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Biophysical Chemistry 104 (2003) 13–19

Biophysical Chemistry

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Acid–base equilibria at interface separating electrolyte solution and lipid bilayer formed from phosphatidylcholine

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Received 13 May 2002; received in revised form 18 September 2002; accepted 23 September 2002

Abstract

The dependence of the interfacial tension of a lipid membrane formed from phosphatidylcholine on the pH of the aqueous solution has been studied. The model describing the H^+ and OH^- ions adsorption in the bilayer lipid surface has been presented in this work. We take suitable equations to describe the dependence of interfacial tension of a lipid bilayer membrane on H^+ and OH^- ion concentrations. A theoretical equation is derived to describe this dependence in the whole pH range.

Keywords: Interfacial tension; Bilayer lipid membrane; Phosphatydylcholine

1. Introduction

Biological membranes are organised, layered macromolecular systems. They are mainly composed of lipids and proteins and they carry out functions necessary for life. They confer individuality to a cell by separating it from its environment and their structure is complex. Experiments are carried out with simple models for a better understanding of properties of natural membranes; synthetic phospholipid membranes, also called equivalent or model membranes, are used to this aim. The equivalent membranes are prepared in such a way that they model some properties of biological membranes. The composition and structure of a model membrane should be well known but, on the other hand, they should effectively reproduce properties of a living cell membrane. In most cases, either flat lipid bilayers spread in a Teflon diaphragm orifice or bubbles (liposomes) are used [1,2].

An important property of a cell membrane is its interfacial tension which determines rigidity of the membrane and, therefore, its stability. A cell membrane is a very complex system; it contains various components influencing its interfacial tension. Interfacial tension is affected by some factors, e.g. by pH of the medium or by the presence of substances able to form hydrophilic pores in the membrane. For this reason, a study of the effect of pH of the medium on interfacial tension of a lipid membrane formed of phosphatidylcholine has been the purpose of this work. It should result in

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PII: S0301-4622(02)00324-1

a better understanding of cell membrane function, equilibria and processes which can occur in it.

Phosphatidylcholine (PC) is a known lipid, often used in measurements. Its molecule is a zwitterion; it takes part in equilibria with both H^+ and OH^- ions. Adsorption of the H^+ and OH^- ions at the PC bilayer surface was described in the paper [3,4], in which the effect of pH on interfacial tension of the PC membrane has been described in terms of the Gibbs isotherm. Maximal interfacial tension value was 3.53 mN/m at pH equal to 4.15. However, the dependence presented there is correct in a narrow pH range only, i.e. near to the isoelectric point of the membrane. For this reason, in this work it has been attempted to describe adsorption of the H^+ and OH^- ions at the PC bilayer in the whole pH range. It should explain the effect of pH of electrolyte solution on interfacial tension of a membrane formed of PC.

2. Theory

The effect of pH on interfacial tension of lipid membranes formed of phosphatidylcholine has been studied.

The H^+ and OH^- ions are adsorbed at phospholipid surface.

Thus, adsorption equilibria can be presented in the form:

$$A^- + H^+ \Leftrightarrow AH \tag{1}$$

$$B^+ + OH^- \Leftrightarrow BOH \tag{2}$$

The lipid is present in the membrane only. Therefore, its concentration can be defined as its amount related to volume of solution or to membrane surface area. These concentrations and hydrogen and hydroxyl ion concentration by volume yield acid–base stability constants according to Eqs. (3) and (4):

$$K_A = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}} \tag{3}$$

$$K_B = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \tag{4}$$

Denoting surface concentration of the lipid by s, the following equations can be written in terms of acid–base equilibrium [Eq. (1)or Eq. (2)]:

$$a_{AH} + a_{A^-} = s \tag{5}$$

$$a_{BOH} + a_{B^+} = s \tag{6}$$

It was assumed in the paper [3] that surface excess of the H^+ and OH^- ions is equal to their surface concentration. This assumption is common in describing adsorption phenomena [5,6] but it is correct in the case only where the adsorption is strong and the concentration of the adsorbed ion in the solution is low. In the present case, the surface excess definition resulting from deduction of the Gibbs equation [7] should be strictly respected.

