Peptide-chain secondary structure of bacteriorhodopsin. *Biophys. J.* 43:81–89.
12. Salemme, F. R. 1981. Conformational and geometrical properties of β-sheets in proteins III. Isotropically stressed configurations. *J. Mol. Biol.* 146:143–156.
13. Hayward, S., and R. M. Stroud. 1981. Projected structure of purple membrane determined to 3.7 Å resolution by low temperature electron microscopy. *J. Mol. Biol.* 151:491–517.
14. Engelman, D. M., R. Henderson, A. D. McLachlan, and B. A. Wallace. 1980. Path of the polypeptide in bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA.* 77:2023–2027.
15. Cortijo, M., A. Alonso, J. C. Gomez-Fernandez, and D. Chapman. 1982. Intrinsic protein-lipid interactions. *J. Mol. Biol.* 157:597–618.
16. Rothschild, K. J., and N. A. Clark. 1979. Anomalous amide I infrared absorption of purple membrane. *Science (Wash., DC).* 204:311–312.
17. Terwilliger, T. C., L. Weissman, and D. Eisenberg. 1982. The structure of melittin in the form I crystals and its implications for melittin's lytic and surface activities. *Biophys. J.* 37:353–361.
18. Engelman, D. M., and G. Zaccai. 1980. Bacteriorhodopsin is an inside-out protein. *Proc. Natl. Acad. Sci. USA.* 77:5894–5898.
19. Engelman, D. M., and T. A. Steitz. 1981. The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. *Cell.* 23:411–422.
20. Steitz, T. A., A. Goldman, and D. M. Engelman. 1982. Quantitative application of the helical hairpin hypothesis to membrane proteins. *Biophys. J.* 37:124–125.

SINGLE CHANNELS OF VARIOUS GRAMICIDINS

Voltage Effects

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It is now well established that with concentrated aqueous solutions of alkali ions, the single channel conductance of Gramicidin A HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-ValL-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHC_2H_4OH (1) is almost independent on the transmembrane potential (2, 3). Recently, we showed that substitution of the four tryptophyl residues by phenylalanyle leads to an analogue called Gramicidin M which has a single channel behavior strongly different from that of the natural product, although both peptides probably have the same backbone conformation (4). We report here further investigations on this analogue, in particular the voltage effect on the cesium and potassium currents together with the blocking effect of the divalent cations Ca^{++} on the Gramicidin A channel, which also depends on the voltage.

RESULTS

Gramicidin M

Fig.1 shows the Λ–V curves obtained at two different CsCl concentrations. They are in accord with the curve previously reported (4), showing a voltage dependence of the single-channel conductance except in the low-voltage region, where the conductance was underestimated. In Fig. 2 we report the limiting conductance vs. the electrolyte concentration. The shape of the curve strongly suggests that the Gramicidin M channel has a single occupancy state when the Cs^+ concentration is increased up to 3 M, while it becomes doubly occupied for higher salt concentrations. Further, on the basis of the current responses of voltage jump measurements made on highly doped membranes, the experimental values of the current (Fig. 3) are given by the relation $I_o = A \sinh 0.38\, FV/RT$. This means that the electrical distance from the aqueous side to the binding site is 0.12, a value that has to be compared to that reported by Eisenman and Sandblom (5) for Gramicidin A (0.18). Such a result suggests that in both Gramicidin A

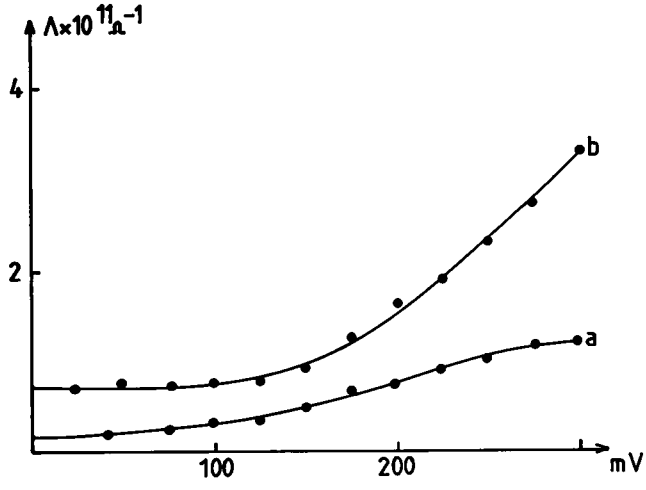

FIGURE 1 Variation with the voltage of the single channel conductance of Gramicidin M (*a*) in CsCl 0.5 M, (*b*) in CsCl 6 M GMO/Decane membranes.

