A biochemical model for characterising the surface–active phospolipid bilayer of articular cartilage relative to acid-base equilibrium

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ABSTRACT

Purpose: This paper, addresses the question of how changes in acid - base equilibrium influence change in the charge density of the phospholipid bilayer on articular cartilage surfaces during lubrication.

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 take place. The charge density of the membrane was determined as a function of pH and electrolyte concentration from the microelectrophoretic method. Liposome membrane was 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 model the phospholipids interface in articular cartilage.

Findings: The adsorbed ions (H^+, OH^-, Na^+, Cl^-) which are present in the electrically charged solutions of liposome membrane comprising phosphatidycholine (PC), were found to exhibit pH-responsive quasi-periodic behavior.

Research limitations/implications: We have established that the acid-base dissociation behavior in phospholipid bilayers of articular cartilage is a key to understanding biolubrication processes. For example, previous investigators found that the formation of the multilayer of polyisopeptide/hyaluronic acid depends on surface properties such as film thickness, surface friction, surface wetability; wetness and swelling behavior. Future work should consider the adsorption of polyelectrolyte ions, e.g., the glycoprotein lubricin and hyaluronan, on the liposome membrane surface in the presence of H^+ and OH^- ions.

Originality/value: A novel model of the joints' phospholipid bilayers has been created using liposome membrane This model can be applied in the investigation of polyelectrolyte ions such as lubricin, in articular cartilage. We have demonstrated that the acid-base processes on charged surfaces is a key mechanism in facilitating lubrication in human joints.

Keywords: Biomaterials; Electrical properties; Lubrication; Articular cartilage; Surface charge density; Phospholipids membrane; pH

MATERIALS

1. Introduction

The articular cartilage lubricant is phospholipid-based

Phospholipid (PL) molecules bind amino acid groups that contain the protein chains in glycoprotein as lubricin [1]. The most compelling argument supporting phospholipids as the main lubricant in the joint is that, lubricin, the often proposed alternative lubricant, would need to be adsorbed to the surface as a hydrophilic molecule and yet act as an effective lubricant, which is difficult if not impossible to achieve. In our opinion, PLs by chemical association with lubricin, act as large water-soluble molecules.

Using non-biological surfaces namely mica and silica covered with phospholipids and normal articular cartilage surfaces [2] measured the friction characteristics of the materials in contact with biosurfaces of native and modified cartilage surface (lipids removed from surface). The method adapted for grafting hyaluronan to lipid bilayer head groups consisted of a amine supported on mica surfaces which resulted in a friction coefficient of 0.19. The authors concluded that hyaluronan (HA) is not responsible for the low friction of cartilage. A negatively charged hyaluronan as polyelectrolyte molecule, HA, in aqueous solution does not adsorb onto negatively charged surfaces (of mica) or articular cartilage. HA did not lubricate the mica surfaces, and under relatively low loads and low sliding velocities exhibited a high friction coefficient. A certain on- purpose role of HA could be viewed, in turn, as to help immobilizing surface-active PLs dispersed in the synovial fluid after some frictional load, which may draw its special role as a promoter of PLs containing (reverse) micelles, emerging therein under a water excess accompanied by some mainly water-assisted mutual replacement between the PLs mentioned and lubricin molecules. The latter molecules are then adsorbed at the solid surfaces of the articular cartilage, thus causing the biolubrication to proceed efficiently [3].

Joint lubrication mechanism

The biolubrication mechanism between two phospholipid (PL) bilayers located on articular cartilage surfaces has been studied for decades [4], however, a further insight into the molecular interactions taking place at the joint interface as the phospholipid layers contact under load delivering biolubrication is still required for us to fully understand the mechanisms involved in the process. Using electron microscopy and fixation procedures phospholipids have been identified in both articular cartilage (oligolamellar surface active and intramatrix phospholipids) and synovial fluid [5,6]. More specifically, a study has shown that when the phospholipid bilayers are removed by a lipid solvent, the surface of articular cartilage becomes very hydrophobic [7], leading to an increase in friction of 150% [8]. An analysis of the fluid extracted from rinsing the surface of articular cartilage with a solvent further reveals that joint surfactant consists of about 61% phospholipids with major sub-fraction of phosphatidycholine [9,10]. It has also been proposed that the components of the synovial fluid namely, lubricin (a glycoprotein), hyaluronan which are "held" on cartilage via their interaction with the proteoglycans, in association with the phospholipid molecules are responsible for the almost frictionless biolubrication of the mammalian joint [11,12]. We hypothesize in this paper that biolubrication of joint surfaces is enabled by the interactions between water (the solvent) which is normally under pressure during physiological function [13], "additives" i.e., ionic salts and other macromolecular components, and the nature of the acid-base equilibrium occurring in the joint space. Note also that the ionic salts may, due to the electrostatic (Debye) screening cause additionally a formation of nuclei containing PLs, which in the confined aqueous ambient phase may start crystallization to be switched on; its thorough emergence can then be hampered by means of friction. Some (un)dissolved nuclei may also contribute to facilitate the biolubrication [14].

