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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 Ion Concentration

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ABSTRACT

The conductance-voltage (G-V) characteristic of a single-filing, multi-barrier, multi-occupancy channel depends in the limit of low ion concentration upon only two parameters: the voltage dependence of the entry step and the ratio of the rate constant for leaving the channel to that for crossing its middle (14,17,20). We show that the G-V shape in this low concentration limit can be measured accurately using a triangular wave, many-channel technique and demonstrate that the observed shape is incompatible with that expected if the only important rate limiting barrier at low concentration were at the channel mouth. Instead the central barrier turns out, surprisingly, in view of the markedly sublinear I-V shape at low concentration, to be even slightly larger than the exit barrier. Additionally, we find that it is not possible to fit both the G-V shape and the concentration dependence of the zero-current conductance simultaneously with a 3-barrier 2-site model. However, by adding additional sites to yield a 3-barrier 4-site model either of the type 3B4S" where the extra site in each channel half is external to the mouth of the channel or of the type 3B4S' where the extra site is internal to the mouth of the channel, we obtain good agreement. Additionally, using the flux ratio data of Procopio and Andersen (19) to discriminate between 3B4S' and 3B4S" models, we find the 3B4S" model to be the only satisfactory one.

INTRODUCTION AND THEORETICAL EXPECTATIONS

The shape of the current-voltage (I-V) characteristic of an ion channel gives information on its energy profile (i.e. the heights and the locations in the electrical potential field of the barriers); and the changes in G-V shape with ion concentration give information about the depths and locations of the wells (e.g. the binding constants of the sites) as well as about shifts in energy profile for different loading states. Läuger (17), analyzing the con-

centration dependence of the G-V characteristic for singly-occupiable multi-site, multi-barrier channels, was the first to point out that if the surface barriers were lower than the internal barriers, the current would increase "supralinearly" (i.e. faster than the voltage); whereas if the surface barriers were sufficiently high compared with the internal barriers, the current would increase "sublinearly" (i.e. at a lower rate than the voltage). Extending these considerations to channels occupiable by a second cation, Hladky et al. (15,16, 25) explained the dependence of the shape of the G-V characteristic of the gramicidin channel (sublinear at low ion concentrations and supralinear at high) by assuming that at low ion activities the entry step was rate determining (the flux being limited by the frequency with which ions arrive at the channel); whereas at high ion activity transfer across the middle of the channel was assumed to become the slow step. These authors concluded (16) that there was good agreement between measured single channel currents and those theoretically calculated according to a 3-barrier 2-site (3B2S) model. However, as will become evident below, the concentration range over which this comparison was carried out was too restricted to define the true limiting shape. Indeed, because the changes in G-V shape are not particularly striking for the usual concentrations (3,4,16), it was initially believed (17) that the G-V characteristic of the single channel was only "slightly sublinear at low ion concentrations and linear or slightly superlinear at saturating ion concentration". This conclusion is erroneous since we have recently shown (8,13) that the concentration dependence is as strong for the gramicidin channel as for typical carriers (cf. Fig. 1A).

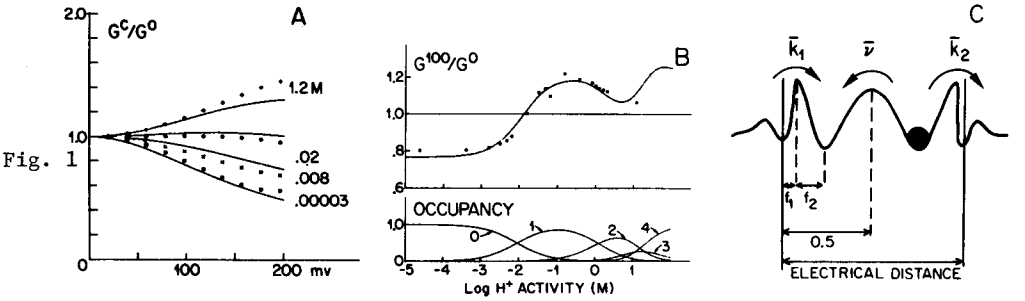

Fig. 1

Although the details of the concentration dependence of G-V, when taken together with the concentration dependence of single channel conductance and of the flux ratio exponent, can enable one in principle to discriminate between alternative models (8,13), many adjustable parameters are involved. A drastic simplication results in certain limiting cases. In particular, it can be shown quite generally (14,20) for single-filing, multi-barrier, multi-occupancy channels that in the limit of low ion concentration the shape of the G-V characteristic is given simply by

$$\left(\frac{G^c}{G^o}\right)_{C\to 0} = \frac{1 + \bar{k}_2/2\bar{\nu}}{\cosh\,(0.5-f_1)\,U + \bar{k}_2/2\bar{\nu}} \cdot \frac{\sinh\,U/2}{U/2} \qquad (1)$$

