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

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Abstract

The dependence of the interfacial tension of a lipid membrane formed from phosphatidylserine on the pH of the aqueous solution has been studied. The model described 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 range of pH, i.e. from 2 to 12.

Keywords: Interfacial tension; Bilayer lipid membrane; Phosphatydylserine

1. Introduction

Biological membranes are characterised by markedly ordered structure. For this reason, they can be considered to be a distinct phase from the physical point of view, separated from surrounding cytoplasm or intermolecular biological fluid. Thus, the membrane surface can be approximated to an interface. The membrane–medium interface is the site where some physicochemical processes occur which are characteristic to a typical interface, e.g. unsymmetric distribution of electric charge.

Experiments are carried out with simple models, i.e. with synthetic phospholipid membranes, in order to understand properties of natural membranes. In most cases, flat lipid bilayers and liposomes are used. Such membranes are usually formed of substances occurring in biological membranes. Many functions of cell membranes could be explained and reproduced with model membranes.

An important property of a cell membrane is its interfacial tension. Interfacial tension of lipid bilayers has been determined before [1,2]; reported values ranged from 0.2×10^{-3} to 6.0×10^{-3} N/m [3,4]. The interfacial tension of lipid bilayer was also determined by measuring energy of membrane formation. It was independent of the nature and concentration of the electrolyte, and its value was $3.4\times10^{-3}\pm0.6\times10^{-3}$ N/m [5].

The effect of pH on the interfacial tension of phosphatidylcholine bilayer was studied in the paper [6] where the existence of equilibria of

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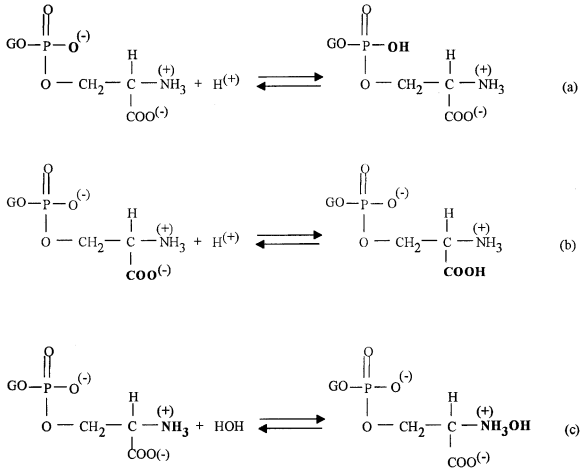
uniformly distributed $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups with the H^+ and OH^- ions was assumed and experimentally confirmed. An alternative model in which the equilibria of the lecithin molecules with the H^+ and OH^- ions was checked but it proved to be in disagreement with the experimental results. Phosphatidylserine (PS) has been chosen for further studies because of its molecular structure: it has the $-N^{(+)}(CH_3)_3$ and the groups situated close to one another and the third one, $-PO^{(-)}$ spaced from them. $-COO^{(-)}$

It has been proved in earlier studies [6] that interfacial tension of lipid membranes formed of lecithin is affected by pH. Maximal interfacial tension of such membranes was attained at pH equal to 4.15. The lecithin molecule has two electric charges, one positive and one negative, on its surface. It is interesting to examine the effect of pH on interfacial tension of a membrane formed of a polar lipid of different molecular structure. PS has been chosen; its molecule has two negative groups and one positive, its net charge being negative. PS molecules are in equilibria with H^+ and OH^- ions; the equilibria can be written in the form:



In the above equilibria, G is the doubly acylated glycerol group [7,8].

2. Theory

The H^+ and OH^- ions are also adsorbed at the phosphatidylserine surface.

$$A_1^- + H^+ \Leftrightarrow A_1H \quad (1)$$

$$A_2^- + H^+ \Leftrightarrow A_2H \quad (2)$$

$$B^+ + OH^- \Leftrightarrow BOH \quad (3)$$

Thus, adsorption equilibria can be written in the form:

$$K_{A_1} = \frac{a_{A_1H}}{a_{A_1^-} \cdot a_{H^+}} \quad (4)$$

$$K_{A_2} = \frac{a_{A_2H}}{a_{A_2^-} \cdot a_{H^+}} \quad (5)$$

$$K_B = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \quad (6)$$

The surface concentration of the lipid is denoted by *s*. Acid–base equilibria can then be written as Eqs. (1)–(3):

$$a_{A_1H} + a_{A_1^-} = s \quad (7)$$

$$a_{A_2H} + a_{A_2^-} = s \quad (8)$$

$$a_{BOH} + a_{B^+} = s \quad (9)$$

The Gibbs equation assumes the form:

$$d\gamma = -\sum \Gamma_i d\bar{\mu}_i \quad (10)$$

If the H^+ and OH^- ions are adsorbed at the lipid surface then the Gibbs equation assumes the form:

$$d\gamma = -\Gamma_{A_1H} d\bar{\mu}_{H^+} - \Gamma_{A_2H} d\bar{\mu}_{H^+} - \Gamma_{BOH} d\bar{\mu}_{OH^-} \quad (11)$$