The equations describing surface excess of the H^+ and OH^- ions in terms of the definition resulting from the Gibbs equation can be presented in the form:

$$\Gamma_{OH^-} = a_{BOH} - V_{H^+} a_{AH} a_{OH^-} - V_{OH^-} a_{BOH} a_{OH^-} \tag{7}$$

$$\Gamma_{H^+} = a_{AH} - V_{H^+} a_{AH} a_{H^+} - V_{OH^-} a_{BOH} a_{H^+} \tag{8}$$

V_{H^+}[m^3]-H^+ ion volume in the adsorption layer; V_{OH^-}[m^3]-OH^- ion volume in the adsorption layer.

The Gibbs equation assumes the form:

$$d\gamma = -\sum \Gamma_i d\bar{\mu}_i \tag{9}$$

If the H^+ and OH^- ions are adsorbed, then the above equation assumes the form:

$$d\gamma = -\Gamma_{H^+} d\bar{\mu}_{H^+} - \Gamma_{OH^-} d\bar{\mu}_{OH^-} \tag{10}$$

Substitution for Eqs. (7) and (8) to the Gibbs equation yields:

$$\begin{aligned} d\gamma = &- RT a_{BOH} \frac{da_{OH^-}}{a_{OH^-}} + RT V_{H^+} a_{AH} da_{OH^-} \\ &+ RT V_{OH^-} a_{BOH} da_{OH^-} - RT a_{AH} \frac{da_{H^+}}{a_{H^+}} \\ &+ RT V_{H^+} a_{AH} da_{H^+} \\ &+ RT V_{OH^-} a_{BOH} da_{H^+} \end{aligned} \tag{11}$$

Then Eqs. (3) and (4) and Eqs. (5) and (6) are used to determine a_{AH} and a_{BOH} resulting in equations:

$$a_{AH} = \frac{K_A s a_{H^+}}{1 + K_A a_{H^+}} \tag{12}$$

$$a_{\mathrm{BOH}} = \frac{K_{\mathrm{B}} s a_{\mathrm{OH}^-}}{1 + K_{\mathrm{B}} a_{\mathrm{OH}^-}} \tag{13}$$

Eqs. (12) and (13) are substituted for Eq. (11) and a_{AH} and a_{BOH} are eliminated; the equation can then be integrated for the conditions $\gamma = \gamma_{\max}$, $a_{\mathrm{H}^+} = a_{\mathrm{H}^+}^{\max}$ and $a_{\mathrm{OH}^-} = a_{\mathrm{OH}^-}^{\max}$ yielding the equation:

$$\begin{aligned}\gamma = \gamma_{\max} &- sRT\left(1 + \frac{V_{\mathrm{H}^+}}{K_{\mathrm{A}}} - V_{\mathrm{H}^+} K_{\mathrm{W}} K_{\mathrm{A}}\right) \\ &\times \ln\frac{1 + K_{\mathrm{A}} a_{\mathrm{H}^+}}{1 + K_{\mathrm{A}} a_{\mathrm{H}^+}^{\max}} - sRT\left(1 + \frac{V_{\mathrm{OH}^-}}{K_{\mathrm{B}}}\right. \\ &\left. - V_{\mathrm{OH}^-} K_{\mathrm{W}} K_{\mathrm{B}}\right) \ln\frac{1 + K_{\mathrm{B}} a_{\mathrm{OH}^-}}{1 + K_{\mathrm{B}} a_{\mathrm{OH}^-}^{\max}} \\ &- sRTV_{\mathrm{H}^+} K_{\mathrm{W}} K_{\mathrm{A}} \ln\frac{a_{\mathrm{H}^+}}{a_{\mathrm{H}^+}^{\max}} \\ &- sRTV_{\mathrm{OH}^-} K_{\mathrm{B}} \ln\frac{a_{\mathrm{OH}^-}}{a_{\mathrm{OH}^-}^{\max}} \\ &+ sRTV_{\mathrm{H}^+}(a_{\mathrm{H}^+} - a_{\mathrm{H}^+}^{\max}) \\ &+ sRTV_{\mathrm{OH}^-}(a_{\mathrm{OH}^-} - a_{\mathrm{OH}^-}^{\max})\end{aligned} \tag{14}$$

3. Experimental

3.1. Methods

The interfacial tension, γ, of lipid bilayer was determined by measuring curvature radius, R, of the convex surface formed by applying a pressure difference, Δp, on its sides. The method used was based on the Young's and Laplace's equation [8]

$$2\gamma = R\Delta p$$

3.2. Measurements

The apparatus and the measurement method were described in the paper [3,4,9,10].