Where (C^c/G^o) is the chord conductance divided by the zero current conductance, called hereafter the "normalized chord conductance" and U is the voltage (in RT/F units). G^c/G^o as a function of U is seen to depend on only two parameters: f_1, the voltage dependence of the entry step, and $\bar{k}_2/\bar{\nu}$, the ratio of the rate constant for leaving the channel ($\bar{k}_2$) to that for crossing its center ($\bar{\nu}$), as defined in Figure 1C.*

At somewhat higher (but still low) concentrations (e.g. where the channel is occupied by one ion) the G-V characteristic is given by:

$$\left(\frac{G^c}{G^o}\right)_{C=low} = \frac{1 + \bar{k}_2/2\bar{\nu}}{a + b\cdot\bar{k}_2/2\bar{\nu}} \cdot \frac{\sinh\,U/2}{U/2} \qquad (2)$$

$$a = \cosh\,f_1U\cdot\cosh\,(0.5-f_1-f_2)U$$

$$b = \cosh\,(f_1+f_2)U$$

The voltage dependence of the exit step (f_2) now enters. The transition between the G-V shapes of equations 1 and 2 occurs at half occupancy of the first site (14,20), as can be seen in Fig. 1B by the correspondence in change of G^{100}/G^0 with the occupancy; and the plateau at 1-ion occupancy is very clearly seen for Tl in Fig. 5 (the explicit relation to the occupancy will be found in Fig. 6 of (8)). The high (but not infinite) concentration limiting G-V characteristic is given by:

$$\left(\frac{G^c}{G^o}\right)_{C\to high} = \frac{1 + \bar{k}_1C/2\bar{\nu}}{\cosh\,(0.5-f_2)U + \bar{k}_1C/2\bar{\nu}} \cdot \frac{\sinh\,U/2}{U/2} \qquad (3)$$

which now contains the rate constant of the entry step for a second ion to enter a singly occupied channel ($\bar{k}_1$), as well as the aqueous ion concentration, C. The only voltage dependence is that for the exit step (f_2). At infinitely high concentration equation 3 reduces to the simple limit

$$\left(\frac{G^c}{G^o}\right)_{C\to\infty} = \frac{\sinh\,U/2}{U/2} \qquad (4)$$

where the supralinear behavior reflects the fact that the central barrier is indeed rate-determining because, as noted by Hladky et al. (15,16), the entry rate is infinite.

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*This limit is consistent with the corresponding one in Läuger's single-occupancy (17) treatment, although f_1 and f_2 do not appear explicitly owing to his assumption of a regularly spaced symmetrical barriers. His treatment allows for multiple internal barriers of equal height instead of the single central barrier used here.

It should be realized that a "supralinear" G-V characteristic at high concentrations does not necessarily signify that the central barrier is rate-determining. Consider the situation in eq. (3) where $\bar{v} \to \infty$ (i.e. where the central barrier is negligible), in which case eq. (3) simplifies to

$$\left(\frac{G^c}{G^o}\right)_{C\to high} = \frac{\sinh U/2}{\frac{U}{2}\cosh\,(0.5-f_2)U} \qquad (5)$$

which is supralinear whenever $f_2 > 0.2$ and sublinear when $f_2 < 0.2$. Thus, even a channel with barriers only at its ends can yield a supralinear G-V characteristic for reasons that have nothing to do directly with the central barrier becoming rate-determining. In this paper we concentrate on the limiting behavior at the lowest ion concentration and interpret the results with pure barrier models. We show that the G-V shape in this limit is incompatible with a model in which the only barriers are at the ends of the channel. Instead, our data indicate that the central barrier is larger than the barrier for exit. Since the relative heights of the barriers are found to depend on permeant ion species but are independent of very large ionic strength variations (e.g. as produced by 1M CsCl in the presence of 2M $Ca(NO_3)_2$), we argue that diffusional resistances at the mouth of the channel (2) are unimportant and that pure barrier models are appropriate. Finally, combining the observed G-V shape and its concentration dependence with existing data for single channel conductances, we show that it is not possible to describe these two electrical properties with a 3-barrier 2-site model, in contrast to the contention of Hladky et al. (16,25). However, a 3-barrier 4-site model with the additional sites external to the outermost barrier can reconcile the electrical data.

