Boundary potential of lipid bilayers: methods, interpretations and biological applications

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Abstract. The electric field distribution at boundaries of cell membranes consists of diffuse part of the electrical double layer and the potential drop over the polar area generally attributed to dipole effects. This report focuses on the molecular nature of dipole components of boundary potential and its relation to bilayer structure as it follows from different experimental approaches and molecular dynamic (MD) simulations. Alterations of the total boundary potential (BP) of planar bilayer lipid membranes (BLM) can be detected by the method of Intramembraneous Field Compensation, developed in our laboratory. When combined with traditional electrokinetic measurements in liposome suspension and Volta potential control at lipid monolayers it reveals alterations of the dipole potential induced by multivalent cations (Be^{2+}, Gd^{3+}) about 100-150 mV. It is related to the lipid phase transition detected by isotherm titration calorimetry (ITC) measurements. IFC method combined with perfusion of the cell show reversible electrostatic effects due to lysine adsorption and irreversible binding of polylysines accompanied by fast positive changes of BP as electrokinetic measurements, and slow negative ones attributed to BP dipole component. According to model proposed it related with changes of lipid hydration state varied by incorporation of ions or organic molecules into the lipid monolayer. Molecular dynamic simulations support this idea and relate dipole effects with H-bonded water molecules and lipid lateral coordination. Both effects are assumed as the principal reason of Gd^{3+} blocking effect on *E.coli* mechanosensitive channels.

1. Introduction

Electrostatic phenomena at cell walls are essential for membrane transport commonly studied by their lipid models – liposomes, planar bilayer lipid membranes (BLM) and lipid monolayers. Our studies are motivated by the idea that the dipole component of electric field distribution over a membrane/water interface (boundary potential, BP) reports on significant alterations of membrane structure and is accompanied by changes of membrane elasticity. The same effects in natural cells may modify the conformation state of membrane proteins and control their enzymatic activity. This idea was tested by experiments with bacterial mechanosensitive channels (MscL) of E-Coli incorporated into artificial lipid membranes of different composition (shown in figure 1).

Lipid membranes placed at the tip of glass pipet and exposed to varied hydrostatic pressure increased their lateral tension. Other technical details of the experiment published in the original paper [1]. Transmembrane current demonstrate pulses at high pressure as result of the channel opening by lateral tension applied to membranes. Experiment with membrane made from neutral phosphatidylcholine (PC) demonstrate, that MscL itself is not sensitive to Gd^{3+} (left panel). If some amount of negatively charged phosphatidylserine (PS) molecules are present in the membrane, MscL is completely blocked by Gd^{3+} cation but reactivated when this cation washed out from media (right panel). It proves a key role of phosphatidylserine in blocking effect of lanthanide on mechanosensitive channels. This fact motivates our special attention to anion phospholipids related to their interaction with inorganic cations and polycations of biological interest.

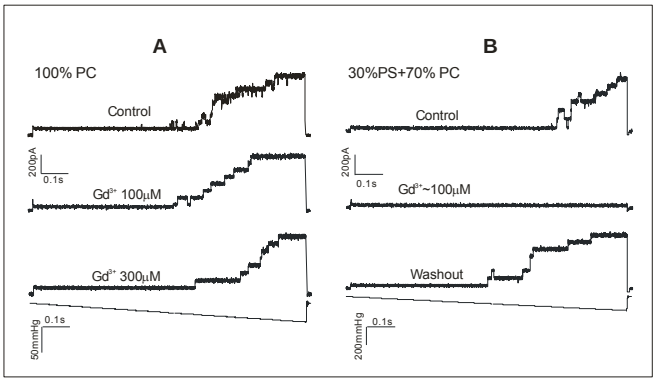

Figure 1. Current traces of MscL reconstituted to lipid membranes from soybean PC (A) and PS (B) placed at the tip of pipet and recorded at the linear pressure pump in the presence of different GdCl3 concentration [1].

