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Adsorption equilibria at interface separating electrolyte solution and phosphatidylcholine–stearylamine liposome membrane

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Abstract

The effect of the pH of an electrolyte solution on the electric surface charge of the liposome membrane was studied. The membrane of vesicles contained egg phosphatidylcholine (PC) with different proportions of stearylamine (ST). The surface charge density of the membrane was determined as a function of pH from electrophoretic mobility measurements. A six equilibria model describing the solution ions adsorption on the PC-ST liposome membrane surface was presented in this paper. The knowledge of the association constants of the $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups of PC with H^+, OH^-, Na^+, Cl^- ions: K_{A_1H}, K_{B_1OH}, K_{A_1Na}, K_{B_1Cl}, that had been presented earlier, allowed to determine the association constants of the $-N^{(+)}H_3$ group of ST with OH^- and Cl^- ions: K_{B_2OH}, K_{B_2Cl}. The proposed model has been proved to be correct by comparing the resulting theoretic charge variation curves of the PC-ST liposomal membrane with the experimental data.

Keywords: Phosphatidylcholine; Stearylamine; Surface charge density; Liposome membrane; Adsorption equilibria; Association constants

1. Introduction

In spite of being complicated, biological membranes are extremely interesting, research systems, contain many elements which influence their electric properties to a considerable extent. Carrying out studies of the complex structure is difficult, because of various kinds of interactions occurring between its components. For this reason, models of the membrane are used, e.g. liposomes which are simplified structures reflecting properties of natural membranes. Liposomes are spherical colloidal particles consisting of one or more concentric bilayers encapsulating part of the aqueous medium in which they float freely. Liposomes are made predominantly from amphiphiles, a special class of surfo-active molecules which are characterized by having a hydrophilic and a hydrophobic group on the same molecule [1]. The properties of liposomes and their subsequent applicability depend on the physical and physico-chemical characteristics of the liposomal membrane. Usually, a zwitterionic or non-ionic lipid is used as the basic lipid for the preparation of liposomes [2]. Phosphatidylcholines are the most widely used liposome-forming molecules because of their relevance to the behavior of these components in cell membranes. They are zwitterionic at physiological of pH because the quaternary ammonium group is neither basic nor acidic in these pH ranges [1]. The bilayer membranes mostly consist of either natural or synthetic phospholipids, but the application of other double-tail surfactants such as dialkyl quaternary ammonium compounds in pharmaceutical applications are also used. In addition, minor amounts of cholesterol, or single-tail surfactants, such as stearylamine (ST), may be added to affect specific characteristics such as the membrane permeability or electric charge density [3].

The electrical properties of liposomal surfaces can be conveniently investigated by microelectrophoresis in which the movement of the liposomes in an electric field is observed. In general, two parameters characterizing the liposomal surface can be calculated from the measured mobilities, firstly the electrical potential at the plane of shear and secondly the surface charge density [4]. An important property of a cell membrane is

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its surface charge density which controls several processes in biological membranes. Thus, it affects membrane-bound enzymes, insertion of newly synthesized proteins into membranes and host-pathogen interactions [5]. Since the surface charge is dependent only on the molecular composition of the cell membrane, the surface charge density is much closer to being an intrinsic property of a given cell than particle's surface potential, making it a more useful parameter for comparing the surface properties of two different cell types [6]. Since biological membranes are charged entities, aqueous solution next to these membranes contain counterions and electrolytes. The interactions between membranes strongly depend on the presence of ions and their specificity [7]. The charge of living cells is due to the dissociation of ionogenic, or charged, groups ($-PO_4^-$, $-NH_3^+$, $-COO^-$,) in the cell surface [8]. These are most often acidic-alkaline properties groups which make the adsorption of many substances and ions on surface of the membrane possible. The equilibria existing at the membrane surface occur between functional groups of the membranes and outer medium components. The equilibria can be affected by e.g. adsorption leading to a membrane surface charge density variation [9]. The change in the pH of the solution induces changes in surface charge of the membrane towards more positive values at lowering pH or towards more negative values at raising pH [10,11]. The surface charge of membranes also depends on ionic strength of the electrolyte and lipid composition of the membrane. The surface charge of liposomes can be modified by the incorporation of positively charged lipids, such as stearylamine, or negatively charged lipids, such as dicetylphosphate, phosphatidylglycerol or phosphatidylserine [2].

