THE "BORN ENERGY" PROBLEM IN BACTERIORHODOPSIN

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The electrostatic energy required to transfer a charged, conducting sphere from one infinite medium to another was first calculated by Born (1). The same macroscopic model has been used to estimate the potential barrier for charge transport across membranes, with suitable corrections being made to account for the finite thickness of the (membrane) dielectric slab (2–4). Similar calculations have also been performed to estimate the reduction in the "Born energy" barrier that is provided by a narrow, water-filled channel (2, 4) or by a suitable "carrier" structure (4) that might surround or "chelate" the charged ion. An important and not necessarily intuitive point is the fact that the Born energy is, to a first approximation, exactly the same for an ion pair as it is for a single charged ion (4). Representative values of the Born energy for different circumstances are illustrated by the graphs shown in Fig. 1.

Bacteriorhodopsin (bR), a transmembrane protein found in *Halobacterium halobium* (5), is an electrogenic ion pump capable of using light energy to establish a pH gradient across the cell membrane (6, 7). Photons are absorbed by a retinal molecule bound to the protein as a protonated Schiff base on lysine 216 (8) and presumably buried in a hydrophobic binding pocket of the protein. Bacteriorhodopsin must therefore solve the Born energy problem in at least two distinct instances, namely the active transport ("pumping") of charged ions across the cell membrane, and the burial of the protonated Schiff base within the membrane interior. Evidently bR must solve the Born energy problem by a strategy different from the incorporation of a water-filled pore, since neutron diffraction difference-Fourier maps exclude the occurrence of a "bulk water" channel (9).

It has been noted previously by Tredgold and Hole (10) that the β-sheet polypeptide conformation is capable of having quite high values of the dielectric constant, and thus might be used to overcome the electrostatic barrier for insertion of charged ions into membranes. Because our own structural studies of bR have resulted in substantial evidence for a transmembrane β-sheet domain (11), we now propose that a possible function for such a transmembrane domain would be to provide an organic phase "channel" or pathway for ion transport.

β-SHEET DOMAINS AS POTENTIAL ORGANIC-PHASE ION CHANNELS

The β-sheet polypeptide conformation is known to be a highly flexible structure (12). The peptide bond has a permanent dipole moment of 3.7 Debye, twice as large as that of water (1.8 Debye). Even a partial rotation of the direction of the permanent dipole moment, in an external field, could therefore result in a relatively large dielectric constant. Experimental measurements made by Tredgold and Hole (10) have confirmed that polypeptides in the β-sheet conformation can have relatively high dielectric constants, provided that the packing of adjacent sheets is a relatively loose one. A linear relationship was found between dielectric constant and the spacing between sheets; for spacings in the range 9.7–10.2 Å the measurements fit on the line $\epsilon = 27.3\ d - 261.3$ where d is the β-sheet spacing in Angstroms.

EVIDENCE FOR A β-SHEET DOMAIN IN BACTERIORHODOPSIN

The suggestion that there might be a transmembrane domain of β-sheet in bR first became evident in the

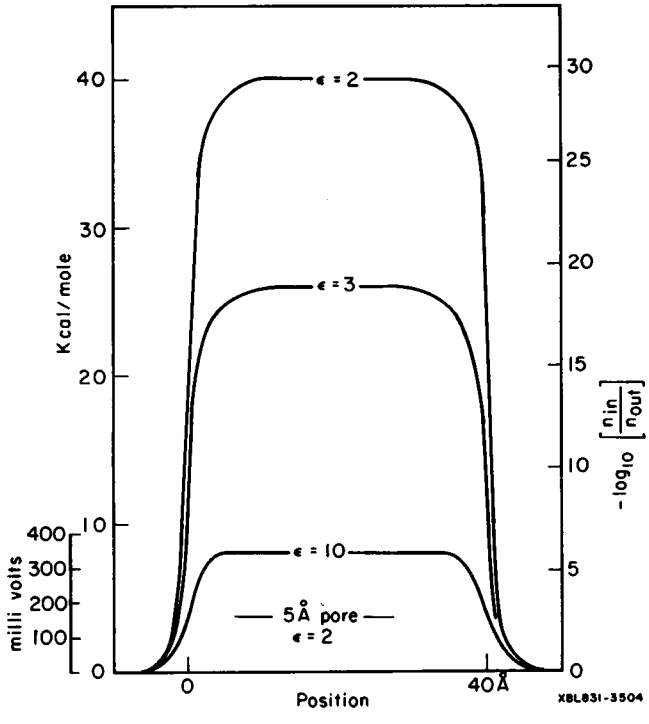

FIGURE 1 The electrostatic potential barrier ("Born energy") for charge transport across a cell membrane. Calculated values are taken primarily from Levitt (2), Läuger and Neumke (3) and Parsegian (4), and apply to a charged sphere (ion) of diameter 4.0 Å. The Born energy is greatly reduced in the case of a water-filled pore ($\epsilon = 80$) that is only 5.0 Å in diameter, even when the surrounding material has a low dielectric constant ($\epsilon = 2$).

high-resolution projection of the crystal structure obtained by Hayward and Stroud (13). Quantitative image-matching calculations (11) confirmed that a portion of this map was inconsistent with a structure composed of two tilted helices, in the positions designated as "helix 1" and "helix 2" by Engelman et al. (14). Infrared spectra show a pronounced shoulder in the Amide I band at ~1,639 cm^{-1} (11, 15, 16) which might be explained by a β-sheet domain (11, 15); ultraviolet circular dichroism spectra also give rather convincing evidence for a major β-sheet domain (11).