The current presentation focuses on the experimental approaches developed in our laboratory to control electrostatic field at membrane/water interface (boundary potential, BP), which consists of two components – electric field drop across the diffuse part of the electrical double layer, and electric potential in polar area of the lipid membrane [2]. Two principal components of the electric field at the membrane/water interfaces qualitatively illustrated by figure 1 where the electric field across the membrane is shown by a red line. The membrane composed of negatively charged lipids and facing to different ionic media with the same potential in the bulk (the short circuit condition). The negative surface potentials (φ_s^1 and φ_s^2) drops at both sides up to zero in the bulk; dipole components (φ_d^1 and φ_d^2) directed to high but different positive values inside of the membrane. The latter fact follows from many experimental works (i.e. [3,4]). Finally, the trans-membrane potential applied to hydrophobic area is defined by the difference between the boundary potentials.

$$\varphi_{in} = \varphi_b^1 - \varphi_b^2 \tag{1}$$

Because of the electrostriction effect on membranes, their electrostatic capacity is sensitive to this potential and becomes zero if compensated by external voltage applied between two sides of planar bilayer lipid membranes (BLM). The method of Intramembranous field compensation (IFC), the

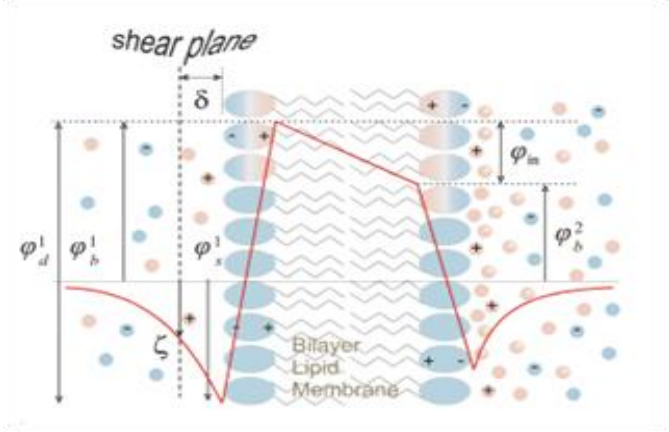

Figure 2. The electric field distribution (red line) at the lipid membrane interfaces.

experimental setup and the procedure of this compensation were developed in our laboratory and described in details in [2]. Two principal points are essential to note. The electric potential at the membrane surface can be found by electrokinetic measurements in liposome suspension. These measurements shown below were performed by dynamic scattering technique. It gives the sign of surface charge and potential but a slightly smaller magnitude, which corresponds to the potential value at some distance, δ, from the surface at the shear plane [5]. One needs alternative electrostatic methods to evaluate this parameter, for instance the IFC method.

2. Results and discussion

The surface potential, φ_s, related to screening the surface charge by ionic media as it well defined by formulas of Gouy-Chapman model. To describe the ion equilibria at the membrane surface it supplied with Langmuir type isotherms. We do not present here details and formulas of this theory, known as Gouy-Chapman-Stern (GCS) and presented in many original paper and reviews [6,7]. The second component of the boundary potential is generally attributed to dipoles oriented across the membrane interface. The dipole potential, φ_d, in spite of fact that electric field in this area is partially created by charged groups of substances or inorganic ions placed under Helmholtz plane assumed, which corresponds to membrane outer surface. Indeed, the molecular nature of dipole potential may differ for many real systems. Several examples of this presentation illustrate this situation by the help of BLM techniques and complementary methods.

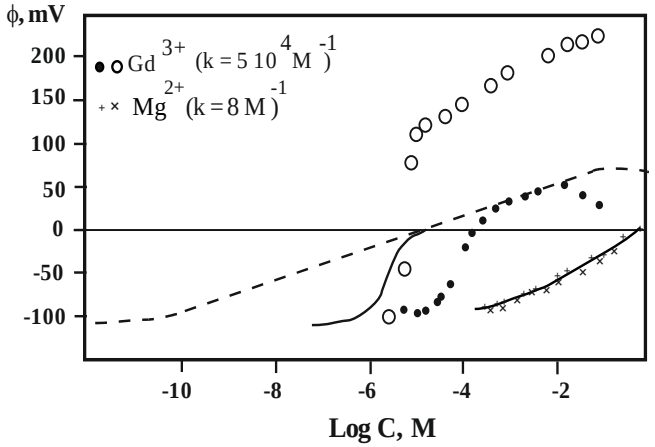

Figure 3. Boundary potential of planar BLM (opened points) and surface potential of liposomes (closed points) at different Gd^{3+} and Mg^{2+} concentration. Solid lines are for Gd^{3+} cations and GCS model with abscissa as total ion amount in the cell and dotted curve as a bulk ion concentration [8].

