BRIEF COMMUNICATION

ON THE POTENTIAL VARIATION IN THE GRAMICIDIN CHANNEL

R. H. TREDGOLD, *Department of Physics, University of Lancaster, Lancaster, United Kingdom.*

ABSTRACT A simple model for the gramicidin ion pore is analyzed in which the potential energy of the ion in the pore can be resolved into three parts: a series of equal potential barriers, the contribution from the applied potential, and a parabolic variation leading to either a potential maximum or a potential minimum at the center of the membrane. For low ion concentrations, the particular case corresponding to a potential minima $2kT$ below the surface potential predicts experimentally observed behavior.

INTRODUCTION

Gramicidin has been extensively studied as a model for membrane ion pores. The single channel behavior first studied by Hladky and Haydon (1) allows one to examine the behavior of individual molecular channels with relative ease. Furthermore the material is easily introduced into black lipid membranes. At the present time no other material is known that has these obvious advantages as a tool to study the basic nature of ion transport through bilayer membranes.

Urry (2) and Veatch et al (3) have put forward two different models for the gramicidin ionophore. Both, however, involve a dimer, and both lead to the existence of a channel 3 nm long and about 0.4 nm in diameter penetrating the hydrocarbon part of the lipid bilayer. Läuger (4) has examined a theoretical model for this material in which the mean energy of the ion throughout the channel is constant (or varies uniformly when a membrane potential exists). Superimposed on this uniform potential are a number of equal potential barriers over which the ion must pass by thermal activation. This model makes predictions in reasonably good accord with the experimental results obtained by Hladky and Haydon (1) for electrolytes having a concentration of less than 0.1 M. This theory may readily be extended to the case when the molarities of the solution on the two sides of the membrane are different, and a more complicated current-voltage curve can be expected. In this laboratory (5) we have recently examined such a system and find excellent agreement between theory and experiment. One is thus inclined to feel that this model for the gramicidin channel must be reasonably correct and that the potential gradient down the channel must be fairly uniform.

However, simple electrostatic considerations lead to a different conclusion. When the ion is within the membrane, there will be an interaction between the charge on it and the images of this charge residing in the aqueous regions on the two sides of the membrane.

The potential arising from the infinite series of images has been calculated by Neumcke and Läuger (6) by numerical means. It is clear from their results that, when no potential is applied across the membrane, a very substantial variation in potential nevertheless exists within it, the potential energy of the ion being highest at the center of the membrane and falling off on either side as the aqueous region is approached. This potential energy may be roughly approximated by an expression of the form

$$U(x) = U_0 - ax^2, \tag{1}$$

where x is the distance from the center of the membrane perpendicular to its plane.

It is possible to visualize other contributions to the energy that vary in an approximately parabolic manner and that may partially cancel this variation. However, it would be truly remarkable if this cancellation process worked with such accuracy for all the ions studied, so as to reduce the energy variation to less than two or three times kT over the whole width of the membrane. One thus asks the question: how will curvature in the potential energy profile influence the current versus voltage characteristic of the channel? Here the discussion is limited to the simple case of dilute electrolytes when ion interaction may be neglected.

ANALYSIS OF CHANNEL BEHAVIOR

To answer this question we examine a model in which there are N potential barriers in the channel, each having a height, E. There are thus $N + 1$ potential minima and it is supposed that the outermost of these on either side of the membrane join smoothly with the uniform potential in the aqueous medium. The electrical potential measured between the outer potential maxima is ϕ, and it is assumed that, when the membrane potential is zero, the mean potential in the channel is symmetrical and parabolic in form. It is further assumed that, under these circumstances, the potential at the center is higher than that in the last potential minima by a factor of $(a(N^2/4))$. These assumptions correspond to a power series expansion of the potential about the membrane center. The only odd term that will appear is of the first order. The first even term neglected is of the fourth order. This model is thus the simplest logical extension of those already examined.