A schematic line-drawing of the high-resolution projection of bR is shown in Fig. 2. Heavy arcs have been drawn in at a radius of ~10.2 Å from the center of helices I, II, and III, to illustrate the fact that about half of the proposed region of β-sheet is located at a relatively "open" portion of the structure.

DISCUSSION

A transmembrane protein made up only of helical segments is unable to overcome the Born energy problem for organic-phase ion transport by any currently known structural mechanism. Excluded from this assertion, of course, would be structural models in which amphiphilic helices, such as those formed by melittin (17), are clustered together to create a water-filled channel rather than an organic-phase channel. Because bR appears to have no water-filled channel, it is not possible to explain how a seven-helix model of the structure of this protein could account for the ability of ionic species to penetrate into the membrane interior. This theoretical difficulty is encountered in connection with burial of the protonated Schiff base of retinal as well as in relation to the electrogenic ion transport that results in creation of a pH gradient across the membrane.

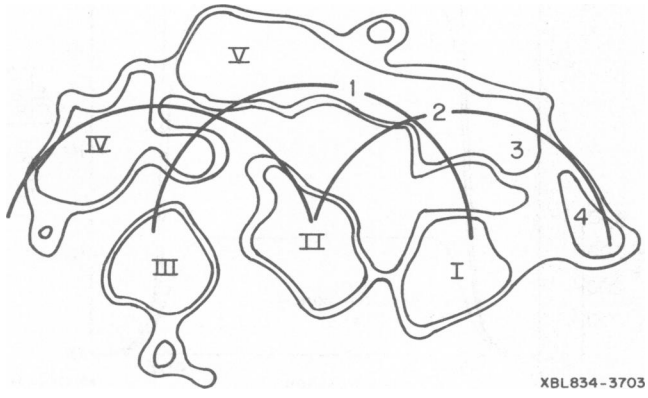

FIGURE 2 Schematic representation of the high resolution projection (13) of the structure of bacteriorhodopsin. Well-defined helices are labeled with Roman numerals, while the positions of possible β-sheet strands are labeled with Arabic numerals. Heavy arcs are drawn in at a radius of 10.2 Å from the centers of helices I, II and III, showing that strands 1 and 2 are located in an especially "open" or "loose" part of the structure.

The requirement for ion-pairing of buried charges has been used as a constraint in efforts to predict the tertiary structure of bR from the amino acid sequence (14, 18–20). However, because these structural models consist of seven transmembrane helices, the dielectric constant will be low, and it can be expected that insertion of charged ions would involve a prohibitive energy barrier even when inserted as ion pairs. Reduction of the estimated energy barrier to only 5 Kcal/mol (20) seems to be quite unrealistic under these circumstances.

A membrane protein that has a transmembrane domain of β-sheet does not face the same electrostatic difficulties, provided that the side-chain packing between the β-sheet domain and its immediate environment is "open" enough to permit a flexible motion of the main-chain residues. The high-resolution projection of the structure of bR and spectroscopic measurements suggest that there is a major β-sheet domain in this transmembrane protein. The attractive feature of this model, as far as the function of the protein is concerned, is that the β-sheet provides a natural environment for insertion of the protonated Schiff base, perhaps as an ion pair with aspartate 212, and it provides a physically reasonable channel for organic-phase transport of charged ions across the cell membrane.

This work was supported by the Director, Office of Energy Research, Office of Health and Environmental Research of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098, and by National Institutes of Health grant GM 23325.

Received for publication 2 May 1983.

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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

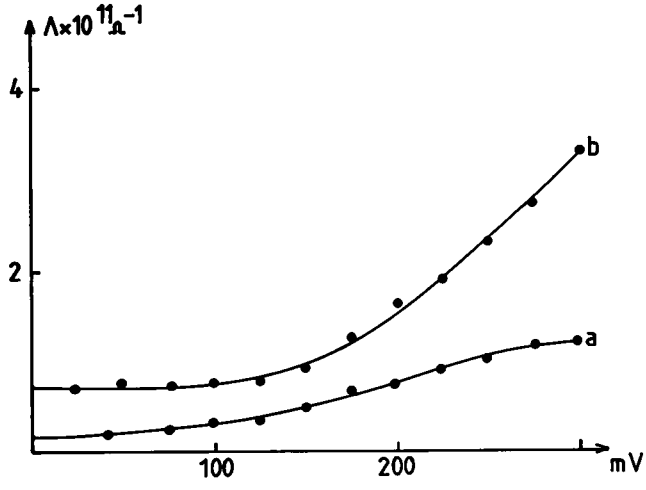

FIGURE 1 Variation with the voltage of the single channel conductance of Gramicidin M (*a*) in CsCl 0.5 M, (*b*) in CsCl 6 M GMO/Decane membranes.