It can be argued that the hydration of ions and macromolecular polyelectrolytes is fundamental to the ability of two charged phospholipid bilayers to function as a lubrication agent on the surfaces of contacting articular cartilage layers. The contacting bilayer phospholipidic articular surfaces in the joint are hydrophilic and it has recently been proposed that their physiological function can be explained using the principles of core reverse micelles [15]. In this regard, we note that the charged core of the reverse micelle is able to solubilize in water molecules forming a lipid semipermeable membrane. The lipid bilayer membrane formed is 6-10 nm thick and act as barrier to the diffusion of polar solutes, with the associated embedded proteins and cholesterol providing the pathways for the charge core of reverse micelle resulting in: (a) organized water molecules and ions on the articular surfaces, (b) stabilization of charged particles and elimination of flocculation, (c) facilitation of electrostatic attachment of polyelectrolyte molecules to the hydrophilic surface and enabling phospholipids to organize into a double layer of electrostatic charges during cartilage-to-cartilage contact, (d) carry out the selective transfer of certain molecular substances through the lipid barrier, and (e) facilitate the transfer of mechanical information from the extracellular matrix (ECM) into the interior of the cell within the cartilage matrix. These bilayers (or sheets) lie in widely separated parallel planes loosely held together by a weak physical force, thus allowing, them to slide over one another with minimal friction. The lipids in articular cartilage are composed of cholesterol, triglycerides, and phospholipids (from 0.3 to 4%) [16]. Furthermore, the electrostatic charges have been identified as a powerful intermediary in the manipulation of the properties of the complex joint fluid system [17,18].

Characteristics of joint fluid-surfactant system

Of importance is the finding that the glycoprotein (GlyPr) with MW of 227,000, namely "lubricin" exhibited remarkable lubricating capabilities when combined with phospholipids. It is also known that water-soluble glycoprotein macromolecules are a carrier for other highly water-insoluble small phospholipid molecules (MW approximately 730) [7]. Lubricin, a component of the synovial fluid, was identified to contain 86% of glycoprotein and 12% phospholipids with 2% remaining unknown [7]. Being a lubricant, lubricin is an active macro-ion in SF which deposits (or adsorbs) the oligolamellar layer of phospholipids that possess the capability to bear high loads [7, 19]. Phospholipid molecules bind amino acid groups that contain the glycoprotein chains forming lubricin [1]. It has also been proposed that self-lubrication of cartilage which is characterized with low lubricity will occur regardless of the type of fluid between contacting cartilage surfaces [11,20]. The hydrophilicity of the surface molecular groups, e.g. lipid head-groups is affected by the electrolyte ions in solutions; such that between negatively charged surfaces, short range-hydration-repulsion increases as more

cations are adsorbed [21,22]. A few molecular layers or 1-2 nm of water in 0.01M KCl solution act as a protective layer against adhesion-induced damage during sliding and a low friction coefficient of 0.02 is maintained under loads of up to 20 MPa [21, 23]. A leading argument regarding the biofluids involved in the biolubrication of articular cartilage is that the glycoprotein lubricin on hydrophilic surface active phospholipids, supported by the hyaluronan that are held together by protoglycans is responsible for the ultra-low friction in the joint [6,19,21].