METHODS

Lipid bilayers were formed on a teflon aperture by the air bubble technique (24) with particular care being taken to add lipid as symmetrically as possible. The lipids used (usually at a concentration of 25 mg per milliliter in n-decane or n-hexadecane (Analabs, Spectroscopic)) were: GMO (glycerol-monooleate, Sigma), GME (glyceryl monoleylether, synthesized by H. Eibl), PE (bacterial phosphatidyl ethanolamine, Supelco). Valine Gramicidin A was a gift from Erhard Gross. Salts were as specified in (18). All measurements were made at 23°C ± 0.5°C.

Previous multi-channel I-V measurements appear to have been largely restricted to the use of brief pulses from which "instantaneous" current voltage curves have been measured (3-5) although the ramp technique has been used as a standard procedure with carriers from the earliest studies (6,22,23). A single triangular ramp of voltage was applied through a pair of chlorided Ag plates

(3 cm^2 area) using an IEC Model F74 function generator via a voltage divider and the resulting current was measured with a Keithley Model 427 amplifier (rise time usually .01 msec.). The transmembrane p.d. between a second pair of chlorided Ag wires close to the membrane was measured with a floating Keithley Model 604 differential amplifier. A magnetic reed switch enabled the current amplifier to be removed from the circuit for measuring the open circuit membrane potential (V^o). Voltage and current were digitized and stored on magnetic tape with a Nicolet Model 1090A digital oscilloscope and a Kennedy Model 9700 tape recorder. Typical I-V records, written directly out from the tape onto an X-Y plotter, can be seen at the left in Figures 2 and 3. The I-V limb from -220 to +220 MV was read into an IBM 3033 computer, "computer filtered" to remove high frequency noise and fitted to a 5th order polynomial for convenient storage using a non-linear least squares regression (6 coefficients sufficed to describe the I-V characteristic indistinguishably from the original record). Using the measured value of V^o and the corresponding current at zero p.d. (I^o) the "normalized chord conductance" G^c/G^o as a function of voltage was easily computed.* Typical G-V curves are seen at the right in Figures 2 and 3 and show symmetry, frequency-independence, and independence of channel density.

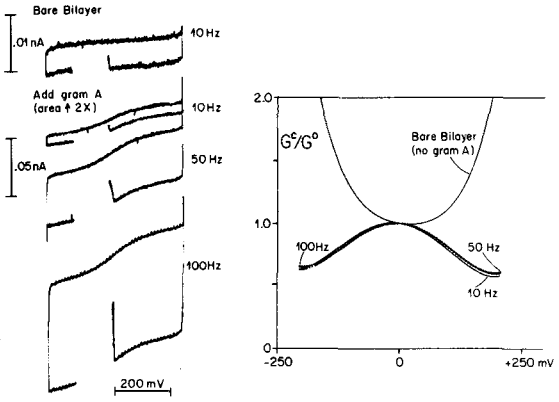

Fig. 2. Effect of gramicidin on the I-V characteristic of the bare bilayer. After the initial record, gramicidin was added to the aqueous solution on both sides of the membrane and the I-V's were recorded successively. Computed curves of G^c/G^o, corresponding to these I-V's, are presented at the right. 0.1 mM KCl, 9 mM $MgSO_4$, PE/Dec.

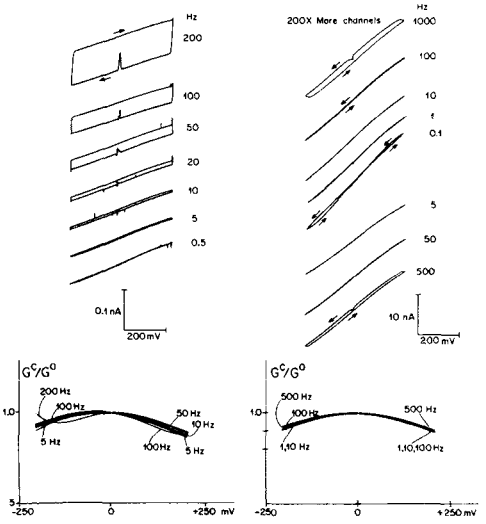

Fig. 3. I-V behavior at two widely differing gramicidin concentrations. Notice at the right that a significant hysteresis, due to an increase in the number of channels (3)) occurs only at frequencies of 0.1 Hz and below. 300 mM KCl, 9 mM $MgSO_4$, GMO/HxDec.