Electrostatic effects accompanied cation adsorption are illustrated in figure 3 by surface and boundary potentials measured in liposome suspension and by IFC method with planar BLM [8]. Experimental data related to membranes made from PS at varied concentration of Gd^{3+} (opened and closed points) and Mg^{2+} (crosses). Theoretical curves were calculated according to GSC model with binding coefficients shown in figure. Note, the adsorption of Mg^{2+} is well defined by the traditional version of the model with share plane position at δ=0.2 nm and experimental data for surface and boundary potentials coincide. The slope of the theoretical curve is about 30 mV per decade as it is predicted for the screening effect by divalent cations. It is not a case with Gd^{3+} cations. A high slope of experimental curves suggests a very high affinity of these cations to the lipid membrane surface, when their bulk concentration decreases (depletion effect). It means that mass balance condition has to be included in the GCS model. Dotted line in the figure corresponds to the traditional GCS model and has the predicted slope about 20 mV per decade for trivalent cations. Solid curve is for modified model

with the same binding constant and abscissa for total ion amount in the experimental cell with undefined bulk concentration [8]. Because of high affinity of Gd^{3+} cation it became "potential determined" ion at bulk concentration of 10^{-10} M [5]. Independently on the depletion effect, a very significant difference between the surface and boundary potential was detected with these cations. They neutralize and overcharge the surface with a surface potential from -100 to +50 mV but naturally, they have no proper dipole moment to create the dipole effect about +150 mV. This effect is an intrinsic property of lipid membrane, which consists of negatively charged PS molecules. This conclusion follows from result of two experimental series depicted in figure 4 [8].

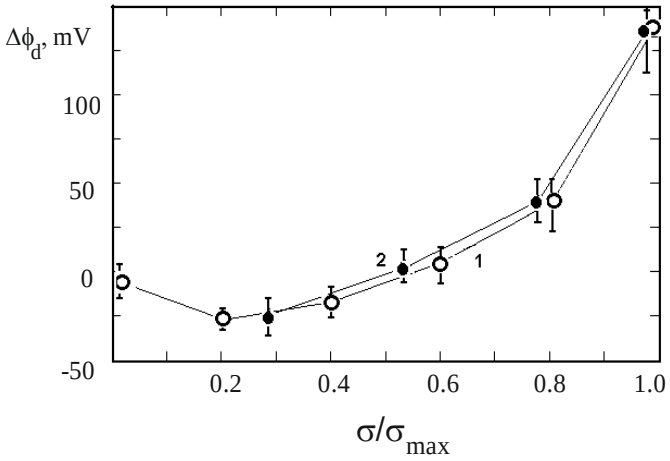

Figure 4. Dipole component of boundary potential induced by Gd^{3+} adsorption at membranes of varied PS/PC composition (opened points) or made from PS at different pH (black points) [8].

A series of measurements done with lipid membranes of varied surface charge density, σ, contains of PS in PS-PC mixture (opened points). Alternative variation of σ is realized by pH control in the media. Each point in figure 4 represents the surface potential of liposomes subtracted from the boundary potential of planar BLM measured at the same Gd^{3+} concentration in the bulk. This procedure removed all electrostatic effects due to changes in the surface charge and its screening by

