

Effect of monovalent ion adsorption on the electric charge of phosphatidylcholine - decylamine liposomal membranes

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Abstract We examined the effect of adsorbed monovalent ions on the surface charge of phosphatidylcholine (PC) – decylamine (DA) liposomal membranes. Surface charge density values were determined from electrophoretic mobility measurements of lipid vesicles performed at various pH levels. The interaction between solution ions and the PC-DA liposomal surface was described by a six component equilibrium model. The previously determined association constants of the $-\text{PO}^{(-)}$ and $-\text{N}^{(+)}(\text{CH}_3)_3$ groups of PC with H^+ , OH^- , Na^+ and Cl^- ions (K_{A1H} , K_{B1OH} , K_{A1Na} , K_{B1Cl}) were used to calculate K_{B2OH} , and K_{B2Cl} , the association constants of the $-\text{N}^{(+)}\text{H}_3$ group of DA with OH^- and Cl^- ions, providing an experimental verification for the proposed model.

Keywords Membranes · Vesicles · Phosphatidylcholine · Decylamine · pH measurement · Surface charge density · Association constants

Introduction

Due to their amphiphilic character, lipids frequently self-organize into spherical structures composed of a bilayer membrane encapsulating a core composed of the aqueous

solution in which they are formed. These structures are known as lipid vesicles or liposomes and are a popular model system for membrane studies owing to their similarity to biological membranes (Lasic 1993; Sabin et al. 2006; Liu et al. 2001; New 1990). The chemical and physical properties of liposomes depend on the composition and concentration of their phospholipid constituents, their capability to bind other chemical species, the ionic strength of aqueous medium, and the method of preparation. Mixtures of egg phosphatidylcholine (PC) are commonly used because of their low cost, neutral charge, and chemical inertness. Phosphatidylcholines are molecules, in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains with a zwitterionic hydrophilic head group. The net charge of PC liposomes may be modified by incorporating additional components possessing a negative (such as phosphatidylserine) or positive (such as decylamine) charge (Frézard 1999).

The binding of ions to phospholipid membranes has been extensively studied (Eisenberg et al. 1979; Akutsu and Seelig 1981; Cohen and Cohen 1981; Tatulian 1987; Cunningham et al. 1988; Gurtovenko et al. 2005; Petrache et al. 2006; Garcia-Celma et al. 2007). The phenomenon is extremely interesting from the standpoint of biophysical chemistry because ion-membrane interactions significantly influence the functional and structural characteristics of biomembranes. When lipid membranes are exposed to solution ions, the electric properties of the membrane are altered due to interactions between the charged groups of the lipid molecules and the surrounding ions. The equilibria occurring at the membrane surface can be altered by adsorption processes, leading to variations in membrane surface charge density. The electric charge of the membrane is not a constant value, but rather depends strongly on a number of factors such as pH, lipid composition and the

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ionic strength of the electrolyte (Deshiikan and Papadopoulos 1998). Changes in solution pH alter the surface charge of the membrane with the membrane becoming more positive at lower pH and more negative at higher pH (Mullet et al. 1997). We examined the influence of solution pH on the electric charge of liposomal membranes in order to better demonstrate and describe the effect of electrolyte ions on membrane surfaces.

This paper examines the interactions between monovalent ions and liposomal membranes. Phosphatidylcholine-based membranes were modified by decylamine, a primary aliphatic amine containing a 10-carbon chain. We describe the influence of electrolyte ion adsorption on the electric charge of the PC-DA liposome surface over the pH range 2–11. Mathematical calculations based on a six-component equilibrium model describing H^+ , OH^- , Na^+ and Cl^- ions adsorption on the PC-DA liposomal surface enabled us to determine the association constants of the membrane functional groups with solution ions.

Experimental

Materials

L- α -phosphatidylcholine (PC) from egg yolk was purchased from Fluka. Decylamine (DA), used as a cationic charge-inducing agent, was supplied by Sigma. HPLC grade chloroform was obtained from Aldrich. All solutions and cleaning procedures were performed with water purified using a Milli – Qll system (18.2, Millipore, USA).

