Streaming Potentials in Gramicidin Channels Measured with Ion-Selective Microelectrodes

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ABSTRACT Streaming potentials have been measured for gramicidin channels with a new method employing ion-selective microelectrodes. It is shown that ideally ion-selective electrodes placed at the membrane surface record the true streaming potential. Using this method for ion concentrations below 100 mM, approximately seven water molecules are transported whenever a sodium, potassium, or cesium ion, passes through the channel. This new method confirms earlier measurements (Rosenberg, P. A., and A. Finkelstein. 1978. Interaction of ions and water in gramicidin A channels. *J. Gen. Physiol.* 72:327–340) in which the streaming potentials were calculated as the difference between electrical potentials measured in the presence of gramicidin and in the presence of the ion carriers valinomycin and nonactin.

INTRODUCTION

Gramicidin in lipid bilayers forms narrow channels that are permeable to small monovalent cations and water but not to anions or to urea. As the channel interior is a narrow pore, ions and water can pass through only in single file and ion binding reduces water permeation through the channel (Dani and Levitt, 1981; Wang et al., 1995). A description of the kinetics of ion permeation must therefore take into account the water molecules solvating the ions and water molecules present inside the channel. Gramicidin is thus a valuable model for studying the mechanisms that underlie coupling of transport of ions and water through transmembrane channels.

At sufficiently low ion concentrations, cations must pass through the channel independently of each other; however, for K^+, Rb^+, Cs^+, and Tl^+, at higher concentrations the exit of one cation from the channel is made more likely by the entry of another (see, e.g., Finkelstein and Andersen, 1981; Hladky and Haydon, 1984; Hladky, 1988 for reviews). Whether ion-ion interaction also occurs for Na^+ is still unclear. Within experimental error the flux ratio exponent for Na^+ equals one in both diphytanoylphosphatidylcholine membranes (Procopio and Andersen, 1971) and monoglyceride membranes (M. Jones, D. S. Game, S. P. Moule, and S. B. Hladky, unpublished observations) suggesting the absence of interaction and that ion binding to the channel can be described as simple competition. However, it remains unclear why Na^+ should differ in a qualitative manner from K^+. Furthermore, if Na^+ obeys simple competition, the dissociation constant required to explain the conductance-activity relation for gramicidin in monoglyceride membranes is 370 mM, while the dissociation constant calculated from the ability of Na^+ to decrease the water permeability of the channels (Dani and Levitt, 1981) is only 80 mM (Wang et al., 1995).

Levitt (1984) reported, based on streaming potential measurements, that at low concentrations the number of water molecules accompanying each ion, η, is >9 for Na^+, but only 7 for K^+. Levitt suggests that either Na^+, which is smaller and more strongly hydrated, drags extra water of hydration along with it, or that Na^+ enters and leaves by moving the entire pore contents of water while K^+ enters and leaves by exchanging for a single water molecule. These are attractive, plausible suggestions, either of which implies that the interactions of Na^+ and K^+ with the pore do indeed differ in a qualitative sense. However, the earlier streaming potential measurements by Rosenberg and Finkelstein (1978) concluded that η was the same for both ions ($\eta = 6.1$ for Na^+ and 6.4 for K^+ at 10 mM, 6.5 and 6.6 at 100 mM). Levitt (1984) criticized both this study and his own earlier work (Levitt et al., 1978) on methodological grounds, but none of the suggested errors explain why η for the two ions was found to be the same in one study but different in the other. We have therefore sought to reinvestigate ion-water coupling in gramicidin channels using a new technique employing ion-selective microelectrodes (ISM) to measure the streaming potential.

MATERIALS AND METHODS

Experimental chamber and measurement of streaming potentials

In an earlier paper (Wang et al., 1995), we reported the development of ion gradients in the unstirred layers next to a membrane as a consequence of osmotic water flow. Although it was possible to measure the changes in salt concentration of the bulk solutions on both sides of the bilayer by puncturing the bilayer with silanized ISMs filled with hydrophobic ion-exchanger cocktails, it became obvious that ISMs that touched a membrane became unstable and noisy, presumably because of admixture of lipid and/or solvent with the components of the ion-exchanger. Whereas the tip resistance of silanized electrodes filled with aqueous solutions only is easily restored by clearing the tips with pressure pulses, recovery of

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function of ISMs was only sometimes achieved with potassium electrodes and rarely so with sodium electrodes. Therefore, a new arrangement was designed to position an ISM and an Ag/AgCl electrode on each side of the membrane without losing the advantages of accurate membrane and microelectrode positioning with inverted optics.

