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Role of electrolyte in the occurrence of the voltage induced phase transitions in a dioleoyl phosphatidylcholine monolayer on Hg

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ABSTRACT

The present study investigates the effect of electrolyte ions on the occurrence of potential induced phase transitions at ca. -0.93 V and -0.99 V vs Ag/AgCl respectively of dioleoyl phosphatidylcholine (DOPC) monolayers on the mercury (Hg) surface. The interaction of both inorganic and organic electrolytes with DOPC was studied and it was shown that the alterations in the nature of the capacitance peaks representing the successive phase transitions depend on the ionic strength and the interfacial properties of the electrolyte ions. Higher ionic strength and cations with stronger binding affinity to DOPC give rise to steeper electric fields across the phospholipid layer shifting the apparent potential characterising capacitance peak-1 to more positive values. Conversely the anion, I^-, which binds to DOPC, lessens the field gradient across the lipid and shifts the potential characterising capacitance peak-1 to more negative potentials. At more negative potentials to those characterising capacitance peak-1, electrolyte penetrates the DOPC. As a consequence a higher ionic strength and smaller hydrated cations with higher charge density enhance the screening of Hg, leading to stronger electric fields at the Hg surface. This effect shortens the onset and increases the rate dependence on the potential of the adsorption of bilayer patches to Hg by a nucleation and growth (N & G) process.

1. Introduction

Ionic interactions with phospholipids have been studied extensively using various biomimetic models such as supported bilayers [1–3] monolayers [4–7], vesicles [8,9] and tethered bilayers [10,11] and are important for investigating the effect of the ions on biomembrane function [12–36]. Studies on the interaction of metal cations with dioleoyl phosphatidylcholine (DOPC) have shown that the strength of alkali metal cation binding to the head group follows the order: $Li^+>Na^+>K^+>Rb^+>Cs^+$ in accordance with the reverse Hofmeister series with no significant adsorption by anions excepting the larger Br^- and I^- [9,36]. This ion specificity can be based on several different physical mechanisms determined by ion pairing, hard and soft nature of ions, membrane defects and their geometry and membrane properties. Force spectroscopic studies support the findings of Vacha [36] and reveal that a higher force is needed for an AFM tip to penetrate the DOPC membrane in solutions with higher ionic strength and with cations such as Na^+ and Ca^{2+} demonstrating that penetration/binding of the ions on to phospholipid head groups is related:- (a) linearly: to an increase in their solution concentration and (b) inversely: to the size of the unhydrated cations [37]. It has also been shown that the water structure around the ions and lipid head groups is distorted within the ion-lipid association [38–40].

As a model membrane, the phospholipid monolayer on mercury (Hg) system has received a lot of interest over the past thirty years [41–54]. Of particular significance are the electric field-induced lipid phase transitions [42,51–53]. Owing to the association between electrolyte ions and phospholipids as described above [12–36], the role of electrolyte ions in the mechanism of the potential-induced lipid phase transitions is of great interest. The present study is an attempt to answer this question by observing the effect of concentration and compositional changes of the electrolyte on the field-induced lipid phase transitions [42,48–51]. These phase transitions give rise to characteristic peaks on the capacitance-potential plot (Fig. 2a). Capacitance peak-1 occurring at about -0.93 V vs Ag/AgCl is indicative of the ingression of electrolyte ions into the dioleol phosphatidylcholine (DOPC) monolayer assembled on the surface of Hg [48]. At potentials negative to the phase transition underlying capacitance peak-1, electrolyte ions approach the Hg surface concurrent with the formation of a structured phospholipid-electrolyte emulsion [52,53]. This is supported by AFM force-distance studies conducted on the DOPC coated Hg electrode showing an increase in the thickness of the monolayer to about 4.6 nm [54]. Capacitance

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peak-2 occurring at the more negative potential of ~ -0.99 V vs Ag/AgCl represents the adsorption by a nucleation and growth (N & G) mechanism [52] of lipid bilayer patches on the Hg surface to give a bilayer of thickness 6.3 nm [54]. In addition to these transitions, a further phase transition occurs at more negative applied potentials represented by a series of capacitance peaks associated with the formation of semi-vesicles followed by a collapse of these structures at even more negative potentials to form an ill-defined monolayer on the electrode surface. The nature of these phase transitions are unique to the phospholipid on Hg system and represent a subtle interplay between the applied electrode potential and the phospholipid, water and electrolyte interactions [50,53,54]. The phases existing at more negative applied potentials are associations of phospholipid with water with different conformations of the phospholipid as described in ref [54]. The field-driven phase transitions of DOPC on Hg are mechanistically different from the classical systems of 2D potential-induced phase transitions [55–59]. These classical phase transitions involve film condensations on Hg and are dependent on temperature. In contrast, the potential-induced phase transitions of DOPC on Hg are relatively insensitive to temperature [48]. This present work is an attempt to relate the origin of the DOPC transitions underlying capacitance peaks-1 and -2 to the involvement of electrolyte ions in their mechanism.