The lipid membranes were formed by the Mueller–Rudin method [11]. They were formed in a Teflon diaphragm of 1.5 mm outer diameter containing an orifice along its axis. Some electrolyte solution was present on both sides of the orifice.

The convexity of the lipid membrane cap was measured with 0.05 mm precision instrument reading. This value together with the Teflon element diameter corresponding to the lipid cap diameter yielded the radius of curvature.

The interfacial tension was measured on freshly created membrane 12–15 times for each pH electrolyte solution. For each membrane approximately 10 instrument readings of the lipid cap diameter caused by pressure difference applied on both sides were made. These measurements were made in the whole range: from very low values of the lipid cap diameter to those almost equal to the Teflon element radius. From all of the instrument readings (100–150) the arithmetic mean and standard deviation were enumerated, marked in Fig. 1. Each measurement, together with preparation of the electrolyte solution, was made two to three times in order to test the repeatability of measurements.

The forming solution contained 20 mg/cm^3 of lipid phosphatidylcholine in *n*-decane. Lecithin was dissolved in chloroform to prevent oxidising; the solvent was evaporated in the atmosphere of argon and the residue was dissolved in *n*-decane (POCh), which had been additionally purified by distillation yielding $\varepsilon = 1.991$ (293.15 K). The pH of electrolyte was controlled during the measurements.

In the measurements the bilayer lipid membranes were also used in the form of liposomes. These can be formed owing to the fact that most phospholipids undergo spontaneous aggregation in water or in aqueous electrolyte solutions if shaken or subjected to ultrasounds. Bubbles of spherical or cylindrical shape, sized from less than 0.1 μm to a fraction of millimetre, are then formed [12,13]. They were formed as follows: [14]: 10 mg of lecithin (99%, Fluka) were dissolved in 1–2 cm^3 of chloroform and the solvent was evaporated in the atmosphere of argon until 25–50 μm^3 of lipid film remained in the beaker. 15 cm^3 of 0.9% NaCl were then added and the beaker was placed in water bath (at approx. 280.15 K). The head of UD-20 ultrasound generator was then immersed in the solution and the solution was subjected to ultrasounds five times for 1.5 min. The liposomes of 10–20 nm were obtained [15].

3.3. Materials

3-*sn*-phosphatydylcholine (99%) from Fluka of molecular formula $C_{40}H_{80}NO_8P.H_2O$ was used in

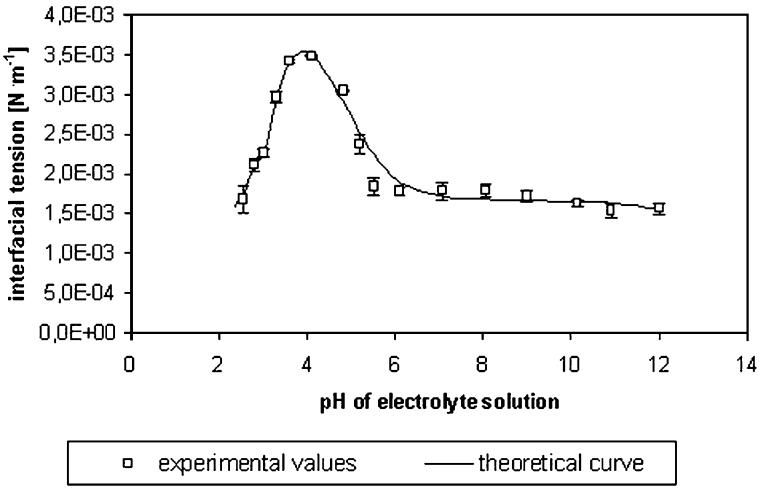

Fig. 1. The dependence of the interfacial tension of lipid membrane formed from phosphatidylcholine on the pH of the electrolyte solution.

the experiment; it had been isolated from hen egg yolk and had the fatty acids composition: 16:0 ~33%, 18:0 ~4%, 18:1 ~30%, 18:2 ~14%, 20:4 ~4%.

Buffers of 2–12 pH ranges prepared according to Britton and Robinson [16] were used as the electrolyte. They were prepared by adding 0.2 M sodium hydroxide to 100 cm^3 of solution having the composition: 0.04 M acetic acid (80%, POCh), 0.04 M phosphoric acid (POCh) and 0.04 M boric acid (POCh). A suitable pH of the buffer was imposed depending on the amount of added sodium hydroxide (at 291.15 K).