The case of phosphatidylserine can be treated like that of lecithin.

Dependences of surface excesses of the H^+ and OH^- ions are the following:

$$\Gamma_{OH^-} = a_{BOH} - V_{H^+}(a_{A_1H} + a_{A_2H}) \times a_{OH^-} - V_{OH^-} a_{BOH} a_{OH^-} \quad (12)$$

$$\Gamma_{H^+} = a_{A_1H} + a_{A_2H} - V_{H^+}(a_{A_1H} + a_{A_2H}) \times a_{H^+} - V_{OH^-} a_{BOH} a_{H^+} \quad (13)$$

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

Substitution of Eqs. (12) and (13) to the Gibbs equation yields:

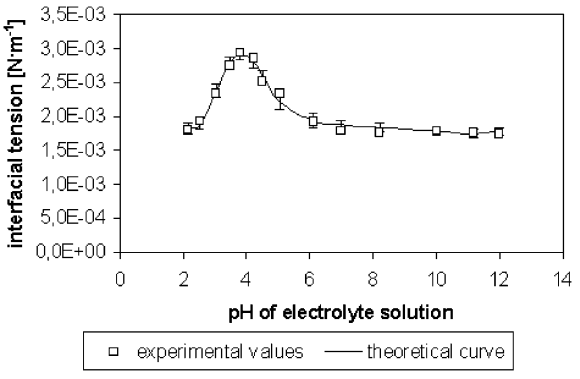


Fig. 1. The dependence of the interfacial tension of a bilayer lipid membrane formed from PS on the pH of the electrolyte solution.

$$
\begin{aligned}
d\gamma = &- RTa_{A_1H}\frac{da_{H^+}}{a_{H^+}} - RTa_{A_2H}\frac{da_{H^+}}{a_{H^+}} \\
&+ RTV_{H^+}a_{A_1H}da_{H^+} \\
&+ RTV_{H^+}a_{A_2H}da_{H^+} \\
&+ RTV_{OH^-}a_{BOH}da_{H^+} - RTa_{BOH}\frac{da_{OH^-}}{a_{OH^-}} \\
&+ RTV_{H^+}a_{A_1H}da_{OH^-} \\
&+ RTV_{H^+}a_{A_2H}da_{OH^-} \\
&+ RTV_{OH^-}a_{BOH}da_{OH^-} \qquad (14)
\end{aligned}
$$

Equations Eqs. (4)–(9) are then used to determine a_{A_1H}, $a_{A_2H}$and a_{BOH} which are expressed by equations:

$$a_{A_1H} = \frac{K_{A_1}sa_{H^+}}{1 + K_{A1}a_{H^+}} \qquad (15)$$

$$a_{A_2H} = \frac{K_{A_2}sa_{H^+}}{1 + K_{A2}a_{H^+}} \qquad (16)$$

$$a_{BOH} = \frac{K_{B}sa_{OH^-}}{1 + K_{B}a_{OH^-}} \qquad (17)$$

Equations Eqs. (15)–(17) are substituted for Eq. (14); a_{A_1H}, a_{A_2H}, and a_{BOH} being hereby eliminated. The equation is then integrated taking the conditions $\gamma = \gamma_{max}$, $a_{H^+} = a_{H^+}^{max}$, and $a_{OH^-} = a_{OH^-}^{max}$ resulting in the equation:

$$
\begin{aligned}
\gamma = \gamma_{max} &- sRT\left(1 + \frac{V_{H^+}}{K_{A_1}} - V_{H^+}K_WK_{A_1}\right) \\
&\times \ln\frac{1 + K_{A_1}a_{H^+}}{1 + K_{A_1}a_{H^+}^{max}} - \\
&- sRT\left(1 + \frac{V_{H^+}}{K_{A_2}} - V_{H^+}K_WK_{A_2}\right) \\
&\times \ln\frac{1 + K_{A_2}a_{H^+}}{1 + K_{A_2}a_{H^+}^{max}} - sRT \\
&\times \left(1 + \frac{V_{OH^-}}{K_B} - V_{OH^-}K_WK_B\right) \\
&\times \ln\frac{1 + K_Ba_{OH^-}}{1 + K_Ba_{OH^-}^{max}} \\
&- \left[sRTV_{H^+}K_W(K_{A_1} + K_{A_2})\right] \times \pm \ln\frac{a_{H^+}}{a_{H^+}^{max}} \\
&- sRTV_{OH^-}K_WK_B\ln\frac{a_{OH^-}}{a_{OH^-}^{max}} \\
&+ 2sRTV_{H^+}(a_{H^+} - a_{H^+}^{max}) \\
&+ sRTV_{OH^-}(a_{OH^-} - a_{OH^-}^{max}) \qquad (18)
\end{aligned}
$$

3. Experimental

3.1. Methods

The interfacial tension, γ, of a 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 [9].

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

3.2. Measurements

The apparatus and the measurement method were described previously [6,10–12].

The lipid membranes were formed by the Mueller–Rudin method [13]. 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 solution used to form the model membrane contained 20 mg/ml of PS in *n*-decane:butanol mixture (3:1). The PS was dissolved in chloroform to prevent oxidation; the solvent was evaporated in the atmosphere of argon and residue was dissolved in *n*-decane:butanol mixture. The pH electrolyte was carefully controlled during the measurements.

In the measurements the bilayer lipid membranes were also used in the form of liposomes. These could 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 [14,15]. They were formed as follows: [16]: 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. A 15-cm^3 aliquot of 0.9% NaCl was then added and the beaker was placed in water bath (at approx. 280.15 K). The head of a UD-20 ultrasound generator was then immersed in the solution and the solution was subjected to ultrasound five times for 1.5 min. Liposomes of 10–20 nm were obtained [17].

3.3. Materials

3-*sn*-phosphatidyl-L-serine from bovine brain (99%) from Fluka was used in the experiment. Buffers of 2–12 pH ranges were prepared according to Britton and Robinson [18] and used as the electrolyte. They were prepared by adding 0.2 M sodium hydroxide to 100 ml of solution having the following 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 established depending on the amount of added sodium hydroxide.

4. Results and discussion

The effect of pH on interfacial tension of lipid bilayer formed of PS has been studied. Britton and Robinson buffer was used as the electrolyte. The measurements were carried out at room temperature in the whole pH range. The dependence of interfacial tension a membrane formed of phosphatidylserine on pH of electrolyte solution is presented in Fig. 1. The maximal interfacial tension value is 2.94 mN/m at pH equal to 3.80.

It was attempted to describe the effect of pH on interfacial tension of lipid bilayer formed of PS in terms of the Gibbs isotherm equation on the basis of a previous paper [6]. In that work, surface concentration was assumed to be equal to surface excess. However, no agreement of experimental data and theoretical results was obtained even in vicinity of the isoelectric point. Therefore, it could be supposed that not all phenomena occurring on the surface of lipid bilayer formed of PS were taken in account. Therefore, rigorous surface excess definition resulting from deduction of the Gibbs equation [19,20] should be applied in interpretation of the pH effect on interfacial tension.

The points in Fig. 1. present the experimental data concerning the interfacial tension of the membrane formed from PS depending on pH of the electrolyte solution.

Eq. (18) is of the type:

$$y = m_1x_1 + m_2x_2 + m_3x_3 + m_4x_4 + m_5x_5 + m_6x_6 + b$$

The m_1, m_2, m_3, m_4, m_5, m_6, and b coefficients were calculated by EXCEL 97 linear regression. The interfacial tension values of membrane formed of PS were calculated by substituting calculated coefficients to Eq. (18), the result is presented in Fig. 1 as solid line. It can be observed that the theoretical results agree well with the experimental data.

Association constants of the membrane with PS were needed for calculations. Their determination was complicated as phosphatidylserine is insoluble in water. For this reason, liposomes were used to determine them as uniform distribution of PS molecules in water was then attained. It was assumed in calculations that the PS molecules present in the outer liposome layer only took part in acid–base equilibria. Therefore, the PS concentration used in calculation was a half of that present in the solution.