*When capacitative distortion (1,3,22) is present it is possible to find the symmetry point of the curve around the true V^o by correcting for capacitative currents. An effective procedure was to compute the symmetry point for data between $\pm$20 mV and recalculate G^o and V^o for this. Occasionally a true membrane asymmetry was observed but such I-V's have not been used. Small asymmetries in our usual records (cf. the typical records of Figures 2 and 3) were corrected for by using the geometric mean of the negative and positive limbs.

The usual useful range of frequency independence for GMO/HXDec and GME/HXDec was 1->200 Hz. For GMO/Dec and PE/Dec 20->200 Hz proved satisfactory. Usually ionic strength was maintained at 9 millimolar with $MgSO_4$ which not only screened surface charge but also lowered the electrical resistance of the solution. We have found (10) that the "blocking" divalent cation Ca^{++} (4), in fact, actually merely reduces the effective concentration of permeant cation to its low concentration limit as judged by the ability of the calcium ion to cause the G-V characteristic at any permeant ion concentration to become that characteristic of the permeant ion in its low concentration limit (cf. Δ's in Fig. 5), presumably by electrostatic repulsion. This provided an alternative way of measuring the limiting shape at low effective permeant ion concentration. The G-V characteristic in GMO bilayers was found to be identical whether decane or hexadecane was used, and was also identical for GME (cf. Cs in Fig. 5). Significant differences existed in PE bilayers at higher salt concentrations (cf. K in Figs. 5 vs. 4), which will be analyzed elsewhere (10), but not in the limiting shape at low concentration (cf. Fig. 6). Freshly formed membranes and membranes aged more than 30' showed virtually identical G-V characteristics up to 200 mV.

RESULTS

The most important control of the correctness of single-channel I-V shape inferred using the many-channel technique is, of course, the direct comparison with single-channel measurements at comparable ionic concentrations. Fortunately, a number of such measurements exist in the literature, although not, of course, at the lowest ionic concentrations; and excellent agreement between our new many-channel measurements and existing single channel data for Cs^+, K^+, and Na^+ can be seen in Figs. 4 and 5.

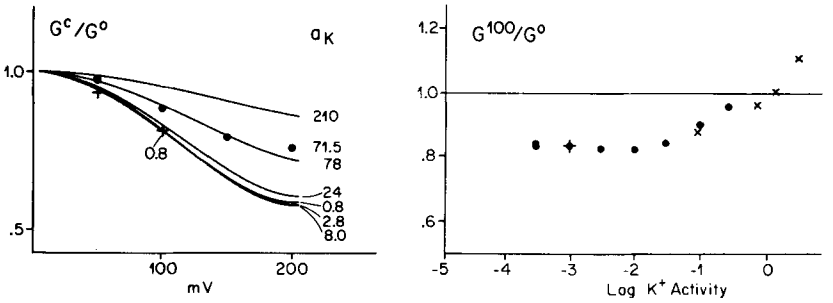

Fig. 4.

The I-V behavior of the gramicidin A channel for K in GMO/HXDec bilayers is summarized in Figure 4, which compares at the left our many-channel measurements (curves) of normalized chord conductance vs. voltage vs. published values (dots) for the single channel of Hladky et al. (16) at 71.5 mM. Note that for measurements at comparable concentrations (cf. 71.5 and 78 mM), the shape of

the G-V characteristic is the same by both many-channel and single-channel techniques. The right-hand figure gives the K concentration dependence of the G-V shape, in terms of the "normalized chord conductance" at 100 millivolts. The dots are our many-channel data; the X's those of Hladky et al. (16) for single channels; and the +'s in both portions of the figure present noise measurements of single-channel G-V shape at a_K = 0.8 mM by Neher, Eisenman and Sandblom (unpublished), which can be seen to substantiate the G-V shape deduced by the many-channel technique at this low concentration.

A similar agreement is illustrated for Cs and Na in Figure 5, which for reasons of space presents only the G^{100}/G^{o} data. Figure 5 also shows, as in Fig. 4, that it is indeed possible using the many channel technique to find a concentration below which the shape of the G-V characteristic is independent of ion concentration.

