WATER TRANSPORT AND ION-WATER INTERACTION IN THE GRAMICIDIN CHANNEL

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ABSTRACT The diffusive permeability and the diffusion coefficient of water \( (D_w) \) in the gramicidin channel is determined from the osmotic water permeability of the channel and “single file” pore theory. \( D_w \) is about 7% of the self-diffusion coefficient of bulk water. The diffusion coefficient of a single water molecule alone in the channel is also determined and is about equal to the value in bulk water. This provides an estimate of the mobility of water on the channel walls in the absence of water-water interaction. Since the gramicidin channel walls should be representative of uncharged polar protein surfaces, this result provides direct evidence that water has a relatively high mobility on such protein surfaces. In addition, it is shown that the presence of a cation in the channel reduces the hydraulic water permeability by a factor ranging from 60 for \( \text{Tl}^+ \) to 5 for \( \text{Na}^+ \). The diffusion coefficient of a cation \( (D_c) \) in the channel is estimated and compared with \( D_w \). For \( \text{Na}^+ \) it is found that \( D_c \approx D_w \), which implies that the movement of the row of water molecules through the channel determines the local mobility of \( \text{Na}^+ \). Thus, it seems that short range ion-wall interactions are not important in determining the channel conductance for \( \text{Na}^+ \). In contrast, for \( \text{Li}^+ \), local ion-wall interactions probably do limit the conductance.

INTRODUCTION

Water molecules are inherently involved with the transport of ions through very narrow channels. In single-file transport, when an ion passes through the channel a row of water molecules is constrained by the channel walls and must move in front of the ion. This mode of transport is of biological importance because the selectivity filter of the K\(^+\) channel found in nerves is \( \approx 3 \) Å in diameter (Hille, 1975), and evidence indicates that antidiuretic hormone-induced channels in collecting tubules are \( \approx 4 \) Å or less in diameter (Finkelstein, 1979).

It is thought that gramicidin forms a cylindrical channel 26 Å long and \( \approx 4 \) Å in diameter (Urry, 1971; Urry et al., 1971; Urry et al., 1975; Koeppe et al., 1979) through which single-file transport occurs (Levitt et al., 1978; Rosenberg and Finkelstein, 1978 a, b; Dani and Levitt, 1981). Thus, gramicidin may be used as a model of biological channels in the study of single-file transport. Our purpose is to present a detailed analysis of water transport and ion-water interaction in the gramicidin channel.

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**TABLE 1**

PROPERTIES OF THE GRAMICIDIN PORE

<table>
<thead>
<tr>
<th>Property</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore length</td>
<td>Urry et al., 1975; Koepppe et al., 1979</td>
</tr>
<tr>
<td>Pore diameter</td>
<td></td>
</tr>
<tr>
<td>Number of water molecules in pore</td>
<td></td>
</tr>
<tr>
<td>Hydraulic water permeability</td>
<td>Urry et al., 1975; Koepppe et al., 1979</td>
</tr>
<tr>
<td>Diffusive water permeability</td>
<td>Levitt et al., 1978 and unpublished results</td>
</tr>
<tr>
<td>Pore water diffusion coefficient ($D_\text{w}$)</td>
<td>Dani and Levitt, 1981</td>
</tr>
<tr>
<td>&quot;Intrinsic&quot; water diffusion coefficient ($D_\text{i}$)</td>
<td>This paper</td>
</tr>
</tbody>
</table>

**Hydraulic (Osmotic) and Diffusive Water Permeability**

If the channel is so narrow that two water molecules cannot get past each other, than the diffusive ($P_D$) and hydraulic ($P_F$) water permeability are related by (Levitt, 1974)

$$\frac{P_F}{P_D} = N$$

where $N$ is the number of water molecules in the channel. $N$ has been determined from streaming potential measurements to be $\sim 9$ (Levitt et al., 1978, and unpublished data)\(^1\) and $P_F$ is $6 \times 10^{-14} \text{ cm}^3/\text{s}$ (Dani and Levitt, 1981). The diffusive water permeability of the channel calculated from Eq. 1 is about $6.6 \times 10^{-15} \text{ cm}^3/\text{s}$ (the results are summarized in Table 1).