The pH value can distinguish normal from osteoarthritic condition. In previous studies of samples of aspirated synovial fluid, the pH of normal synovial fluid was found to be between 7.3 and 7.43 [22, 24]. In contrast the pH values of synovial fluid in various inflammatory conditions from joints with osteoarthritis (OA) and rheumatoid arthritis (RA) were 7.4 - 8.1 (mean of 7.9) for 16 joints with OA, and 7.4 -7.6 (7.5) for the six joints with RA [25]. It is known that multilayer film prepared by sequential electrostatic adsorption of poly(L-lysine) and hyaluronic acid, (PLL/HA) onto charged silicon surfaces can provide an insight into the understanding of surface friction and wettability. In particular, studies have shown that surface friction can be altered by a factor of 10 and the degree of swelling by a factor of 8 for films composed of the two polyelectrolytes, by simply varying the pH [26,27].

Therefore, in this paper, we will examine via micro-electrophoresis the adsorption of ions (H$^+$, OH$^-$, Na$^+$, Cl$^-$) on the phosphatidycholine (PC) membrane which have also been found to exhibit pH-responsive behavior. Mathematical calculations of association constants for liposome membrane surface in contact with ions in solution (K_{AH}, K_{ANA}, K_{BOH}, K_{BCL}) will also be carried out, leading to a model for adsorption of other ions, such as lubricin at the liposome membrane surface.

2. Experimental methodology

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 [28]. Phospholipid vesicles were prepared according to the method proposed in [29]. The electrophoretic mobility of the phospholipid vesicle suspension was obtained by performing a.n 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 the range 10^{-5} to 0.155 M or in DI water. [39]

The electrophoretic behavior of the particle is strongly influenced by the size of the electrical double layer layer (DL) of Stern type [30]. Mathematically, we can express the viscosity, velocity and mobility, equation (1) below as [31]:

$$q = \frac{\eta \mu}{d} \tag{1}$$

$$q = \frac{\varepsilon \varepsilon_0 \zeta}{4\pi d} \tag{2}$$

$$\mu = \frac{\varepsilon \varepsilon_0 \zeta}{4\pi \eta} \tag{3}$$

where, q is the charge per unit area on the plate.

Making use of the electrostatic expression (2), and introducing the mobility by (1), we have Smoluchowski's equation (3) [31], where η is viscosity of solution; d is thickness of diffuse double layer; μ is electrophoretic mobility; ε is relative permittivity of electrolyte; ε_0 is vacuum absolute permittivity, ζ is zeta potential. The Smoluchowski equation stands for an intriguing example of the empirical fluctuation-dissipation relation, where the fluctuation is due to directional diffusion of ions in the microelectrophoresis whereas the kinetic-energy dissipation is subjected to a deceleration in the quasi-directional motion of ions due to presence of the damping medium viz solvent. Notice that a proportionality factor in Eq. (3), namely $\frac{\varepsilon \varepsilon_0 \zeta}{4\pi}$, must be temperature-adjustable, pointing this way to a proper temperature value reflecting, for example, the physiological or experimental conditions of interest.

Assuming that the H$^+$, OH$^-$, Na$^+$, and Cl$^-$ ions are adsorbed at the phosphatidylcholine surface the adsorption equilibrium are then described by the equations:

A$^-$+H$^+$↔AH; B$^+$+OH$^-$↔BOH;
A$^-$+Na$^+$↔ANa B$^+$+Cl$^-$↔BCl (4)
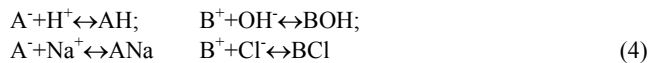
where: A$^-$ is group –PO$^{(-)}$, B$^+$ is group –N$^{(+)}$(CH$_3$)$_3$.

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

$$K_{AH} = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}}$$

$$K_{BOH} = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}}$$

$$K_{ANa} = \frac{a_{ANa}}{a_{A^-} \cdot a_{Na^+}} \tag{5}$$

$$K_{BCl} = \frac{a_{BCl}}{a_{B^+} \cdot a_{Cl^-}}$$

The surface concentration of the phosphatidylcholine is denoted by C$_L$, so that:

$$a_{A^-} + a_{AH} + a_{ANa} = C_L$$
$$a_{B^+} + a_{BOH} + a_{BCL} = C_L \tag{6}$$

where a_{A^-}, a_{AH}, a_{ANa}, a_{B^+}, a_{BOH}, a_{BCl} are surface concentrations of membrane components [mol/m²], a_{H^+}, a_{OH^-}, a_{Na^+}, a_{Cl^-} are volume concentrations of ions in solution [mol/m³] and association constants: K_{BOH}, K_{BCL}, K_{AH}, K_{ANA}.

