{kT}{a^2\left(\frac{\partial \Pi}{\partial a}\right)_T}} \qquad \text{(II-4)}$$

where Π is the lateral force exerted in the system per unit length. This reasoning can be extended to a lipid monolayer (see below in which conditions) where the available area per molecule a and the derivative of the force with respect to this area $(\partial\Pi/\partial a)_T$ can be obtained from the phase diagram (Π vs a) obtained experimentally with a Langmuir balance. It can be observed experimentally, Figure 1a, that, in the gel−liquid-crystalline phase transition, $(\partial\Pi/\partial a)_T$ can be quite low. As a consequence, high fluctuations in particle number are expected. Data for performing Figure 1a were extracted from ref[18] but are available in many other works.

In Figure 1b is plotted the compressibility factor $z = \Pi a/kT$ vs Π; this coefficient is a measure of the deviations of the system from ideal behavior. Equation 4 gives the fluctuation in particle number relative to a system without interparticle interaction (ideal gas) with the same number

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$\bar{N}$ of particles (Figure 1c). $\sigma_{\bar{N}}/\sigma_{\bar{N}}^{\text{ideal}}$ is a measurement of the correlations between the particles. This quotient is less than unity, meaning that, due to interparticle interaction, $\sigma_{\bar{N}} < \sigma_{\bar{N}}^{\text{ideal}}$, and consequently, the distribution given by eq II-2 is more localized. From Figure 1c at $a = 50$ Å^2, we obtain $\sigma_R^{-1} = 1.28$. This ratio will be used as a *correction factor* to extend the results obtained from ideal gas to real monolayers (see Results).

Equation II-2 can be expressed through the basic Einstein formula:[19]

$$w(N) = \frac{1}{\sqrt{2\pi}\sigma_{\bar{N}}} \exp\left(-\frac{\Delta F_t}{kT}\right) \qquad \text{(II-5)}$$

with ΔF_t given by

$$\Delta F_t = \frac{1}{2}\left(\frac{N-\bar{N}}{\sigma_{\bar{N}}}\right)^2 kT \qquad \text{(II-6)}$$

Equation II-6 as such is not valid at the critical point ($\sigma_{\bar{N}} = \infty$) and in its neighborhood; another term which takes into account the spatial variation of the density, $\nabla\delta$ ($\delta = \bar{N}/A$), has to be added.[17b] If the chosen area A of the bidimensional gas is sufficiently small, the number of particles is correspondingly small, but the relative fluctuation still can be considerable, and eq II-2 is no longer valid. In this small system, it is not permissible to make associations between statistical mechanical quantities and thermodynamical functions, since many of the thermodynamic functions will not be well-defined macroscopic experimental quantities. In this case, it is a valid Poisson's probability distribution for this kind of fluctuation.[17c] For a bidimensional gas, we have

$$w(N) = \frac{N_0!}{N!(N_0-N)!}\left(\frac{A}{A_0}\right)^N\left(1-\frac{A}{A_0}\right)^{N_0-N} \qquad \text{(II-7)}$$

where A_0 and N_0 are the gas total area and particle number, respectively, and A is the area inside A_0 which contains N particles. Equation II-7 for $N = 0$ gives

$$w(0) = \left(1-\frac{A}{A_0}\right)^{N_0} = \left(1-\frac{1}{n}\right)^{n\bar{N}} \qquad \text{(II-8)}$$

where N_0A/A_0 is the mean value $\bar{N}$ of particle number in A and $n = A_0/A$. For n sufficiently large, eq II-8 transforms into

$$w(0) = \exp(-\bar{N}) = \exp\left(-\frac{A}{a}\right) \qquad \text{(II-9)}$$

Equation II-9 can also be derived from the Gibbs distribution,[17b] remembering that, for each state of thermodynamic equilibrium, we have a determined available area a per molecule affecting, in this way, the value of $\bar{N}$. We observe also that the probability does not depend of A_0, for n larger than approximately 2000, meaning that, beyond an area A_0 of about $2000A$, the influence of the surrounding particles on the particle distribution in A is practically negligible. We then define the term *membrane domain* as the area $A_0 = 2000A$. As a consequence of the uncorrelation between domains, each domain can be considered as an "autonomous" submembrane that functions in complete independence from the rest. If we extend this reasoning to the corresponding contralateral monolayer, we can consider a macroscopic bilayer membrane as an in-parallel collection of independent membrane patches, each patch constituting a domain. This also implicates that, for each defect size considered, there is an associated domain area. As a consequence of the above assumption, the macroscopic bilayer membrane conductance can be expressed as the sum of the individual domain conductances.