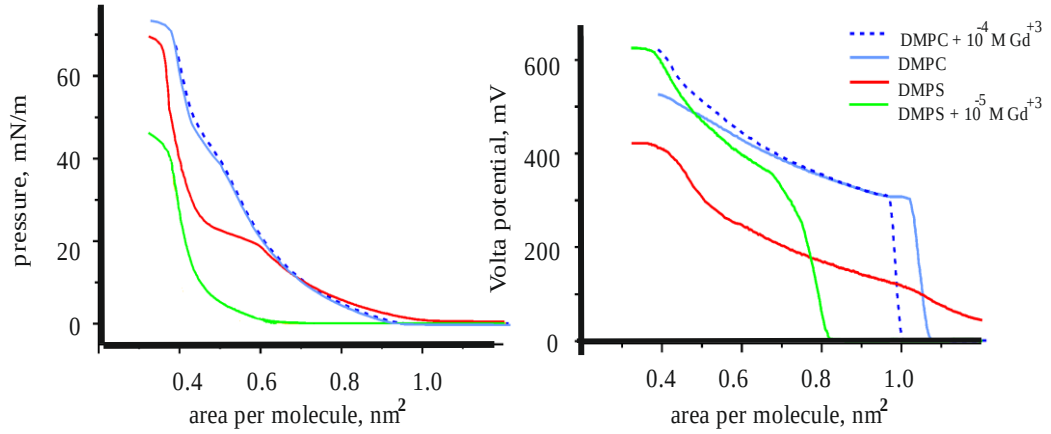

Figure 5. Lateral pressure (left panel) and Volta potential (right panel) measured simultaneously by compression of DMPC (blue lines) or DMPS monolayer at 10 mM of KCl and in the presence of sown $GdCl_3$ concentrations [1].

the electrolyte. The result is attributed to the change of the dipole component of the boundary potential $\Delta\phi_d$. An important conclusion follows from the data in figure 4, that negatively charged PS molecules, but not PC are a source of the dipole effect. This conclusion is supported by independent measurements with the technique of Langmuir monolayers (figure 5) [1].

Pressure-area diagrams and Volta potential of monolayers from dimyristoyl derivatives of PC and PS measured simultaneously are shown in the figure 5 by the same color. The shape of the diagrams with DMPC (blue lines) shows no sensitivity to adsorption of Gd^{3+}. Volta potential of these monolayers detects the cation adsorption as a positive shift about several tens of mV at the area below 0.6 nm per one PC molecule. The difference between "pure" PS monolayers and PS+Gd^{3+} is significant as in the pressure-area diagram and Volta potential measurements (compare red and green curves). Monolayers of PS over subphase of 10 mM KCl reveals a phase transition at a pressure about 20 mN/m with compressibility increased in solid (gel) state and Volta potential deflects to positive side at the same area. The same slope was observed in PS monolayer over subphase with Gd^{3+} concentration 10^{-5} M (green curve at left panel), which close to its bulk concentration at zero charge point in electrokinetic data for PS liposomes (solid theoretical curve in figure 3). The corresponding curve of Volta potential shifted to positive direction for about 200 mV as result of surface neutralization and the additional dipole effect. It leads to conclusion about gel state of lipid monolayer initiated by cation adsorption. Isotherm titration calorimetry reveals interesting details of this process [1].

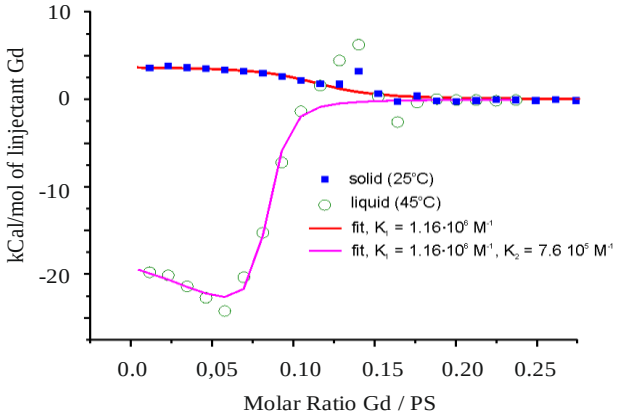

Figure 6. Integrated ITC data measured in DMPS suspension [1].

