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# POSSIBLE EXISTENCE OF ION PAIRS AT THE MOUTHS OF ION CHANNELS

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**An electrostatic calculation suggests that when an ion is bound near the mouth of a channel penetrating a low-dielectric membrane, a counter ion may form an ion pair with this ion. The tendency towards ion-pair formation is remarkably enhanced at channel mouths by forces (image forces) arising from the charges induced on the boundaries between different dielectrics. The binding constant for the formation of ion-pairs of monovalent ions is estimated under the assumption that local interactions between the counter ion and the channel wall are negligibly small. It is of the order of 1–10 molal$^{-1}$ or more for the binding of a $Cl^-$ ($F^-$) counter ion to an $Na^+$ ($Li^+$) ion if appropriate conditions are fulfilled. The binding constant depends on the position of the binding site, the dimensions and geometries of the channel and channel mouth, and the state of ion loading of the channel, as well as the ionic species. The present results also indicate that when cation (anion) channels have anionic (cationic) groups as integrant parts of their channel walls, interactions between these charged groups and permeant ions are markedly enhanced by the image forces.**

The hydrophobic inner portion of cell membranes and of artificial lipid bilayer leaflets is far less polarizable than aqueous solutions outside. Ions near the surface of, or within, the membranes are known to be subjected to image forces *, which are caused by the electric charges induced on the boundaries between different dielectrics. We wish to suggest that when an ion is bound near the mouth of a channel penetrating a low-dielectric membrane, a counter ion may form an ion pair with this ion. (The term ion pair used here refers to two ions that are in contact through their ionic radii or are close together with no water molecules between them.) When local interactions between the counter ion and the channel wall are supposed to be negligibly small, the binding constant is of the order of 1–10 molal$^{-1}$ or more for the binding of a $Cl^-$ (or $F^-$) counter ion to an $Na^+$ (or $Li^+$) ion under favorable conditions. This increased tendency towards ion-pair formation is mainly attributable to image forces. We have reached these conclusions by means of the numerical computation of electrostatic potential energies due to image forces.

This work was motivated by a paper of Eisenman et al. [1], who reported modulating effects of anions on the transport properties of the channel formed by gramicidin A. They postulated the binding of an anion to the entrance of a cation-loaded channel in order to explain their experimental data. Some consequences of anion binding at a channel mouth were also discussed by Levitt [2].

## *The substitute charge method*

Electrostatic problems involving a pore of finite length is rather difficult to solve analytically. The

* In simple dielectric problems, these forces are often calculated by the method of electrical images, and hence the name image forces. Although this method is not employed in the present work, this name will be used for convenience.

substitute charge method (Ersatzladungsmethode), developed by Steinbigler [3], is used in this work for the numerical calculation of image-force energies. In this method, continuously distributed charges on the boundaries are displaced by a finite number of discrete charges with appropriate geometries. These charges are put outside the space where potentials are to be calculated. Three types of fictitious charges are usually used: point charges, line charges, and ring charges. The number, the locations, and the magnitudes of the ficitious charges have to be determined so that given boundary conditions can be satisfied exactly at a finite number of points on the boundaries (contour points) and can be satisfied approximately (within a given error) at points on the boundaries other than contour points. Once these parameters are determined, image-force energies can be calculated.

For the interface between dielectrics 1 and 2, the boundary conditions are known to be, in the absence of true charges on the interface,

$$\phi_1 = \phi_2 \tag{1}$$

$$D_{1n} = D_{2n} \tag{2}$$

where $\phi_1$ and $\phi_2$ ($D_{1n}$ and $D_{2n}$) are the electric potentials (the normal components of the electric displacements) in spaces 1 and 2, respectively.

The systems to be considered have a cylindrical symmetry with respect to the pore axis, which will be taken as the $z$-axis of the cylindrical coordinates. The potential $\phi(i)$ and the radial and axial components, $E_\rho(i)$ and $E_z(i)$, of the field at point $i$ in a space are given by

$$\phi(i) = \sum_j P(i,j)Q(j) \tag{3}$$

$$E_\rho(i) = \sum_j F_\rho(i,j)Q(j) \tag{4}$$

$$E_z(i) = \sum_j F_z(i,j)Q(j) \tag{5}$$

where $Q(j)$'s represent (i) the fictitious charges outside the space and (ii) the true charges (other than those displaced by fictitious charges, if any), $P(i,j)$ is the potential coefficient of charge $j$ with respect to point $i$, and $F_\rho(i,j)$ and $F_z(i,j)$ are the radial and axial components of the field coefficient of charge $j$ with respect to point $i$. The potential and field coefficients of ring charge $j$ with respect to point $i$ in a dielectric $\varepsilon$ are expressed (when the ring charge is coaxial with respect to the $z$-axis) as

