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Independent adsorption of monovalent cations and cationic polymers at PE/PG lipid membranes

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Abstract. Synthetic cationic polymers constitute a wide class of polymeric biocides. Commonly their antimicrobial effect is associated to their interaction with bacterial membranes. In the present study we analyze the interaction of various cationic polymers with model bacterial membranes comprised of a mixture of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). We describe a polymer-membrane interaction as a process of modification of the surface charge. It is well known that small monovalent inorganic cations (Na^+, K^+) cannot overcharge the surface of a bilayer containing anionic lipids. In contrast, polycations are able to overcharge anionic membranes and demonstrate a very large input to the electric field distribution at the membrane-water interface. We aimed here to study the electrostatic effects associated with the interaction of polycations of different types with a model lipid membrane whose composition closely resembles that of bacterial membranes (PE:PG = 1:4). Four different cationic polymers (polyvinylamine, polyallylamine, poly-L-lysine and polyethylenimine) were adsorbed at a model PE/PG bilayer in MD simulations. Adsorption of sodium cations was inspected separately for PE/PG bilayers of different composition and cation's binding parameters were determined. From computational experiments and consequent theoretical analysis we concluded that sodium adsorption at anionic binding sites does not depend on the presence of polycations. Therefore, we hypothesize that antimicrobial activity of the studied cationic polymers should depend on the ionic composition of the medium.

1. Introduction

There are many substances that are able to provide antimicrobial activity and a large number of these biocides are cationic polymers. Their bactericidal effect depends on the length of the polymer chain and is followed from their interaction to the bacterial membrane [1].

Prokaryotic membrane usually carry a negative surface charge as it contains anionic and zwitterionic lipids. Bactericidic effect of polycation can be associated with membrane neutralization, thus it is important to create a quantitative description of polymer-membrane interaction and corresponding change in membrane surface charge. Such interactions may be studied on model systems, especially on phosphatidilethanolamine/phosphatydilglycerol (PE/PG) mixtures in the case of prokaryotic membranes [2]. Molecular dynamic technique looks very attractive and powerful for such investigation.

Polycations, contrary to monovalent ions, demonstrate a great contribution to electric field distribution when adsorbed at the membrane-water interface [3]. Neutralization of negatively charged surface with a polycation can be described quantitatively considering irreversible nature of polymer adsorption. Monovalent cations that are always presented in background electrolyte are also able to affect the surface charge of a bilayer containing anionic lipids. When both polycation and monovalent cation interact with anionic surface it can puzzle the situation. We performed a series of computational experiments and proved that adsorption of polycation and sodium ions can be considered as independent. We analyzed the interaction of the membrane with monovalent ions individually before studying their joint action. First part of the work is devoted to interaction of sodium cations to PE/PG bilayers of different lipid compositions. Next we analyze interaction of four cationic polymers to PE/PG bilayer in the presence of sodium as a background electrolyte. As a result the quantitative description of polycation adsorption is introduced and verified over the set of computational experiments.

2. Adsorption of monovalent cations

2.1. Theoretical background

All of monovalent cations have a non-zero affinity to negatively charged phospholipids [4], whereas K^+ and Na^+ is almost not adsorbed on neutral (zwitterionic) phospholipids [5]. All the ions create an electric double layer (EDL) through their interaction to anionic lipids, wherein ions adsorbed at lipid/water interface penetrate the membrane at certain depth. Thickness of the dense layer is determined by the heads of phospholipids and their interaction to adjacent water [6]. Relation between ion adsorption, membrane surface potential and surface charge in such systems can be described with sufficient accuracy by means of Gouy-Chapman-Stern theory (GCS).

Following Ermakov [4] we describe cation equilibrium between membrane and adjacent solution with the Langmuir adsorption isotherm. Also we assume only anionic lipids to be the binding centers for cations. Surface filling θ can be expressed as a ratio of the surface density of these centers, free from the cations (σ), and a their total density (σ_{max}):

$$1 - \theta = \frac{\sigma}{\sigma_{max}} = \frac{1}{1 + KC(0)} \ , \tag{1}$$

where K is the adsorption constant and $C(0)$ is the concentration of the cation at the outer Helmholtz plane (the border beyond which ions have a Boltzmann distribution is called 'outer Helmholtz plane'). Note that the amount of ions adsorbed at the interface depends on the concentration of ions at the outer Helmholtz plane $C(0)$ and not on the ion concentration in bulk. Both center densities σ, σ_0 are expressed as charge densities.