The experimental cell is shown in Fig. 1. The overall chamber was made of acrylate (Perspex, Plexiglass, Lucite) with a glass coverslip as the floor. A bimolecular lipid membrane was formed on the end of the poly(tetrafluoroethylene) (PTFE) tube (ID 2.3 mm; OD 4.1 mm) that projects into the right, open compartment. The left compartment is closed. An ion-selective microelectrode is introduced into the closed compartment through a PTFE sleeve sealed with petroleum jelly. The membrane is viewed in phase contrast or dark field modes with an inverted microscope for accurate positioning. When an osmotic gradient is imposed across the membrane by adding urea to the open compartment, water leaves the closed compartment and the membrane tends to move into the tube. The membrane can be held stationary or positioned by delivering/withdrawing fluid from the closed compartment with a 250-μl syringe under micrometer control. The cation activity profile perpendicular to the membrane is measured on the left side by moving the membrane with the left electrode fixed. The profile on the right is measured by moving the right microelectrode with the membrane fixed. Membrane and ISM positioning were done under micromanipulator control using an eyepiece graticule with an accuracy of better than 10 μm.

Solutions and cleaning

All glass or PTFE parts were cleaned with dilute detergent and degreased in petroleum ether if necessary. They were then washed in dichromate-sulfuric acid mixtures or in nitric acid followed by copious rinsing with clean water. Acrylate parts were cleaned in isopropanol. Chemicals were of analytical grade. All solutions were made up in MilliQ-PF (Millipore, Bedford, MA) water. Hexadecane was passed through a column of alumina. To remove organic impurities, salts were heated in a muffle furnace for 3 h at 600°C in quartz vessels. Bimolecular lipid membranes were formed from a 10 mM solution of 1-monooleoyl-*rac*-glycerol (Sigma M7765) in *n*-hexadecane (Koch-Lite, puriss). Gramicidin (Sigma G5002) was added to the aqueous phase from a 10^{-4} M stock solution in ethanol (Aldrich); typically 1 μl was added and mixed into the chamber (typical volume 11–12 ml). Osmotic gradients were imposed by adding one-seventh of the measured bath volume of 4 M urea (Sigma) made up in the respective bath solution to impose a final driving force of 0.5 M urea without producing any change in the molality of the salt in the solution. Experiments were carried out at 22 ± 1°C.

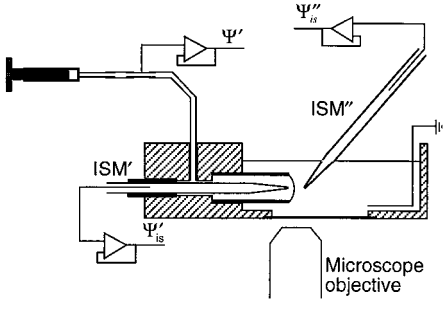

FIGURE 1 Experimental cell. Ψ' is the potential of the Ag/AgCl tube with respect to the Ag/AgCl earth wire. Ψ'_{is} and Ψ''_{is} are the potentials of the liquid ion exchanger microelectrodes (ISMs). The left, singly primed compartment is closed by the membrane, the seal around the microelectrode and the piston of the syringe are used to position the membrane. The right, doubly primed compartment is open. The entire cell is mounted on the stage of an inverted microscope.