$$P(i,j) = \frac{1}{2\pi^2\varepsilon}\int_0^{\pi/2}\frac{d\theta}{D_{ij}^{1/2}} \tag{6}$$

$$F_\rho(i,j) = \frac{1}{2\pi^2\varepsilon}\int_0^{\pi/2}\frac{(\rho_i + a_j) - 2a_j\cos^2\theta}{D_{ij}^{3/2}}d\theta \tag{7}$$

$$F_z(i,j) = \frac{1}{2\pi^2\varepsilon}\int_0^{\pi/2}\frac{z_i - z_j}{D_{ij}^{3/2}}d\theta \tag{8}$$

$$D_{ij} = (\rho_i + a_j)^2 - 4\rho_i a_j\cos^2\theta + (z_i - z_j)^2 \tag{9}$$

where $\rho_i$ and $z_i$ are the radial and axial coordinates of point $i$, $a_j$ is the radius of ring charge $j$, and $z_j$ is the axial coordinate of the center of ring charge $j$. The right-hand sides of Eqns. 6–8 are known to be transformed to types involving the complete elliptic integral of the first and/or the second kind.

The total image-force energy $E_{\text{image}}$ of the system in the presence of true point charges $Q_t(j)$ is

$$E_{\text{image}} = \tfrac{1}{2}\sum_j Q_t(j)\phi_{\text{image}}(j) \tag{10}$$

where $\phi_{\text{image}}(j)$ is the electric potential at the position of $Q_t(j)$ due to the fictitious image charges.

The most laborious step in the substitute charge method is to find an optimum arrangement of fictitious charges and contour points. Errors of calculated potentials are critically dependent on the arrangement employed. In the present calculation, 164 or 200 contour points are usually placed on the interface, corresponding to the presence or absence of dimples at the pore mouths (see below). The same number of ring fictitious charges are placed coaxially with respect to the $z$-axis on each side of the interface up to a radial distance of about 160 Å. Details of the arrangements used and the evaluation of the errors in potential calculations will be reported elsewhere. The relative errors of calculated image-force energies are esti-

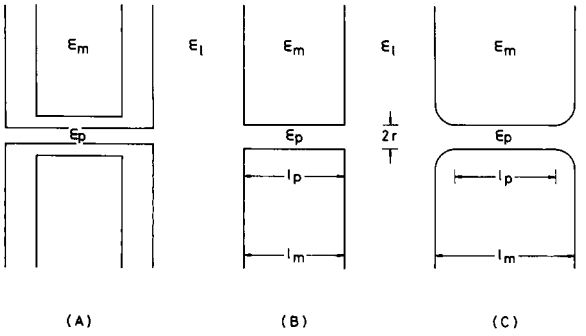


Fig. 1. Simple dielectric pictures of channels through low-dielectric membranes.

mated to be of the order of 0.1%, at most, or not to greatly exceed 0.1%.

*Model*

A continuum dielectric model will be used. Fig. 1A shows a simplified dielectric picture of membranes equipped with channels. A cylindrical pore of dielectric constant $\varepsilon_p$ penetrates a planar membrane, which is bounded on two sides by semi-infinite liquid phases of dielectric constant $\varepsilon_l$. The membrane is composed of a low-dielectric inner portion of dielectric constant $\varepsilon_m$ and more polarizable superficial layers contiguous to the bulk aqueous phase and to the space within the pore. Assume that $\varepsilon_m = 2$ ($\approx$ dielectric constant $\varepsilon$ of hydrocarbon) and $\varepsilon_l = 78$ (a bulk $\varepsilon$ value of water). $\varepsilon_p$ has been taken to be equal to $\varepsilon_l$ in precedent works [2,4–6] in this field. We follow this treatment.

Little data are available on the $\varepsilon$ value of superficial polar layers, especially that of channel walls, which has a primary significance in the energetics of ionic movement through channels. The wall of the gramicidin channel consists of peptide backbones of the drug. Tredgold and Hole [7] measured $\varepsilon$ of dry synthetic polypeptides in a $\beta$-sheet structure, a structure similar to that of the gramicidin channel [8–13]. The value of $\varepsilon$ for polyalanylglycine was found to be about 3.5–5, depending on the frequency, at physiological temperatures. Assuming that the nonpolar side groups have $\varepsilon$ similar to that of hydrocarbon (approx. 2), we can safely estimate that $\varepsilon$ of the polypeptide backbone in this conformation is not more than 10. In the present calculations, a pore and its wall (2 Å in radius and 3 Å in thickness, respectively, for the gramicidin channel) will be approximated by a fictitious pore whose radius (effective radius) $r$ is greater than the intrinsic pore radius and less than the pore radius plus the wall thickness. Calculations will be made with $r = 2$–$4$ Å. Similarly, the whole membrane will be approximated by a homogeneous slab whose thickness (effective thickness) $l_m$ is greater than that of the hydrophobic core and less than the whole thickness of the membrane. Thus we get Fig. 1B. The hydrocarbon thickness of lipid bilayer membranes is known to be mostly between 25 and 50 Å.