2. Experimental

2.1. Materials and methods

The inorganic electrolytes KCl, KNO_3, LiCl, NaCl, $CaCl_2$, $BaCl_2$, NaF, KF, RbF, CsF, LiOH, NaOH, KOH, KBr and KI were calcined in the muffle furnace and oven as appropriate to oxidise organic impurities depending on their decomposition temperature and melting points for a minimum of 4–6 hours. Inorganic electrolyte solutions were prepared of ionic strength 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 mol dm^{-3} KCl and 0.1 mol dm^{-3} of all other inorganic electrolytes using 18.2 MΩ MilliQ water. Organic electrolytes such as ionic liquids were not calcined because of their decomposition even at low temperatures. 1-butyl-3-methylimidazolium chloride (BMIMCl), tetraethylammonium chloride (TEAC) and choline chloride (ChoCl) were added to 0.1 mol dm^{-3} KCl. All electrolyte solutions were purged with argon gas for 15–20 minutes prior to experiment. After deaeration, a blanket of argon gas was maintained above the fully deaerated solutions to avoid penetration of any oxygen into the solution during all experiments. A DOPC solution (Avanti Lipid) of 2 mg cm^{-3} was prepared in pentane (HPLC grade, Fisher Scientific Chemicals Ltd.) in a clean glass vial and was stored in the freezer. A glass syringe was used to transfer the lipid solution to the electrolyte surface in the electrochemical cell. DOPC monolayers were prepared by spreading about 15 μdm^3 of the DOPC working solution at the argon-electrolyte interface in the electrochemical cell [60–63]. A period of about 5–10 minutes was required for the pentane to evaporate.

Both a Kemula-type hanging Hg drop electrode (HMDE) and fabricated Hg film electrode (MFE) were used for the experiments. The HMDE assembly (Polish Academy of Sciences, Warsaw-Poland) with extruded Hg drop was lowered on to the lipid-electrolyte surface slowly so that the drop touched the DOPC covered electrolyte surface for a sufficient time to allow for monolayer deposition. Lipid transfer to the apolar Hg resulted in the formation of a DOPC monolayer on Hg. The HMDE was coated with a fresh DOPC layer prior to each series of experiments by lifting the Hg above the interface, tapping the electrode to knock the used drop off, forming a new drop by turning the scaled sleeve and lowering the electrode down into the solution across the lipid-electrolyte interface.

The MFE was prepared on a silicon wafer based microfabricated platinum (Pt) electrode (Tyndall National Institute, Ireland) with a Pt disc diameter of 1 mm. Embedded on the same wafer was a Pt rectangle as a contact pad. The Pt disc was connected to the contact pad by a 0.5 mm thick Pt trace interconnect that was insulated with approximately 1.5 mm of Si_3N_4 deposited by plasma enhanced chemical vapour deposition (PECVD). The Pt electrode was cleaned prior to the Hg deposition using a solution of H_2SO_4 (Fisher Scientific) and 30% H_2O_2 (Fluka) mixture (piranha solution) and rinsed with Milli-Q 18.2 MΩ water (Millipore, U.K.) before drying. The Pt electrode was then subjected to rapid cyclic voltammetry (RCV) from -0.2 to -3.0 V vs Ag/AgCl at 40 Vs^{-1} to remove any remains of organic film followed by the manual deposition of Hg using an Eppendorf microliter pipette on the dried Pt disk to form the MFE. The MFE is advantageous in terms of its reusability utilizing a small amount of Hg because it can be cleaned similarly to the Pt surface by voltammetric cycling with negative potential excursion between -0.2 and -3.0 V vs Ag/AgCl at 40 Vs^{-1}. Strongly adsorbed impurities can be removed from the Hg surface by cycling to a more negative potential of -4.0 V vs Ag/AgCl without causing any damage to the Hg or to the Pt underneath.

The electrochemical cell and all other glass apparatus were washed with piranha solution to remove organic contamination. Piranha solution was kept in the cell for at least an hour for efficient cleaning before performing experiments. Also, the counter electrode and reference electrode were immersed in the same solution for a few seconds to remove any organic contamination before each experiment. Cleaning the ceramic frit of the reference electrode is necessary because a blocked or dirty frit will cause erroneous results in the electrochemical measurements. The apparatus and the electrodes were then rinsed with Milli-Q water to remove any piranha residue since the presence of trace piranha in the electrolyte can damage the lipid.

2.2. Electrochemical techniques and set-up

An Ag/AgCl electrode with porous sintered glass frit separating the inner 3.5 mol dm^{-3} solution from the outer electrolyte solution was employed as reference electrode and all potentials in the paper are quoted versus this. A Pt rod was used as counter electrode. The electrochemical apparatus was contained in a Faraday cage. RCV was used as a preliminary screen of the monolayer properties by recording a fast scan “fingerprint” at a scan rate (ν) of 40 Vs^{-1}. RCV measurements were carried out with a PGSTAT 30 Autolab potentiostat (Ecochemie, Utrecht, Netherlands) interfaced to PowerLab 4/25 signal generator (AD Instruments Ltd.) controlled by Scope software. The integrity of the DOPC monolayer was checked at the beginning and end of each experiment.

Alternate current voltammetry (ACV) was used to characterize the capacitance peaks on the capacitance–potential (C_{sp}-E) profile by measuring the out-of-phase current in response to the application of a sine wave of 0.005 V amplitude and 75 Hz frequency added to a negatively increasing DC potential from -0.2 to -1.2 V. ACV measurements were carried out using the Autolab system: GPES with PGSTAT 30 and controlled with Autolab software. The specific capacitance is related to the current from ACV scans by the Eq. (1):

$$C_{sp} = I/\omega A \Delta V \tag{1}$$

Where $\omega = 2\pi f$ is the angular frequency and ΔV is the amplitude of the sine wave.

Double potential step chronoamperometry was used to study the kinetics of the phase transition underlying capacitance peak-2. Chronoamperometric measurements were carried out using the same electrochemical apparatus as employed for RCV. Each series of potential step experiments was carried out on a freshly deposited DOPC monolayer on the Hg surface. In double potential step experiments, current (I)-time (t) transients were recorded in