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 Tl^+ to 5 for Na^+. The diffusion coefficient of a cation (D_c) in the channel is estimated and compared with D_w. For 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 Na^+. Thus, it seems that short range ion-wall interactions are not important in determining the channel conductance for Na^+. In contrast, for 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 ~3 Å in diameter (Hille, 1975), and evidence indicates that antidiuretic hormone-induced channels in collecting tubules are 4 Å or less in diameter (Finkelstein, 1979).

It is thought that gramicidin forms a cylindrical channel 26 Å long and ~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 I
PROPERTIES OF THE GRAMICIDIN PORE

		Reference
Pore length	26 Å	Urry et al., 1975; Koeppe et al., 1979
Pore diameter	4 Å	Urry et al., 1975; Koeppe et al., 1979
Number of water molecules in pore	9	Levitt et al., 1978 and unpublished results
Hydraulic water permeability	6×10^{-14} cm^3/s	Dani and Levitt, 1981
Diffusive water permeability	6.6×10^{-15} cm^3/s	This paper
Pore water diffusion coefficient (D_w)	1.7×10^{-6} cm^2/s	This paper
"Intrinsic" water diffusion coefficient (D_0)	1.5×10^{-5} cm^2/s	This paper

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)

$$P_F/P_D = N \tag{1}$$

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 N has been determined from streaming potential measurements to be ~9 (Levitt et al., 1978, and unpublished data)[1] and P_F is 6×10^{-14} cm^3/s (Dani and Levitt, 1981).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

Diffusion Coefficient of Water

The diffusion coefficient of water in the channel (D_w) is defined by the following expressions for the diffusive flux (J):

$$J = P_D \Delta c = D_w \Delta p / L \tag{2}$$

where Δc is the concentration difference of the tracer water in bulk solutions, Δp is the difference in the probability per unit length of finding a tracer water molecule at the two ends of the channel (Δp is equivalent to a one dimensional "concentration") and L is the channel length. An expression for Δp 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 (1978*a*,*b*) have reported an N of ~5 or 6. Continued work on this problem has led us to believe that the best value for N is ~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:

$$X_1 = c_1\overline{V}_w; \qquad X_2 = c_2\overline{V}_w \tag{3}$$

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

$$p_1 = X_1/(L/N)/N_A = c_1\overline{V}_wN/(LN_A), \qquad p_2 = c_2\overline{V}_wN/(LN_A)$$
$$\Delta p = p_1 - p_2 = \Delta c\overline{V}_wN/(LN_A) \tag{4}$$

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:

$$D_w = P_DL^2N_A/(\overline{V}_wN). \tag{5}$$

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

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):

$$D_0/D_w = N. \tag{6}$$

D_0 is equal to $1.5 \times 10^{-5}\ \mathrm{cm^2/s}$ 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).

$$D_K = (2/3)(d - b)(2kT/\pi M)^{1/2} \tag{7}$$

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 $2 \times 10^{-4}\ \mathrm{cm^2/s}$ is obtained. This indicates that the mobility of water on the gramicidin pore wall (D_0) is ~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 ($2.3 \times 10^{-5}\ \mathrm{cm^2/s}$; 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.

TABLE II

PROPERTIES OF A GRAMICIDIN PORE THAT CONTAINS ONE ION

Ion	Maximum electrical conductance (G_{max})	Hydraulic water permeability (P_1)*	Minimum cation diffusion coefficient (D_c)‡
	(10^{-12} *Siemens*)	(10^{-15} cm^3/s)	(10^{-7} cm^2/s)
Li^+	5.5	2	2.3
Na^+	34	13	14.3
K^+	20	5	8.4
Tl^+	3.0	1	1.4
Ion free	—	60	$D_w = 17$

*Data determined from Eq. 8.

‡Data determined from Eq. 12; $g = 0$.

Hydraulic Permeability of a Channel that Contains One Cation[2]

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

$$\overline{V}_w GRT(N/zF)^2 = f_1P_1 + f_2P_2 + \dots \tag{8}$$

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 Li^+, Na^+, K^+ and Tl^+ are listed in Table II.[3] The presence of an ion in the channel reduces the hydraulic permeability by a factor ranging from ~60 for Tl^+ to 5 for 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, 1978*a*). (*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

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[2] Dr. Alan Finkelstein suggested to us that this type of calculation could be made.