Integrated heat-production curves in figure 6 illustrate the result of $GdCl_3$ injection to suspension of DMPS liposomes performed by ITC technique at temperatures below and above the lipid phase transition (about 37° C). The molar ratio Gd/PS between 0.1 and 0.15 corresponds to zero charge point in the electrokinetic data. Note that Gd^{3+} binding to solid DMPS is an exothermic reaction, whereas it binding to liquid membranes causes massive heat release. However, at the end the heat effect changes its sign to positive because DMPS liposomes come to solid state. Simulation of this system by molecular dynamics reveals probable reasons of its behavior. It visualized the effect of multivalent cations (balls in figure 7, left panel) interaction with polar heads of lipids, which coordinates 2-3 lipid molecules into nano-clusters [9]. Some of them are colored in right panel and looks stable for long MD-trajectories (typical 100 ns). This fact explains their effect on the membrane elasticity revealed by experiments with MscL, incorporated in artificial membranes (figure1). The same effect is assumed important for any proteins imbedded to natural membranes.

It is necessary to mention that conclution about potential-elasticity relation does not depend on the presence of multivalent cations. We found correlation of these parameters for any systems studied by Langmuir monolayers [10]. One example is shown in figure 8.

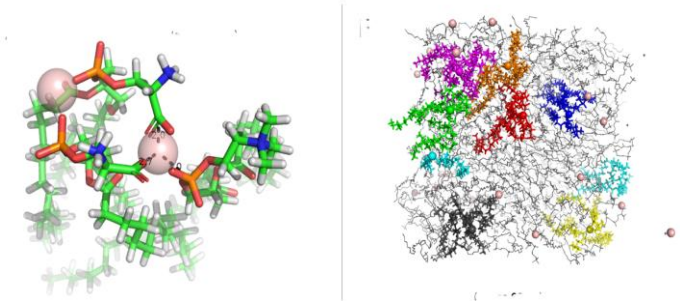

Figure 7. Coordination of PS molecules by multivalent cations (left) and their stable nanoclusters colored in right panel according to MD simulation.

Two derivatives namely the Area and Volta potential in respect of pressure defined for experimental data of figure 5 (red lines) and depicted by open and closed points in figure 8. The scales of both parameters rescaled to get a similar position of ordinates. It gives a coefficient E correlated these parameters as $\Delta\phi/\Delta p = E\,(\Delta A/\Delta p)$. The same correlation revealed for DMPS monolayers placed at different concentration of electrolyte KCl: 10^{-4}, 10^{-3}, 0.01, 0.1, and 1.0 M, and leads to values of E equal to 0.54, 0.55, 0.57, 0.49 and 0.88 V nm^{-2}, respectively. This fact clearly demonstrates the direct relationship between electrostatic and elastic phenomena in these systems. These experimental curves depicted in figure 9 in special scale as the work applied to compress monolayer (upper) and Volta potential (upper) versus to lateral pressure. It presents electrostatic-elastic relation in the clearest way [11].

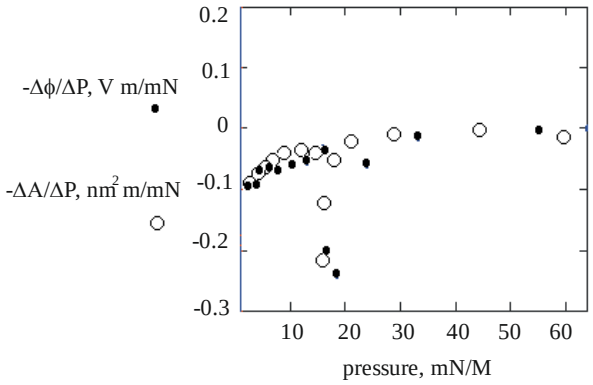

Figure 8. Increment of elasticity and Volta potential vs lateral pressure [10].