Ion-selective microelectrodes

The activities of cations in the electrolyte solutions in the aqueous unstirred layers close to the bilayer were measured by microelectrodes filled with liquid ion-exchanger as described earlier (Tripathi et al., 1985). Both borosilicate and aluminosilicate glasses were used, but there was no evidence that the choice of glass affected the measurements. One microelectrode attached to a holder with a side arm was inserted horizontally via a PTFE sleeve into the closed compartment of the chamber. A small positive pressure set by the height of the liquid column in the side arm was applied to the interior of the electrode to prevent the pressure of the solution within the closed compartment displacing the ion-exchanger from the electrode tip. This microelectrode was made with 1.5 mm OD glass, with a closely fitting PTFE sleeve that was used to protect the tip during coaxial positioning and to seal against the acrylate chamber. The ISM in the chamber on the other side of the membrane was 2.0 mm OD. Capillary glass tubings were from Frederick Haer & Co. (Bowdoinham, ME) or Clark Electromedical (Pangbourne, Reading, England). The electrodes were pulled in two stages and silanized by 50 μl dimethyl-dichlorosilane vapor in an all-glass oven. Liquid ion-exchanger (Fluka) was introduced into the tip and the electrode was backfilled with a 300 mM solution of the respective salt. K^+-ion exchanger was used for K^+ and Cs^+ measurements and Na^+-exchanger was used for Na^+ measurements. The K^+-exchanger has a lower resistivity and selects for all cations of interest. The microelectrodes were pulled with large tips of 2–5 μm to decrease response times and noise levels. Electrode resistances were typically 1–2 GΩ. Measurements were made with only one cation present at any one time, and had Nernst responses to the cations relevant for this study as measured at the end of the experiment by a 10-fold change in the concentration of the solution in the chamber. Since the earth wire was an Ag/AgCl electrode, the change in potential was twice the Nernst potential. The selectivity of the exchanger among cations was not relevant, as all measurements were made in solutions containing only a single salt.

All individual potentials were measured relative to an Ag/AgCl wire electrode positioned in the open compartment far from the membrane. The true streaming potential is the difference in potentials between two ideally cation-selective ISMs placed at the membrane surfaces (see Appendix). The transmembrane potential measured by the Ag/AgCl tube electrode in the left compartment is larger, as it includes potentials resulting from concentration polarization and diffusion potentials in the aqueous phases. The potential profiles measured by the ISMs (closest approach was 25 μm to avoid the risk of the tips touching the membrane) are extrapolated to the membrane surfaces. In each experiment it was confirmed with a clean bilayer that the left compartment was electrically isolated from ground and that prior to the addition of gramicidin the electrical conductance between the compartments was negligible. The potentials of the ion-selective microelectrodes were usually measured with a dual high impedance electrometer (WPI, Sarasota, FL, model FD223); in a few initial studies a pair of patch clamp amplifiers (List, Darmstadt, Germany, model EPC-7) were used in the current clamp mode. The potential of the Ag/AgCl tube was measured with another electrometer (WPI, model M707). All signals were filtered at 10 Hz, preamplified and digitized with a 12-bit A-to-D converter, recorded on a computer, and displayed in real time. The data acquisition system was calibrated with a precision DC voltage source.

Statistical comparisons are based on the two-tailed *t*-test for the hypothesis that a mean equal to the value in question would occur by chance.

RESULTS

A representative experimental trace is shown in Fig. 2. After a gramicidin-containing membrane had been formed, the ISM and Ag/AgCl potentials were checked to be stable. The chamber was made hyperosmolar with urea (final concentration 500 mM) added during the time interval 250–270 s. The bottom trace shows the potential recorded between the Ag/AgCl tube and Ag/AgCl earth; the middle trace the

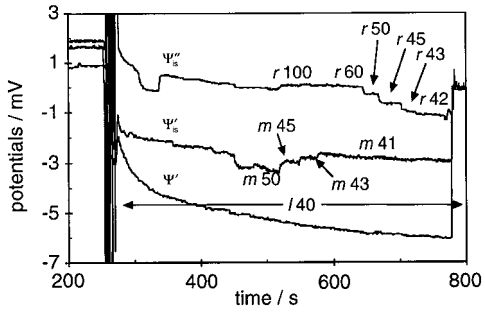

FIGURE 2 *Bottom trace:* potential between the Ag/AgCl electrodes; *middle trace:* the ISM′ potential from the left, closed compartment (′); *top trace:* potential from the ISM″ in the right, open compartment (″). The left microelectrode was positioned at 40 on the graticule (1 unit = 25 μm) immediately after the addition of urea and held at 40. Between 400 and 600 s the membrane position was adjusted to the values shown to obtain a profile of potential in the closed compartment (graticule positions prefixed by *m*). Between 620 s and membrane breakage the position of the right microelectrode was adjusted to obtain a profile in the open compartment. At 780 s the membrane was broken using a suction microelectrode. The potential traces have been offset to superimpose after membrane breakage when the two ISMs are recording the same potential and ion activity.