[3] To determine P_1 for Na^+ the maximum conductance of the channel containing one Na^+ was used, in which case $f_1 = 1$ and $N = 9$.

the channel. If $D_c \simeq D_w$, 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

$$G^{-1} = G_e^{-1} + G_c^{-1} \tag{9}$$

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

$$G = G_c/(1 + g); \qquad g = G_c/G_e. \tag{10}$$

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, 1978*a*):

$$G_c = 2.4 \times 10^{-5} f_1 D_c \tag{11}$$

where f_1 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

$$D_c = 4.2 \times 10^4 G_{max}(1 + g) \tag{12}$$

where G_{max} is the conductance when the channel contains one and only one cation ($f_1 = 1$). G_{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_{max} used to calculate the minimum value (with $g = 0$) of D_c for Li^+, Na^+, K^+ and Tl^+. The values listed are minimum values because they were calculated with $g = 0$. That is the case where the conductance (G_{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_{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_{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, 1978*b*; 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 Tl^+ 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 Na^+ is about four-fifths that of D_w. Since the maximum value of D_c is D_w, and there is general agreement that g for Na^+ is ~0.5 or larger, it can be concluded that $D_{Na} \simeq D_w$ (see Eq. 12). Thus, the local mobility of Na^+ is limited by the friction between the wall and the column of nine water molecules which are directly coupled to the Na^+ ion by the single-file nature of the channel. The local interaction between the Na^+ ion and the channel wall is small enough that it does not influence the channel conductance.[4] Depending on which published values of r are used, this same conclusion could also apply to K^+ and Tl^+.

In contrast, it is probable that the conductance of Li^+ is limited by the local ion-wall interaction. 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 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 Li^+.

All ions which have $D_c = D_w$ (e.g. 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 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 (~6×10^{-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—~10% of the self-diffusion coefficient of bulk water (Table I). This clearly demonstrates

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[4] Recently, 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 ($\Delta\pi$):

$$J_V = (\bar{V}_w P_F/RT)\Delta\pi + L_{12}\Delta\psi \tag{A1}$$

$$I = L_{21}\Delta\pi + G\Delta\psi \tag{A2}$$

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):

$$(\Delta\Psi/\Delta\pi)_{I=0} = -L_{21}/G = -\bar{V}_w N/zF \longrightarrow L_{21} = L_{12} = \bar{V}_w NG/zF \tag{A3}$$

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:

$$P_F = f_0 P_0 + f_1 P_1 + f_2 P_2 + \ldots . \tag{A4}$$

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):

$$(J_V)_{I=0} = (J_0)_{I=0} + (J_1)_{I=0} + \cdot \cdot \cdot = (J_0)_{I=0} = \bar{V}_w f_0 P_0 \Delta\pi/RT \tag{A5}$$

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:

$$(J_V)_{I=0} = (\bar{V}_w P/RT - L_{12}^2/G)\Delta\pi \tag{A6}$$

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

$$\bar{V}_w GRT\,(N/zF)^2 = f_1 P_1 + f_2 P_2 + \ldots . \tag{A7}$$

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REFERENCES

Andersen, O. S., and J. Procopio. 1980. Ion movement through gramacidin A channels. On the importance of the aqueous diffusion resistance and ion-water interactions. *Acta Physiol. Scand.* (In press).

Borisova, M. P., L. N. Erishkin, and A. Ya. Sibersteine. 1979. Mechanism of blockage of amphotericin B channels in a lipid bilayer. *Biochim. Biophys. Acta.* 553:450–459.

Bryant, R. G., and W. M. Shirley. 1980. Dynamical deductions form nuclear magnetic resonance relaxation measurements at the water protein interface. *Biophys. J.* 32:3–11.

Dani, J. A., and D. G. Levitt. 1980. Binding constants of Li^+, K^+ and Tl^+ in the gramicidin channel determined from water permeability measurements. *Biophys. J.* 35:485–499.

Eisenman, G., J. Hagglund, J. Sandblom, and B. Enos. 1980. The current-voltage behavior of ion channels: important features of the energy profile of the gramicidin channel deduced from the conductance-voltage characteristic in the limit of low concentration. *Ups. J. Med. Sci.* (In press).

