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Cholesterol favors the emergence of a long-range autocorrelated fluctuation pattern in voltage-induced ionic currents through lipid bilayers

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ABSTRACT

The present paper was aimed at evaluating the effect of cholesterol (CHO) on the voltage-induced lipid pore formation in bilayer membranes through a global characterization of the temporal dynamics of the fluctuation pattern of ion currents. The bilayer model used was black lipid membranes (BLMs) of palmitoyloleoylphosphatidylethanolamine and palmitoyloleoylphosphatidylcholine (POPE:POPC) at a 7:3 molar ratio in the absence (BLM_0) or in the presence of 30 (BLM_{30}), 40 (BLM_{40}) or 50 (BLM_{50}) mol% of cholesterol with respect to total phospholipids. Electrical current intensities (I) were measured in voltage (ΔV) clamped conditions at ΔV ranging between 0 and ± 200 mV. The autocorrelation parameter α derived from detrended fluctuation analysis (DFA) on temporal fluctuation patterns of electrical currents allowed discriminating between non-correlated ($\alpha = 0.5$, white noise) and long-range correlated ($0.5 < \alpha < 1$) behaviors. The increase in $|\Delta V|$ as well as in cholesterol content increased the number of conductance states, the magnitude of conductance level, the capacitance of the bilayers and increased the tendency towards the development of long-range autocorrelated (fractal) processes ($0.5 < \alpha < 1$) in lipid channel generation. Experiments were performed above the phase transition temperature of the lipid mixtures, but compositions used predicted a superlattice-like organization. This leads to the conclusion that structural defects other than phase coexistence may promote lipid channel formation under voltage clamped conditions. Furthermore, cholesterol controls the voltage threshold that allows the percolation of channel behavior where isolated channels become an interconnected network.

1. Introduction

One of the prominent membrane functions is the regulation of the transport of ions or other molecules. Ions have substantial energetic barrier to be transferred from water into the membrane. However, the energy of ions in a membrane decreases due to the presence of restricted water domains between lipids [1] and pores (channels) of high dielectric permittivity [2]. In textbooks [3], channels are mostly conceived as intrinsic membrane proteins gated by specific ligands [4], voltage-gated [5] or mechanically activated [6]. The ability of the lipid environment to modulate the activity of proteinaceous channels has been acknowledged [7], however, the lipid part of the membrane is mainly considered a permeability barrier for polar compounds and mere electrical insulator [3]. Conversely, for more than 40 years it has been known that pure lipid membranes in their chain melting regime and phase coexistence can be highly permeable to water, small molecules and ions [8,9].

Voltage-induced currents across pure lipid membranes exhibit discrete ion conduction events similar to proteins [10,11]. Hence, the concept of lipid ion channels (pores) has been coined to described this behavior in pure lipid bilayers where they were induced experimentally [2,12–15] and simulated through molecular dynamics [16]. Based on the conductance value and its dependence of the ion size, the radius of the average lipid pore formed in phospholipidic membranes was estimated as 1 nm [17]. Conversely, lipid mixtures containing ceramide are able to form channels large enough to translocate proteins [18]. A working model describes ceramide channels as columns of ceramide monomers that span the membrane and assemble to form a barrel-like structure [19].

The similarity between the quantized conductance and ionic current fluctuation pattern exhibited by protein free membranes and that what is considered to be due to protein channels led to propose that "the theoretical description of channels would lie in the thermodynamics of the membrane and its cooperative phase behavior rather than in the geometry of individual protein" [2]. On the same line of reasoning, the remarkable cholesterol concentration dependency of the activity of the human erythrocyte glucose transporter [20] has been considered an example of a regulatory effect of membrane lateral order, but it might also be suspected as a case of membrane permeability increase due to membrane packing defects at a phase coexisting condition.

Abbreviations: BLM, black lipid membrane; bSM, synaptosomal membranes purified from bovine brain cortex; CHO, cholesterol; DFA, detrended fluctuation analysis; POPC, palmitoyloleoylphosphatidylcholine; POPE, palmitoyloleoylphosphatidylethanolamine

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Lipid ion channels appear in an asymmetrically stressed membrane. Charge imbalance [16] or the application of low-voltage, long-duration electrical pulses induce rearrangements of the membrane components that ultimately lead to the formation of aqueous hydrophilic transient pores [17]. However, for some high values of transmembrane voltage, irreversible electrical breakdown occurs with the consequent irreversible membrane rupture, by one or more supercritical pores formation that expand to the membrane boundary [21].

Currently, the phenomenon of voltage-induced lipid ion channels is vigorously debated among researchers who are interested in the study of specific ion channel conductivity and those who are attracted mainly by the study of natural and artificial membranes. For both the black lipid membrane (BLM) represents a useful model and in some cases is the model of choice. Thus, BLM electrophysiology provides several distinct advantages for these studies, including control of the constituents on either side of the membrane, manipulation of lipid composition, and the ability to study rare or challenging channels inaccessible to patch-clamp methods. In addition, studies on the BLM model may contribute to the understanding of several biologically relevant processes, including fusion, lysis, and apoptosis of cells that, as well as electrical cell activity, also involve an opening of a lipid pore and interestingly, have been shown to involve non-lamellar structures [22].