ISM′ potential from the left, closed compartment (′), and the top trace the potential from the ISM″ in the right, open compartment (″). The left microelectrode was positioned at 40 on the graticule (1 unit = 25 μm) immediately after the addition of urea. Between 400 and 600 s the membrane position was adjusted to the values shown to obtain a profile of potential in the closed compartment. Between 620 s and membrane breakage the position of the right microelectrode was adjusted to obtain a profile in the open compartment. At 780 s the membrane was broken using a suction microelectrode. The potential traces have been offset to superimpose after membrane breakage when the two ISMs are recording the same potential and ion activity. The total potential reported in the bottom trace is thus the difference between the membrane potential and the liquid junction potential immediately after membrane breakage (for K^+ the diffusion potential in the aqueous phases is negligible). The profiles are used to extrapolate (by 25 μm) the measured potentials to the membrane surfaces. The difference between these surface potentials is the estimate of the streaming potential, $\Delta\Psi_e$.

The number of water molecules transported per cation, η, is calculated from the streaming potential using

$$\left(\frac{\Delta\Psi_e}{\Delta\pi}\right)_{I=0} = -\frac{\bar{V}_w}{zF}\,\eta \qquad (1)$$

where

$$\Delta\pi = RT\Delta m_i \qquad (2)$$

is the osmotic pressure difference, Δm_i, is the difference in molality of the impermeant solute, R is the gas constant, T is the absolute temperature, $\bar{V}_w$ is the partial molar volume of water, F is the Faraday, and z_i (=1) is the charge on the permeant ion. For practical calculation using a gradient of 0.5 M urea,

$$\eta = \left(\frac{\Delta\Psi_e}{0.23\ \text{mV}}\right) \qquad (3)$$

The mean values of η are shown in Fig. 3 and the individual data values are listed in Table 1. For Na^+ the mean of the values at 3 and 30 mM is 7.1 ± 0.3 (mean ± SE, n = 7), for K^+ the mean of the values at 10 and 30 mM is 6.6 ± 0.6 (n = 10), while for Cs^+ the mean of the values at 3 and 10 mM is 7.3 ± 0.8 (n = 6). There is no significant difference between these values. The value for Na^+ is less than the value 9.5 reported by Levitt ($p < 0.001$) but greater than the 6.1 reported by Rosenberg and Finkelstein ($p < 0.02$) for 10 mM. It does not differ significantly ($p > 0.06$) from the consensus value, 6.5, reported by Rosenberg and Finkelstein for all their data at and below 100 mM. The value for potassium does not differ significantly from either of the previously reported values (7.1 and 6.4, respectively) while that for cesium does not differ significantly from the 6.7 reported by Rosenberg and Finkelstein.

DISCUSSION

When an osmotic gradient is imposed across a membrane, there is a flow of water through both the lipid bilayer and any pores embedded in it. Flow through the pores will tend

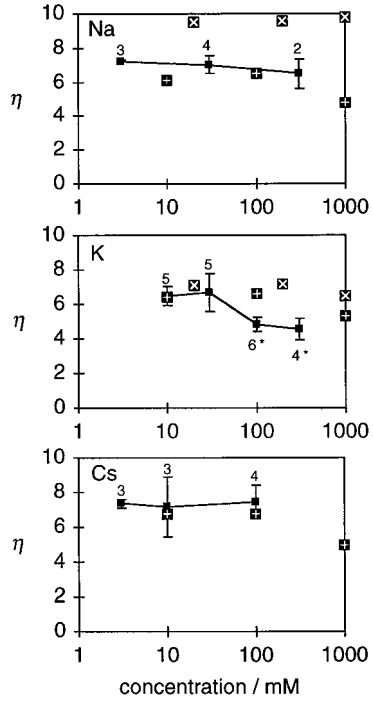

FIGURE 3 The number of water molecules transported per cation in gramicidin channels. *Top,* Na^+; *middle,* K^+; *bottom,* Cs^+. ■, Error bars and connecting lines: data of the present study presented as mean ± SE. The numbers near the error bars indicate the number of experiments. ⊠, Values reported by Levitt (1984). ✚, Values reported by Rosenberg and Finkelstein (1978).