**Diffusion Coefficient of Water**

The diffusion coefficient of water in the channel ($D_\text{w}$) is defined by the following expressions for the diffusive flux ($J$):

$$J = P_D \Delta c = D_\text{w} \Delta p / L$$

where $\Delta c$ is the concentration difference of the tracer water in bulk solutions, $\Delta p$ is the difference in the probability per unit length of finding a tracer water molecule at the two ends of the channel ($\Delta p$ is equivalent to a one dimensional "concentration") and $L$ is the channel length. An expression for $\Delta p$ can be derived by assuming that the ends of the channels are in equilibrium with the bulk solutions. The probability that the water molecule at each end is a tracer is equal to the fraction ($X$) of the water molecules in the adjacent bulk solution that are

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\(^1\)Rosenberg and Finkelstein (1978a,b) have reported an $N$ of $\sim 5$ or 6. Continued work on this problem has led us to believe that the best value for $N$ is $\sim 8$ or 9, which is more consistent with the proposed structure of gramicidin (Urry, 1971), also see footnote 1 of Dani and Levitt (1981).
tracers:

\[ X_1 = c_1 \overline{V}_w; \quad X_2 = c_2 \overline{V}_w \]  

where 1 and 2 denote the two sides of the membrane, \( c_1 \) and \( c_2 \) are the concentrations of tracer and \( \overline{V}_w \) is the molar volume of water. Since the length occupied by the water molecule at the channel end is \( L/N \), the probability per unit length is

\[
p_1 = X_1/(L/N)/N_A = c_1 \overline{V}_w N/(LN_A), \quad p_2 = c_2 \overline{V}_w N/(LN_A)
\]

\[
\Delta p = p_1 - p_2 = \Delta c \overline{V}_w N/(LN_A)
\]

where Avogadro's number \( (N_A) \) has been introduced, since \( p \) should be in units of moles per unit length. The substitution of Eq. 4 into Eq. 2 gives the final expression for \( D_w \):

\[ D_w = P_0 L^2 N_A/(\overline{V}_w N). \]

When the known values of the parameters (Table 1) are substituted into Eq. 5, \( D_w \) is found to be \( 1.7 \times 10^{-6} \) cm\(^2\)/s.

The \( D_w \) of Eq. 5 is the diffusion coefficient of a water molecule in a gramicidin channel that is filled with water. Since the channel is so narrow that the water molecules cannot get around each other, \( D_w \) is the "effective" diffusion coefficient of the entire row of \( N \) water molecules. From the theory for a single-file pore, \( D_w \) can be related to the hypothetical diffusion coefficient \( (D_0) \) that a water molecule would have if it were alone in the pore and the rest of the channel was a vacuum (Levitt and Subramanian, 1974):

\[ D_0/D_w = N. \]

\( D_0 \) is equal to \( 1.5 \times 10^{-5} \) cm\(^2\)/s for \( N \) equal to 9. This \( D_0 \) is a measure of the intrinsic resistance presented by the pore wall without any water-water interactions.

It is of interest to compare this \( D_0 \) with the value that would be expected if the pore wall simply adsorbed the water molecule and instantaneously readmitted it in a random direction. This is the case described by the Knudsen diffusion coefficient \( (D_K; \ Levitt \ and \ Subramanian, \ 1974) \):

\[ D_K = (2/3)(d - b)(2kT/\pi M)^{1/2} \]

where \( d \) is the diameter of the channel, \( b \) is the diameter of the water molecule, \( k \) is Boltzmann's constant and \( M \) is the mass of a water molecule. If it is assumed that \( d = 4 \) Å and \( b = 3 \) Å, then a value of \( D_K \) equal to \( 2 \times 10^{-4} \) cm\(^2\)/s is obtained. This indicates that the mobility of water on the gramicidin pore wall \( (D_0) \) is \( \sim 13 \) times less than that for the ideal \( (D_K) \) wall described by the Knudsen equation.

Water movement in the gramicidin channel provides a specific example of the diffusion of water on the surface of an uncharged polar protein. The value of \( D_0 \) is about equal to the self-diffusion coefficient in bulk water \( (2.3 \times 10^{-5} \) cm\(^2\)/s; Wang et al. 1953) and is, therefore, relatively fast. Clearly, the water is not "frozen" or immobilized by the peptide surface provided by the inner wall of the gramicidin channel.