There are evidences that the presence of non-bilayer forming phospholipids (i.e. PEs or cardiolipin) is also essential for the activity of peripheral and integral membrane proteins [23–25]. It has been proposed that these phospholipids might modulate the protein function since they exert different lateral pressure at different depth of the bilayer [25]. In particular, the binary mixture composed of phospholipid mixture 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) has been widely used to build up BLMs for protein channel reconstitution [26–28] but, up to our knowledge, the ion permeability exhibited by this type of lipid matrix perturbed through the application of a transmembrane voltage potential, has not received deep attention. The polar group of PEs forms intermolecular hydrogen bonds which provide unique properties to the hydration network at the lipid water-interface [29,30] and may play an important role in defining the pore formation tendency and consequently the conducting properties of these bilayers.

Beyond the membrane model and composition, in studies on membrane pore formation tendency and conducting behavior, the type of data analysis may be crucial to display the emergence of the complexity underneath the phenomenon under investigation. It is well established that the shape and duration of biological membrane electrical events fluctuate in time [31]. Focus has been centered on fluctuations from individual proteinaceous ionic channels, in particular, the temporal sequence of open and close times. Traditionally, it is considered that proteinaceous ionic channels present few possible states and that the probability of switching from one state to another depends only on the present state of the channel (i.e. is random; not autocorrelated) [32]. Frequently, fluctuations on different time scales may appear to be self-similar, in other words, the magnitude and rate of events may fluctuate over many temporal scales [31], show time series with fractal characteristics and memory, and could be considered fractal noise [33]. A large body of experimental and computational evidence [31,32,34–39] indicate that at least some proteinaceous ionic channels and other proteins exhibit significant memory effects (long-range autocorrelations) and fractal dynamics (for reviews see [31,32]). Moreover, fluctuations in membrane conductance (in the absence of proteinaceous ionic channels) also can present non-random dynamics [40] mainly under certain conditions, for example by applying a voltage step of sufficiently high amplitude [17]. To our knowledge, the conditions that favor the appearance of long-range correlations and fractal dynamics in lipid membranes, associated with voltage changes and cholesterol concentration, have not been systematically studied.

Thus, in the present work we studied voltage-induced ion current fluctuations in a phospholipid binary mixture of POPE:POPC in a 7:3 molar ratio, in the absence or in the presence of cholesterol (CHO). To get closer to the molecular complexity of a biomembrane, the behavior of a complex mixture of lipids extracted by the Folch–Lees method [41] from bovine brain cortex was also analyzed. The main objective was to achieve a global characterization of the temporal dynamics of the ion current fluctuation pattern under the hypothesis that it encodes some aspects of the membrane structural dynamics.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesterol (CHO) were obtained from Avanti Polar Lipid, Inc. (Alabaster, Alabama). HEPES was from AppliChem GmbH (Darmstadt, Germany), n-decane from Mallinckrodt (Paris, KY, USA), KCl was from Merck (Darmstadt, Germany) and solvents (chloroform and methanol, HPLC grade) were from Sintorgan S.A. (Argentina).

2.2. Lipid source and solution preparation

Lipids used were either synthetic or became the lower phase of a Folch's partition [41] of synaptosomal membranes purified from bovine brain cortex (bSM).

bSM were obtained as described previously [42]. Briefly, meninges were eliminated, the cortex dissected, and the bSM were purified essentially according to the method of Enna and Snyder, modified by Perillo and Arce [43], lyophilized and stored at −20 °C. Immediately before use, membranes were resuspended in 20 volumes of 2:1 (v/v) chloroform/methanol. Then, 0.2 volumes of water were added to achieve a 2-phase separation. The upper phase and the interface were eliminated. The solvent of the lower phase was evaporated and the residual lipids were resuspended in n-decane.

Stock solutions of synthetic lipids in 2:1 v/v chloroform/methanol stored at −20 °C were mixed immediately before used to obtain a 7:3 mol/mol POPE: POPC binary mixture with 0, 30, 40 or 50 mol% of cholesterol (CHOL) with respect to total phospholipids. Solvent was evaporated under a stream of nitrogen and n-decane was added to reach a ~20–25 mg/ml final total lipid concentration.

Controls made in Langmuir films evidenced that n-decane was expelled from a monolayer when it reached ~30 mM/m. This lateral surface pressure is close to what is considered the equilibrium lateral pressure of bilayers ([44], see Supporting information).

2.3. Planar lipid bilayers

Planar lipid bilayers (BLMs) composed of phospholipids with different cholesterol content (BLM_0, BLM_{30}, BLM_{40} and BLM_{50}) or bSM (BLM_{bSM}) were formed, with the aid of a thin glass rod, by painting with the lipid solution over a circular hole (150 μm diameter) sculpted in the polystyrene cuvette of a bilayer chamber (model BCH-13A, Warner Instruments Inc., Hamden, CT). The cuvette was inserted into a polyvinylchloride holder, thus defining a wall and two aqueous compartments separated by a planar lipid film as was described previously [5,45]. Prior to bilayer formation, the hole was "coated" with a small quantity of the lipid cocktail on the *cis* side of the wall and was allowed to dry before adding solutions to the chambers. Both compartments, *cis* and *trans*, were filled symmetrically with 10 mM HEPES, 150 mM KCl and pH 7.4 electrolyte solution. Electrical connections were made via 2% (w/v) agar salt bridges in 200 mM KCl into each chamber, and silver–silver chloride electrodes. High ionic strength salt bridges are used to minimize liquid junction potentials [46]. To avoid mechanical vibrations and interference from electric fields during the measurement, the bilayer chamber with two Ag/AgCl electrodes in the *cis* and *trans* compartment