TABLE 1 The number of water molecules transported per cation in gramicidin channels

Concentration (mM)	Permeant Ion		
	Sodium	Potassium	Cesium
3	7.3, 7.3, 7.2		(7.1, 7.8), 7.1
10		5.1, 6.6, 6.3, 8.4, 6.0	10.6, 5.4, 5.4
30	6.3, 6.3, 7.0, (7.0), 8.5	10.0, (7.5, 4.0), 7.6, 4.4	
100		[4.3, 4.3, 3.9, 5.8, 4.2, 6.4]	10.2, 7.0, 5.7, 7.0
300	8.5, 5.7, 7.4	[3.5, 4.2, 4.3, 6.3]	

Values in parentheses were obtained shortly (e.g., 1 min) after addition of urea with electrodes positioned 25 μm from the membrane surfaces, but they have not been corrected by extrapolation of the potential profile. Values in brackets were calculated from potentials measured using the cell described by Wang et al., 1995. With this cell an ISM is advanced across the colored membrane and allowed to "clear" before potentials are measured.

to push permeant ions in the same direction. The streaming potential is the electrical potential that can just balance this effect leading to zero current through the pores. The number of water molecules, η, coupled to ion transfer through the pores can be calculated directly from the streaming potential.

Although the origin of the streaming potential is conceptually simple, it is difficult to measure because the imposition of the osmotic gradient produces two other effects that also change the measured potential. The impermeant solute added to create the osmotic gradient alters the activity of the permeant ion in the solution to which it is added, and the flow of water across the membrane leads to accumulation of solutes on one side of the membrane and depletion on the other. The resulting concentration gradient of permeant ion leads to a potential of the same polarity as the streaming potential. This potential can be much larger than the streaming potential itself.

In this study streaming potentials have been measured using cation-selective microelectrodes placed close to the membrane. If the electrodes were ideally cation-selective, these measurements extrapolated to the membrane surfaces would yield the true streaming potential. Non-ideal behavior would lead to an overestimate of the streaming potential and hence to an overestimate of η (see Appendix).

Between 3 and 300 mM the values for η show no systematic trend, nor are there clear differences between Na^+, K^+, and Cs^+ (see Fig. 3). Our value for K^+, 6.6, is close to those previously reported: 6.4 (Rosenberg and Finkelstein, 1978) and 7.1 (Levitt, 1984) as is that for Cs^+, 7.3 compared to 6.7 (Rosenberg and Finkelstein, 1978). As reported by Rosenberg and Finkelstein (1978) but not by Levitt (1984), the value for Na^+ is similar to that for K^+, 7.1 and 6.6, respectively. There are now three different methods for determining η. Levitt et al. (1978) and Levitt (1984) based their calculations on an extrapolation of the measured potential difference backward in time to the instant at which osmotic flow begins across the membrane. They reasoned that the concentration changes in the unstirred layers develop progressively over time, while the streaming potential is produced immediately. Levitt et al. extrapolated backward over minutes. Levitt (1984) improved the technique so that measurements could be taken within seconds. He also used an ion-selective electrode to correct for the change in ion activity in the bulk solution caused by the addition of the impermeant solute.

Rosenberg and Finkelstein do not mention any time dependence of the measured potentials even though initially these must have varied. They measured potential differences across small membranes separating two comparatively large aqueous phases. With their geometry stirring by natural convection should allow the concentrations in the unstirred layers to reach an apparent steady state; thus their measured potential in the presence of gramicidin should represent the sum of at least three terms, the streaming potential, a change in the cation activity on one side caused by the addition of urea, and the concentration changes near the membrane caused by the volume flow. They corrected for the additional contributions by measuring the potentials in separate experiments in which the membrane was made conducting by the carriers valinomycin or nonactin instead of gramicidin. If, as is widely believed, these carriers transport bare cations without water, then the streaming potential in these control experiments is zero. Their value for the streaming potential is obtained by subtracting the potential measured with the carrier from that measured with gramicidin.

