Impact of the charge density of phospholipid bilayers on lubrication of articular cartilage surfaces

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Received 04.04.2007; published in revised form 01.07.2007

ABSTRACT

Purpose: We attempt to answer the question how some changes in acid-base equilibrium have an impact on the charge density of a phospholipid bilayer formed during lubrication occurring at articular cartilage surfaces.

Design/methodology/approach: Liposomes have been used to mimic biological phospholipid membranes on articular cartilage surface where proteins are bound. Ions are transported, energy is transduced, and cellular processes are taking place. The charge density of the membrane was determined as a function of pH and electrolyte concentration from the microelectrophoretic method. Liposome membrane has been prepared as an aqueous solution of NaCl under various pH conditions. Microelectrophoresis was used to examine the local acid-base equilibrium of the electrolytes with the membrane surface, which can be considered to be an interface of phospholipids in articular cartilage.

Findings: The effects of the adsorption of ions (H+, OH−; Na+, Cl−), which are present in solution upon electric charge of the liposome membrane assembled of phosphatidylcholine (PC), have also been found to exhibit pH-responsive (quasi-periodic) behavior.

Research limitations/implications: We hypothesized that the acid-base dissociation behavior in phospholipid bilayers of articular cartilage is a key to understanding biolubrication processes. For example, similar previous investigators found that the behavior of a multilayer made of polysaccharide/hyaluronic acid depends on some of the surface properties such as film thickness, surface friction, surface wetness and swelling conditions. Future work should consider the adsorption of polyelectrolyte ions, e.g., the glycoprotein lubricin and hyaluronic acid, at the liposome membrane surface involved, assumed that besides the H+ and OH− ions, the polyelectrolyte ions were also engaged.

Originality/value: This liposome membrane is a model for phospholipid bilayers and will be applied for the investigation of polyelectrolyte ions, e.g., lubricin, in articular cartilage conditions. We demonstrate that knowledge on the acid-base processes on charged surface is the key to understanding phenomena occurring at interfaces in human joints lubrication, thus pointing to the biolubrication as a charged interface-controlled process

Keywords: Electrical properties; Biomaterials; Lubrication; Articular cartilage

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2. Experimental

Egg PC (99%) from Fluka was used in the experiment and it had the following lipid-acid composition: 16:0 ~ 13%, 18:0 ~ 14%, 18:1 ~ 30%, 18:2 ~ 14%, 20:4 ~ 4%. The size of phospholipid vesicle suspension was determined at 25°C by Dynamic Light Scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments, UK) and was between 10 and 20 nm in diameter [20]. Small unilamellar vesicles were prepared according to the method proposed by [20].

The electroocytotic mobility of the phospholipid vesicle suspension was obtained by performing an electroocytotic experiment on the surface of the particles using Laser Doppler Velocimetry (LDV) with the Zetasizer Nano ZS (Malvern Instruments, UK). The measurements were carried out as function of hydrogen ion concentration in sodium chloride solution within limit range of 10^-3 to 0.155 M or DI water.

The electroocytotic behavior of the particle is strongly influenced by the size of the electrical double layer (DL) of Stern type [25]. Let the plates have a charge q per unit area. From the definitions of viscosity, velocity and mobility, we obtain [26]:

\[
q = \frac{\eta d}{4\pi} \quad (1)
\]

\[
q = \frac{E e^2 \varepsilon_0}{4\pi d} \quad (2)
\]

\[
\mu = \frac{E e^2 \varepsilon_0}{4\pi d} \quad (3)
\]

Making use of the electrostatic expression (2), and further, introducing the mobility by (3), we have obtained a Smoluchowski's equation (3) [26], where: \(\eta\) - viscosity of solution; \(d\) - thickness of diffuse double layer; \(\mu\) - electroocytotic mobility; \(\varepsilon\) - relative permittivity of electrolyte; \(e_0\) - vacuum absolute permittivity; \(C\) - zeta potential.

Let us assume that the H^+, OH^-, and Na^+ ions are adsorbed at the PC surface. The adsorption equilibria are described by the equations:

\[
A^+ + H^+ \leftrightarrow AH; \quad B^- + OH^{-} \leftrightarrow BOH; \quad A^+ + Na^+ \leftrightarrow ANa; \quad B^- + Cl^- \leftrightarrow BCi
\]

(4)

where: \(A^+\) is a -PO_4^2- group, H^+ is a -NO_3^2 group, Cl^- is a -Cl^- group.

Association constants (K) are determined in a suitable temperature range by surface concentrations of the membrane components and volume concentrations of the ions present in the solution:

\[
K_{AH}, K_{BOH}, K_{ANa}, and K_{BCl}
\]

(5)

The surface concentration of the PC is denoted by \(C_L\)

\[
a^+_A + a^-_A + a^+_{ANa} + a^-_{ANa} = C_L; \quad a^-_B + a^+_{BOH} + a^-_{BCl} = C_L
\]

(6)

where \(a^+_A, a^-_A, a^+_{ANa}, a^-_{ANa}, a^-_B, a^+_{BOH}, a^-_{BCl}\) - surface concentrations of membrane components \([\text{mol/l}^2]\), \(a^-_A, a^-_B, a^-_{ANa}, a^-_{BCl}\) - volume concentrations of ions in solution \([\text{mol/m}^3]\) and association constants: \(K_{AH}, K_{BOH}, K_{ANa}, K_{BCl}\). The degree of coverage values of the PC membrane surface, theta, with the H^+, OH^-, Na^+, Cl^- ions were determined from the relationship:

\[
a^-_A = x \cdot a^-_L
\]

(7)

where \(x = A^+, AH, ANa, B^-, BOH, BCi\). The surface conc. \(a^+_{ANa}\) were determined from Eqs. 5 - 6.