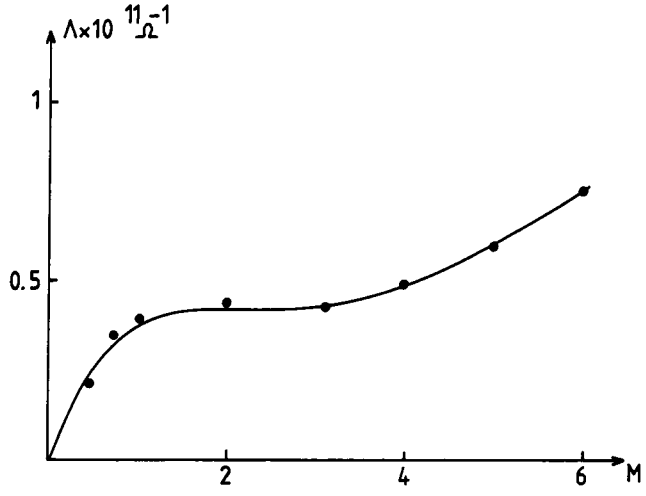

FIGURE 2 Variation with CsCl concentration of the limiting conductance for Gramicidin M.

and M, the internal binding sites are located at comparable distances from the mouth of the channel. Combining both the single channel and the voltage-jump experiments leads to the conclusion that for Gramicidin M, the rate constant of the crossing step is almost the same as that of Gramicidin A, while the rate constant of the exit step of Cs^+ ions from the internal binding site to the aqueous side is increased; the binding of Cs^+ is weaker for the synthetic peptide. Therefore, as it has a potential dependent single-channel conductance, the crossing step becomes rate-determining.

Ca^{++} Blocking Effects

To account for the blocking effect of Ca^{++} ions on the transfer of alkali ions such as Cs^+ and K^+ through the Gramicidin A channel, a model with two Ca^{++}-binding sites is proposed, one site located inside the channel and the other outside, near the mouth. On the basis of the results obtained on symmetrical systems, the ratio of the single

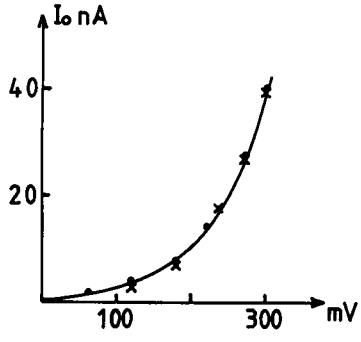

FIGURE 3 Variation with the voltage of the transmembrane current obtained on highly doped membranes (Gramicidin M), • experimental values; x calculated from $I_o = 0.907 \sinh (0.38\ FV/RT)$.