Preparation of phospholipid vesicles

Liposome dispersions were prepared by sonication of neutral PC and positively charged DA. Dry PC and DA were weighed, dissolved in chloroform (10 mg/ml), and mixed in various ratios of PC:DA (3:1, 2:1, and 1:1). The solvent was evaporated in a stream of argon to obtain 25 – 50 μm^3 of lipid film in a beaker. The film was hydrated with 15 mL isotonic saline solution (0.9% NaCl) and the beaker was placed in a water bath at approx. 7° C. The head of a UD 20 ultrasound generator (Techpan, Poland) was immersed in the solution and the solution was subjected to ultrasound for five 1.5 min periods.

Microelectrophoretic mobility measurements

The electrophoretic mobility of phospholipid vesicles in suspension was measured using Laser Doppler Velocimetry (LDV) and a Zetasizer Nano ZS (Malvern Instruments, UK)

apparatus. The measurements were carried out as a function of pH. The liposomes were suspended in NaCl solution and titrated to the desired pH using HCl or NaOH. The reported values represent the average of at least six measurements performed at a given pH.

From electrophoretic mobility measurements the surface charge density was determined using the equation (Alexander and Johnson 1949):

$$\delta = \frac{\eta \cdot u}{d} \quad (1)$$

where: η – viscosity of solution, u – electrophoretic mobility, d – diffuse layer thickness

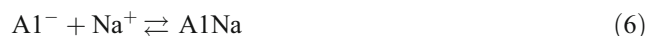
The diffuse layer thickness was determined from the formula (Barrow 1996):

$$d = \sqrt{\frac{\varepsilon \cdot \varepsilon_0 \cdot R \cdot T}{2 \cdot F^2 \cdot I}} \quad (2)$$

where: R is the gas constant, T is the temperature, F is the Faraday number and I is the ionic strength of 0,9% NaCl, $\varepsilon\varepsilon_0$ – permeability of the electric medium.

Theory

The dependence of the surface charge density of PC:DA liposome membranes on the pH of the electrolyte solution can be described using six equilibrium equations. Four are connected with positive groups (phosphatidylcholine, decylamine and sodium and hydrogen ions), and two concern the negative species of phosphatidylcholine and hydroxide and chloride ions. The H^+ , OH^- , Na^+ , and Cl^- ions are adsorbed at the PC:DA surface, and the adsorption equilibria can be presented in the form:



where: $A1^-$ is group $-PO^{(-)}$, $B1^+$ is group $-N^{(+)}(CH_3)_3$, of phosphatidylcholine and $B2^+$ is group $-N^{(+)}H_3$ of decylamine.

Therefore the association constants are expressed in the following manner:

$$K_{A1H} = \frac{a_{A1H}}{a_{A1^-} \cdot a_{H^+}} \tag{9}$$

$$K_{B1OH} = \frac{a_{B1OH}}{a_{B1^+} \cdot a_{OH^-}} \tag{10}$$

$$K_{B2OH} = \frac{a_{B2OH}}{a_{B2^+} \cdot a_{OH^-}} \tag{11}$$

$$K_{A1Na} = \frac{a_{A1Na}}{a_{A1^-} \cdot a_{Na^+}} \tag{12}$$

$$K_{B1Cl} = \frac{a_{B1Cl}}{a_{B1^+} \cdot a_{Cl^-}} \tag{13}$$

$$K_{B2Cl} = \frac{a_{B2Cl}}{a_{B2^+} \cdot a_{Cl^-}} \tag{14}$$

Surface concentrations of PC and DA are given by C_L and C_{DA} :

$$a_{A1^-} + a_{A1H} + a_{A1Na} = c_L \tag{15}$$

$$a_{B1^+} + a_{B1OH} + a_{B1Cl} = c_L \tag{16}$$

$$a_{B2^+} + a_{B2OH} + a_{B2Cl} = c_{DA} \tag{17}$$

Surface charge density of PC:DA membrane is given by the equation:

$$\delta = (a_{B1^+} + a_{B2^+} - a_{A1^-})F \tag{18}$$

Elimination of a_{A1H} , a_{A1Na} , a_{B1OH} , a_{B2OH} , a_{B1Cl} , a_{B2Cl} , a_{B1^+} , a_{B2^+} , a_{A1^-} values from above equations yields the following formula:

$$\frac{\delta}{F} = \frac{c_{DA}}{1 + K_{B2OH}a_{OH^-} + K_{B2Cl}a_{Cl^-}} + \frac{c_L}{1 + K_{B1OH}a_{OH^-} + K_{B1Cl}a_{Cl^-}} - \frac{c_L}{1 + K_{A1H}a_{H^+} + K_{A1Na}a_{Na^+}} \tag{19}$$

Determination of the association constants requires a simplification of the above equation to a linear form at high H^+ ($a_{H^+} \rightarrow \infty$) and low H^+ ($a_{H^+} \rightarrow 0$) concentrations. In the former case Eq. 19 was rewritten as a decreasing

exponential function of H^+ concentration (Eq. 20) and in the latter case as an increasing exponential function of H^+ concentration (Eq. 21).

$$\frac{\delta}{F} = \frac{c_{DA}}{a_{H^+} (1 + K_{B2Cl}a_{Cl^-}) + K_{B2OH}K_W} + \frac{c_L}{a_{H^+} (1 + K_{B1Cl}a_{Cl^-}) + K_{B1OH}K_W} - \frac{c_L}{K_{A1H}a_{H^+} + (1 + K_{A1Na}a_{Na^+})} \tag{20}$$

$$\frac{\delta}{F} = \frac{c_{DA}}{K_{B2OH}K_W + a_{H^+} (1 + K_{B2Cl}a_{Cl^-})} + \frac{c_L}{K_{B1OH}K_W + a_{H^+} (1 + K_{B1Cl}a_{Cl^-})} - \frac{c_L}{(1 + K_{A1Na}a_{Na^+}) + K_{A1H}a_{H^+}} \tag{21}$$

The numerator of each term in Eq. 20 was divided by the denominator to yield two terms. These operations resulted in a linear equation in the a_{H^+} and $\frac{\delta a_{H^+}}{F}$ coordinate system which was correct for high hydrogen ion concentrations ($a_{H^+} \rightarrow \infty$).

$$\frac{\delta a_{H^+}}{F} = \left(\frac{c_{DA}}{1 + K_{B2Cl}a_{Cl^-}} + \frac{c_L}{1 + K_{B1Cl}a_{Cl^-}} \right) a_{H^+} - \left(\frac{c_L K_{B1OH}K_W}{(1 + K_{B1Cl}a_{Cl^-})^2} + \frac{c_{DA} K_{B2OH}K_W}{(1 + K_{B2Cl}a_{Cl^-})^2} + \frac{c_L}{K_{A1H}} \right) \tag{22}$$

Applying the same procedure to Eq. 21 resulted in a linear equation in the $\frac{1}{a_{H^+}}$ and $\frac{\delta}{F a_{H^+}}$ coordinate system which was correct for low hydrogen ion concentrations ($a_{H^+} \rightarrow 0$):

$$\frac{\delta}{F a_{H^+}} = - \left(\frac{c_L}{(1 + K_{A1Na}a_{Na^+})} \right) \frac{1}{a_{H^+}} + \left(\frac{c_L}{K_{B1OH}K_W} + \frac{c_{DA}}{K_{B2OH}K_W} + \frac{c_L K_{A1H}}{(1 + K_{A1Na}a_{Na^+})^2} \right) \tag{23}$$

The coefficients describing these linear functions may be easily obtained using linear regression and subsequently applied to calculate the parameters. Knowledge of the association constants K_{A1H} , K_{B1OH} , K_{A1Na} , K_{B1Cl} (Dobrzyńska et al. 2007) is useful in determining the association constants K_{B2OH} , and K_{B2Cl} . Defining the value of these parameters permits calculation of the

theoretical liposome membrane surface charge from Eq. 19 for comparison to experimental data.