III. Results

If we consider a bilayer of area A_{BLM}, the number of domains of area A_0 (in which defects occur) will be $\Omega = A_{BLM}/A_0$. This number can be quite considerable in a macroscopic system like a bilayer. We partition now a domain in a number χ of *subdomains* having each subdomain the area of a defect. Thus, $\chi = A_0/A$. The probability that a defect corresponding to the removal of $\bar{N}$ molecules occurs in one of the two monolayers within a domain is given by eq II-9 as

$$w(0)_{\text{domain}} = \exp(-\bar{N}) \qquad \text{(III-1)}$$

Since $w(0)$ in eq II-9 refers to complete absence of particles, we have that $\Delta N = \bar{N}$. In the presence of intermolecular interaction, $\Delta N_{\text{ideal}}/\Delta N_{\text{real}}$ will be the correction factor of 1.28 derived in the Theory section. In a real system, the probability for a fluctuation ΔN will then be equivalent to the probability of a fluctuation $1.28\Delta N$ in the ideal system.

Correcting for intermolecular interaction and then eq III-1, we obtain

$$w(0)_{\text{domain}}^{\text{int}} = \exp(-\bar{N}) = \exp(-1.28\bar{N}) \qquad \text{(III-2)}$$

The probability that the defect occurs at a given subdomain inside the domain will then be

$$w(0)_{\text{local}} = \frac{w(0)_{\text{domain}}^{\text{int}}}{\chi} \qquad \text{(III-3)}$$

This refers to the probability of defect occurrence in one of the two monolayers of the domain. The joint probability that a defect occurs also in the corresponding subdomain of the contralateral monolayer is then

$$P_{\text{open}} = \left[\frac{w(0)_{\text{domain}}^{\text{int}}}{\chi}\right]^2 = \left[\frac{\exp(-1.28\bar{N})}{\chi}\right]^2 \qquad \text{(III-4)}$$

where P_{open} defines then the probability of occurrence of a bilayer-spanning defect inside a domain. The conductance of a unitary bilayer spanning defect, referred to as G_{defect}, is calculated assuming the defect as a solution-filled cylinder of sectional area A and length d, where d is equal to the bilayer thickness. In this case, we have

$$G_{\text{defect}} = \left(\frac{A}{d}\right)\varrho \qquad \text{(III-5)}$$

where ϱ is the conductivity of the electrolyte solution bathing the membrane. The macroscopic (or total) conductance of the bilayer membrane is then given by

$$G_{\text{BLM}} = P_{\text{open}}G_{\text{defect}}\Omega \qquad \text{(III-6)}$$

The total bilayer conductance can be then expressed as a function of the defect radius as

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Table 1. Conductance of a 1-cm² Lipid Bilayer Membrane Based on Defect Formation, as a Function of Defect Radius

defect radius, Å	defect molecules $\bar{N}$	defect conductance G_{defect}, S	relaxation time τ, ns	defect probability P_{open}	macroscopic conductance G_{BLM}, S·cm^{-2}
1	0.03	7.020×10^{-11}	5	2.128×10^{-7}	2.378×10^{-5}
2	0.25	2.808×10^{-10}	20	1.313×10^{-7}	1.467×10^{-5}
3	0.57	6.318×10^{-10}	45	5.878×10^{-8}	6.567×10^{-6}
4	1.00	1.123×10^{-9}	80	1.906×10^{-8}	2.130×10^{-6}
5	1.57	1.755×10^{-9}	125	4.482×10^{-9}	5.008×10^{-7}
6	2.26	2.527×10^{-9}	180	7.640×10^{-10}	8.536×10^{-8}
7	3.08	3.439×10^{-9}	245	9.440×10^{-11}	1.054×10^{-8}
8	4.02	4.492×10^{-9}	320	8.455×10^{-12}	9.447×10^{10}
9	5.09	5.686×10^{-9}	405	5.490×10^{-13}	6.134×10^{-11}
10	6.28	7.020×10^{-9}	500	2.584×10^{-14}	2.887×10^{-12}
11	7.60	8.494×10^{-9}	605	8.817×10^{-16}	9.852×10^{-14}
12	9.05	1.010×10^{-8}	720	2.181×10^{-17}	2.436×10^{-15}
13	10.61	1.186×10^{-8}	845	3.910×10^{-19}	4.369×10^{-17}
14	12.32	1.375×10^{-8}	980	5.083×10^{-21}	5.679×10^{-19}
15	14.14	1.579×10^{-8}	1125	4.790×10^{-23}	5.351×10^{-21}
16	16.08	1.797×10^{-8}	1280	3.271×10^{-25}	3.655×10^{-23}
17	18.16	2.028×10^{-8}	1445	1.620×10^{-27}	1.810×10^{-25}
18	20.36	2.274×10^{-8}	1620	5.815×10^{-30}	6.497×10^{-28}
19	22.68	2.534×10^{-8}	1805	1.513×10^{-32}	0
20	25.13	2.808×10^{-8}	2000	2.854×10^{-35}	0

