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Binding of monovalent alkali metal ions with negatively charged phospholipid membranes

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ABSTRACT

We have systematically investigated the effect of various alkali metal ions with negatively charged phospholipid membranes. Size distributions of large unilamellar vesicles have been confirmed using dynamic light scattering. Zeta potential and effective charges per vesicle in the presence of various alkali metal ions have been estimated from the measured electrophoretic mobility. We have determined the intrinsic binding constant from the zeta potential using electrostatic double layer theory. The reasonable and consistent value of the intrinsic binding constant of Na^+, found at moderate NaCl concentration (10–100 mM), indicates that the Gouy–Chapman theory cannot be applied for very high (>100 mM) and very low (<10 mM) electrolyte concentrations. The isothermal titration calorimetry study has revealed that the net binding heat of interaction of the negatively charged vesicles with monovalent alkali metal ions is small and comparable to those obtained from neutral phosphatidylcholine vesicles. The overall endothermic response of binding heat suggests that interaction is primarily entropy driven. The entropy gain might arise due to the release of water molecules from the hydration layer vicinity of the membranes. Therefore, the partition model which does not include the electrostatic contribution suffices to describe the interaction. The binding constant of Na^+ ($2.4 \pm 0.1\ M^{-1}$), obtained from the ITC, is in agreement with that estimated from the zeta potential (~$2.0\ M^{-1}$) at moderate salt concentrations. Our results suggest that hydration dynamics may play a vital role in the membrane solution interface which strongly affects the ion–membrane interaction.

1. Introduction

Unilamellar vesicle is an enclosed lipid bilayer shell and serves as an excellent model system of biological membranes. Apart from model systems of biological membrane, these vesicles can be used as microreactors for enzymatic RNA synthesis [1] and also to form nanoparticles of controlled size distributions [2,3]. Vesicles are also extensively used as carriers of bioactive agents, including drugs, vaccines, and cosmetics [4,5]. The effect of various types of ions on model membranes has enticed significant interest due to their immense biological applications [6]. For example, the transmembrane potential may arise from the asymmetric binding of Na^+ and Cl^- ions which in turn affects the ion transport across the bilayer [7]. In 1888, Hofmeister proposed a series which qualitatively arranged ions according to their propensity to precipitate proteins [8]. The presence of ions in the aqueous medium plays a vital role in modulating the bilayer properties, such as, surface potential [9,10], the dipole potential, [11] structure and dynamics of lipid molecules [12–16], inter-membrane forces, [17] the transition from micelles to vesicles [18] and the swelling of vesicles [19]. It is known that restructuring of macromolecules, such as proteins, and peptides, at the membrane–solvent interface is greatly influenced by the ion binding to the membranes. Among all ions present in the intra- and extra-cellular media, the alkali metal ions such as Na^+, and K^+ are the most abundant cations in eukaryotic cells. In the cellular fluid, Na^+ and K^+ are present in the concentration ~100 mM. Influence of other alkali metal ions, such as Li^+, Rb^+, and Cs^+ are also physiologically relevant and are of bio-medical importance.

A variety of experimental as well as simulation studies have been employed in order to gain some insights into the binding affinity of various ions and their effect on physico–chemical properties of phospholipid membranes. Differential scanning calorimetry studies have shown the decrease in enthalpy of chain melting transition from 4.95 to 4.59 kcal/mol of dimyristoyl phosphatidylcholine vesicles in the presence of salts [10]. The addition of NaCl can also alter the bilayer properties. For example, membrane thickness and order parameter of lipid tails tend to increase, whereas, area per molecule increases in the presence of NaCl [20,21]. Lateral self diffusion of POPC lipids within the bilayer was found to decrease with increasing NaCl concentration [22]. All these techniques are aimed at quantifying binding affinity of ions with phospholipid membranes. Electrophoretic mobility is widely used to characterize the surface charge, surface potential of the

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membranes and the qualitative features of binding phenomenon of ions [9,23]. Other experimental techniques, such as nuclear magnetic resonance [24], Fourier transforms infrared spectroscopy [25,26], fluorescence correlation spectroscopy, [22], and atomic force microscopy were also used to characterize the physico–chemical properties of membranes in the presence of ions [12,27]. Recently, molecular dynamic simulations and fluorescence solvent relaxation measurement have been extensively used to understand the kinetics and mechanism of interaction of both cations as well as anions with phospholipid membranes [15,28]. It was found that chloride adsorption is caused by the electrostatic attraction to an adsorbed Na^+, i.e., it is a counter ion effect, whereas, iodide affinity to the membrane is independent of the presence of counterions [15]. Therefore, the adsorption of cations has significantly been influenced by their counterions [28,29]. For example, iodide seems to penetrate deep into the headgroup regions, whereas, chloride accumulates at the membrane surface which in turn screened the effect of cations. These simulation studies have also revealed that the membrane affinity of a monovalent cation increases with decreasing size of the bare cation. However, recent isothermal titration calorimetry (ITC) and computer simulation studies on phosphatidylcholine (PC) vesicles have found a very similar apparent binding constant (~1.2 M^{-1}) for Li^+, Na^+, K^+, Rb^+ and Cs^+ (size increases from Li to Cs) [10,30]. Interestingly, it was suggested by Klasczyk et al. that binding of chloride ions was almost as strong as sodium ions and this observation was supported by their ITC as well as electrophoretic mobility results studied on the PC–NaCl interaction [30]. Klaszcyk et al. have also suggested that the binding affinity of both anions and cations with phosphocholine membranes found in many simulation studies have to be reconsidered and revised. The discrepancy of results was due to the artifact in the force field used in simulation. Although there are several studies on the interaction of monovalent ions with zwitterionic membranes, the effect of monovalent ions on the negatively charged vesicles was not studied systematically. The previous studies on the interaction of phosphatidylserine (PS) vesicles with different ions have estimated the intrinsic association binding constant, *K*, of ions [9]. For example, *K* was found to be ~0.6 M^{-1} for Na^+ and 0.15 M^{-1} for K^+ at decimolar concentration. Liposomes composed of PC-cholesterol and phosphatidylinositol have shown much higher binding constant of ~4.6 M^{-1} [31]. Surprisingly, the binding constant of monovalent ions with PC (~1.2 M^{-1}) was found to be comparable to those obtained from charged vesicles. This is contradictory to the fact that cations should interact significantly with negatively charged membranes. Many conflicting results on liposomes–ion interaction have led us to study systematically the effect of monovalent ion on the charged membranes.