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The following relation can be derived by a simple application of irreversible thermodynamics (see Appendix):

\[ \bar{V}_w GRT \frac{(N/zF)^2}{z} = f_1P_1 + f_2P_2 + \ldots \]  

where \( P_1, P_2, \) etc. are the hydraulic permeability coefficients of a channel that contains one, two, etc., ions; \( f_1, f_2, \) are the fraction of channels that contain one or two ions; \( G \) is the single-channel conductance and \( N \) is the number of water molecules transported per ion—all evaluated at the same ion concentration. In the limit of low-ion concentration, \( f_2 \) and higher terms can be neglected; \( f_1 \) is known (Dani and Levitt, 1981), as is \( N \) (Levitt et al., 1978 and unpublished results), and \( G \) (Neher et al., 1978) so \( P_1 \) (the hydraulic permeability coefficient of a channel that contains one ion) can be determined. The results for \( \text{Li}^+, \text{Na}^+, \text{K}^+ \) and \( \text{Tl}^+ \) are listed in Table II.\(^1\) The presence of an ion in the channel reduces the hydraulic permeability by a factor ranging from \(~60\) for \( \text{Tl}^+ \) to \(~5\) for \( \text{Na}^+ \). This large reduction indicates that in this single-file channel it is the large resistance to the movement of the ion which essentially limits the water permeability when an ion is in the channel.

**Diffusion Coefficient of Cations in the Channel**

The high resistance to ion transport found in the preceding section is the result of at least three factors: \( a \) The electrostatic energy barrier in the center of the membrane. This is a purely continuum effect and should be the same for all ions with the same charge (Levitt, 1978a). \( b \) The rate of unbinding from the end of the channel and moving into the bulk solution. This rate should decrease as the affinity of the channel increases. \( c \) The local mobility or diffusion coefficient of the ion in the channel. This is determined by the short range ion-wall and water-wall interactions (as opposed to long range electrostatic effects). The purpose of this section is to obtain an estimate of this cation diffusion coefficient \( (D_c) \) in the channel.

Since the channel is single file, the cation cannot diffuse faster than the row of water molecules that moves along with the cation. Thus, an upper limit for the diffusion coefficient of a cation \( (D_c) \) is provided by the diffusion coefficient of the row of water molecules \( (D_w) \) in

<table>
<thead>
<tr>
<th>Ion</th>
<th>Maximum electrical conductance ( (G_{max}) ) ( (10^{-12} \text{ Siemens}) )</th>
<th>Hydraulic water permeability ( (P_1)^* ) ( (10^{-15} \text{ cm}^2/\text{s}) )</th>
<th>Minimum cation diffusion coefficient ( (D_c)^\dagger ) ( (10^{-7} \text{ cm}^2/\text{s}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Li}^+ )</td>
<td>5.5</td>
<td>2</td>
<td>2.3</td>
</tr>
<tr>
<td>( \text{Na}^+ )</td>
<td>34</td>
<td>13</td>
<td>14.3</td>
</tr>
<tr>
<td>( \text{K}^+ )</td>
<td>20</td>
<td>5</td>
<td>8.4</td>
</tr>
<tr>
<td>( \text{Tl}^+ )</td>
<td>3.0</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Ion free</td>
<td>—</td>
<td>60</td>
<td>( D_w - 17 )</td>
</tr>
</tbody>
</table>

\*Data determined from Eq. 8.
\^Data determined from Eq. 12; \( g = 0 \).

\(^1\)Dr. Alan Finkelstein suggested to us that this type of calculation could be made.

\(^2\)To determine \( P_1 \) for \( \text{Na}^+ \) the maximum conductance of the channel containing one \( \text{Na}^+ \) was used, in which case \( f_1 = 1 \) and \( N = 9 \).
the channel. If \( D_c \approx D_w \), then the short range ion-wall interactions are not a significant factor in determining the channel conductance. In that case, friction between the ion and the channel walls is small compared to the friction between the row of water molecules and the wall.

The channel conductance \( (G) \) can be written (approximately) in the form

\[
G^{-1} = G_e^{-1} + G_c^{-1}
\]

where \( G_e \) is the conductance barrier presented by the two channel ends and \( G_c \) is the conductance through the center of the channel. Eq. 9 can be written in the form

\[
G = G_c/(1 + g); \quad g = G_c/G_e.
\]

The ratio \( g \) represents the ease of movement of a cation through the center of the channel, relative to the ease of movement into and out of the channel. If the rate of translocation through the channel is very slow compared with the rate at which a cation enters and leaves the channel, then \( g \) will be small.