The gramicidin channel is about 26 Å in length [9,14]. Let $l_p$ (pore length) be 26 Å in our model as well. When $l_m$ is greater than this pore length, a deformation, or dimpling, of the membrane is required to expose the mouth of the channel [15]. For simplicity, the dimple is supposed to be a quarter circle on a plane containing the channel axis (Fig. 1C). Calculations will be made for dimples of 5 Å in depth; this corresponds to $l_m = 36$ Å.

The contour point cannot be placed at singular points on such a rectangular edge at a pore mouth as shown in Fig. 1B, and errors in the boundary conditions are extremely large at these points. The pore mouth is thus supposed to be a quarter circle with a radius of 1 Å on a plane containing the pore axis.

*Theory*

It will be supposed that the binding site near the channel end is for a cation. Let us consider the exchange reaction between an anionic counter ion and a water molecule for a cation which has already been bound at the site:

$$(\text{anion})^l + (\text{water})^b \rightleftharpoons (\text{anion})^b + (\text{water})^l \qquad (11)$$

where superscripts l and b represent the bulk liquid phase and the binding to the bound cation, respectively. We have [16]

$$\frac{x^b_{\text{anion}} x^l_{\text{water}}}{x^l_{\text{anion}} x^b_{\text{water}}} = \exp(-\Delta F/RT) \qquad (12)$$

where $\Delta F$ is the free energy change, and $x^l_{\text{anion}}$,

TABLE I

SEVERAL NUMERICAL VALUES

| | | Ref. |
|---|---|---|
| Ionic radius of $Na^+$ | 0.97 Å | 17 |
| Ionic radius of $Cl^-$ | 1.81 Å | 17 |
| Ionic radius of $Li^+$ | 0.68 Å | 17 |
| Ionic radius of $F^-$ | 1.33 Å | 17 |
| Distance between $Na^+$ and hydrated $H_2O$ [a] | 2.4 Å | 18 |
| Distance between $Cl^-$ and hydrated $H_2O$ [b] | 3.1 Å | 19 |
| Distance between $Li^+$ and hydrated $H_2O$ [c] | 2.0 Å | 19 |
| Hydration number of $Cl^-$ in the bulk [d] | 6 | 19 |
| Dipole moment of $H_2O$ (debyes) | 1.84 | 17 |

1 cal ≡ 4.18605 J

[a] Na-O distance for primary waters.
[b] Cl-O distance for primary waters.
[c] Li-O distance for primary waters.
[d] Nearest neighbor waters.

$x^l_{water}$, $x^b_{anion}$, and $x^b_{water}$ are the equilibrium mole fractions of the anion and water present in the bulk phase and bound to the cation. When the anion concentration is equal to the inverse of the binding constant $K$ of the anion, $x^b_{anion}$ is equal to $x^b_{water}$, so that

$$K = (\exp(-\Delta F/RT))/55.5 \qquad (13)$$

Suppose that local interactions between the bound anion and the channel wall are negligibly small. Only electrostatic interactions between ions, between ions and waters hydrated to ions, and between ions (or waters hydrated to ions) and induced charges on the interface will be considered. The energy change $\Delta F$ involves (I) detachment of the water molecule coordinated to the cation, (II) partial dehydration of an anion in the bulk, and (III) binding of the anion to the cation. The energy changes of steps I and III are calculated by the substitute charge method. Ions are treated as point charges placed on the axis of the channel. Let the bound anion be in contact with the cation through their ionic radii. Only primary waters of hydration are considered. Hydrated waters are taken as dipoles collinearly placed at the positions of the oxygen atoms, and within the channel they are supposed to be on the channel axis. In calculating the energy required in step II, it is assumed that the effective hydration number of the bound anion is one (i.e., the energy is equal to that required for removing all but one hydrated water in the bulk).

The present calculation will be restricted to cases in which $Na^+$ and $Cl^-$ ions (or $Li^+$ and $F^-$ ions) are the only ions concerned. Values of parameters used are listed in Table I. Numerical calculation is performed with a double (18-figure) precision on a ACOS Series 77 NEAC System 1000 digital computer (Computer Center of Tohoku University).