A degree of the coverage of the liposomal surface occupied by DA with the OH^- , Cl^- ions were determined from the relationship:

$$\theta_x = \frac{a_x}{c_{\text{DA}}}, \quad (24)$$

where: $x = \text{B2}^+, \text{B2OH}, \text{B2Cl}$

The degree of the coverage of the PC membrane surface with H^+ , OH^- , Na^+ , Cl^- ions were determined previous (Dobrzyńska et al. 2007).

Results and discussion

The electrophoretic mobility measurements of the PC:DA liposome system formed the basis for investigations of ion/membrane association phenomena. The experiments were performed at several pH values using 0.155 M NaCl as a supporting electrolyte. The electrophoretic mobility values were converted to surface charge density using Eq. 1. The theoretical values of the surface charge density were determined by applying Eq. 19 to the experimental data. The association constants of PC surface groups with solution ions ($K_{\text{A1H}}=7.172 \times 10^2$, $K_{\text{A1Na}}=0.230$, $K_{\text{B1OH}}=3.351 \times 10^9$, $K_{\text{B1Cl}}=0.076$ [m^3/mol]) have been previously reported (Dobrzyńska et al. 2007). Equations 22 and 23 were used to define the association constants of the amine group of DA with OH^- and Cl^- ions (K_{B2OH} , K_{B2Cl}). The calculated association constants were substituted into Eq. 19 to produce a theoretical curve. The surface charge densities of the pure PC and PC:DA liposomal membranes at molar ratios of 3:1, 2:1, and 1:1 are plotted as a function of pH in Fig. 1. The points denote the experimental values

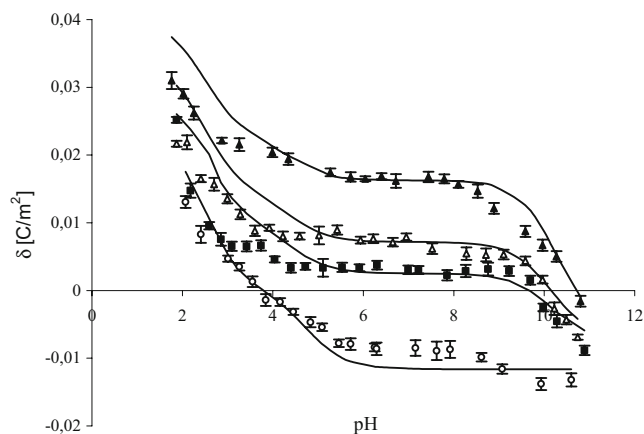


Fig. 1 The surface charge density of the pure PC and PC:DA liposomal membranes vs. pH of the electrolyte solution. The points denote experimental values; \circ – PC, \blacksquare – PC:DA 3:1, \triangle – PC:DA 2:1, \blacktriangle – PC:DA 1:1, continuous lines present the theoretical values

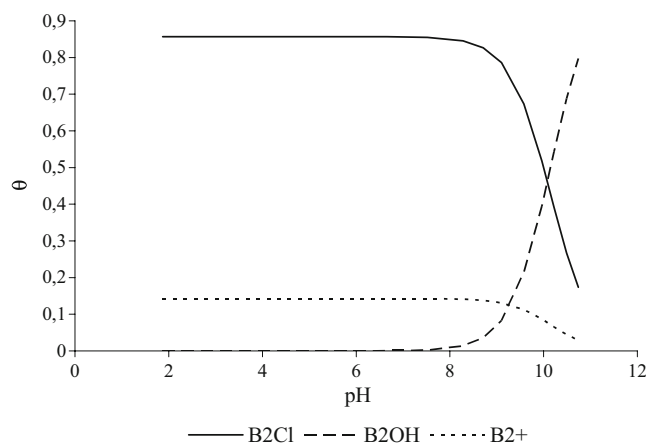


Fig. 2 A degree of the coverage of the DA membrane (θ) with the OH^- and Cl^- ions, as a function of pH of 0.155 M NaCl solution

