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

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Properties

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 bounded, ions are transported, energy is transducted, 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 phosphatidycholine (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 polyisopeptide/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 hyaluronan, at the liposome membrane surface involved, assumed that besides the H^+ and OH^- ions, the polyelectolyte 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

1. Introduction

The biolubriction mechanism between two phospholipid (PL) bilayers on articular cartilage surfaces has been studied for decades [1] and still requires a better knowledge of the tissues and molecular interactions [2, 3]. The solvent (water) as a lubricant determines the biolubrication of surfaces, by the additives as ions and macromolecules components and acid-base equilibrium. The two normal articular bilayer phospholipidic surfaces are very hydrophilic and perform all principles of core of reverse micelles [4]. The charged core of reverse micelle is powerful enough to solubilize water molecules, and do the selective transfer of certain molecular substances through the lipid barrier. [5-7].

The phospholipids have been recognized in tissue of articular cartilage and synovial fluid. Using electron microscope and fixation procedure the surface layer oligolamellar phospholipid was confirmed [5, 8]. It was than proven when the phospholipid bilayers are removed by lipid solvent, than articular cartilage surface becomes very hydrophobic [9, 10], which leads to friction increase by 150% [11]. The major components are about 61% of phospholipids with major sub-fraction of phosphatidycholine (PC) [12,13].

Interestingly, the glycoprotein (GlyPr) with MW of 227000 was named a "lubricin" performed the remarkable lubricating capabilities with PLs participation. The water-soluble macromolecules of glycoprotein is carrier for very insoluble in water small phospholipid molecules (MW approximately 730) [10]. Lubricin, a component of the synovial fluid, was identified to contain 86% of glycoprotein and 12% of phospholipids with 2% remaining unknown [10]. Being a lubricant, lubricin is an active macro-ion in SF which deposits (or adsorbs) the oligolamellar layer of phospholipids that possess the capabilities of high-load bearings [9, 10]. Phosphlipids molecules bind amino acid groups that contain the protein chains in glycoprotein as lubricin [14]. Self-lubrication of cartilage is expressed in low lubricity regardless of the type of fluid between cartilage surfaces [2, 15]. The hydrophilicity of the surface molecular groups, e.g. lipid head-groups is affected by the electrolyte ions in the solutions. Between negatively charged surfaces the short - range hydration repulsion usually increases as more cations adsorb [16,17]. Concerning complex biofluids involved in the biolubrication of articular cartilage it is the glycoprotein lubricin on hydrophilic surface phospholipids with a support of hyaluronan held together by protoglycan [1, 8, 9]. In previous studies of samples of aspirated synovial fluid, the pH of normal synovial fluid was between 7.3 and 7.43 [18, 19]. The pH value of synovial fluid in various inflammatory conditions from joints with osteoarthritis (OA) and rheumatoid arthritis (RA) obtained were 7.4 - 8.1 (mean of 7.9) for the 16 joints with OA, and 7.4 - 7.6 (7.5) for the six joints with RA [20]. It is known that multilayer film prepared by a sequential electrostatic adsorption of poly(L-lysine) and hyaluronic acid, (PLL/HA) onto charged silicon surfaces is a key to learn about surface friction and wetness. In particular, studies have shown that the surface friction can be altered by a factor of 10 and the degree of swelling by a factor of 8 for film composed of the two polyelectrolytes, simply by varying the pH [21, 22]. In this paper, we will examine by micro-electrophoresis the adsorption of ions (H^+, OH^-, Na^+, Cl^-) on the PC membranes which have also been found to exhibit pH-responsive behavior. The idea was confirmed by mathematical calculations of association constants of the liposome membrane surface with ions of solution (K_{AH}, K_{ANa}, K_{BOH}, K_{BCl}). However, one may ask a question whether only these ions are adsorbed at the phospholipid surface. It is the aim of this work to present the model for adsorption of other ions, e.g., lubricin at the liposome membrane surface.

2. Experimental

Egg PC (99%) from Fluka was used in the experiment and it had the following fatty acid composition: 16:0 ~ 33%, 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 [23]. Phospholipid vesicles were prepared according to the method proposed by [24].

The electrophoretic mobility of the phospholipid vesicle suspension was obtained 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 function of hydrogen ion concentration in sodium chloride solution within limit range of 10^{-5} to 0.155 M or in DI water.

The electrophoretic 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]**:**

$$q = \frac{\eta\mu}{d} \quad (1)\,; \qquad q = \frac{\varepsilon\varepsilon_0\zeta}{4\pi d} \quad (2); \qquad \mu = \frac{\varepsilon\varepsilon_0\zeta}{4\pi\eta} \quad (3)$$

Making use of the electrostatic expression (2), and further, introducing the mobility by (1), we have obtained a Smoluchowski's equation (3) [26], where: η – viscosity of solution ; d - thickness of diffuse double layer; μ - electrophoretic mobility; ε - relative permittivity of electrolyte; ε_0 – vacuum absolute permittivity, ζ- zeta potential.

