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ATYPICAL GRAMICIDIN A CHANNELS APPEAR TO HAVE INCREASED FIELD STRENGTH AT ONE BINDING SITE

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The population of discrete transmembrane channels formed by gramicidin A, whether purified or synthetic, exhibits a heterogeneous distribution of conductances characterized by a single narrow peak typically containing 65% of the channels, together with a broad dispersion of the remaining channels encompassing all conductance values below the main peak. The "standard" channels in the narrow main peak appear to result from NH_2-terminus to NH_2-terminus dimerization of left-handed, single-stranded $\beta^{6.4}$ helices (Urry et al., 1983, Bamberg et al., 1978, Weinstein et al., 1980). The "variant" channels in the low-conductance range were initially ignored (Hladky, 1972) and later attributed to the presence of double-stranded helical conformers (Ovchinnikov and Ivanov, 1977; but see Bamberg et al., 1978). Consideration of hydrogen-bonding patterns in the various dimer structures indicates that double-stranded channels should have much longer lifetimes than single-stranded, end-to-end dimer channels (Bamberg et al., 1978). We have shown that the variants have approximately the same lifetimes as standard channels, implying that variants must also be comprised of end-to-end combinations of single $\beta^{6.4}$ helices. That standard and variant channels are structurally homologous is further supported by our observation that standard channels may spontaneously change to a variant conductance state and revert back to the standard state (Busath and Szabo, 1981). It is difficult to imagine double-stranded to single-stranded conformational changes taking place so rapidly and without loss of channel conductance. Thus it is likely that variants result from secondary conformational changes which do not substantially alter the basic helical structure. At present it is not known, however, whether the arrangements of the peptide backbone or the side chains are altered. The hypothesis of altered side-chain conformation is particularly plausible because it readily allows for multiplicity of channel states with discrete interconversions, which are rare due to substantial steric hindrance (Urry et al., 1981).

This paper presents preliminary results concerning the mechanisms by which the energetics of ion permeation are altered for channels in low-conductance states. Specifically, we present evidence that most variants are caused by an increased binding affinity for cations at one end of the channel. The increased binding shows ion specificity that appears to be in qualitative agreement with the predictions of Eisenman's theory (Eisenman, 1962).

METHODS

To assess the transport energetics of individual variant channels, we measured their current voltage (*I-V*) relation. A lipid bilayer was formed on the aperture of a pipette as previously described (Prasad et al., 1982). Val^1-gramicidin A, purified from gramicidin D (ICN Pharmaceuticals, Cleveland, OH) by HPLC and stored in reagent-grade methanol at 3°C, was added to the chamber. Transmembrane current was measured while applying voltage ramps to the membrane from a dispersion (50 mg/ml) of glyceryl monooleate (GMO) (Nucheck) in squalene (Eastman Organic Chemicals Div., Rochester, NY) or hexadecane (Aldrich Chemical Co., Inc., Milwaukee, WI). The single channel *I-V* was obtained by subtracting the *I-V* measured before and after the opening of a single channel from that measured while the channel was open. For any given channel, increasing and decreasing voltage ramps gave identical *I-V*, as expected. These were therefore combined.

In symmetric bathing solutions (i.e., the same solution on both sides of the membrane) all channels have vanishingly small reversal potentials. Standard channels have symmetric *I-V* whereas variant channels, randomly interspersed between standard channels, always have asymmetric *I-V* (Busath and Szabo, 1981). We made use of the frequently employed three-barrier/two-site version of Eyring rate theory (Begenisich and Cahalan, 1980; Urban and Hladky, 1979) to assess variations in the energetics of ion transport through variant channels. In this model, ions are not allowed to cross paths within a channel. Steric restraints, energetic contributions of the aqueous solvent, and repulsion between ions are implicit in the barrier height and ion-ion interaction terms of the model.