[a] Conductivity of membrane bathing solution was considered to be 11.173 Ω^{-1}·m^{-1}, which is the conductivity of a KCl solution at 25 °C. [b] Defect relaxation time was calculated for a DMPA monolayer at a lateral pressure of 18.06 dyn·cm^{-1} corresponding to $a = 50$ Å² with $D_{PL} = 10^{-8}$ cm²·s^{-1}.

$$G_{BLM} = \left[\frac{\exp\left(-\frac{1.28\pi r^2}{a}\right)}{\chi}\right]^2 \left(\frac{\pi r^2}{d}\right) \varrho \frac{A_{BLM}}{2000\pi r^2} \qquad \text{(III-7)}$$

In Table 1 are shown the values of G_{BLM} given by eq III-7, together with the probability of occurrence of bilayer spanning defects (P_{open}) and defect relaxation times, as a function of the defect radius. From the results in Table 1, it is clear that there is a narrow range of defect radii compatible with the experimentally observed values of lipid bilayer membrane conductance. If we consider a typical experimental value of the electrical conductance of unmodified egg lecithin BLMs as $G_{BLM} = 10^{-10}\ \Omega^{-1}$·cm^{-2}, this puts the typical average defect radius, according to Table 1, at about 9–10 Å. If we consider the range of experimentally determined bilayer conductances 10^{-9}–$10^{-10}\ \Omega^{-1}$·cm^{-2},[11] this implicates average defects having radii between 6 and 11 Å. The relaxation time of a fluctuational defect with area $A = \pi r^2$, in the monolayer, can be obtained by application of the Einstein formula $\tau = r^2/2D_{PL}$, which can be put as

$$\tau = \frac{A}{2\pi D_{PL}} = \frac{\bar{N}a}{2\pi D_{PL}} \qquad \text{(III-8)}$$

where D_{PL} is the self-lateral diffusion coefficient of the phospholipid molecules.[16] Table 1 also presents values of τ as a function of defect radius. Relaxation times of defects of the radius between 6 and 11 Å fall in the 200–600-ns mark. This implies conductive defects of extremely short duration, probably not detectable experimentally (see Discussion).

IV. Discussion

The mechanism of permeability of phospholipid bilayers to ions is of considerable importance from the point of view of charge transfer through biological membranes. Despite considerable evidence that charge translocation occurs essentially through definite specialized structures inserted in the plasma membrane matrix, the role of conductive membrane defects to that process seems to be gaining increasing acceptance in restricted cases. The evidence for that kind of mechanism comes mainly from the fact that bare bilayers, even when formed under careful experimental conditions and free of impurities present finite resistivities, are not explainable simply by the passage of "dissolved" ions across the matrix.

The recent observation of lipid domains[21] containing enormous numbers of lipid molecules points to the existence of degrees of molecular organization in lipid bilayer membranes not suspected a few decades ago. The recent field of membrane electroporation reveals that the lipid barrier can be broken in a reversible way provided the right combination of deterministic (electrical field) and stochastic (fluctuational density changes) influences impinge upon the membrane.[22] In this way, fluctuational defects, even occurring in half-bilayer, could be eventually amplified and sustained by the energy of the applied electric field.

Our analysis of defect formation is based on lateral compressibility data from monolayers at the air–water interface but is derived from fluctuational parameters from an ideal gas. In effect, at a range of lateral pressures, monolayers do present very small lateral intermolecular interactions, with $(\partial\Pi/\partial a)_T$ values as low as 2×10^{16} N/m^3. We have explored the possibility that, at least in regions of minimal intermolecular interactions, fluctuational voids could account for the observed conductivity of unmodified BLMs.