In the present study it is shown that the streaming potential can be measured using the potentials measured with ion exchange electrodes extrapolated to the membrane surfaces. If the electrodes were ideal, this measurement could be made at any time after the imposition of the osmotic flow; however, minimization of the effects of non-ideality requires that the measurement be made before the potentials due to concentration changes are large. A major reason for not crossing the membrane with ion-selective electrodes for most of the data presented in this study was the possible shunting of the streaming potential by the thin glass wall of the ISMs near their tips, particularly for the high resistivity of Na^+ liquid ion-exchanger (see Tripathi et al., 1985). It is possible that the slightly lower values obtained for 100 and 300 mM K^+ could be on account of this shunt.

The observations with the ion-selective electrodes and those based on subtraction of potentials measured in separate experiments with gramicidin and the carriers are in good agreement. The observations based on rapid measurement of the electrical potential agree for K^+, but do not for Na^+. At present the reason for the discrepancy is unknown. Our data and those of Rosenberg and Finkelstein suggest that Na^+ entry and exit from the channel occurs by a mechanism that is qualitatively similar to that for K^+ and Cs^+.

In the limit of low ion concentrations, ions are transported independently of each other and η must reflect the number of water molecules transported per ion by this mechanism. At higher ion concentrations at which the pore is almost always occupied by at least one ion, η should equal the

number of water molecules trapped between the ions in a doubly occupied pore (Levitt et al., 1978; Rosenberg and Finkelstein, 1978; Wang et al., 1995). The transition between these two limits is expected to occur (Hladky and Haydon, 1984; Wang et al., 1995) in the concentration range investigated here for cesium and potassium. Over this range there is little change in the observed values of η in any of the studies. The origins of the previously reported decrease in η at higher concentrations are still unknown.

Streaming potentials have been measured in a variety of membrane channels either reconstituted into lipid bilayers (Miller, 1982; Alcayaga et al., 1989; Pottosin, 1992; Tu et al., 1994; Ismailov et al., 1997) or in membrane patches (Homblé and Véry, 1992). The primary motivation of many of these studies has been to obtain an indication of the length and width of the selectivity filter in these channels from the measurements of η. Most have used the procedure introduced by Miller (1982) in which valinomycin is introduced at the end of the experiment to allow correction for the potentials other than the streaming potential that result from the imposition of an osmotic gradient. The demonstration here that similar results are obtained using a valinomycin correction and direct measurement of the potential using ISMs lends support to the validity of this procedure.

APPENDIX

Calculation of the number of water molecules transported per cation, η, from potentials measured using ion-selective microelectrodes

Levitt et al. (1978) have provided the equations for the calculation of η from measured electrical potentials when the molalities of the permeant cation are kept constant. In the present method ion-selective microelectrodes (ISMs) are used to measure the driving potential for the permeant cations rather than the electrical potentials. By using a minor extension of the notation of Levitt et al. (1978) and closely following their derivation, the differences in electrochemical potential for the cations and water can be written as

$$\Delta\mu_c = zF\Delta\Psi + \frac{RT}{zF}\ln\frac{a''}{a'} \equiv zF\Delta\Psi_e \tag{4}$$

and

$$\Delta\mu_w = RT\ln\frac{X''_w}{X_w} \cong RT\ln(1 + \bar{V}_w\Delta m_i) \cong \bar{V}_w\Delta\pi \tag{5}$$

where $\Delta\Psi$ is the electrical potential difference, a'' and a' are the ion activities, $\Delta\Psi_e$ is the driving potential (which would be measured by ideal ion-selective electrodes), X''_w and X_w are the mole fractions of water, $\bar{V}_w$ is the partial molar volume of water, Δm_i is the difference in molalities of the impermeant solute (here urea), and $\Delta\pi$ is the difference in osmotic pressure. Still following Levitt et al., the dissipation function and linear flux equations of irreversible thermodynamics can be written