Total density σ_{max} is linearly related to the concentration of the anionic lipid in the mixture (α):

$$\sigma_{max} = Q \cdot \alpha \ . \tag{2}$$

Next, we transform (1):

$$C(0) = \frac{\alpha}{\sigma} \cdot \frac{Q}{K} + \frac{1}{K} \ . \tag{3}$$

If the computational experiment data are represented in coordinates $C(0)$ and α/σ it is possible to determine the parameters of adsorption (Q and K) as parameters of linear fit.

2.2. Computational details

Simulation was performed using the MD method in all-atom force field SLipids [7]. Length of the trajectories was 1 microsecond. Last 0.3 μs of every trajectory was analyzed as a stationary state. Membranes for experiments with monovalent cations were modeled at various ionic strengths, for systems having an ionic strength of 50 mM size of membrane was 20 lipid per monolayer (a total of 40 molecules) for the rest of 10 lipid per monolayer. Size of the system averaged 20000 atoms for systems of 40 lipid molecules and 10000 atoms for systems of 20 lipid molecules.

We determined the localization of the outer Helmholtz plane in order to determine the potential and the charge on the surface of the membrane. According to distribution of components at the lipid-water interface, for our systems the position of this plane was defined at 9 Å from average C_α atoms of phospholipid glycerol.

The distribution of the electric potential in the Gouy-Chapman model described by potential of mean force (Ψ_{PMF}) [6]:

$$\Psi_{PMF} = \frac{zRT}{F} \cdot \frac{C(0)}{C(\infty)}\ , \tag{4}$$

where z — ion charge number, $C(0)$ — the concentration of ion at the outer Helmholtz plane defined above, $C(\infty)$ — concentration in the bulk solution.

2.3. Computational results

The adsorption of sodium cations to (PE/PG) model system was observed using molecular dynamics simulation. We compared the area per lipid and the thickness of simulated membranes to corresponding experimental values [8] in order to validate the molecular model. At room temperature the model membrane made of pure POPG has average area per lipid of 67.7 Å^2 whereas the natural membrane of the same composition has the experimental value of 66.1 Å^2. At 65°C corresponding values are 72.2 and 71.3 Å^2. Full thickness of membrane (D_{HH}) is defined by many authors as the distance between the peaks on the plot of electron density whereas the thickness of the layer of hydrophobic hydrocarbon chains of lipids ($2D_C$) can be calculated from the distance between peaks in the derivative of the last plot. The computed values ($D_{HH} =$ 38 Å, $2D_C = 25$ Å) are also in a good agreement with experimentally measured: 37.4 and 27.8 Å, respectively.

We use equation (3) to evaluate the adsorption parameters, such as adsorption constant K and the surface density of binding sites Q, in accordance with the selected model. We simulated series of lipid bilayers in different ionic solutions and determined $C(0)$ and the ratio α/σ. $C(0)$ was calculated from the concentration profile, averaged over the trajectory (example of such profile is presented at figure 1b). Surface charge was calculated with Graham equation from the surface potential Ψ_{surf}, which is well described by the potential mean force (PMF). Cation concentration near the surface $C(0)$ and its concentration in the bulk solution $C(\infty)$ were obtained from trajectories and substituted into (4) to find PMF (see 'Computational details'). α is known initially as the amount of anionic lipid in the mixture prepared for simulation. For each simulated lipid bilayer we put the point $\{C(0), \alpha/\sigma\}$ on the coordinate plane and then approximated all points by equation (3) (figure 1a). The result was: K = 2.16 M^{-1}, Q = -17.3 μC/cm^2 (for T = 300 K) and K = 7.15 M^{-1}, Q = -25.5 μC/cm^2 (for T = 340 K).

The adsorption of sodium was found to be more intensive under high temperature. Position of the main plane of the adsorption of sodium cations (figure 1b) was found on the distribution of the ions. The presence of the second peak on the chart the distribution of Na indicates the complex nature of sodium binding sites.