*Binding constant*

Calculated values of $K$ are given in Table II. The decrease in the effective pore radius and the increase in the distance from the channel end markedly favor the formation of ion pairs. $K$ is greatly enhanced in the presence of dimples. When the binding site is 1.0–3.5 Å from the end of a channel with an effective radius of 2–4 Å and dimples of 5 Å in depth, $K$ is calculated to be 0.25–41 $molal^{-1}$ for the binding of a $Cl^-$ counter ion to an $Na^+$ ion which has already been bound at the channel mouth. $K$ is greatly increases when a second cation is present at the opposite mouth. $K$ for the $Li^+$-$F^-$ pair is approximately twice the corresponding value for the $Na^+$-$Cl^-$ pair. In these calculations, $\varepsilon_p$ is taken to be 78. It is likely that $K$ tends to increase when smaller values of $\varepsilon_p$ are used.

*Conclusion*

Although several simplifications are made in the present calculations, the results indicate that when an ion is bound at a channel mouth, the tendency of a counter ion to form an ion pair with this ion is remarkably enhanced owing to image forces, and suggest that the ion pair of these ions may be formed if appropriate conditions are fulfilled. The binding constant depends on the position of the binding site, the dimensions and geometries of the channel and channel mouth, and the state of ion loading of the channel, as well as the species of ions concerned. It also depends on the energy of local interactions between the coun-

TABLE II

*K* FOR THE BINDING OF AN ANIONIC COUNTER ION TO A CATION WHICH HAS ALREADY BEEN BOUND AT THE MOUTH OF A CHANNEL

$l_p = 26$ Å, $\varepsilon_l = \varepsilon_p = 78$, and $\varepsilon_m = 2$. The Na-Cl and the Li-F distance are taken to be 2.8 and 2.0 Å, respectively. The hydration number of $F^-$ in the bulk and the distance between $F^-$ and coordinated $H_2O$ (F-O distance) are assumed to be 6 and 2.7 Å, respectively.

| Ions | Dimple | Effective pore radius (Å) | Position (Å from end) | Energy change (kcal/mol) | *K* ($molal^{-1}$) |
|---|---|---|---|---|---|
| $Na^+$-$Cl^-$ | 5-Å dimple | 4.0 | 1.0 | −1.57 | 0.25 |
| | | | 2.5 | −1.88 | 0.42 |
| | | | 3.5 | −2.08 | 0.59 |
| | | 3.0 | 1.0 | −2.09 | 0.60 |
| | | | 2.5 | −2.62 | 1.5 |
| | | | 3.5 | −2.95 | 2.6 |
| | | 2.0 | 1.0 | −3.07 | 3.1 |
| | | | 2.5 | −3.95 | 13.6 |
| | | | 3.5 | −4.61 | 41.4 |
| | None | 3.0 | 1.0 | −1.04 | 0.10 |
| | | | 2.5 | −1.46 | 0.21 |
| | | | 3.5 | −1.82 | 0.38 |
| $Na^+$-$Cl^-$ [a] | 5-Å dimple | 3.0 | 1.0 | −2.74 | 1.8 |
| | | | 2.5 | −3.71 | 9.1 |
| | | | 3.5 | −4.44 | 31.2 |
| $Li^+$-$F^-$ | 5-Å dimple | 3.0 | 1.0 | −2.53 | 1.3 |
| | | | 2.5 | −3.10 | 3.3 |
| | | | 3.5 | −3.45 | 5.9 |

[a] In the presence of a second cation at the opposite mouth, the distance from the end being the same as for the first cation.

ter ion and the channel wall. This energy must not be so unfavorable as to cancel out the effect of image forces. Thus the presence of an anionic group in the vicinity of the end of a cation channel (as is in fact the case for some ion channels) is expected to be very unfavorable for the formation of ion pairs. It should be noted here that the ion-pair formation of this species is not limited to the neighborhood of a channel end if the counter ion can enter deep into the channel without significantly unfavorable interactions with the channel wall.

In the above calculation, we considered the binding of an ion to an oppositely charged ion that has already been bound to a channel. This case is essentially equivalent to the binding of a cation (anion) to a negative (positive) fixed charge that might be present on the wall of a cation (anion) channel. the present results thus indicate that when a cation (anion) channel has an anionic (cationic) group as an integrant part of its wall, interactions between this fixed charged group and a permeant ion are remarkably enhanced by image forces, and suggest that even a monovalent ion may form an ion pair with this charged group.

The relevance of the present calculations to ion permeation in ion channels will be reported in a subsequent paper. Some consequences of anion binding at the mouth of the gramicidin channel has been discussed [1,2,6]. It has not escaped our notice that the implications of ion binding of this type are not restricted to cases in which ion channels are involved.

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