Amino acid substitutions and ion channel function

Model-dependent conclusions

Murray D. Becker,* Roger E. Koeppe II,‡ and Olaf S. Andersen*

*Department of Physiology and Biophysics, Cornell University Medical College, New York, New York 10021; and ‡Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas 72701 USA

INTRODUCTION

Two questions arise when molecular biological and electrophysiological methods are combined to study channel function: are the mutant and native channels structurally equivalent (cf. Durkin et al., 1990), such that a sequence alteration can be regarded as a "point perturbation" (which usually will be a prerequisite for mechanistic analysis); and, if the answer is affirmative, what is an appropriate molecular interpretation of the functional alterations? We will address the latter question.

The parent compound in these studies, [Val^1]gramicidin A (gA), has the sequence: Formyl-L-Val^1-Gly^2-L-Ala^3-D-Leu^4-L-Ala^5-D-Val^6-L-Val^7-D-Val^8-L-Trp^9-D-Leu^{10}- L-Trp^{11}-D-Leu^{12}-L-Trp^{13}-D-Leu^{14}-L-Trp^{15}-ethanolamine. The alternating L,D sequence permits gA to form channels that are Formyl-NH-to-Formyl-NH dimers of $\beta^{6.3}$-helices (intramolecular helices, in which the peptide groups line the pore whereas the side chains form the exterior surface).

The importance of the Trp residues was shown by Heitz et al. (1982). We pursued this question further by examining gramicidins with one, two, or three Trp → Phe substitutions. The results suggest that each of the Trp side chains has an approximately equal and independent effect on the channel's Na^+ conductance (Becker et al., 1991). To obtain a more detailed understanding, we used a four-state model, denoted 3B2S2I, as the framework for the analysis (Fig. 1; cf Finklestein and Andersen, 1980; Urban et al., 1980). This model was augmented by incorporating interfacial polarization (IP) and aqueous access limitation (DL) (cf. Andersen 1983*a*, *b*). Four models were thus examined: 3B2S2I, 3B2S2I-(IP), 3B2S2I-(DL), and 3B2S2I-(DL,IP).[1]

RESULTS AND DISCUSSION

The initial model selection was based on a gA data set (0.01–5.0 M NaCl, 25–500 mV) with a total of 119 independent data points. We then investigated two sequence-substituted gramicidins: W9FgA and W15FgA, where the numeral denotes the substitution's position (0.1–5.0 M NaCl, 25–500 mV; $n = 83$ for W9FgA, $n = 78$ for W15FgA). The lack of results < 0.1 M NaCl for these channels was due to their lower single-channel conductances. The models were fit to the data by nonlinear least squares. A comparison of the fits shows that using a model that neglects DL and IP not only affects the "goodness of fit," but also the "best fit" rate constants.

3B2S2I-(DL,IP) provides the best description of the gA data set (Table 1; Fig. 2). The smaller W9FgA and W15FgA data sets could not be used to distinguish between the various models because the lack of results below 0.1 M NaCl caused there to be multiple minima with widely varying DL and IP parameters when fitting with 3B2S2I-(DL,IP). Even setting DL and IP to the values from the gA best fit did not allow us to distinguish between 3B2S2I and 3B2S2I-(DL,IP) [Table 2 and Fig. 2]. Based on the gA results, however, 3B2S2I-(DL,IP) is the most appropriate model.

As expected, estimates for κ_{10}^{00} and κ_{00}^{01} are especially sensitive to an incorrectly modeled entry step. By including DL, the ion entry step becomes a composite of two processes: the ion's movement from the bulk phase to the entrance (access limitation), and the ion's direct interaction with the channel's entrance. 3B2S2I-(DL) and 3B2S2I-(DL,IP) assign separate rate constants to each of these steps. In gA channels, ion access is the rate limiting step for ion entry ($\kappa_0 < \kappa_{10}^{00}$). When access limitation is neglected κ_{10}^{00} is largely determined by the access limitation, $\kappa_{10}^{00}(3B2S2I) \approx \kappa_0(3B2S2I\text{-}(DL,IP))$, and one loses the ability to estimate the intrinsic rate constant for ion entry. This is particularly important

Address correspondence to O. S. Andersen, Department of Physiology and Biophysics, Cornell University Medical College, 1300 York Avenue, New York, NY 10021-4896.

