Studies of \( \text{L-}\alpha\)-phosphatidylcholine impregnated parchment paper model membrane

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Measurements of bi-ionic potential (BIP) across \( \text{L-}\alpha\)-phosphatidylcholine impregnated parchment paper model membrane have been carried out in a cell of the type,

\[
\text{SCE} / |A^+ M^-| \text{membrane} / |B^+ M^-| / \text{SCE}
\]

The magnitude of the BIP depends upon the concentration and nature of the cations. Theories based on the principles of irreversible thermodynamics as proposed by Tasaka et al. [*J. Membr. Sci.*, 24 (1985) 29] and Toyoshima et al. [*J. Phys. Chem.*, 74 (1970) 2704] have been compared to obtain a relationship which has been used for the evaluation of theoretical values of bi-ionic potentials. The close agreement between the theoretical and the observed values confirms the applicability of the derived relationship to the membrane-electrolyte systems used in these investigations.

A potential difference is observed between the two sides of a membrane when the membrane separates two electrolyte solutions of the same molar concentrations with a common co-ion but different counter ions. The membrane potential is called the bi-ionic potential (BIP). Extensive studies have been initiated to understand the complex behaviour of biological membranes by developing various artificial membranes such as parchment supported or cellophane supported systems, which may mimic some properties of biological membranes. However, investigation of the physicochemical processes occurring in biological membranes is frequently discouraged by the complex structure and multicomponent nature of these membranes. This fact is responsible for the great interest in the study of properties of simple model systems, among which bilayer lipid membranes (BLMs) and liposomes have been most commonly studied.

Some authors have studied model membranes in the form of porous substrates, such as ultrafilters impregnated with organic solvents and lipid dissolved therein. In the present study, we have prepared \( \text{L-}\alpha\)-phosphatidylcholine (\( \text{L-}\alpha\)-lecithin) parchment supported membrane and measured the BIP across the membrane. The conductance behaviour of this lipid membrane with respect to some common electrolytes has been studied at different temperatures.

**Materials and Methods**

\( \text{L-}\alpha\)-Phosphatidylcholine was purified as described earlier and kept in vacuo. Chloroform and methanol were twice distilled. Doubly distilled water (over potassium permanganate) was used in the present experiments. The lipid solution was prepared by dissolving the pure lipid in chloroform-methanol mixtures (2:1, v/v) to which \( n \)-decane (20% of the lipid amount) was added so as to prevent solidification of the lipid. One milliliter of this solution was used to form a thin lipid layer on parchment paper. For doing this, the cell containing the parchment paper was clamped vertically and the lipid solution was added dropwise onto the parchment paper with a pipette. After the addition of a drop, the solvent mixture was quickly evaporated vigorously before the addition of the next drop. This resulted in the complete evaporation of the liquid, leaving a thin layer of the lipid covering the whole of parchment paper in the cell.

The potential developed by setting up a concentration cell (A) was measured using a potentiometer at 25°C ± 1°C and saturated calomel electrode (SCE). The cell potential was taken as a measure of BIP.

| SCE | Solution A | \( |A^+ M^-| \text{membrane} \) | Solution B | \( |B^+ M^-| \) | SCE |
|-----|------------|-------------------------------|------------|----------------|-----|
|     | I          | \( \text{L-}\alpha\)-phosphatidylcholine model membrane | II         | \( \text{L-}\alpha\)-phosphatidylcholine model membrane |

For conductometric measurements, the membrane was first equilibrated with an electrolyte solution. The latter was then replaced by purified mercury without removing the adhering surface liquid. Platinum electrodes dipping in mercury were used to es-
Results and Discussion

By definition, the BIP is the algebraic sum of the two potentials arising at the two solution-membrane interfaces and the liquid junction potential arising because of the membrane.

On the basis of flow equations based on the thermodynamics of irreversible processes, Toyoshima and Nozaki\(^1\) derived the following equation for the steady electromotive force, called the bi-ionic potential, of a bi-ionic cell, containing two electrolytes (with a common anion but different cations) separated by a membrane,

\[
\Delta \phi_{\text{BIP}} = \left[ 2 \ln \left( \frac{K_{A^+}}{K_{B^+}} \right) + \ln \left( J V_{A^+} + 1 \right) / \left( J V_{B^+} + 1 \right) \right] \times (F/RT)\]

where \(K_{A^+}/K_{B^+}\) is the selectivity constant of a membrane for positive ions A\(^+\) and B\(^+\) and the terms \(V_N\) and \(J\) are defined as,

\[
V_N = 1 + \frac{u^0_+}{u^0_-} (N = A^+, B^+) \quad \ldots \quad (2)
\]

\[
(2J + 1) \ln \left( g_{A^+} + 2J \right) / \left( g_{B^+} + 2J \right) - \ln \left( J V_{A^+} + 1 \right) / \left( J V_{B^+} + 1 \right) \quad \ldots \quad (3)
\]

where \(g_N = 1 + [1 + (2 K_N C/X)^2]^{1/2} \quad \ldots \quad (4)\]