4. Results and discussion

The effect of pH on interfacial tension of a PC lipid bilayer was studied using the Britton and Robinson buffer. The studies were carried out at room temperature in the whole pH range.

The points in Fig. 1 present the experimental data concerning the interfacial tension of the membrane formed from PC depending on pH of the electrolyte solution. Eq. (14) is of the $y = m_1x_1 + m_2x_2 + m_3x_3 + m_4x_4 + m_5x_5 + m_6x_6 + b$ type. The m_1, m_2, m_3, m_4, m_5, m_6, and b coefficients were calculated by EXCEL 97 linear regression. Then the interfacial tension values were calculated by substituting calculated coefficients to Eq. (14); the results are presented in Fig. 1 as solid line. As it is seen, the experimental data agree with the calculation results. Eq. (14), deduced theoretically, describes the experimental results in the whole pH range.

The m_1, m_2, m_3, m_4, m_5, m_6, and b coefficients contain the equilibrium constants and volumes of the adsorbed H^+ and OH^- ions. Thus, having the equation coefficients calculated and using the equilibrium constants from papers[3,4] we can calculate the volumes of adsorbed H^+ and OH^- ions. The ions are solvated from the bilayer surface. Assuming that H^+ and OH^- ions are hemispheres can calculate the radius of such a hemisphere.

We assume that the radius of solvated ions does depend on pH.

Eq. (14) can be used to calculate volumes occupied by the H^+ and OH^- ions; they amount to 2026 $Å^3$ and 3190 $Å^3$, respectively.

Assuming the most dense packing of PC molecules in two-dimensional space the surface occupied by a molecule corresponds to two equilateral triangles of side equal to the intermolecular distance. Thus, the mean intermolecular distance of the PC molecules on the bilayer surface can be determined if the area per molecule is known. Assuming this area to be 85 $Å^2$ as determined in

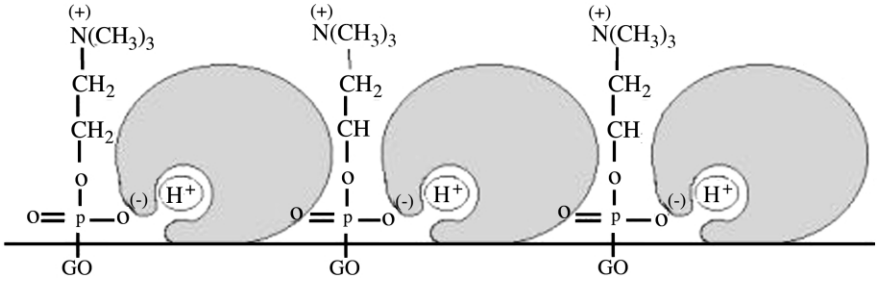

Fig. 2. The diagram of a H^+ ions adsorption in the bilayer lipid membrane formed from phosphatidylcholine, pH < 2.5.

the work [3] the mean H^+-OH^- ion adsorption centres distance amounts to 9.9 Å.

As it has been mentioned, the volume occupied by H^+ ion can be calculated from Eq. (14). The solvated H^+ ion in bulk solution is spherical but, if adsorbed at an active center, it loses a part of its solvation layer as it is shown in Fig. 2. Therefore, the volume of the adsorbed solvated ion can be expected to be smaller than that of the ion in the bulk. The radius of adsorbed H^+ ion was determined from the experimental volume assuming its hemispherical shape; the result was 9.89 Å. This value is in agreement with the estimated distance between PC molecules in the membrane, 9.9 Å.

The volume and radius of the solvated OH^- ion on the lipid membrane surface can be determined in a similar way from Eq. (14). However, the OH^- ion is adsorbed by an active center at the end of the hydrophilic phosphatidylcholine chain. The chain can be bent and the active center can appear at various distances from the interface. The