The association constants were determined by titration of prepared liposomes with hydrochloric

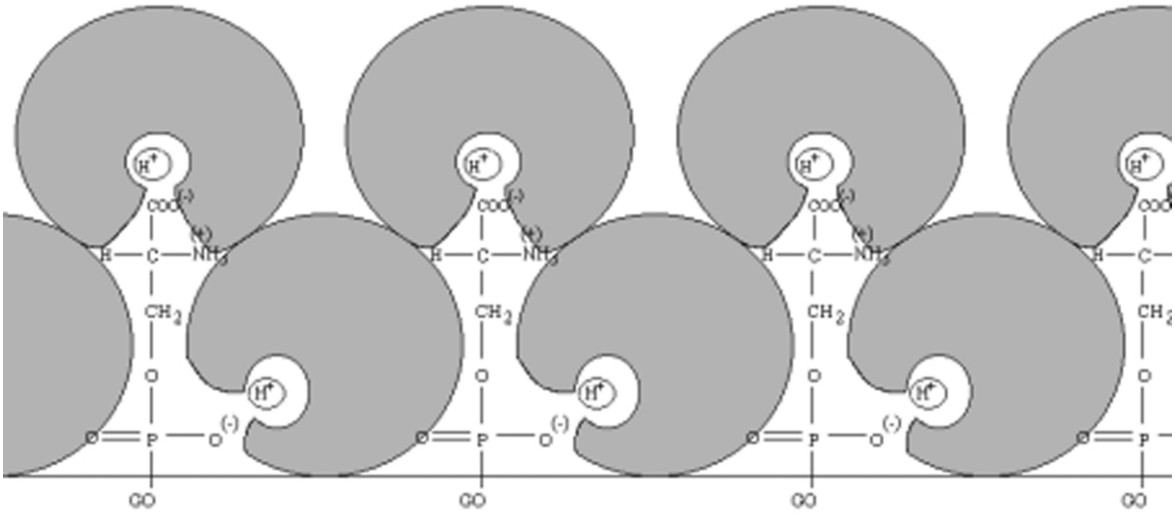


Fig. 2. The diagram of a H^+ ion adsorption in the bilayer lipid membrane formed from phosphatidylserine, pH < 2.5.

acid or sodium hydroxide; a 736GP Titrino from Metrohm (Switzerland) was used for titration.

The association constant values obtained in this way were $K_1 = 10^{3.36}$ and $K_2 = 10^{9.55}$, respectively. As two association constants only could be determined in this way and three were needed, the association constant corresponding to the $-PO^{(-)}$ group, $K_{A_1} = 10^{2.581}$ was taken from the work [6]. Thus, the K_1 constant determined by titration was the geometric mean of the K_{A_1} and K_{A_2} constants. On this ground, the $K_{A_2} = 10^{4.139}$ could be determined. and K_{A_1}. K_B constant $= 10^{9.55}$ was determined titrimetrically and it was found to be $10^{9.55}$.

The deduced theoretical Eq. (18) describes the experimental results in the whole pH range.

The m_1, m_2, m_3, m_4, m_5, m_6, m_7, m_8 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 paper [11] 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.

Volumes occupied by the H^+ and OH^- ions can be deduced from Eq. (18); they amount to 18 844 $Å^3$ and 28 889 $Å^3$, respectively.

Assuming most dense packing of PS molecules in two-dimensional space, the surface occupied by a molecule corresponds to two equilateral triangles of side equal to the intermolecular distance. If the area occupied by a PS molecule is known then mean intermolecular distance of phosphatidylserine on the bilayer surface can be estimated. Assuming the area occupied by a PS molecule to be 68.5 Å, the mean distance from an H^+ ion to an OH^- ion adsorption centre is approximately 11 Å.

As it has been mentioned earlier, the volume occupied by an H^+ ion can be determined from Eq. (18). The solvated H^+ ion is spherical in the bulk solution but it loses a part of its solvation shell when adsorbed at an active centre as it is shown in Fig. 2. For this reason, volume of the adsorbed solvated H^+ ion can be assumed to be smaller than that of the solvated ions in the bulk. The radius of adsorbed H^+ ion was calculated from its volume assuming its hemispherical shape; the result was approximately 10 Å. It is in agreement with the estimated distances of PS molecules in the membrane—11 Å.