The degree of coverage values of the phosphatidylcholine membrane surface, θ with the H^+, OH^-, Na^+, Cl^- ions were determined from the relationships:

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

where x = A⁻, AH, ANa, B⁺, BOH, BCl. The surface concentration, a_{A^-}, a_{B^+} were determined using Eqs. 5 - 6, such that,

$$A_{A-} = \frac{C_L}{1 + K_{AH}a_H + K_{ANa}a_{Na}}$$

$$a_{B+} = \frac{C_L}{1 + K_{BOH}a_{OH} + K_{BCl}a_{Cl}} \qquad (7a)$$

Where as a_{AH}, a_{ANa}, a_{BOH} a_{BCl} were obtained by transforming Eqs. 4, 5 and 6.
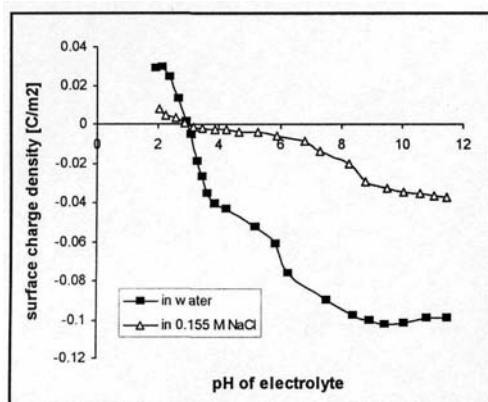

Fig. 1. The pH dependence of the surface charge density of liposomal membrane formed from phosphatidylcholine in deionized water and 0.155 M NaCl solution

3. Results and discussion

Microelectrophoresis was used to follow the mobility of liposome membrane formed from phosphatidylcholine as a function of pH in DI water and 0.155 M sodium chloride solution, to test the central idea of this paper, namely the potential adsorption of sodium and chloride ions to the surfaces of phospholipids. To obtain a complete set of measurements, the effect of the concentration of electrolyte solution was also studied. 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 the methodology from [28]. The surface charge density (q) of a phosphatidylcholine membrane is described by the equation: q = $(a_B^+-a_A^-)$ F, (where: F- Faraday constant) and by elimination of a_{OH}, a_{ANa}, a_{BOH}, a_{BCl} (from Eqs. 6) and of a_A^-, a_B^+ from Eq. q = $(a_B^+ - a_A^-)$ F, yields the equation (8):

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

The pH dependence of the surface charge of the liposomal membrane in 0.155 M NaCl solution and deionised water (control curve) is plotted in Fig. 1. It can be seen that in decrease in the negative charge occur in the saline environment. $-N^{(+)}(CH_3)$ groups of phosphatidylcholine 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. $-(PO)^{(-)}$ groups are covered by H^+ ions, whereas $-N^{(+)}(CH_3)$ groups are uncovered. This result indicates adsorption of Cl^- ions.

Association constants of the surface groups with the solution ions were determined from Eq. (17) and Eq. (18) using linear regression (for details see paper) [28]. The association constants determined in this way are equal to K_{BOH} = 5.35 x 10⁹ ± 1.56 x 10⁸, K_{BCl} = 0.218 ± 0.011, K_{AH} = 5.58 x 10⁵ ± 2.03 x 10⁴, K_{ANa} = 0.051 ± 0.002 [m³/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. However, in acidic solution, in the absence of the OH^- ions, the adsorption of the Cl^- ions is observed. In basic solution, in very low concentration of the H^+ ions, the adsorption of Na^+ is observed (Fig. 1).

The degree of coverage of the phosphatidylcholine membrane surface ions as a function of the pH of the 0.155 M NaCl is presented in Fig. 2. Aside from 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 high enough to affect the phosphatidylcholine 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 where 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. Thus, the adsorption of the Na^+ and Cl^- ions must be taken into account as the electric charge is affected by this phenomenon. Let us conclude that a type of quasi-periodicity can be attributed to such a behavior of competitive nature, cf. Fig. 2. It is characteristic of many natural systems under friction [32].