Data presented in figure 9 suggests a simple empirical description. The relative increment of monolayer compressibility, *dA/A*, assumed proportional to increment of lateral pressure with modulus k_p:

$$dA/A = dP/k_p\,. \tag{2}$$

This relation similar to Hook's law leads to integral form

$$A = A_0 + A_e \exp(-\frac{P}{k_p})\,. \tag{3}$$

It allows to determine the mechanical work applied to compress the monolayer of DMPS as:

$$W = A_0 P + A_e P \exp(-\frac{P}{k_p})\,. \tag{4}$$

Here A_0 and A_e – fixed and varied components of area per molecule. If electrostatic potential

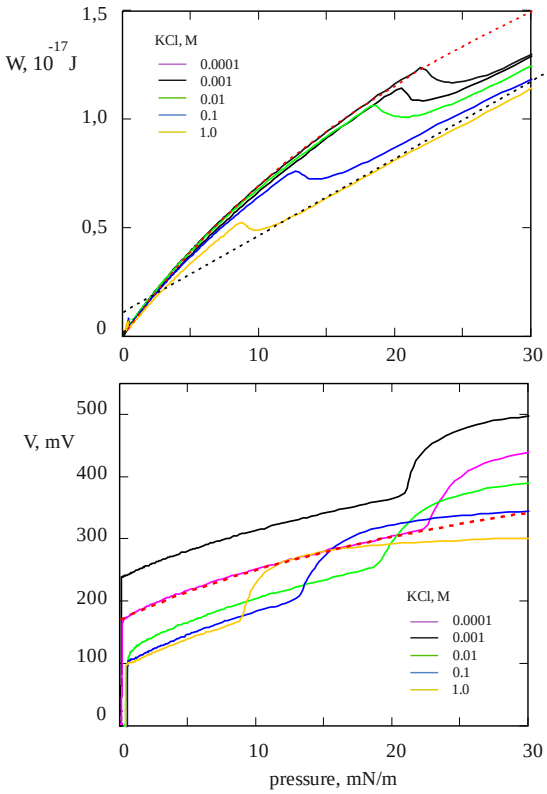

Figure 9. Data of compression DMPS monolayers at the subphase of different KCl concentration. Ordinate in upper panel is area per molecule multiplied by pressure (see equation (4)).

changes assumed proportional to increased compression energy, it gives the expression for changes of Volta potential with fitting coefficient N_d and f_0 for parallel shift in ordinate:

$$\Delta V = f_0 + N_d[A_0 P + A_e P \exp(-\frac{P}{k_p})]. \quad (5)$$

Dotted red curves in figure 9 corresponds to one of the experimental curve (violet) measured at 10^{-4} M KCl. It well described the shape as the pressure-area diagram and Volta potential below the point of phase transition with empirical parameters A_0=34 $Å^2$, A_e=58 $Å^2$, k_p= 20 m/mN and N_d = 0.12 V/N nm^2. After phase transition all experimental curves in upper panel move to the same asymptote with a linear dependence between W and lateral pressure:

$$W=109+35.5\ P \quad (6)$$

These parameters slightly vary for other curves. It means that the elasticity and electrostatics of DMPS monolayer nearly the same at different KCl concentration. In contrast, the presence of electrolyte is essential for critical value of pressure when monolayer transformed from liquid to solid state. This value became smaller when increased concentration of KCl and surface change of negatively charged PS molecules decreased due to K^+ adsorption. The same conclusion is true for series of experiments, when ionization state and surface charge of PS varied by pH of electrolyte (not shown). In both cases empirical relations listed above are well applied. All experimental curves presented in scale of upper panel positioned between two asymptotes/ which corresponds to liquid and solid states of lipids in monolayer.

3.General speculations and conclusions.

The biological importance of phosphatidylserines is related to their ability to control membrane elasticity and by this conformation mobility of proteins as result of PS interaction with inorganic actions in the media. Intrinsic properties of PS revealed in by Langmuir technique have the same molecular nature related with water molecules associated with the lipid polar heads. It depends on the cation type adsorbed at the membrane surface. Inorganic cations, especially multivalent ones, facilitate lipid phase transition to solid, gel state. Dipole component of boundary potential reports on this transformation and results from cation modification of lipid lateral interaction is accompanied by cluster formation in lipid membranes. The initial reason of this phenomenon may be the reorganization the net of hydrogen bonds between lipids. This hypothesis follows from the preliminary data of molecular dynamics and is currently under detailed studies.

Acknowledgements

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