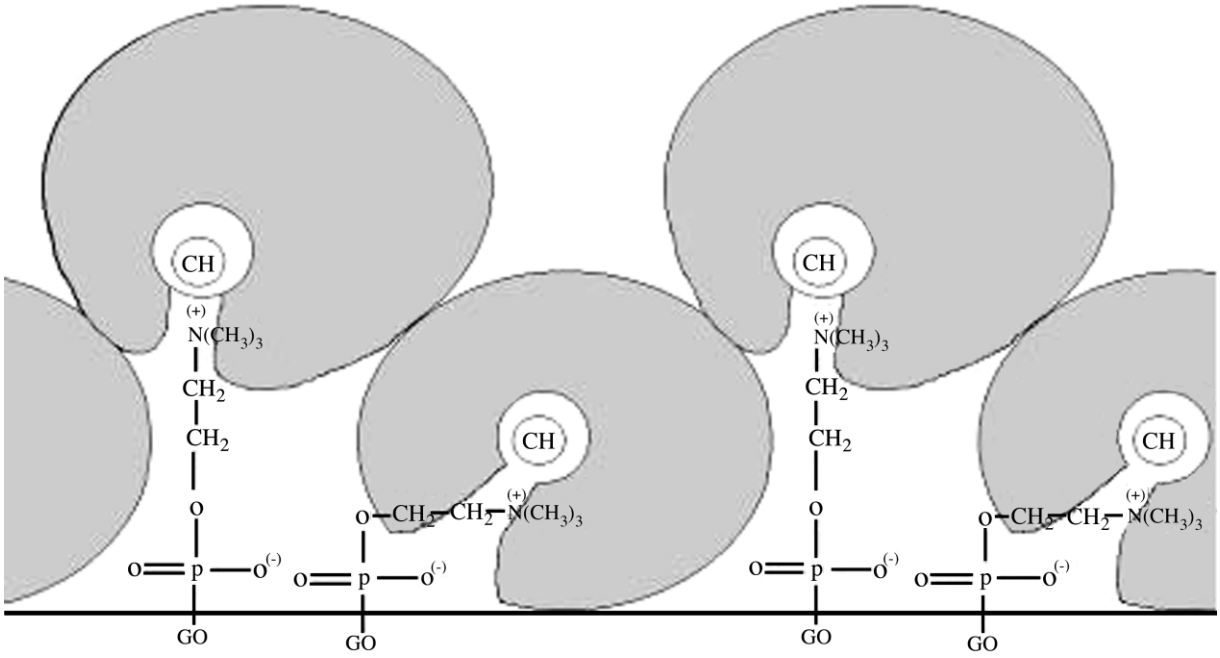

Fig. 3. The diagram of a OH^- ions adsorption in the bilayer lipid membrane formed from phosphatidylcholine, pH > 5.

chain length amounts to 5–7,5 Å [17]. Therefore, the adsorbed OH^- ions can be imagined to be present in two layers: a layer of ions bonded to active centres at the ends of straight chains and a layer of molecules situated at the interface at the ends of bent chains, as it is presented in Fig. 3. Assuming that a half of hydrophilic phosphatidylcholine chains are straight and a half are bent to the surface, the surface concentration of straight chain PC is a half of the total phosphatidylcholine surface concentration and the distances between straight-chain PC molecules are approximately 14 Å. Similar estimates can be made for the chains bent to the membrane surface.

In this case, too, the radius of partly desolvated OH^- ion can be evaluated from its volume determined by Eq. (14) assuming its hemispherical shape; it amounts to approximately 20 Å. This value is in only approximate agreement with the distance of adsorption centres, which is equal to 14 Å (as already presented).

The above numbers are rough estimates only because of doubtlessly strong deformation of the OH^- ion solvation shell due to dense packing of the layers.

The above discussed radii of hydrated H^+ and OH^- ions are longer than the literature values [18–20] which were determined from hydration energies and corresponding to the ions with one hydration shell. Actually, the number of hydration shells can be greater because of hydrogen bondings between water molecules. Thus, the radius of an ion adsorbed at the membrane can be longer than that determined by formation energy of single solvation layer.

5. Conclusions

The phosphatidylcholine layer observed from the aqueous solution side has uniformly distributed $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups, because it is built of molecules each having one $-PO^{(-)}$ group and one $-N^{(+)}(CH_3)_3$ group. The adsorption of the H^+ and OH^- ions was described as the Gibbs isotherm. Thanks to the theoretical equation can be used to calculate volumes occupied by the H^+ and OH^- ions; they amount to 2026 $Å^3$ and 3190 $Å^3$, respectively.

Acknowledgments

This work was supported by a grant from Polish Committee of Scientific Research No. 3 T09A 006 15.

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