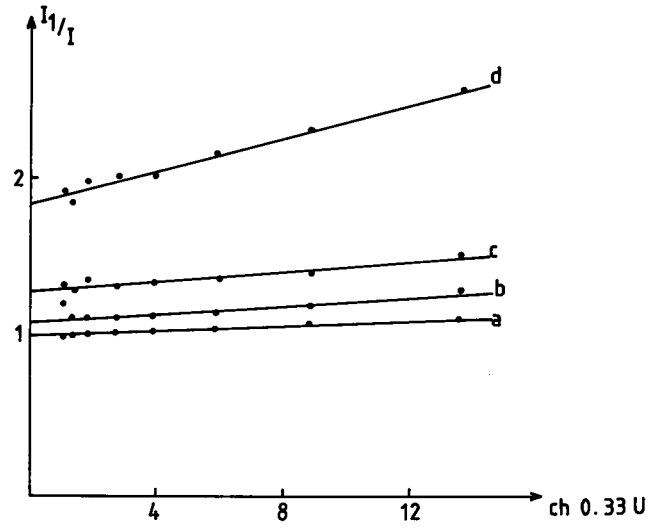

FIGURE 4 Variation with the voltage of the ratio I_1:I (Gramicidin A). (*a*) CsCl 0.25 M + $CaCl_2$ 0.25 M; (*b*) KCl 1 M + $CaCl_2$ 0.25 M; (*c*) KCl 1 M + $CaCl_2$ 0.5 M; (*d*) KCl 1 M + $CaCl_2$ 1 M.

channel current without (I_1) and with (I) calcium is given by

$$I_1/I = 1 + a_{Ca^{++}}(K_1 + K_2 \cosh 2\frac{FV}{RT}\alpha_{Ca^{++}})$$

where $\alpha_{Ca^{++}}$ is the electrical distance of the calcium-binding site in the channel. Its value (0.165) indicates that its location is close to that of the alkali ions.

Examination of Fig. 4 reveals that the Ca^{++}-blocking effect depends on the nature of the alkali ion. At low Cs^+ concentration (0.25 M), $K_1 = 0$, indicating that Ca^{++} does not bind the external site while it does in the case of potassium ($K_1 = f(C_{Ca^{++}}) \neq 0$). This conclusion is in accord with the energy profiles given by Eisenman and Sandblom (5) and is corroborated by the study of highly concentrated (3 M) Cs^+ systems ($K_1 \neq 0$) (6).

For Gramicidin M, the Ca^{++}-blocking effect is qualitatively the same as described above but it cannot be analyzed in detail owing to the low amplitudes of the signals.

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REFERENCES

1. Sarges, R., and B. Witkop. 1965. V. The structure of valine- and isoleucine-Gramicidin A. *J. Am. Chem. Soc.* 87:2011–2020.
2. Hladky, S. B., and D. A. Haydon. 1972. Ion transfer across lipid membranes in the presence of Gramicidin A. *Biochim. Biophys. Acta.* 274:294–312.
3. Bamberg, E., K. Noda, E. Gross, and P. Läuger. 1976. Single channel parameters of Gramicidin A, B, and C. *Biochim. Biophys. Acta.* 419:223–228.

4. Heitz, F., G. Spach, and Y. Trudelle. 1982. Single channels of 9, 11, 13, 15 destryptophyl-phenylalanyl-Gramicidin A. *Biophys. J.* 40:87–89.
5. Eisenman, G., and J. P. Sandblom. 1983. Energy barriers in ionic channels: data for Gramicidin A interpreted using a single-file (3B4S") model having 3 barriers separating 4 sites. *In* Physical Chemistry of Transmembrane Ion Motions. G. Spach, editor. Elsevier/North Holland, Amsterdam. 329–348.
6. Heitz, F., and C. Gavach. 1983. Ca^{++}-Gramicidin A interactions and blocking effects on the ionic channel. *Biophys. Chem.* 18:153–163.

ELECTROSTATIC ACTIVATION ENTHALPY FOR ION TRANSPORT THROUGH A MEMBRANE CHANNEL

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The temperature dependence of the conductance of a membrane channel offers a means of obtaining information about the electrostatic energy barrier that opposes the entry of ions into a channel. Quantitative calculations of the magnitude of this barrier show that it may be low enough to allow ion transport to occur at observed rates (1). However, the activation enthalpies obtained from Arrhenius plots of channel conductances are often close to zero, once they are corrected for the temperature dependence of water viscosity. The temperature dependence of channel conductance is usually only slightly steeper than that of ion mobilities in water. The temperature dependences of these mobilities all closely follow the temperature dependence of the viscosity of water with an apparent activation enthalpy near 3.8 kcal/mol (2).