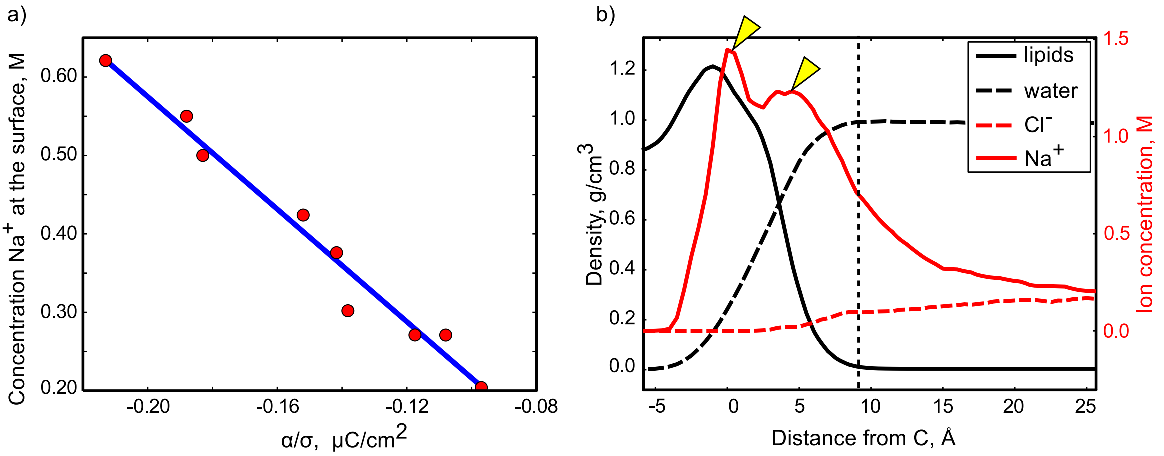

Figure 1. **(a)** Fitting the experimental values of charge density σ and near-surface cation concentration $C(0)$ with equation (3). Every marker corresponds to a separate simulation of PE/PG bilayer with PG content denoted by α. **(b)** The distribution of components at the lipid-water interface in the system POPE/POPG (2/3), I (in bulk) = 130 mM, T = 300 K. Black curves are plotted in density units (left axis), red curves are plotted in concentration units (right axis). Yellow arrows indicate the localization of the peaks which correspond to the planes of sodium ions adsorption. The vertical dashed line indicates the position of the membrane surface (outer Helmholtz plane).

3. Adsorption of polycations

3.1. Theoretical background

As we shown that GCS theory describes well the adsorption of sodium cations at pure membrane we can develop the similar theory in order to estimate sodium effect in the presence of polymer at the membrane surface. Let us derive the dependency between surface potential, adsorbed surface charge and concentration of monovalent electrolyte. The complete theory is presented in [9].

The near-surface concentration of monovalent cation, $C(0)$, is linked with the surface electric potential through the Boltzmann equation:

$$C(0) = C_{bulk} e^{-F\Psi_s/RT} \ . \tag{5}$$

Combining equations (1) and (5) provides us with an equation that links the binding site occupation θ and the surface potential Ψ_s:

$$\theta = \frac{K C_{bulk} e^{-F\Psi_s/RT}}{1 + K C_{bulk} e^{-F\Psi_s/RT}} \tag{6}$$

Ψ_s depends in turn on the overall surface charge density σ; the corresponding relation is known as the Grahame equation. In case of a symmetrical monovalent electrolyte one has:

$$\sigma = \sqrt{8 C_{bulk} \varepsilon \varepsilon_0 RT} \sinh\left(\frac{F}{2RT}\Psi_s\right) \ . \tag{7}$$

Next we should define surface charge dependency on occupation, $\sigma(\theta)$, and combine it with (6) and (7). The resulting expression will link the surface charge with the ionic concentration and adsorption parameters. First, let us consider a polymer-free membrane system. As we assumed previously (1), if the anionic lipids are the binding sites of sodium ions one has:

$$\sigma = -\sigma_{max} * (1 - \theta) \ . \tag{8}$$

Next, let us introduce the polymer adsorption into equation (8) (we assume that polycation adsorption is irreversible which is a good approximation for our systems). If an ion and a polymer do not compete for the same binding center, one has:

$$\sigma = -\sigma_{max} * (1 - \theta) + \sigma_p \ , \tag{9}$$

where σ_p is the density of the adsorbed positive charge (charge of a polycation divided on the membrane surface area).

A combination of (6), (7) and (9) gives:

$$\sqrt{8C_{bulk}\varepsilon\varepsilon_0 RT} \sinh\left(\frac{F}{2RT}\Psi_s\right) + \frac{\sigma_{max}}{1 + KC_{bulk}e^{-F\Psi_s/RT}} = \sigma_p \tag{10}$$

This equation can be validated in an experiment where polymer charge (and σ_p) is varied and surface potential Ψ_s is measured. In the next section we describe such a series of computational experiments.