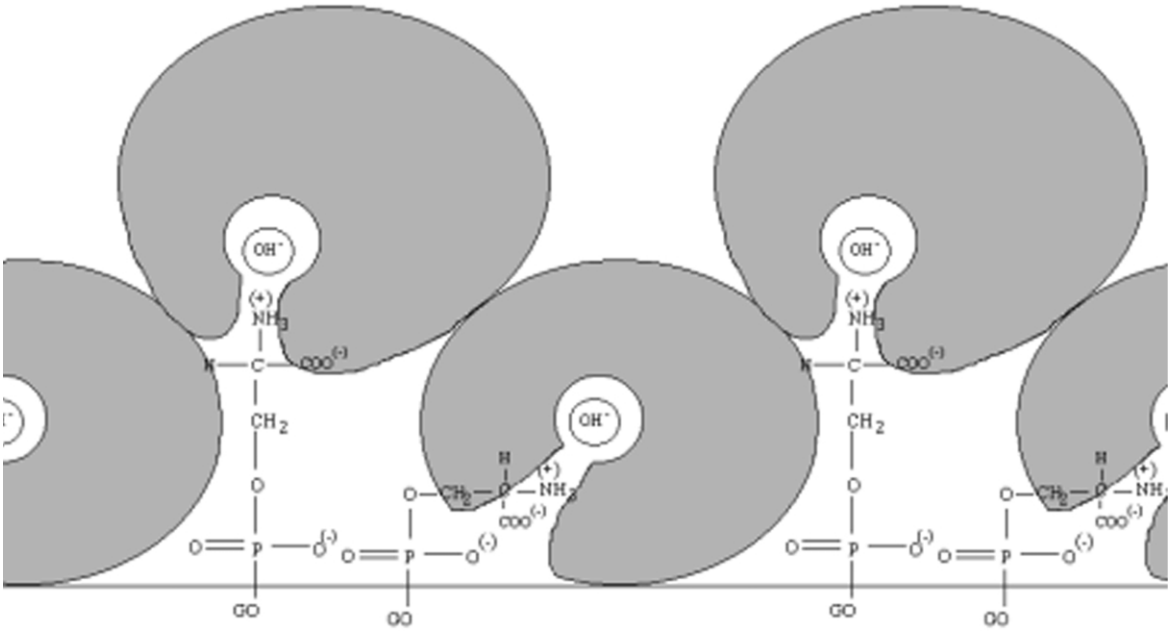


Fig. 3. The diagram of a OH^- ion adsorption in the bilayer lipid membrane formed from phosphatidylserine, pH > 5.

The volume and radius of the solvated OH^- ion at the lipid membrane surface can be determined in similar way from Eq. (18). However, the OH^- ion is adsorbed in an active centrum which is present at the end of the hydrophilic phosphatidylserine chain. The chain can appear in various conformations resulting that the active centres in various distances to the interface. The chain length is 5–7.5 Å [21]. Thus, the adsorbed OH^- ions are present in two layers: a layer of adsorption centres at the end of straight chains and the other layer with the centres at the ends that the absorb OH^- ion to stay at the interface; the situation is presented in Fig. 3. It was assumed that a part of PS molecule hydrophilic chains at the absorb OH^- ions are straight and a part are bent to the surface. For this reason, the straight-chain PS molecule surface concentration is half that of total phosphatidylserine concentration and distances between straight hydrophilic chains are approximately 16 Å. Similar estimates can be carried out for the chains bent to the membrane surface.

Radius of partly desolvated OH^- ion could be determined in this case, too, from its volume calculated from Eq. (18) assuming its hemispherical shape; it is equal to approximately 24 Å. It only approximately agrees with the above presented 16 Å distance between the adsorption centres.

The above numbers are rough estimates because of doubtlessly strong deformation of the OH^- ion solvation layer caused by dense packing of two layers.

The above discussed radii of hydrated H^+ and OH^- ions are greater than those given in literature [22–24] which have been determined from hydration energy and correspond to ions having one hydration layer. Actually, the ions can be covered by more hydration layers due to hydrogen bonds acting between water molecules. Thus, the radius of an ion adsorbed by the layer can be greater than the value determined by formation energy of single hydration layer.

5. Conclusion

The phosphatidylserine layer observed from the aqueous solution side has uniformly distributed $-PO^{(-)}$, $-NH_3^{(+)}$ and $-COO^{(-)}$ groups, because it

is built of molecules each having one $-PO^{(-)}$ group, one $-NH_3^{(+)}$ group and one $-COO^{(-)}$ group. The adsorption of the H^+ and OH^- ions was described as the Gibbs isotherm. The theoretical equation can be used to calculate volumes occupied by the H^+ and OH^- ions; they amount to 18 844 $Å^3$ and 28 889 $Å^3$, respectively.

Acknowledgments

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