Ermishkin, L. N., Kh. M. Kasumov, and V. W. Potseluyev. 1977. Properties of amphotericin B channels in a lipid bilayer. *Biochim. Biophys. Acta.* 470:357–367.

Finkelstein, A., and O. S. Andersen. 1981. The gramicidin A channel: a review of its permeability characterisitcs with special reference to the single-file aspect of transport. *J. Membr. Biol.* 59:155–171.

Finkelstein, A., and P. A. Rosenberg. 1979. Single-file transport: implications for ion and water movement through gramicidin A channels. *In* Ion Permeation Through Membrane Channels: Membrane Transport Processes, C. F. Stevens and R. W. Tsien, editors. Raven Press. New York. 73–88.

Hille, B. 1975. Ionic selectivity of Na and K channels of nerve membranes. *In* Membranes, vol. 3. Lipid Bilayers and Biological Membranes: Dynamic Properties. G. Eisenman, editor. Marcel Dekker, Inc., New York. 255–323.

Hille, B. 1978. Ionic channels in excitable membranes: current problems and biophysical approaches. *Biophys. J.* 22:283–294.

Holz, R., and A. Finkelstein. 1970. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotic nystatin and amphotericin B. *J. Gen. Physiol.* 56:125–145.

Koeppe, R. E., II, J. M. Berg, K. O. Hodgson, and L. Stryer. 1979. Gramicidin A crystals contain two cation binding sites per channel. *Nature (Lond.).* 279:723–725.

Levitt, D. G. 1974. A new theory of transport for cell membranes pores. I. General theory and application to red cell. *Biochim. Biophys. Acta.* 373:115–131.

Levitt, D. G. 1978*a*. Electrostatic calculation for an ion channel. I. Energy and potential profiles and interactions between ions. *Biophys. J.* 22:209–219.

Levitt, D. G. 1978*b*. Electrostatic calculation for an ion channel. II. Kinetic behavior of the gramicidin A channel. *Biophys. J.* 22:221–248.

Levitt, D. G., and G. Subramanian. 1974. A new theory of transport for cell membrane pores. II. Exact results and computer simulation (molecular dynamics). *Biochim. Biophy. Acta.* 373:132–140.

Levitt, D. G., S. R. Elias, and J. M. Hautman. 1978. Number of water molecules coupled to the transport of sodium, potassium and hydrogen ions via gramicidin, nonactin or valinomycin. *Biochim. Biophys. Acta.* 512:436–451.

Neher, E., J. Sandblom, and G. Eisenman. 1978. Ionic selectivity, saturation, and block in gramicidin A channels. II. Saturation behavior of single channel conductances and evidence for the existence of multiple binding sites in the channel. *J. Membr. Biol.* 40:97–116.

Rosenberg, P. A., and A. Finkelstein. 1978*a*. Interaction of ions and water in gramicidin A channels. Streaming potentials across lipid bilayer membranes. *J. Gen. Physiol.* 72:327–340.

Rosenberg, P. A., and A. Finkelstein. 1978*b*. Water permeability of gramicidin A treated lipid bilayer membranes. *J. Gen. Physiol.* 72:341–350.

Urban, B. W., S. B. Hladky, and D. A. Haydon. 1980. Ion movements in gramicidin pores. An example of single-file transport. *Biochim. Biophys. Acta.* 602:331–354.

Urry, D. W. 1971. The gramicidin A transmembrane channel: a proposed π (L,D) helix. *Proc. Natl. Acad. Sci. U. S. A.* 68:672–676.

Urry, D. W., M. C. Goodell, J. D. Glickson, and D. F. Mayers. 1971. The gramicidin A transmembrane channel: characteristics of head-to-head dimerized helixes. *Proc. Natl. Acad. Sci. U.S.A.* 68:1907–1911.

Urry, D. W., M. M. Long, M. Jacobs, and R. D. Harris. 1975. Conformation and molecular mechanisms of carriers and channels. *Ann. N.Y. Acad. Sci.* 264:203–220.

Wang, J. H., C. V. Robinson, I. S. Edelman. 1953. Self-diffusion and structure of liquid water. III. Measurement of the self-diffusion of liquid water with H^2, H^3 and O^{18}, as tracers. *J. Am. Chem. Soc.* 75:466.