The dependence of the surface charge of the PC membrane on the concentration of NaCl for physiological pH is presented in Fig. 3. The negative value of surface charge density of PC

membrane at higher concentrations of sodium and chloride ions is reduced in a predictable manner. As expected for low concentrations of electrolyte solution (H$^+$ and OH$^-$ ions, also very low), meaningful changes in the surface charge density values are observed. The increase of the Na$^+$ ion concentration causes a decrease in the concentration of negative charges, thereby indicating the adsorption of Na$^+$ ions. In our experiment, the pH range 6.4 to 8.4 (7.4 is physiological condition of synovial fluid) is of the most interest. In this regime, 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 the membrane by the OH ions is close to one. The adsorption of the chloride ions, which occurs as a very weak base is not observed in pH range 6.4 to 8.4. Our results do indeed indicate that the surface charge strongly influences the acid-base equilibrium of the adsorbing species. Similarly to other experiments [33-35], 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 the phospholipid bilayer. This liposome bilayer is a model for phospholipid bilayers and will be applied for the investigation of the lubrication in general of contacting articular cartilage.
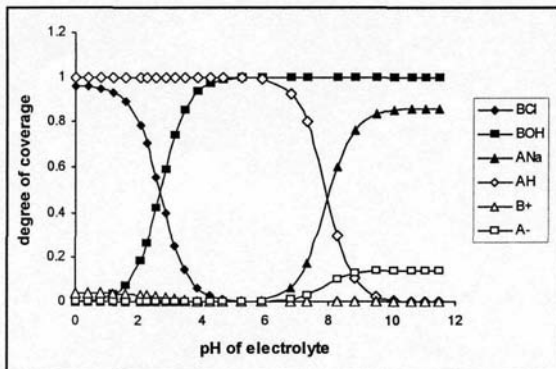

Fig. 2. The degree of coverage, θ, of the phosphatidylcholine membrane surface with the H$^+$, OH$^-$, Na$^+$, Cl$^-$ ions, as a function of pH of the 0.155 M NaCl solution. The adsorption equilibriums are described by the equations, where: A$^-$ is group –PO$^{(-)}$, B$^+$ is group -N$^{(+)}$(CH$_3$)$_3$: A$^-$ + H$^+$ ↔ AH ; B$^+$ + OH$^-$ ↔ BOH; A$^-$ + Na$^+$ ↔ ANa; B$^+$ + Cl$^-$ ↔ BCl. The physiological pH coverage is in range 7.3 to 7.5. Realize a quasi-periodic and mutually competing character of the curves drawn for two main ionic groups under consideration, AH + BOH and ANa + BCl, respectively

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 quasiequilibria, 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 occuring in the ions-involving prone-to-friction viscoelastic membrane [32], also causing the ions to flow [36]. Our analysis can also be extended to modern biomaterials-involving applications, especially when invoking orthopaedic implants, or specifically, some stent- oesophagus systems [37, 38]. The equilibrium could then be restored when direct interactions between charged groups of PLs and/or lubricin molecules, with the dissociated water dipoles (and salts' ions), would become effective. It may lead to have the polyelectrolite ions in an extended (swollen) state because in equilibrium conditions the water-based ions (and, possibly, the others) will reside with in non-excluded volume spaces of the polyelectrolyte molecules-such a scenario seems plausible, and can most likely be revealed by applying the dissipative dynamical (tribomicellization) system invoked above [32].
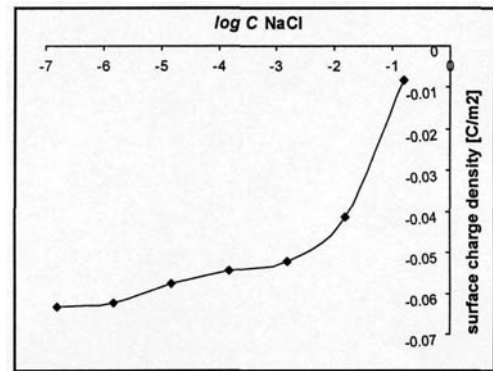

Fig. 3. The surface charge density of the phosphatidylcholine membrane as a function of concentration of sodium chloride within the range 10^{-5} to 0.155 M in physiological pH condition

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