Bilayer membranes and the corresponding half-bilayers do behave differently, as has been demonstrated by many workers. The problem of obtaining information about bilayer behavior from monolayer experimental studies is controversial. Blume[14] has undertaken an analysis of this problem. Unfortunately, most of the information can be summarized in the fact that there is a range of lateral pressures in which bilayers and monolayers behave similarly. The measurement of a working lateral pressure for a bilayer system is not also trivial, being considered to be about 30 dyn/cm for DPPC membranes.[13]

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The problem of calculating the probability of a bilayer-spanning defect was dealt with by assuming that the two monolayers constituting the bilayer have a reasonable degree of independence. Thus, defect formation on one half would not be influenced by the behavior of the other half. This assumption is based on the somewhat controversial issue of the degree of interaction between the two opposing monolayers that constitute a bilayer. Pink[13] reports that the intermonolayer interaction is about 2% of that within each monolayer, for PC membranes. On the other hand, studies by Tien and Dawidowicz[25] suggest that there is an appreciable intermonolayer interaction, due to van der Waals forces, and that this effect is pivotal to the mechanism of bilayer formation, working in analogy to a "zipperlike" action.

The "filling" of a defect with aqueous solution is probably critical for the analysis of fluctuational defects in bilayers. Since water and phospholipid headgroups interact strongly, it is difficult to predict what happens in a defect. Since water has a well-known stabilizing effect on the membrane, it is reasonable to suppose that the formation of a defect is counteracted by the lipid/water interaction (see ref 5, for example). In larger defects, adjoining water will probably tend to penetrate into the defect, importantly modifying its evolution.

It is well-known that water permeates bilayers[24] at a rate not always explainable by a simple diffusion mechanism. Defects or pores have been proposed to explain the high rate of water translocation in the bilayers. This means that the energies involved in defect formation and water "inclusion" are of the same order for a range of conditions.

Also, the known fact that monolayers formed at the air–water interface have enormous transverse water permeability, not explainable simply by their half-thickness as compared to bilayers, can be taken as evidence that mechanisms other than simple diffusion hold for water movement.

The experimental finding that the current vs voltage curve of nonmodified bilayers is ohmic up to about 100 mV[11] does not rule out the possibility that, at higher voltages, fluctuational defects play a role. Indeed, Chernomordik et al.[23] have undertaken a careful study of cell and bilayer membrane electrical breakdown. These authors clearly detect the existence of reversible breakdown, which they explain by the formation of membrane defects.

Fluctuational defects can be amplified or even sustained in the presence of a strong applied electric field (2×10^7 V/m) as in a common experimental setup (or normally existing across living cell membranes). This raises the possibility that a defect occurring in one of the monolayers constituting a bilayer might be amplified or even sustained by the field, leading to a bilayer-spanning pore (see for instance, ref 20 or ref 23).

The lifetimes of the fluctuational membrane defects, as calculated here, are exceedingly small (on the order of nanoseconds) to permit experimental detection with the presently available amplifiers. Only when summed in parallel over a great number of bilayer domains would these defects result in a detectable average membrane conductance.

Conductive events in unmodified lipid bilayers experimentally observed in many conditions, as unitary current steps, are probably the result of spontaneous density fluctuations, amplified or stabilized by the applied electric field. In this case, very brief hydrophobic pores could be changed, due to the intense electric field, into hydrophylic structures having lifetimes many orders of magnitude larger. This might explain the poor reproducibility of such events and their modification by even slight impurities in the system. It is a quite common observation in bilayers studies that the presence of conductive flickers signal the rupture of the membrane at voltages above the 200-mV mark. It is generally agreed (but not consistently demonstrated) that certain impurities greatly increase flickering and lead to membrane rupture. This possibility was considered by Antonov et al.,[2] who concluded, however, that impurities are not the cause of their channellike conductive events. Unfortunately, there is not sufficient controlled experimental evidence for a quantitative analysis of this effect. Chernomordik et al.[23] resorted to UO_2^{2+} ions as a modifier/impurity to promote electrical breakdown in membranes.

An important issue related to impurities, which may have relevance for the present study, is their effect on the phase transitions of bilayers. This problem has been dealt with by Pink,[13] who modeled the effect of integral proteins (viewed as "impurities") on the phase-transition temperatures of bilayers. This question was also treated by Albon and Sturtevant,[26] who quantified the extent of "melting" fraction of a membrane as a function of the impurity mole fraction in the mixture. In this context, impurities can play a role on the probability the occurrence of defects.

The experimental evidence for our proposal comes mainly from the comparison between the calculated values of bilayer conductance (from Table 1) and the values experimentally reported in the literature. Along this reasoning, and in order for the values of bilayer conductance reported in Table 1 to fall within the range of experimentally reasonable values, defects with average radii between 6 and 11 Å are required. This is within the order of defect sizes obtained by Nagle and Scott.[7]

Acknowledgment. This work was partially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil).

LA9409541

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