In this article, we present systematic investigations on the binding affinity of various monovalent alkali metal cations with the negatively charged phospholipid membranes. We discuss the results on the interaction of Na^+ with both negatively charged and neutral vesicles starting from low concentration to high concentration. We have estimated the intrinsic binding constant of various ions using the Gouy–Chapman theory and compared the results with those obtained from the ITC study and earlier experimental and simulation studies on similar systems. We have also tried to explore the effect of emission spectra on the membrane sensitive probe nile red. Interestingly, the absorption of ions with the phospholipid membranes has been envisaged from fluorescence spectra of nile red. Finally, we suggest the appropriate concentration range in which membrane electrostatics can be best described by the Gouy–Chapman theory.

2. Materials and methods

Phospholipids, such as, dioleoyl phosphatidylcholine (DOPC), dioleoyl phosphatidylglycerol (DOPG) were purchased from Sigma-Aldrich and were used for vesicle preparation without further purification. Lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl), rubidium chloride (RbCl), cesium chloride and HEPES buffer were obtained from Merck.

2.1. Preparation of LUV

Alkali metal chloride solutions of different concentration (100–500 mM) were prepared in 1 mM HEPES buffer, pH was adjusted to 7.4 with 1 M solution of KOH. In particular, NaCl solutions in the concentration range from 10 to 500 mM were prepared. DOPC, DOPG and mixture of DOPC and DOPG (4:1) were used for vesicle preparation. Large unilamellar vesicles (LUV) were prepared using an extrusion technique as described by Hope et al. [32]. An appropriate amount of lipid in chloroform (concentration of stock solution is 10 mg/ml) was transferred to a 10 ml glass bottle. Organic solvent was removed by gently passing dry nitrogen gas. The sample was then placed in a desiccator, connected to a vacuum pump, for a couple of hours to remove the traces of the left over solvent. Required volume of 1 mM HEPES was added to the dried lipid film so that the final desired concentration (1.18 mM) was obtained. The lipid film with the buffer was kept overnight at 4 °C to ensure the better hydration of phospholipid heads. Vortexing of hydrated lipid film for about 30 min produces multilamellar vesicles (MLV). Sometime long vortexing was required to make uniform lipid mixtures. LUV were prepared by extruding the MLV with LiposoFast from AVESTIN (Canada). MLV suspensions were extruded through a polycarbonate membrane having a pore diameter of 100 nm. This results in the formation of well defined size of LUV (average diameter ~100 nm), as measured by dynamic light scattering. Vesicle solution was degassed prior to all measurements, as air bubbles introduced in the sample during the extrusion may lead to artifacts.

2.2. Zeta potential and dynamic light scattering

Zeta potential and size distribution were obtained at room temperature (~25 °C) with the Zetasizer Nano ZS (Malvern Instruments, UK). The Zetasizer Nano uses 4 mW He–Ne Laser of wavelength 632.8 nm. The detector is positioned at scattering angle 173°. The detected scattered light is sent to signal processing correlator. Extruded vesicles, prepared with varying salts, were loaded in a folded capillary cell for both zeta and size measurements. Each zeta potential measurement consisting of 100 runs and size measurement 10–100 runs has been performed. Zeta potential was measured from the electrophoretic mobility μ using a model described by the Smoluchowski and Hückel equation [33].

$$\zeta = \frac{3\mu\eta}{2\varepsilon f(\kappa a)}, \tag{1}$$

where, η and ε are the coefficient of viscosity and the permittivity of the aqueous medium, respectively. The Henry function, $f(\kappa a)$, depends on the inverse Debye length $\kappa = \sqrt{\frac{2e^2 C_{ion}}{\varepsilon kT}}$ and the radius of the vesicle *a*, where *e* is the electronic charge, *k* is the Boltzmann constant, and *T* is the temperature. As the κ, is a function of ion concentration (C_{ion}) only, it was calculated for different concentration of ions and hence $f(\kappa a)$ was estimated using the following equation.

$$\begin{aligned} f(\kappa a) &= 1, \textit{ for } \kappa a < 1 \\ &= \frac{1}{6}\, log(\kappa a) + 1, \textit{ for } 1 < \kappa a < 1000 \\ &= 1.5, \textit{for } \kappa a > 1000 \end{aligned} \tag{2}$$

The hydrodynamic radius of the vesicles was measured from the dynamic light scattering (DLS). In DLS, intensity fluctuations of the scattered light were measured and intensity auto correlation function was fitted to an exponential decay function to obtain the diffusion constant. Einstein–Stokes relation, $a = \frac{kT}{6\pi\eta D}$, was used to determine the hydrodynamic radius of the vesicle. D is the diffusion constant and *kT*