The average current through one channel will then be given by:

$$J = \nu e e^{-\frac{E}{kT}}\left[y_n e^{\frac{1}{kT}\left[\frac{\phi e}{2N} + an + \frac{a}{2}\right]} - y_{n+1} e^{-\frac{1}{kT}\left[\frac{\phi e}{2N} + an + \frac{a}{2}\right]}\right] \tag{2}$$

for all n.

Here y_n is the probability of finding an ion at the nth potential minima from the center. ν has the dimensions of frequency and a magnitude of the order 10^{13} s^{-1}, e is the electronic charge, and the other symbols have their usual meanings. If a is put equal to zero, the problem degenerates to the one already given explicitly by Läuger (7). Then

$$J = \nu e c e^{-(E/kT)} \sinh(\phi e/2NkT) \tag{3}$$

where c is the probability of finding an ion in the outermost potential minima. This quantity is assumed to be independent of membrane potential, an assumption justified by Läuger (7).

For values of $N \sim 10$ or greater and values of ϕ less than 200 MV, Eq. 3 yields ohmic conduction to a good approximation.

One may now solve Eq. 2 for the case when c is the same on both sides of the membrane and a is not zero. Using standard techniques (Hildebrand (8)) for solving difference equations and particularizing to the case $N = 10$, one finds that

$$J = \delta e^{-\frac{25a}{kT}} \sinh\left[\frac{\phi e}{2kT}\right] \Bigg/ \sum_{n=0}^{4} e^{-\frac{a}{2kT}[n^2 + (n+1)^2]} \cosh\left[\frac{\phi e}{10kT} \quad \left[N + \frac{1}{2}\right]\right] \qquad (4)$$

where $\delta = evce^{-(E/kT)}$. In Fig. 1 J/δ is plotted for various values of $(a(N^2/4))$ for ϕ in the range 0 to 200 MV. In each case the value of δ has been adjusted to give an initial slope equal to that obtained by Hladky and Haydon (1) for 0.1 M KCl. Note that the curve corresponding to a potential at the center of the membrane $2kT$ less than that at the edge gives very good agreement with the experimental points. Clearly this does not prove that the experimental results can only be explained by exactly the potential profile studied here. However, it is a strong indication that they are best explained by a profile that has a potential minimum at the center of the membrane and that this minimum lies about $2kT$ below the potential at the edge of the membrane. This result may appear surprising in view of the electrostatic arguments given above. However, other contributions to the potential must exist, such as the radial polarization of the peptide chains. It is quite possible that these will "overcompensate" the other electrostatic terms and lead to the form of potential profile discussed here. It can be verified that a model involving a large number of smaller potential barriers leads to essentially the same result. However, if the number is decreased below ten, the predicted behavior varies significantly.

DISCUSSION

We have shown that there is strong reason to believe that the potential profile in the gramicidin channel is constant to within $2kT$ over most of its length. (There exists, of course, the series of barriers superimposed on this potential.) We have also shown the small likelihood of this situation being brought about within the limits of the conventional model,

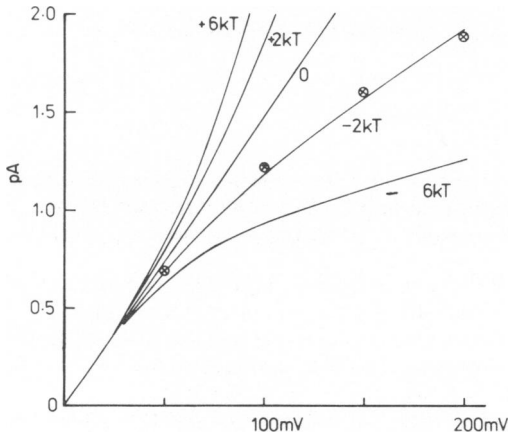

FIGURE 1 Current versus membrane potential for the model for the gramicidin pore discussed in the text. The curves are all normalized to have the slope at the origin corresponding to the experimental results obtained by Hladky and Haydon (1) using 0.1 M KCl. These results are indicated thus ⊗. The potential at the membrane center, compared with the edge, is shown after each curve.

in which an unhydrated ion passes through a channel of radius 0.4 nm. If however, the ion passes through the membrane in a hydrated state, its effective radius would be increased considerably, and thus the overall effect of change in hydration energy with passage through the membrane would be proportionately decreased. (The hydration energy is inversely proportional to the effective radius.) If one uses the Pauling radius, this quantity for unhydrated potassium, for example, is 0.133 nm. What value should be take for the hydrated ion?