The interactions between lipid membranes and surroundings are nowadays intensively developed. McLaughlin et al. [12] studied the adsorption of ClO_4^- and SCN^- ions on the phospholipid membranes; it was proved that the adsorption of these ions causes an increase of the negative surface potential. Grasdalen et al. [13], Gabrielska et al. [14], MacDonald and Seeling [15] studied the adsorption of multivalence ions, such as La^{3+}, Pr^{3+}, on the liposome surface. In our previous paper we presented the adsorption of Na^+ and Cl^- ions on the phosphatidylcholine (PC) surface [9]. The association constants (K_{A_1H}, K_{A_1Na}, K_{B_1OH}, K_{B_1Cl}) of the functional groups ($-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$) with the solution ions (H^+, Na^+, OH^-, Cl^-) for PC membrane were determined. The aim of this work is to examine and describe the phenomena occuring on the PC liposomal membrane surface modified by ST. The stearylamine molecules consist of two parts, a hydrophobic domain and a ionizable nitrogen atom positively charged at physiological pH [16]. We present changes of the electric charge caused by the solution ions adsorption on the PC-ST liposome surface. We also propose a six equilibria model describing the H^+, OH^-, Na^+, Cl^- ions adsorption on the PC-ST liposome membrane surface. The mathematical calculations enabled to determine the association constants of the $-N^{(+)}H_3$ group of ST with OH^- and Cl^- ions (K_{B_2OH}, K_{B_2Cl}).

Data presented in this work, obtained in result of mathematical derivation and confirmed experimentally, are of great importance for the interpretation of the phenomena occurring in lipid membranes. The knowledge of adsorption equilibria lets us understand the processes that take place on liposomal surface. The obtained results can be used in quantitative description of physical and chemical properties of biological membranes and, in our opinion, can help in a better understanding of biological membranes and in their biophysical studies.

2. Experimental

2.1. Materials

L-α-phosphatidylcholine from egg yolk was purchased from Fluka. Stearylamine, used as cationic charge-inducing agent, was supplied from Sigma. Chloroform was chromatographic standard grade (Aldrich). Water purified by Milli-Qll (18.2, Millipore, USA) was used to make all solutions and in all cleaning procedures.

2.2. Preparation of the phospholipid vesicles

Phospholipid vesicles were prepared by sonication. The lipids used were neutral PC and positively charged ST. Dry PC and ST were weighed, dissolved in chloroform (10 mg/1.4 ml), and mixed in molar ratios of PC-ST (10-1, 5-1, 3-1). Then 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 the 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 five times for 1.5 min each time.

2.3. Microelectrophoretic mobility measurements

The electrophoretic mobility of the phospholipid vesicle suspensions was determined by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using Laser Doppler Velocimetry (LDV) with the Zetasizer Nano ZS (Malvern Instruments, UK). The measurements were carried out as a function of hydrogen ion concentration. Every result is a mean of six measurements at the given pH value. Liposomes formed earlier were suspended in sodium chloride solution and were titrated either with hydrochloric acid or with sodium hydroxide to obtain the series of different pH solutions. Then the solutions were put into the measuring vessel one by one and the electrophoretic mobility was measured. All experiments were performed at least three times.

3. Theory

The measurement of electrophoretic mobilities is relatively straightforward, but their interpretation is more problematical. The parameter characterizing the liposomal surface is the surface charge density which can be calculated from the measured mobilities [4].