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

$A^- + H^+ \leftrightarrow AH$; $B^+ + OH^- \leftrightarrow BOH$; $A^- + Na^+ \leftrightarrow ANa$; $B^+ + Cl^- \leftrightarrow BCl$ (4)

where: A^- is a $-PO^{(-)}$ group, B^+ is a $-N^{(+)}(CH_3)_3$ 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

$a_{A^-} + a_{AH} + a_{ANa} = C_L$; $a_{B^+} + a_{BOH} + a_{BCl} = C_L$ (6)

where a_{A^-}, a_{AH}, a_{ANa}, a_{B^+}, a_{BOH}, a_{BCl} – surface concentrations of membrane components [mol/m^2], a_{H^+}, a_{OH^-}, a_{Na^+}, a_{Cl^-} - volume concentrations of ions in solution [mol/m^3] and association constants: K_{BOH}, K_{BCl}, K_{AH}, K_{ANa}. The degree of coverage values of the PC membrane surface, theta θ, with the H^+, OH^-, Na^+, Cl^- ions were determined from the relationship:

$$\theta_x = \frac{a_X}{C_L} \quad (7)$$

where x = A^-, AH, ANa, B^+, BOH, BCl. The surface conc. a_{A^-}, a_{B+} were determined from Eqs. 5 – 6.

3. Results and discussion

The experimental values of electrophoretic 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: $q = (a_{B^+} - a_{A^-})\,F$, and by elimination of a_{AH}, a_{ANa}, a_{BOH}, a_{BCl} (from Eqs. 6) and of a_{A^-}, a_{B^+}, from the equation $q = (a_{B^+} - a_{A^-})\,F$, yields Eq. (8):

$$\frac{q}{F} = \frac{C_L}{1 + K_{BOH}a_{OH^-} + K_{BCl}a_{Cl^-}} - \frac{C_L}{1 + K_{AH}a_{H^+} + K_{ANa}a_{Na^+}} \quad (8)$$

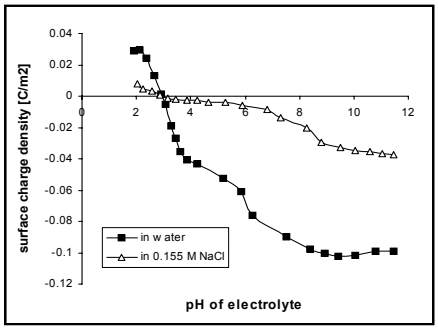

Fig 1. The pH dependence of the surface charge density of liposomal membrane formed from PC. The experimental values are those obtained for deionized water and for 0.155 M NaCl solution.

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 $-N^{(+)}(CH_3)$ groups of PC molecules are covered by OH^- ions, whereas $-(PO)^{(-)}$ 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 $-(PO)^{(-)}$ groups are covered by H^+ ions, whereas $-N^{(+)}(CH_3)$ groups are uncovered. This fact indicates adsorption of Cl^- 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 $K_{BOH} = 5.35 \times 10^9 \pm 1.56 \times 10^8$, $K_{BCl} = 0.218 \pm 0.011$, $K_{AH} = 5.58 \times 10^5 \pm 2.03 \times 10^4$, $K_{ANa} = 0.051 \pm 0.002$ [m^3/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 Cl^- 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 $-(PO)^{(-)}$ (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.

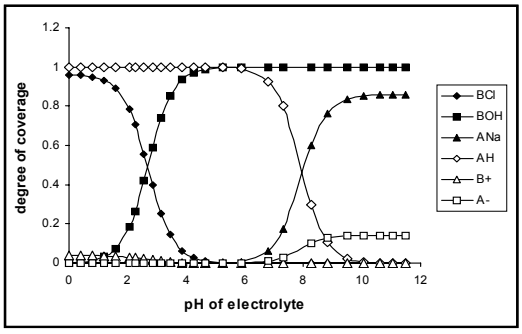

Fig. 2. The degree of coverage, θ, of the PC membrane surface with the H^+, OH^-, Na^+, Cl^- ions, as a (quasi-periodic) function of pH of the 0.155 M NaCl solution. The adsorption equilibria are described by the equations, where: A^- is the $-PO^{(-)}$ group , B^+ is the $-N^{(+)}(CH_3)_3$ group: $A^- + H^+ \leftrightarrow AH$; $B^+ + OH^- \leftrightarrow BOH$; $A^- + Na^+ \leftrightarrow ANa$; $B^+ + Cl^- \leftrightarrow BCl$. The physiological pH coverage is in range 7.3 to 7.5.

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.

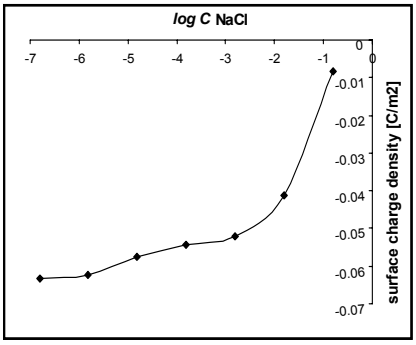

Fig. 3. The surface charge density of the PC membrane as a function of concentration of sodium chloride within limit range of 10^{-5} to 0.155 M in physiological pH condition.

If acid-base quasiequilibria are kept/recovered by the system, it is more resistive to wear (when static-friction 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 salts 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].

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