RESULTS AND DISCUSSION

Using a nonlinear least-squares algorithm, we first determined parameters which provide good fits to the *I-V* of standard channels in aqueous 3 M, 1 M, and 0.1 M KCL solutions. These are given in Table I. The positions of the barriers and wells in the electric field determine the voltage dependence of the rate constants for ion translocation as suggested by Eyring et al. (1949). In the symmetric standard channel, $\alpha_4 = 1 - \alpha_2$ and $\alpha_5 = 1 - \alpha_1$. The simple two-site/three-barrier model gave a qualitatively reasonable but statistically unacceptable fit ($P < 0.005$). Nevertheless, for the purpose of qualitatively determining the nature of the energetic changes occurring in the variant

TABLE I
STANDARD CHANNEL PARAMETERS

G_1, G_5*	10.8 RT	entry barrier height
G_2, G_4	−1.39 RT	binding site depth
G_3	10.1 RT	central barrier height
α_1	0.0605	entry barrier position in field
α_2	0.154	binding site position
α_3	0.500	central barrier position
DG_1, DG_5	0.272 RT	added entry barrier height when opposite site occupied
DG_w	0.907 RT	decreased well depth during double occupancy

*Refer to the left-hand (G_1) and right-hand (G_5) entry barriers. Other positions are listed analogously.

channels, the simplicity of this model made it preferable to more complicated ones. The standard channel model with initial parameters shown in Table I and allowing only simple combinations of one or two parameters to be altered, was used to fit 21 variant *I-V*. Typical results are illustrated in Fig. 1. The observed *I-V* of a single low-conductance variant channel is shown as circles. Also shown are best-fitting theoretical predictions of the locally modified standard channel. The solid curve shows the effect of deepening the energy minimum on one side and shifting its position in the membrane electric field. Deepening the well alone produces the conductance decrease and

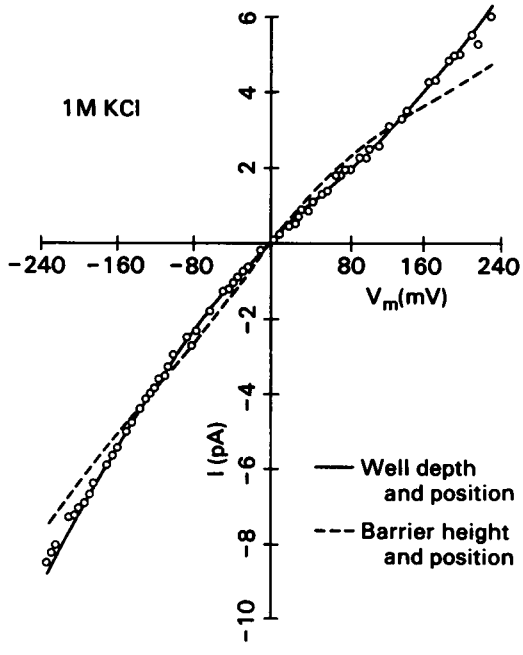

FIGURE 1 Comparison of the measured *I–V* relationship of a variant channel (o) with the theoretical predictions of the standard channel's three-barrier/two-site model, modified only by allowing a single set of physically related parameters to vary. Note the excellent fit resulting from an increased strength of ion binding (—). Altering the height or location of the energy barrier to ion entry could not produce the proper *I-V* shape (---). Similar results were obtained for other atypical *I-V*. GMO-squalene membranes.

rectification. Shifting the well position somewhat improves the approximation to the *I-V* shape. The dashed line shows the best fit when only the entry barrier height and its position are allowed to vary. Again, the predicted *I-V* rectifies in the same direction as the observed *I-V*. It is evident, however, that raising the entry barrier (G_1) does not approximate the shape of the observed *I-V* data as well as the deepened-well fit does.

The difference in the shapes of the fits was observed for all of the twenty other channels examined. This is shown in Table II, which compares the mean-reduced χ-square (the average of the reduced χ-square values from each of the 21 channels) for the two types of fits. The values should be compared to the corresponding goodness-of-fit index of 16.3 for the standard channel model. It is evident that varying the strength of ion binding for each channel (G_2, α_2) provided much better fits, on average, than varying the entry barrier (G_1, α_1). Note that varying the well and its position results in an overall fit equal in quality to the fit of the model to the standard channel.