and the continuous lines represent the theoretical values obtained from Eq. 19. The theoretical and the experimental surface charge density values agree between pH 5 and 11 but diverge slightly in the low pH range. The deviations from the theoretical curve may be caused by the interactions between the functional groups of the two membrane components. The association equilibria depend on pH due to changes in the ionic forms of the functional groups involved in the equilibria. It is difficult to determine exactly which interactions may cause the deviation outside the reliable pH range.

If we consider an acid solution, an increased positive charge is observed with increasing DA content in the membrane. This is caused by shielding of the negative phosphate groups ($-\text{PO}^{(-)}$) of PC molecules by H^+ ions coupled with exposure of the positively charged groups ($-\text{N}^{(+)}(\text{CH}_3)_3$ and $-\text{N}^{(+)}\text{H}_3$) of PC and DA and the association of Cl^- ions with these groups. In basic solutions the increasing monoamine content in the PC membrane causes a decrease in negative charge and a shift of the isoelectric point of the membrane to high pH values. The amine groups of PC and DA are shielded by OH^- ions while the negative groups of PC are exposed and are associated with Na^+ ions. The association constants of the $-\text{N}^{(+)}\text{H}_3$ group of DA with OH^- and Cl^- ions (K_{B2OH} , K_{B2Cl}) were calculated from Eq. 22 and Eq. 23. These were 5×10^1 and 0.051 [m^3/mol] respectively.

The degree of coverage of the DA membrane surface by ions as a function of pH of 0.155 M NaCl solution is depicted in Fig. 2. The coverage of the PC membrane surface was described previously (Dobrzyńska et al. 2007). The coverage of both components with OH^- and Cl^- ions demonstrates that interaction of Cl^- ions with the membrane surface has a significant influence on the membrane electric charge. The association of Cl^- ions with the DA surface begins when the number of OH^- ions starts to decrease (at

pH \sim 11). The coverage of $-\text{N}^{(+)}\text{H}_3$ groups by Cl^- ions remains unchanged in the pH range below 9, with the degree of coverage remaining approximately 0.87. While some free $-\text{N}^{(+)}\text{H}_3$ groups remain at the DA membrane, the surface is covered to a considerable degree. The number of free $-\text{N}^{(+)}\text{H}_3$ groups also remains unchanged between pH 2–9, with a degree of coverage value of approximately 0.13. In comparison the surface coverage by free $-\text{N}^+(\text{CH}_3)_3$ groups on the PC surface is approximately 0.05 (Dobrzyńska et al. 2007). The surface distribution is reflected in the association constants of the PC:DA membrane groups with OH^- and Cl^- ions: $K_{\text{B1Cl}}=0.076$, $K_{\text{B1OH}}=3.351 \times 10^9$, $K_{\text{B2Cl}}=0.051$, and $K_{\text{B2OH}}=5 \times 10^1$ [m^3/mol].

Conclusions

The interaction between lipid membranes and solution ions has been well characterized. The dependence of the surface charge density of the PC:DA liposomes on pH was described using a mathematical model derived from experimental electrophoretic data. The theoretical estimates of electric charge enabled the determination of association constants for the functional groups of DA and electrolyte ions. Our study demonstrates that the adsorption of Na^+ and Cl^- ions should be taken into consideration when examining phenomena occurring on the membrane surface. The agreement of the experimental and theoretical charge variation curves of the PC:DA liposomal membrane is good between pH 5 and 11. The

deviation observed at lower pH may be caused by disregarding interactions between the functional groups of PC and DA.

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