In order to evaluate \(V_N\) and \(X/K_N\) with the help of data of apparent transferable number of anion (\(t_{\text{app}}\)) and thermodynamically effective fixed charge density (\(X\)) of the membrane, the following expression has been proposed,

\[
1/t_{\text{app}} = V_N + (V_N - 1) / \ln \left( \frac{X}{K_N} \right) \left( \frac{1}{C_N} \right) + \quad \ldots \quad (5)
\]

Equation (5) indicates that the intercept and slope of a straight line plot of \(1/t_{\text{app}}\) against \(1/C_N\) at fixed \(\gamma\) (Fig. 1) will give the values of \(V_N\) and \(X/K_N\) respectively. The values of \(V_N\) were found to be 2.60, 2.54 and 2.48 for KCl, NaCl and LiCl respectively, while the \(X/K_N\) values were 0.16, 0.15 and 0.12 respectively for these salts. These values may then be utilized to evaluate \(g_N\) with the help of Eq. (1). With the help of these parameters for a membrane-electrolyte system, the values of \(J\) and \(\Delta \phi_{\text{BIP}}\) can be calculated using Eqs (3) and (1), respectively.

The observed and calculated values of bi-ionic potentials for the \(L-\alpha\)-phosphatidylcholine (\(L-\alpha\)-lecithin) model membrane in contact with different pairs of electrolytes are plotted against log C in Fig. 2. It is apparent from the figure that there is a good agreement between the observed and theoretical values of bi-ionic potentials and it may be concluded that the theory of Toyoshima and Nozaki\(^1\) is fairly well applicable to \(L-\alpha\)-phosphatidylcholine model membrane.
Table 1—Values of mobility ratio of various alkali metal ion pairs for the t-α-phosphatidylcholine membrane

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>$U_{K^+}/U_{Na^+}$</th>
<th>$U_{Na^+}/U_{Li^+}$</th>
<th>$U_{K^+}/U_{Li^+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1/0.1</td>
<td>1.31</td>
<td>1.41</td>
<td>1.84</td>
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<tr>
<td>0.05/0.05</td>
<td>1.37</td>
<td>1.47</td>
<td>1.95</td>
</tr>
<tr>
<td>0.02/0.02</td>
<td>1.49</td>
<td>1.62</td>
<td>2.25</td>
</tr>
<tr>
<td>0.005/0.005</td>
<td>1.72</td>
<td>2.08</td>
<td>2.76</td>
</tr>
<tr>
<td>0.002/0.002</td>
<td>1.86</td>
<td>2.21</td>
<td>2.95</td>
</tr>
</tbody>
</table>

Table 2—Values of selectivity constant ($K_{ij}$) of various alkali metal ion pairs for the t-α-phosphatidylcholine model membrane

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>$K_{K^+}/Na^+$</th>
<th>$K_{Na^+}/Li^+$</th>
<th>$K_{K^+}/Li^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1/0.1</td>
<td>1.01</td>
<td>1.01</td>
<td>0.96</td>
</tr>
<tr>
<td>0.05/0.05</td>
<td>1.02</td>
<td>1.04</td>
<td>1.00</td>
</tr>
<tr>
<td>0.02/0.02</td>
<td>1.03</td>
<td>1.05</td>
<td>1.08</td>
</tr>
<tr>
<td>0.005/0.005</td>
<td>1.04</td>
<td>1.07</td>
<td>1.16</td>
</tr>
<tr>
<td>0.002/0.002</td>
<td>1.05</td>
<td>1.08</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Theoretical values of $\Delta \Phi_{BIP}$ can also be obtained with the help of Eq. (6), derived on the basis of a recent theory of Tasaka et al. using the principle of irreversible thermodynamics as,

$$\Delta \Phi_{BIP} = \frac{RT}{Z_{A^+}F} \ln \left( \frac{l_{A^+}}{l_{B^+}} + \frac{l_m}{l_1} \right) ... (6)$$

where $l_{A^+} = l_{A^+} + l_{B^+} - l_{m}$

$l_{B^+} = l_{A^+} + l_{B^+} - l_{m}$

$l_m = l_{A^+} + l_{B^+} - l_{m}$

$C$ represents the concentration of counter ions ($A^+$, $B^+$) or co-ion ($m$) in the membrane phase and (1) and (2) refer to the two surfaces of the membrane.