In the limit where there is at most one ion per channel, the conductance through the center of the channel \( (G_c) \) can be estimated from the Nernst-Planck equation and a theoretical calculation of the electrostatic energy barrier (Levitt, 1978a):

\[
G_c = 2.4 \times 10^{-5} f_i D_c
\]

where \( f_i \) is the fraction of channels that contain one cation, \( D_c \) is the diffusion coefficient of the cation in the channel in units of cm\(^2\)/s and \( G_c \) is in Siemens units. Eq. 11 substituted into Eq. 10 gives

\[
D_c = 4.2 \times 10^4 G_{\text{max}} (1 + g)
\]

where \( G_{\text{max}} \) is the conductance when the channel contains one and only one cation \( (f_i = 1) \). \( G_{\text{max}} \) was obtained by extrapolating the low concentration conductance data (the y-intercepts in Fig. 9, Dani and Levitt, 1981).

Listed in Table II are the values of \( G_{\text{max}} \) used to calculate the minimum value (with \( g = 0 \)) of \( D_c \) for \( \text{Li}^+, \text{Na}^+, \text{K}^+, \text{Tl}^+ \). The values listed are minimum values because they were calculated with \( g = 0 \). That is the case where the conductance \( (G_{\text{max}}) \) is completely limited by the rate at which the cation is translocated from one end of the channel to the other. If the rates of entering and leaving the channel are not infinitely fast \( (g > 0) \), then to obtain the same conductance \( (G_{\text{max}}) \), the cation must move more rapidly through the center of the channel and \( D_c \) will be greater than the minimum values estimated in Table II.

**DISCUSSION AND SUMMARY:**

In order to accurately determine the diffusion coefficient of the cation (Eq. 12), it is necessary to know \( g \) (the ratio of the conductance across the center of the channel to the conductance at the channel ends). For the high concentration limit used to estimate \( G_{\text{max}} \) in Table II, the rate of entering the channel is fast compared with the rate of leaving, and \( g \) is approximately equal to the ratio of the rate constants for crossing the center of the channel to that for leaving the channel \( (r) \). This ratio can be determined by fitting the rate constants of an assumed channel model to the observed kinetics (Levitt, 1978b; Anderson and Procopio, 1980; Urban et al., 1980; Eisenman et al. 1980). There is a large variation in the values of \( r \) determined in this
way. For example, estimates of $r$ for $\text{Ti}^+$ range from 0.5 (Eisenman et al., 1980) to 10,000 (Urban et al., 1980).

Fortunately, there are some conclusions about $D_c$ that do not require an accurate knowledge of $g$. It can be seen from Table II that the minimum $D_c$ for $\text{Na}^+$ is about four-fifths of that of $D_w$. Since the maximum value of $D_c$ is $D_w$, and there is general agreement that $g$ for $\text{Na}^+$ is $\sim 0.5$ or larger, it can be concluded that $D_{Na} \approx D_w$ (see Eq. 12). Thus, the local mobility of $\text{Na}^+$ is limited by the friction between the wall and the column of nine water molecules which are directly coupled to the $\text{Na}^+$ ion by the single-file nature of the channel. The local interaction between the $\text{Na}^+$ ion and the channel wall is small enough that it does not influence the channel conductance. Depending on which published values of $r$ are used, this same conclusion could also apply to $\text{K}^+$ and $\text{Ti}^+$.

In contrast, it is probable that the conductance of $\text{Li}^+$ is limited by the local ion-wall interaction. $\text{Li}^+$ has a low affinity (see Table II, Dani and Levitt, 1981), which indicates a relatively high rate of leaving the channel, and a low maximum conductance (Neher et al., 1978), which indicates a relatively slow rate of crossing the center of the channel. Thus $r$ (approximately $g$) for $\text{Li}^+$ should be close to zero so that $D_{Li}$ is approximately equal to the minimum value of Table II, which is about 10 times smaller than $D_w$. This large ion-wall interaction is probably the explanation of the relatively low maximum conductance that is found for $\text{Li}^+$.

All ions which have $D_c = D_w$ (e.g. $\text{Na}^+$) will have the same local mobility and the same rate of moving across the center of the channel. For the same level of ion occupancy, the conductance for the different ions will differ only because of variations in the value of $G_e$ (the conductance at the channel ends). Since the channel conductance is limited by the mobility of the water column, the only way to increase the conductance is to shorten the length of the water column; for example, by having only a short single-file segment in the channel, as is thought to be the case in the $\text{K}^+$ channel (Hille, 1978).