[1]The 3B2S2I model has eight independent parameters: five voltage dependent rate constants, two voltage dependencies, and a trapezoidal shape factor for the translocation rate constant (Andersen, 1989). Interfacial polarization is described by the electrostatic model presented by Andersen (1983*a*); this adds one additional parameter IP, the specific capacitance of the membrane. The aqueous diffusion limitation is described by the kinetic model presented by Schurr (1970); this adds one additional parameter (κ_0, a voltage-independent aqueous access rate constant).

0006-3495/92/04/25/03 $2.00

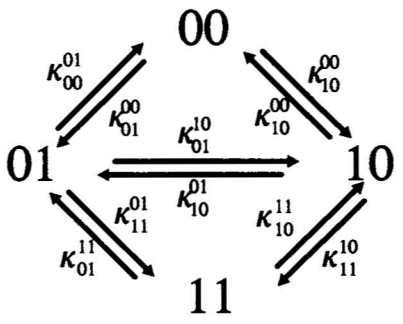

FIGURE 1 State diagram for 3B2S2I. There are four states 00, 10, 01, and 11 corresponding to a channel that is empty, left binding-site occupied, right binding-site occupied, and doubly occupied, respectively. The rate constants are denoted $\kappa_{\text{final state}}^{\text{initial state}}$. Symmetry reduces the number of independent rate constants to five ($\kappa_{10}^{00} = \kappa_{01}^{00}$, $\kappa_{11}^{01} = \kappa_{11}^{10}$, $\kappa_{00}^{10} = \kappa_{00}^{01}$, $\kappa_{10}^{11} = \kappa_{01}^{11}$ and $\kappa_{01}^{10} = \kappa_{10}^{01}$). The rate constants have exponential voltage dependencies that depend on entry (δ_{10}^{00}), exit (δ_{00}^{01}), and translocation ($0.5\text{-}\delta_{10}^{00}\text{-}\delta_{00}^{01}$) electrical distances. The voltage dependent translocation rate constant (κ_{01}^{10}) is modified by a trapezoidal shape factor (e.g., Andersen, 1989).

TABLE 1 Best fits of the 3B2S2I, 3B2S2I-(IP), 3B2S2I-(DL), and 3B2S2I-(DL,IP) models to the gA data set

	gramicidin A			
Parameter	3B2S2I	3B2S2I-(IP)	3B2S2I-(DL)	3B2S2I-(DL,IP)
$\kappa_{10}^{00}/M^{-1}\cdot s^{-1}\cdot 10^7$	7.1	7.4	49.5	41.1
$\kappa_{01}^{10}/s^{-1}\cdot 10^7$	0.7	0.4	0.6	0.7
$\kappa_{00}^{01}/s^{-1}\cdot 10^7$	2.4	0.9	13.9	11.2
$\kappa_{11}^{01}/M^{-1}\cdot s^{-1}\cdot 10^7$	0.2	10.8	2.2	0.7
$\kappa_{10}^{11}/s^{-1}\cdot 10^7$	2.3	119.30	11.1	2.8
$\kappa_0/M^{-1}\cdot s^{-1}\cdot 10^7$	—	—	15.0	8.8
$IP/\mu F\cdot cm^{-2}$	—	1.1	—	1.1
$\delta_{10}^{00} = \delta_{11}^{01}$	0.04	0.01	0.07	0.10
$\delta_{00}^{01} = \delta_{10}^{11}$	0.07	0.10	0.03	0.04
SF	0.03	0.37	0.36	0.15
χ^2	1465	274	1393	140

TABLE 2 Best fits of the 3B2S2I, 3B2S2I-(DL,IP) models to the W9FgA and W15FgA data sets

	W9FgA		W15FgA	
Parameter	3B2S2I	3B2S2I-(DL,IP)	3B2S2I	3B2S2I-(DL,IP)
$\kappa_{10}^{00}/M^{-1}\cdot s^{-1}\cdot 10^7$	8.7	44.6	5.0	7.1
$\kappa_{01}^{10}/s^{-1}\cdot 10^7$	0.2	0.2	0.4	0.5
$\kappa_{00}^{01}/s^{-1}\cdot 10^7$	4.3	2.3	3.2	4.3
$\kappa_{11}^{01}/M^{-1}\cdot s^{-1}\cdot 10^7$	0.02	0.01	0.01	0.1
$\kappa_{10}^{11}/s^{-1}\cdot 10^7$	0.5	0.1	0.2	0.3
$\kappa_0/M^{-1}\cdot s^{-1}\cdot 10^7$	—	8.8	—	8.8
$IP/\mu F\cdot cm^{-2}$	—	1.1	—	1.1
$\delta_{10}^{00} = \delta_{11}^{01}$	0.01	0.03	0.02	0.05
$\delta_{00}^{01} = \delta_{10}^{11}$	0.06	0.05	0.07	0.04
SF	0.03	0.37	0.18	0.08
χ^2	181	185	224	257

when comparing the W9FgA and W15FgA channels with gA channels.