At the limit of low electrolyte concentration, Eq. (6) reduces to,

$$\Delta \Phi_{BIP} = \frac{RT}{Z_{A^+}F} \ln \frac{l_{A^+}}{l_{B^+}} ... (7)$$

while at the limit of high electrolyte concentration Eq. (6) gives,

$$\Delta \Phi_{BIP} = \frac{RT}{Z_{A^+}F} \ln \left( \frac{l_{A^+} + l_m}{l_{B^+} + l_m} \right) ... (8)$$

Comparison of this equation with that of Nozaki for uni-univalent electrolytes gives,

$$\Delta \Phi_{BIP} = \frac{RT}{F} \left[ 2 \ln \frac{S_{A^+} + \ln \left( \frac{J_{B^+}}{J_{B^+}} \right)}{S_{B^+} + \ln \left( \frac{J_{A^+}}{J_{B^+}} \right) \right] ... (9)$$

where $S_{A^+} = \exp \left( 1 - \frac{RT}{F} \right) / \mu_{A^+} / \mu_{A^+} / \mu_{B^+} / \mu_{B^+}$

and $\mu_{A^+}$ and $\mu_{B^+}$ are the standard chemical potentials of counter ions and co-ions, respectively, and symbols with an overbar indicate the membrane phase.

At the limit of low electrolyte concentration the solute flux through a charged membrane, $J_N$, is governed only by the co-ion species, we obtain

$$-j_{A^+}/j_{B^+} = l_{A^+}/l_{B^+} ... (14)$$

On the other hand, at the limit of high electrolyte concentration, we get

$$-j_{A^+}/j_{B^+} = \left( l_{B^+} + l_m \right) / \left( l_{A^+} + l_m \right) ... (15)$$

Comparison of Eqs (2) and (13) gives

$$l_{A^+}/l_m = U_{A^+}/U_{m} ... (16)$$

and

$$l_{B^+}/l_m = U_{B^+}/U_{m} ... (17)$$

On dividing Eq. (16) by (17), we get

$$l_{A^+}/l_{B^+} = U_{A^+}/U_{B^+}$$

Hence, in terms of mobility ratio, Eq. (7) becomes

$$\Delta \Phi_{BIP} = \frac{RT}{Z_{A^+}F} \ln \frac{U_{A^+}}{U_{B^+}} ... (18)$$

Equation (18) affords the calculation of theoretical
value of bi-ionic potential, if the value of the mobility ratio is known. The mobility ratio, $\frac{m_{A}^{+}}{m_{B}^{+}}$, has been expressed as

$$\frac{m_{A}^{+}}{m_{B}^{+}} = \frac{m_{A}^{+}}{m_{A}^{+}}$$

where $m_{A}^{+}$ and $m_{B}^{+}$ represent the steady state equilibrium concentrations of ions $A^{+}$ and $B^{+}$ in the respective junction zones and $\lambda_{A}$ and $\lambda_{B}$ represent the conductivities of the membrane when wholly in the $A^{+}$ and $B^{+}$ forms respectively. It has also been shown that $m_{A}^{+}/m_{B}^{+}$ is nearly equal to $K_{B_{A}}$, the selectivity constant or the ion-exchange equilibrium constant. In terms of $K_{B_{A}}$, Eq. (19) becomes,

$$\frac{m_{A}^{+}}{m_{B}^{+}} = \frac{m_{B}^{+}}{m_{B}^{+}}$$

The selectivity constant is also related to the permeability ratio $P_{A^{+}}/P_{B^{+}}$ as

$$P_{A^{+}}/P_{B^{+}} = K_{B_{A}} \left( \frac{U_{A^{+}}}{U_{B^{+}}} \right)$$

The mobility ratio values (Table 1) evaluated with the help of conductance data and Eq. (19) have been used to calculate (a) the theoretical values of bi-ionic potentials from Eq. (18), (b) the selectivity constant (Table 2) from Eq. (20) and (c) the permeability ratio (Table 3) from Eq. (21). These theoretical as well as observed values of bi-ionic potential have been plotted as a function of log C in Fig. 3. The very close agreement between the theoretical and observed bi-ionic potentials proves the applicability of the derived equation to the $\alpha$-phosphatidylcholine model membrane system.

For checking selectivity of the membrane, the bi-ionic potentials were also measured by keeping the concentration of one of the electrolytes, say $A^{+}M^{-}$, constant and varying the concentration of the other electrolyte say $B^{+}M^{-}$, and again by keeping the concentration of $B^{+}M^{-}$ constant and varying the concentration of $A^{+}M^{-}$. These data are represented in the form of plots of bi-ionic potential against $\log \frac{a_{A^{+}M^{-}}}{a_{B^{+}M^{-}}}$ (Fig. 4). The point of intersection of the straight lines, demanded by the equation,

$$\Delta \phi_{BIP} = \frac{RT}{F} \ln \frac{t_{A^{+}}}{t_{B^{+}}}$$

at the same activity gave the values of transport ratios. The transport ratios thus obtained for different 1:1 electrolyte pairs are:

$$t_{K^{+}}/t_{Na^{+}} = 1.820, t_{Na^{+}}/t_{Li^{+}} = 1.413$$

and

$$t_{K^{+}}/t_{Na^{+}} = 1.312$$

The observed data on membrane potential, bi-ionic potential, transport ratio, mobility ratio (Table 1) and permeability ratio (Table 3) of cations show that phosphatidylcholine model membrane behave as a cation selective membrane.

Acknowledgement

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References

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