3. Results and discussion

The experimental values of electroocytotic mobility were converted to surface charge density using Eq. 1. The calculated values of surface charge density were determined on the basis of Eq. 7. The association constants of the surface groups with the
solution ions were determined using methodology from [23]. The surface charge density (\( q \)) of a PC membrane is described by the equation: \( q = (a^-_N - a^-_A) \) F, and by elimination of \( a^{0}_{Na}, a^{0}_{NH}, a^{0}_{OH} \), \( a^{0}_{Cl} \) (from Eqs. 6) and of \( a^+_A, a^+_N \), from the equation \( q = (a^-_N - a^-_A) \) F; yields Eq. (8):

\[
q = \frac{C_L}{F} + \frac{K_{BOH}a^+_{OH} + K_{BOH}a^+_{A}}{1 + K_{AHA}a^+_{A} + K_{ANa}a^+_{Na}}.
\]

Fig 1. The pH dependence of the surface charge density of liposomal membrane formed from PC. The experimental values are those obtained for deionized water and for 0.155 M NaCl solution.

The pH dependence of the surface charge of the liposomal membrane is plotted in Fig. 1. The experimental control curve, which was made in DI water in the absence of sodium chloride. The other curve was obtained in the presence of 0.155 M NaCl. It can be observed that in basic solution in the presence of the sodium chloride, a decrease of negative charge occurs. The \( -N^i(CH_3)_2 \) groups of PC molecules are covered by OH- ions, whereas \(-PO_3^2^- \) groups are uncovered. The fact indicates adsorption of Na+ ions. A similar tendency can be observed in acidic solution: in the presence of sodium chloride, a decrease of positive charge occurs. The \(-PO_3^2^- \) groups are covered by H+ ions, whereas \(-N^i(CH_3)_2 \) groups are uncovered. This fact indicates adsorption of CT ions.

Association constants of the surface groups with the solution ions were determined by the linear regression method using the Excel 97 program [23]. The association constants determined in this way are equal to \( K_{BOH} = 5.35 \times 10^9 \), 1.56 \times 10^9, \( K_{AHA} = 0.218 \), 0.011, \( K_{ANa} = 5.58 \times 10^9 \), 2.03 \times 10^9, \( K_{ANa} = 0.051 \), 0.002 (m^2/mol). From comparison of the association constants it appears that the H+ ion is more strongly adsorbed than the Na+ ion and the OH- ion is also more strongly adsorbed than the CT ion.

The degree of coverage of the PC membrane surface by ions as function of pH of 0.155 M NaCl is presented in Fig. 2. Beside the coverage with the H+ and OH- ions, the coverage with other ions (Na+ and Cl-) was considered to check if the coverage with these ions is as high as to affect the PC membrane surface charge. As can be seen in Fig. 2 the Na+ ions adsorption starts when the amount of the H+ ions becomes low (at pH > 6). In basic solution the degree of coverage of the membrane by the Na+ ions is over 0.8, e.g., in this pH range the membrane is covered by the Na+ ions. A similar tendency can be observed for the Cl ions: the adsorption of the Cl ions begins when the amount of the OH- ions begins to decrease (at pH < 4). In a strongly acidic solution the degree of coverage of the membrane by the Cl ions is almost one. The measurements of the surface charge of the viscoelastic PC membrane as a function of concentration of NaCl for physiological pH are presented in Fig. 3. The increase of the Na+ ion concentration causes the decrease of the negative charge, and the same is proving the adsorption of the Na+ ions.

In our experiment, the pH range 6.4 to 8.4 (7.4 is physiological condition of synovial fluid) is most of our interest. From these, we can conclude that sodium and hydrogen ions interaction with group-PO_3^2^- (or the degree of coverage of phospholipid membrane surface) is high. Also, in the physiological pH condition, the degree of coverage of membrane by the OH ions is near one. The adsorption of the chloride ions, which is as a very weak base is not observed in pH range 6.4 to 8.4 conditions.

Our results do indeed indicate that the surface charge strongly influences the acid-base equilibrium of the adsorbing species. Similarly to other experiments [27-29], we chose to alter the surface charge by changing the pH of the solution used to assemble the bilayer since the pH affects the degree of dissociation of both polyelectrolytes (if present) and the charge density on phospholipid bilayer. This liposome bilayer is a model for phospholipid bilayers and will be applied for the investigation of lubricin in articular cartilage.

If acid-base quasi-equilibria are kept/recovered by the system, it is more resistive to wear (when static frictional treated); hydration of
phospholipids assures that coagulation becomes ineffective – the layers involving hydrated phospholipids, and being electrostatically adsorbed at the surface(s) of articular cartilage, are also more mechanically robust. The latter gives rise to weak-friction promoting sliding effect, due to electrostatic repulsion, and opposes a (possible) peptization to enter, which, however, depends upon keeping a balance of sits within the system. If the balance is not kept by the system, the coagulation effects may dominate, which leads to loosing one of the desired acid-base quasi equilibria, thus driving the system out of equilibrium. This may spoil a quasi-periodic character of the relations presented in Fig. 2, which would imply an imbalance in the ions-involving prone-to-friction viscoelastic membrane [30], also causing the ions to flow [31].

Our analysis can also be extended to modern biomaterials-involving applications, especially when invoking orthopaedic implants, or specifically, some stent–oesophagus systems [32, 33].

References