$$\begin{aligned}\Phi &= J_w\Delta\mu_w + J_c\Delta\mu_c \\ &= J_v\alpha\Delta\pi + I\Delta\Psi_e \\ J_v &= L_{11}\alpha\Delta\pi + L_{12}\Delta\Psi_e \\ I &= L_{12}\alpha\Delta\pi + L_{22}\Delta\Psi_e\end{aligned} \tag{6}$$

where J_w and J_c are the fluxes of water and permeant cation,

$$J_v = J_w\bar{V}_w + J_c\bar{V}_I \tag{7}$$

is the volume flow, $\bar{V}_I$ is the volume change in solution when a cation passes through the membrane, $I = zFJ_c$ is the current, the L's are the phenomenological coefficients, and using

$$\eta = \frac{J_w}{J_c} \tag{8}$$

$$\alpha = \frac{J_w\bar{V}_w}{J_v} = \frac{\eta}{\eta + \bar{V}_I/\bar{V}_w}. \tag{9}$$

In the absence of an osmotic gradient

$$\left(\frac{J_v}{I}\right)_{\Delta\pi=0} = \frac{J_w\bar{V}_w + J_c\bar{V}_I}{zFJ_c} = \frac{\bar{V}_w}{zF}(\eta + \bar{V}_I/\bar{V}_w) = \frac{L_{12}}{L_{22}}$$

while at zero current

$$\left(\frac{\Delta\Psi_e}{\Delta\pi}\right)_{I=0} = -\frac{L_{12}\alpha}{L_{22}} = -\frac{\bar{V}_w}{zF}\eta. \tag{10}$$

There are three experimental difficulties: the offset of the ISMs must be set in some standard condition, the ISMs are non-ideal, and the electrodes cannot be positioned immediately at the membrane surfaces. A standard condition is conveniently set by breaking the membrane with the electrodes in their final positions.

The potentials reported by ISMs at the two surfaces of the membrane are

$$\Psi_{is} = \Psi' + SF_a\frac{RT}{F}\ln\frac{a'}{a_{refa}} \tag{11}$$

and

$$\Psi''_{is} = \Psi'' + SF_b\frac{RT}{F}\ln\frac{a''}{a_{refb}} \tag{12}$$

where SF_a and SF_b are the calibration slope factors for the electrodes and a_{refa} and a_{refb} are the (unknown) reference activities at which the electrode potentials equal the respective electrical potentials. The difference between these ISM potentials,

$$\Delta\Psi_{is} = \Psi''_{is} - \Psi'_{is}, \tag{13}$$

is measured just before and shortly after breaking the membrane. After breaking the membrane both the electrical potential and the cation activity, a_0, seen by the ISMs will be the same and

$$\Delta\Psi_{is} = SF_b\frac{RT}{F}\ln\frac{a_0}{a_{refb}} - SF_a\frac{RT}{F}\ln\frac{a_0}{a_{refa}} \tag{14}$$

The difference between the values of Ψ_{is} before and after breaking the membrane is then

$$\Delta(\Delta\Psi_{is}) = \Delta\Psi_e + (SF_b - 1)\frac{RT}{F}\ln\frac{a''}{a_0} + (SF_a - 1)\frac{RT}{F}\ln\frac{a_0}{a'} \tag{15}$$

The second and third terms on the right-hand side account for the less than ideal response of the ISMs. The measured slope factors for our electrodes were always >55/58 and were the same for the pairs of electrodes used in these experiments. Thus, for a large accumulation and depletion of the cations with $RT/F\ln a'/a'' = 5$ mV and our worst electrodes, the error in

the estimate of V_s caused by non-ideality of the electrodes would be 0.25 mV, which corresponds to an overestimate of the number of water molecules coupled to transport of an ion, η, by 1. This correction has been ignored.

In practice the ISMs cannot be placed at the membrane surfaces. The potential has therefore been measured 25 and 50 μm from each surface and the potential at the surface estimated as

$$\Psi_{is}(0) = \Psi_{is}(25) + [\Psi_{is}(25) - \Psi_{is}(50)]. \qquad (16)$$

In several experiments it was confirmed that the increment between 75 and 50 μm was the same as that between 50 and 25 μm. In theory the variation should become steeper close to the membrane. Use of a linear extrapolation should therefore underestimate the correction, which in turn would lead to an overestimate of η.

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