The limiting G-V shapes at low concentration of various permeant cations are summarized in Figures 6-8. Figure 6 presents data for K in GMO/HXDec, as well as PE/Dec, bilayers and compares the shape with the expectations of eq. 1 if the central barrier were not significant ($\bar{k}_2/\bar{\nu}$=0), in which case f_1

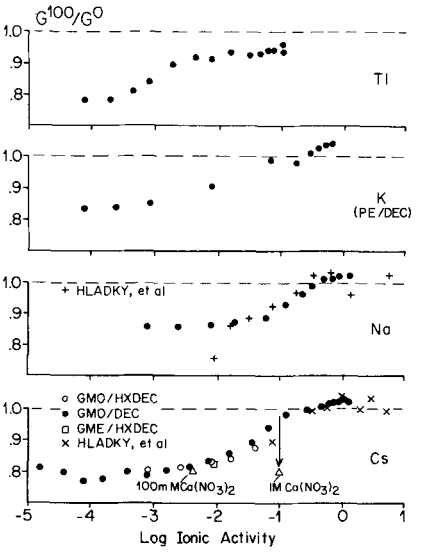

Fig . 5. Many-channel (0, ●, ■) and single channel (+,x) data. Tl (GMO/HXDec). Na (GMO/Dec). For Cs, various lipids can be compared, as well as the effect of the indicated concentrations of Ca (Δ).

is the only adjustable parameter. Clearly, it is not possible to represent the experimental data adequately with a model in which only the outermost barriers are significantly rate determining.

In contrast, Figure 7 illustrates that, by allowing for a significant inner barrier, the experimental data points are nicely representable by theoretical curves drawn according to eq. 1 for the indicated values of $\bar{k}_2/\bar{\nu}$. Except for Na^+ at higher voltages, all data are best fit with no voltage dependence of the entrance step (solid curves, f_1=0). The dashed curve shows the effect of a 3% voltage dependence of the entry step (f_1 = .03) in the case of Na^+. The differing values of $\bar{k}_2/\bar{\nu}$ show that the species differences reflect the ease with which the ions cross the central barrier relative to leaving the channel. Among the alkali cations, the least permeant species, Na, clearly is also the least "sublinear". K and Na find it increasingly easier to leave the channel than to cross it compared to Cs.

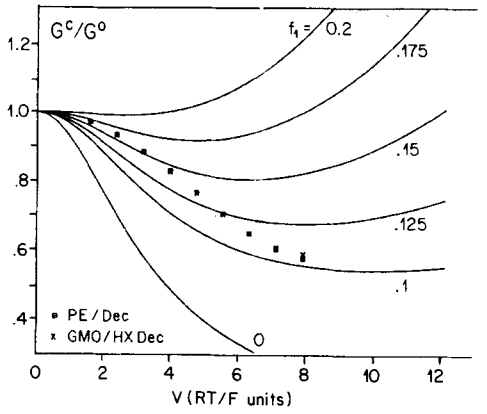

Fig. 6. 10 mM K in PE/Dec GMO/HXDec (X) 0.3 mM K in PE/Dec (□). 9 mM $MgSO_4$.

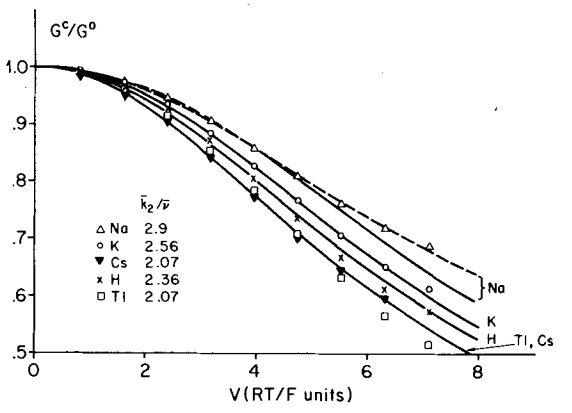

Fig. 7. Solid curves (f_1=0); dashed curve (f_1=.03). Na (3 mM, GMO/Dec), K (10 mM, GMO/HXDec), Cs (0.1 mM, GMO/Dec), Tl (0.25 mM, GMO/HXDec), H (.03 mM, GMO/HXDec). 9 mM $MgSO_4$.