For example, using 3B2S2I, it appears that a position 15 Trp → Phe substitution lowers κ_{10}^{00} by ≈30% (Tables 1 and 2). When using 3B2S2I-(DL,IP), however, it is seen that the position 15 substitution decreases κ_{10}^{00} by approximately sixfold. This larger change is seen because the intrinsic association rate constant is no longer "hidden" by the access limitation. This makes sense because position 15 is the residue closest to the entrance. Replacing the amphipathic Trp by the hydrophobic Phe would constrain the conformational space available to residue 15, which should decrease the rate at which the channel can dehydrate/resolvate incoming ions. Consis-

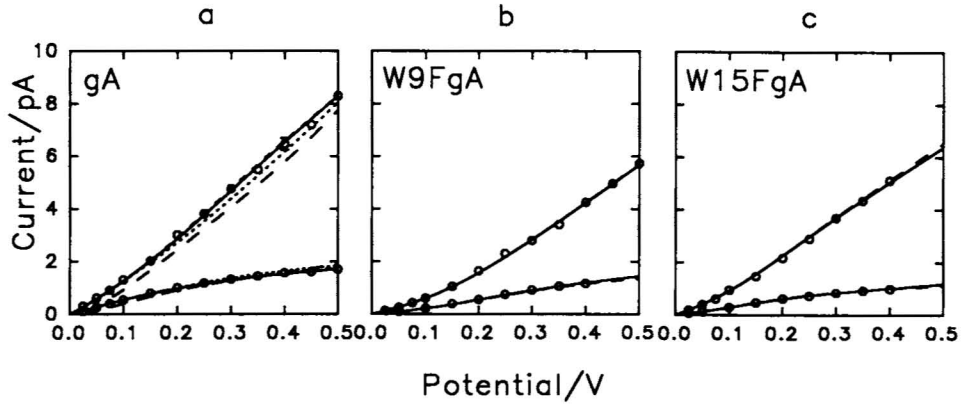

FIGURE 2 Current-voltage relations for (*a*) gA, (*b*) W9FgA, and (*c*) W15FgA in 0.1 and 1.0 M NaCl. Theoretical curves are drawn from the parameters given in Tables 1 and 2. They are denoted 3B2S2I (– – –); 3B2S2I-(IP) (– –); 3B2S2I-(DL) (···); 3B2S2I-(DL,IP) (——).

tent with this picture, the position 9 substitution does not affect entry, in accordance with position 9's greater distance from the entrance.

The estimates for κ_{01}^{10} are much less model-dependent because DL and IP do not directly modify κ_{01}^{10}. The position 9 substitution has a twofold greater effect on the ion's translocation rate constant than the position 15 substitution. Both substitutions decrease κ_{01}^{10} relative to that in gA channels. This is consistent with the notion that the Trp residues facilitate ion movement through the channel by favorable ion-indole electrostatics (Becker et al., 1991).

REFERENCES

Andersen, O. S. 1983*a*. Ion movement through gramicidin A channels. Interfacial polarization effects on single-channel current measurements. *Biophys. J.* 41:135–146.

Andersen, O. S. 1983*b*. Ion movement through gramicidin A channels. Studies on the diffusion-controlled association step. *Biophys. J.* 41:147–165.

Andersen, O. S. 1989. Kinetics of ion movement mediated by carriers and channels. *Methods Enzymol.* 171:62–112.

Becker, M. D., D. V. Greathouse, R. E. Koeppe II, and O. S. Andersen. 1991. Amino acid sequence modulation of gramicidin channel function. Effects of tryptophan-to-phenylalanine substitutions on the single-channel conductance and duration. *Biochemistry.* 30:8830–8839.

Durkin, J. T., R. E. Koeppe II, and O. S. Andersen. 1990. Energetics of gramicidin hybrid channel formation as a test for structural equivalence. Side-chain substitutions in the native sequence. *J. Mol. Biol.* 211:221–234.

Heitz, F., G. Spach, and Y. Trudelle. 1982. Single channels of 9, 11, 13, 15-destryptophyl-phenylalanyl-gramicidin A. *Biophys. J.* 40:87–89.

Schurr, J. M. 1970. The role of diffusion in bimolecular solution kinetics. *Biophys. J.* 10:701–716.

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.