This report points out that the activation enthalpy is not identical with the activation free energy and can be much lower than the total electrostatic free energy of placing an ion within an aqueous cylindrical pore surrounded by a low dielectric medium such as hydrocarbon. Previously, Hille found that a barrier model for the sodium channel was improved when entropic terms were added to the energy barriers (3). Although it is a trivial matter to differentiate a free energy with respect to temperature to obtain the entropy, it is not widely appreciated that electrostatic energies are not purely enthalpic. At 25°C the dielectric constant of water changes by a fraction of 0.0046 of itself per degree Centigrade. Differentiating with respect to temperature, the coulombic potential, G, in water gives an entropy of $S = -0.0046 \times G$. At 25°C, $TS = -1.37 \times G$. Electrostatic interactions in water are therefore entropy-driven (4).

The quantitative calculations of Levitt (1) are numerical and not easily subjected to this kind of analysis. However, Levitt has found that Parsegian's infinite cylindrical pore potential (5) can be corrected for end effects with Parsegian's finite slab potential (5) to give

$$G = \frac{e^2}{\epsilon_h b} P\left(\frac{\epsilon_h}{\epsilon_w}\right) - \frac{e^2}{\epsilon_h \ell} \ln\left(\frac{2\epsilon_w}{\epsilon_h + \epsilon_w}\right) \qquad (1)$$

where e is the charge of an electron, ϵ_h and ϵ_w are the dielectric constants of hydrocarbon and water respectively, b and ℓ are the radius and length, respectively, of the channel, and P is an integral evaluated and tabulated by Parsegian (5). This expression is 26% larger than the result of a more quantitative calculation of the free energy of placing an ion in the center of a pore with the same dimensions as gramicidin (6 Å wide, 25 Å long) (1).

Differentiating Eq. 1 with respect to temperature gives

$$-S = \frac{-e^2 P}{\epsilon_h^2 b}\frac{d\epsilon_h}{dT} + \frac{e^2 P'}{\epsilon_h b}\left(\frac{1}{\epsilon_w}\frac{d\epsilon_h}{dT} - \frac{\epsilon_h}{\epsilon_w^2}\frac{d\epsilon_w}{dT}\right) + \frac{e^2}{\ell}\left[\frac{1}{\epsilon_h^2}\frac{d\epsilon_h}{dT}\ln\left(\frac{2\epsilon_w}{\epsilon_h + \epsilon_w}\right) - \frac{1}{\epsilon_w \epsilon_h}\frac{d\epsilon_w}{dT} + \frac{\frac{d\epsilon_w}{dT} + \frac{d\epsilon_h}{dT}}{\epsilon_h(\epsilon_h + \epsilon_w)}\right]$$

With $\epsilon_h = 2$ and $\epsilon_w = 80$, the appropriate value of P is $P(0.025) = 0.17$. $P'(0.025)$ was estimated from the table of values of P to be 2.1 (5). $d\epsilon_w/dT = -0.368$; $d\epsilon_h/dT = 0.0012$ (for dodecane [6]). With $b = 3$ Å and $\ell = 25$ Å, TS is -4.5 kcal/mol. For these values of b, ℓ, ϵ_w, and ϵ_h, G is determined from Eq. 1 to be 4.8 kcal/mol, so the free energy of the barrier is almost all entropic, and the barrier enthalpy is only 0.3 kcal/mol.

The measured activation enthalpy for the conductance of the gramicidin channel is 1–3.5 kcal/mol larger than that for the viscosity of water. (7, 8). For the excitability-inducing material channel (9), for the acetylcholine channel (10), and for the sodium and potassium channels (11, 12), there is no significant activation enthalpy in excess of that of the free ion mobility. Though precise dimensions are available only for gramicidin, the above calculation suggests that low activation enthalpies of channel conductances do not indicate the absence of an electrostatic barrier.

In conclusion, the low activation enthalpies of channel conductances are consistent with a naive model of a cylindrical water-filled pore through a slab of hydrocarbon, in which macroscopic properties of electrolyte solutions and hydrocarbon are applied. It is not clear whether a more molecular picture would preserve the qualitative predictions of this model, but it is worth bearing in mind