The results of Figures 4-7 indicate, at the very least, that more than one barrier is needed in any model which hopes to account for the shape of the G-V relationship at low ion concentration in the gramicidin channel. Here we have assumed that the additional barrier is likely to be in the middle of the channel. This assumption is not in conflict with Finkelstein's and Andersen's (12) statement "that there is no significant electrostatic barrier to ion movement between the energy wells at the two ends of the channel" because our, purely formal, central barrier can result from the summation of many smaller barriers which could represent not only the energy of moving the ion but also the kinetic limitations involved in the coupled movements of water molecules.*

DISCUSSION

The finding of a significant central barrier even at the lowest concentrations has implications for the behavior of the channel at higher concentrations. This can be seen from Figure 8 which demonstrates for Tl and H the inadequacy of a 3-barrier 2-site model (left and middle figures for each ion) to fit simultaneously the limiting shape of the G-V characteristic at low salt concentration (upper) and the concentration dependence of the single channel conductance at low voltage (lower). One can either fit the G-V shape (upper) or the concentration dependence of conductance (lower), but not both. In contrast,

*Although additional barriers (e.g. for diffusional access to the channel mouth suggested by Andersen (2) cannot be excluded rigorously, a pure barrier model is not only the simplest but is supported by the species differences of relative barrier heights implied by Fig. 7, which are not easily explained by additional diffusional barriers. Also, the same limiting shape is observed at vastly higher salt concentrations in the presence of Ca^{++}. Indeed, the G-V shape characteristic for each ion at its low concentration limit is also observed at a higher permeant ion concentration when the channel is maximally blocked by Ca, as can be seen from the Δ's for Cs in Fig. 5.

adding 2 additional sites enables a very satisfactory fit to both sets of measurements, as can be seen at the right for each ion.* The additional sites can be added either internal to the barriers at the channel ends (3B4S' model (14)), as illustrated here, or external to these (3B4S" model (20)) for which a partial comparison can be found in Fig. 1 and a full comparison has been given (9,11) elsewhere for H.

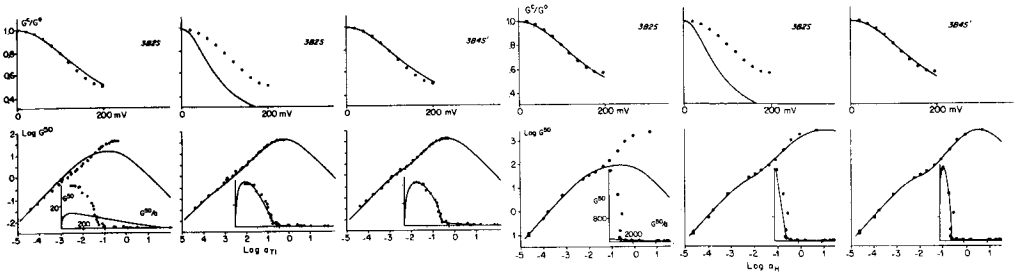

Fig. 8. Each vertical pair of figures compares the observed data points with theoretical curves for the indicated model. The left hand pair for TlF (left) and HCl (right) shows that if the 3B3S model is fitted to the G-V shape, the conductance-concentration behavior is not well fitted. The middle pair shows that if the conductance-concentration behavior is fitted for the 3B2S model, the G-V shape is wrong. In contrast, the right hand pair shows that a 3-barrier 4-site (3B4S') model fits both behaviors satisfactorily.

We therefore conclude that a 3-barrier 2-site model, despite contrary contentions (16,25), is <u>not</u> adequate for the electrical behavior of the gramicidin channel; whereas a 3-barrier 4-site model of either of the types 3B4S" or 3B4S' can account for all existing electrical measurements on all ions studied so far. To choose between these models, the concentration dependence of the flux ratio (21,19) is crucial, the differences between the two 3-barrier 4-site models being the expectation that the flux ratio exponent cannot exceed 2 for 3B4S" whereas for 3B4S' it can be as high as 4. Although we previously concluded (8,13) from Lev's early measurements (21) on Rb that the 3B4S' model was likely to be correct, now, relying upon Procopio's and Andersen's more definitive data (19) for Cs, Rb, and Na in an uncharged lipid, we conclude that the 3B4S" model of Fig. 1C is the only 4-site model which satisfactorily represents these data.

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* The additional sites also give a better fit to the details of shape as seen most easily in the Eadie-Hofstee type plots in the inserts.

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