The osmotic water permeability coefficient can be determined for the ion-free amphotericin B channel by combining the osmotic permeability measurements of Holz and Finkelstein (1970) and the single channel conductance measurements of Ermishkin et al. (1977). This value is nearly identical to that of gramicidin ($\sim 6 \times 10^{-14}$ cm$^3$/s) (Dani and Levitt, 1981). This is a surprising result because amphotericin B has a diameter almost twice as large as gramicidin (Borisova et al., 1979). It suggests that gramicidin has a water permeability that is relatively large for its channel diameter, possibly because of the single-file nature of the water. The similar values of the water permeability for these two channels with markedly different structures leads one to expect that uncharged ion-free biological ion channels should have a similar hydraulic water permeability. The addition of an ion to the gramicidin channel produces a large decrease in the water permeability (Table II). This suggests that the ion channels found in nerve and muscle should have a low water permeability because they normally contain at least one ion.

The diffusion coefficient of the water column ($D_w$) in the gramicidin channel is relatively fast—$\sim 10\%$ of the self-diffusion coefficient of bulk water (Table I). This clearly demonstrates

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4Recently, Finkelstein and Anderson (1981) reached this same conclusion using different experimental data and a different set of arguments. Some of their other results do not agree with the conclusions of this paper.
that water moves rapidly over the uncharged polar surfaces that make up the inner walls of the gramicidin channel. This result is consistent with recent NMR measurements of the rate of water movement on the surface of lysozyme (Bryant and Shirley, 1980).

APPENDIX

Derivation of Eq. 8

It is assumed the system is linear, the volume flux \((J_v)\) and current \((I)\) can be written as a linear combination of the electrical potential \((\Delta \psi)\) and hydrostatic (or osmotic) pressure difference \((\Delta \pi)\):

\[
J_v = (\bar{V}_w P_F/RT) \Delta \pi + L_{12} \Delta \psi \quad (A1)
\]

\[
I = L_{21} \Delta \pi + G \Delta \psi \quad (A2)
\]

where \(P_F\) is the osmotic water permeability \((\Delta \psi = 0)\), \(G\) is the electrical conductance and \(L_{12}\) and \(L_{21}\) are cross coefficients, which are equal by Onsager’s theorem. (It is assumed in Eqs. A1 and A2 that there are equal ion activities on the two sides of the membrane). It can be shown (Levitt et al., 1978) that the streaming potential can be related to \(N\) (the number of water molecules coupled to the transport of each ion):

\[
(\Delta \Psi/\Delta \pi)_{I=0} = -L_{21}/G = -\bar{V}_w N/zF \quad (A3)
\]

The hydraulic water permeability \((P_F)\) can be factored into the permeability of channels that contain zero \((P_0)\), one \((P_1)\), two \((P_2)\) etc. ions:

\[
P_F = f_0 P_0 + f_1 P_1 + f_2 P_2 + \ldots \quad (A4)
\]

where \(f_0, f_1, f_2\) etc. are the fraction of channels that contain zero, one, two, etc. ions. For a single-file channel, under open circuit conditions \((I = 0)\) a volume flux can occur only through those channels that do not contain an ion (see footnote 2, Dani and Levitt, 1981):

\[
(J_v)_{I=0} = (J_0)_{I=0} + (J_1)_{I=0} + \ldots = (J_0)_{\Delta \psi=0} = \bar{V}_w f_0 P_0 \Delta \pi/RT \quad (A5)
\]

where \(J_0\) is the volume flux through the ion-free channel. The last equality in Eq. A5 results from the fact that for an ion-free channel: \((J_0)_{I=0} = (J_0)_{\Delta \psi=0}\). Another expression for \((J_v)_{I=0}\) can be obtained by solving Eq. A2 for \(\Delta \psi\) when \(I = 0\) and substituting into Eq. A1:

\[
(J_v)_{I=0} = (\bar{V}_w P/RT - L_{12}^2/G) \Delta \pi \quad (A6)
\]

Finally, substituting \(L_{12}\) from Eq. A3 and \(P\) from Eq. A4 into Eq. A6 and equating Eqs. A5 and A6 gives:

\[
\bar{V}_w GRT (N/zF)^2 = f_1 P_1 + f_2 P_2 + \ldots \quad (A7)
\]

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REFERENCES