Until recently, discussions of hydration shells were largely hypothetical and were not based on any direct measurement. However, the very elegant neutron diffraction work carried out by Enderby and his collaborators (9) has begun to place our ideas on this topic on a firmer foundation. It is now apparent that the first hydration shell is extremely stable and has a radius of nearly 0.4 nm. It thus seems possible that ions pass through the gramicidin channel accompanied by their first hydration shells. Such a concept would mean modifying the present picture of the behavior of the gramicidin dimer. However, this picture cannot be supported by any direct physical measurements (though it provides a conceptually simple and satisfying explanation of the behavior of gramicidin) and should thus be viewed with an open mind. It is relevant to note that Heitz and his collaborators (10, 11) have investigated both single and double helix structures obtainable from L-D peptide chains such as gramicidin. They have shown that a considerable number of different helical structures are possible.

At this stage it would be unprofitable to speculate further. The very uniform nature of the mean potential in the gramicidin channel has been demonstrated, and the difficulty of reconciling this fact with a simple electrostatic picture has been pointed out. One possible way of resolving this paradox is by assuming that ions travel in the hydrated state. Clearly it is central to the physical problem of understanding the transport of ions through membranes to examine other possible resolutions of this problem.

The author wishes to thank Dr. D.A. Haydon and the Editors of *Biochimica et Biophysica Acta* for permission to reproduce the experimental points shown in the figure.

Received for publication 7 March 1978 and in revised form 18 September 1978.

REFERENCES

1. HLADKY, S. B., and D. A. HAYDON. 1972. Ion transfer across lipid membranes in the presence of gramicidin A; studies of the unit conductance channel. *Biochim. Biophys. Acta.* **274**:294.
2. URRY, D. W. 1971. The gramicidin A transmembrane channel: a proposed $\pi_{(L\text{-}D)}$ helix. *Proc. Natl. Acad. Sci. U.S.A.* **68**:672.
3. VEATCH, W. R., E. T. FOSSEL, and E. R. BLOUT. 1974. The conformation of gramicidin A. *Biochemistry.* **13**: 5249.
4. LÄUGER, P. 1973. Ion transport through pores: a rate theory analysis. *Biochim. Biophys. Acta.* **311**:423.
5. TREDGOLD, R. H., P. N. HOLE, R. C. SPROULE, and M. ELGAMAL. 1977. Single channel characteristics of some synthetic gramicidins. *Biochim. Biophys. Acta.* **471**:189.
6. NEUMCKE, B., and P. LÄUGER. 1969. Nonlinear electrical effects in lipid bilayer membranes. II. Integration of the generalized Nerst-Planck equations. *Biophys. J.* **9**:1160.
7. LÄUGER, P. 1976. Diffusion-limited ion flow through pores. *Biochim. Biophys. Acta.* **455**:493.
8. HILDEBRAND, F. B. 1968. Finite Difference Equations and Simulations. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

9. SOPER, A. K., G. W. NEILSON, J. E. ENDERBY, and R. A. HOWE. 1977. A neutron diffraction study of hydration effects in aqueous solutions. *J. Phys. C:(Solid-State Phys.).* **10**:1793.
10. HEITZ, F., B. LOTZ, and G. SPACH. 1975. α_{DL} and π_{DL} helices of alternating poly-γ-benzyl-D-L-glutamate. *J. Mol. Biol.* **92**:1.
11. LOTZ, B., F. COLONNA-CASARI, F. HEITZ, and G. SPACH. A family of double helices of alternating poly-γ-benzyl-D-L-glutamate, a stereochemical model for gramicidin A. *J. Mol. Biol.* **106**:915.