Some parameters of the standard model are not well constrained by experimental *I-V*. This is particularly true for the well depth, even with the range of concentrations examined. We repeated fits using standard model parameters with deeper wells (down to -6 *RT* units) and arrived at the same conclusions. Thus, despite the approximate nature of the model, our results strongly suggest that the predominant change in variant channel energetics is an increase in ion-binding affinity at one end of the channel.

The equilibrium binding affinity of a site has been related by Eisenman (1962) to its field strength, a useful quantity reflecting electrostatic ion-site interactions. Our fits indicate an increased ion-binding affinity in atypical channels. This may result either from an increase in the partial negative charge of individual carbonyl oxygens that form the binding site or from a closer approach of these to the complexed ion. In either case, the increased field strength would lead to a greater increase in affinity for higher field-strength (i.e., smaller) cations. For instance, Na^+, a high field-strength cation, should experience a larger enhancement in binding strength than Cs^+. To test this expectation, we measured single channel *I-V* under biionic conditions, that is, with 1M NaCl on one side and 1M CsCl on the other side of the membrane. Consideration of the three-barrier/two-site model with the parameters for Na^+ and Cs^+ used by Urban et al. (1980) shows that selective deepening of the sodium well preferentially

TABLE II
GOODNESS OF FIT OF MODIFIED STANDARD CHANNEL MODEL TO VARIANT CHANNEL I-V

Parameter varied	Average χ^2_r
None	3443
G_1, α_1	73
G_2, α_2	17

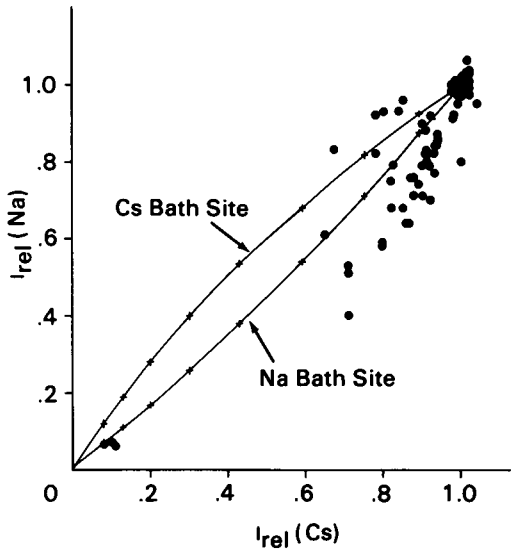

FIGURE 2 The relationship between unidirectional Na and Cs currents in normal and variant gramicidin A channels. Each solid circle represents an individual channel. Solid lines show the locus of points traced by the three-barrier/two-site model for equally increased Na^+ and Cs^+ binding. GMO-hexadecane membranes.

reduces sodium flux. We therefore propose to examine the relative decrease in Na^+-mediated current as compared to Cs^+-mediated current in variant channels as a measure of the ion specificity of the binding-affinity increase. A preferential increase in Na^+ affinity at the site nearest the Na^+ bath has a greater effect on the differential decrease in fluxes than does an increase on the opposite side of the channel. Nevertheless, we show in Fig. 2 validation of the above prediction. The relative Na^+-mediated current measured at 186 mV is plotted against the relative Cs^+-mediated current measured at -194 mV for each of several channels. The majority are grouped together at the value (1,1), these being standard channels. The variants are seen to have lower fluxes for both ionic species. The solid lines are the predictions of the Urban-parameter three-barrier/two-site model when the binding site is deepened equally for sodium and cesium on either the Cs^+ bath (upper curve) or Na^+ bath (lower curve) side of the pore. Points above or below the curves reflect, respectively, a greater increase in cesium or sodium binding provided that the binding affinity was the predominant change, as we concluded above. While a few of the points fall above both curves, the majority (85%) of points falls in a broad group below both curves, consistent with the prediction that sodium binding is increased more than cesium binding. The aberrance of some of the data from this pattern may well reflect an increased Cs^+ preference at one binding site resulting from ion coordination outside the framework of the Eisenman theory. Nevertheless, the presence of the majority of channels in the lower group strongly suggests that for most variant channels, an increased binding affinity on one side of the channel is associated with an increased-site field strength at that location.

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