3.2. Computational results

Several computational experiments were performed in order to test equation (10). Four polymers were adsorbed at the surface of pre-equilibrated dioleoyl-PE/dioleoyl-PG membrane (1:4): polyallylamine (PAA), polyethylenimine (PEI), polyvinylamine (PVA) and poly-L-lysine (PLL). In the experiments 1–3 molecules of a polymer were added to the bilayer in the presence of sodium chloride as a background electrolyte. Simulations were performed with systems of 128 lipids and about 10000 water molecules. The force field for polymers was developed recently [10]. The following protonation levels were used for the polymers: 15% (PAA), 50% (PVA), 50% (PEI) and 100% (PLL). One to three polymer molecule of 20 units were added to the bilayer for simulations. Detailed description of the simulation as well as resulting molecular picture of polymer-membrane interaction is provided in [9].

Polymer has 20 units in length and its protonation level corresponds to its charge at neutral pH. For each system surface potential Ψ_s was calculated as described above and in 'Computational details'. The resulting potential was plotted against polymeric charge (figure 2a) Theoretical curves are also presented at the same figure plotted for $K = 2$–$6\ \mathrm{M}^{-1}$ and $\sigma_{max} = 0.05\ C/m^2$ (this corresponds to 13 PG lipids per 41.5 nm^2). Experimental points could be fitted with equation (10) considering $K = 6\ M^{-1}$. Higher value of adsorption constant then it was obtained in the first section (6.0 vs 2.5 M^{-1}) can be explained as a result of using different acyl tails in these two simulation series (DOPG/DOPE vs POPG/POPE). Another reason could be the following: in our simple model we can not consider all complications of joint mono- and polycation binding.

4. Conclusion

As we found the adsorption of monovalent sodium cations at PE/PG lipid bilayer is perfectly described with Gouy-Chapman-Stern theory. It is the first time this theory is proved for phosphoglycerol containing bilayer. Simultaneous adsorption of monovalent cations and polycations could also be described with similar theory considering irreversible polymer adsorption and independent adsorption of mono- and polycations. The equation of joint

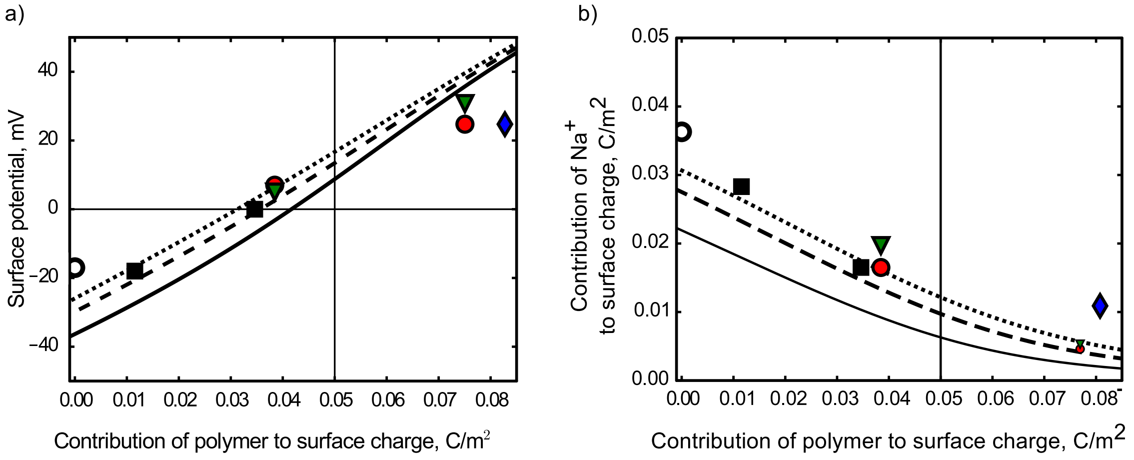

Figure 2. (a) Surface potential dependency on adsorbed polymer surface charge. Black theoretical curves are plotted with equation (10) using C = 100 mM, T = 310 K, $\sigma = 0.05$ C/cm^2 and $K = 2$ (dots), 4 (dashed), 6 (solid). (b) Contribution of sodium to the surface charge found by the same equation. Results of computational experiments are depicted by single markers: open circle (pure bilayer), black squares (PAA), green triangles (PVA), red circles (PEI) and blue diamond (PLL).

adsorption (10) was derived and seems suitable for describing the results of a series of numerical experiments.

According to the theory one may formulate the following predictions: (1) Point of zero charge (concentration of polymer required for neutralizing anionic liposome in a suspension) could be shifted to smaller polymer concentration by adding monovalent cations. (2) Type and amount of monovalent cations could affect biocidic effect of polycations. (3) Polycation binds irreversibly with any bilayer containing zwitter-ionic phosphatidylethanolamine in its composition.

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