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The α-helix dipole in membranes: a new gating mechanism for ion channels

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Abstract. Electric dipoles placed side by side attract each other if antiparallel and repel each other if parallel. The hydrophobic α-helical sections of proteins that span membranes are known to possess large electric dipole moments. The first part of the paper consists of a calculation of the interaction energies between such helices including screening effects. Interaction energies remain comparable with a typical thermal energy of KT up to separations of order 20 Å. In addition it is shown that, due solely to its dipole moment, an α-helix which completely spans the membrane has an energy up to 5 KT lower than one which terminates within the membrane width. The second part of the paper describes the electrical interaction of the charge structure of a membrane channel and the protein helices that surround the pore. The gating charge transfer that is measured when a voltage sensitive ion channel switches, means that the dipole moment of the ion channel changes. This in turn results in a change in the radial forces that act between the pore and the α-helices that surround it. A change in these radial forces which tend to open or to close the pore constitutes an electrically silent gating mechanism that must necessarily act subsequent to the gating charge transfer. The gating mechanism could consist of the radial translation of the neighbouring proteins or in their axial rotation under the influence of the torque that would act on a pair of approximately equidistant but oppositely directed α-helices. An attempt to calculate the interaction energy of a typical pore and a single α-helix spanning the membrane results in an energy of many times KT.

Key words: Channel, gating, α-helix, dipole

Introduction

The structure of most membrane channel proteins seems to consist of clumps of amphipathic protein that surround and define the pore in a barrel stave fashion. The clumps may be separate proteins as in the acetylcholine receptor (Changeux et al. 1984) or helical sections of a single large protein as in the sodium channel of Electrophorus electricus (Noda et al. 1984). In both cases the channel is closely surrounded by helical (probably α-helical) sections of protein that penetrate the membrane perpendicularly. Protein helices are known (Wada 1976; Hol et al. 1978) to possess large electric dipole moments and in this paper I consider some of the consequences of these large electric dipole moments in such close proximity to the ion channels including helix–helix interaction, helix–interface interaction and the interaction between the dipole moments of the helices and that of the pore which leads to a new model of voltage sensitive membrane channel gating.

Results

In Fig. 1 is displayed as a dotted line the calculated electrostatic interaction energy of two parallel α-helices, each of 33 residues, as a function of their separation when they are embedded in a medium of relative dielectric constant $\varepsilon_1 = 3$, typical of lipid or protein. The interaction energies are seen to be large in comparison with a typical thermal energy of $KT = 4.14 * 10^{-21}$ J as has been previously noted (Hol et al. 1981). However for helices that totally penetrate the membrane the calculation is unrealistic in that it does not take into account the electric field screening effects of the high dielectric constant fluid that bathes the two faces of the membrane. For a plane parallel sided slab of homogeneous dielectric it is possible to calculate the effects of screening exactly using the method of electrical images, described in most undergraduate electricity texts, and treated in detail by Smythe (1939). In the case of a real biological membrane which is not homo-

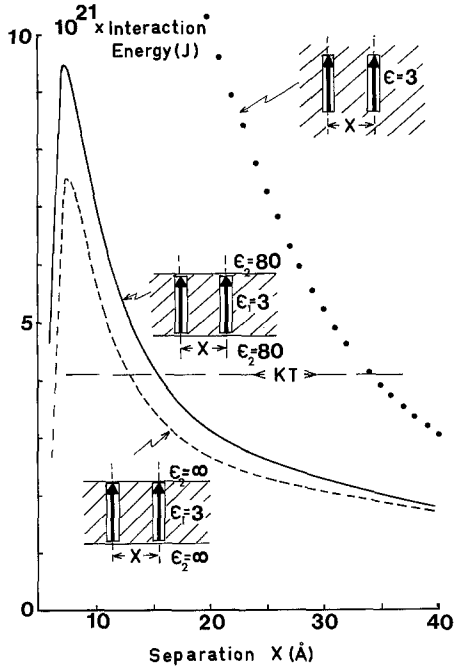

Fig. 1. The dotted line in Fig. 1 shows the electrostatic interaction energy of two parallel α-helices each of 33 residues as a function of their separation X when they are immersed in a fluid of relative dielectric constant $\varepsilon_1 = 3$. The partial charges Q and cylindrical co-ordinates (R, Z, φ) of the atoms in a residue relative to that of the α-carbon atom in the same residue that were used to generate the α-helix are listed below. The increments in the cylindrical co-ordinates between the α-carbon atoms of adjacent residues used were $\Delta Z = 1.479$ Å and $\Delta\varphi = 101.1°$ and no charged side chain atoms were included in the calculation.

The full line in Fig. 1 gives the calculated interaction energy of the same two parallel α-helices embedded perpendicularly across a parallel sided slab of material of relative dielectric constant $\varepsilon_1 = 3$ and thickness 27 Å when surrounded by a fluid of relative dielectric constant $\varepsilon_2 = 80$. The calculation in performed using the method of images utilizing the first two image helices. The broken line in Fig. 1 shows the energy of interaction between the helices when ε_2 is made very large in order to simulate perfect electrical screening of the electric fields at the ends of the helix.

Atom	R [Å]	Z [Å]	φ [Deg]	Q [$\|e\|$]
H	1.492	+1.863	+18.88	+0.2
N	1.505	+0.890	+27.82	−0.2
α-C	2.260	0	0	0
C	1.628	−1.066	−27.99	+0.42
O	1.877	−2.246	−21.60	−0.42

geneous the method provides only an approximate solution which is however likely to prove much more accurate than calculations, such as that represented by the dotted line in Fig. 1, which totally ignore screening. In this case the calculation involves the addition to the electric field of an unscreened helix, of the electric fields of the "image helices" which occupy the positions of the optical virtual images of the original helix as seen in two parallel plane mirrors that occupy the positions of the membrane surfaces. A full calculation involves the sum of an infinite set of image helices at increasing distances from the membrane but, because we are dealing with dipolar fields, the convergence is rapid so that the force calculated using only the first two image helices leads to an approximation as accurate as is warranted in view of the known inhomogeneity of real membranes. Using 4 image helices rather than 2 changes the calculated force by less than 10%. The full line in Fig. 1 shows the calculated interaction energy of the two helices as a function of their separation using the nearest two image helices. The interaction energy is appreciable in comparison with KT up to separations of about 20 Å but is much reduced in comparison with the unscreened calculation. A similar reduction in the calculated electrostatic interaction of α-helices in globular proteins surrounded by water has been noted by Rogers and Sternberg (1984). The force between the helices that results from an interaction energy U is given by $-DU/DX$. It is repulsive for parallel helices and attractive for those antiparallel. These calculations are not much dependent on the dielectric constant chosen for the aqueous phase and the broken line in Fig. 1 gives the results for a bathing fluid of indefinitely large dielectric constant corresponding to perfect screening.

A second consequence of the interaction of the dipole moment of the helix and the screening charge induced by it at the membrane surface, is the interaction energy displayed in Fig. 2, calculated as a function of the distance between the end of the helix and the membrane surface. A helix is seen to lower its energy by about 10^{-20} J (2.5 KT) independent of the helix length in the low dielectric region for each end that terminates in the aqueous phase. An α-helix will, of course, partition between hydrophobic and hydrophillic environments largely depending upon the ability of its surface groups to form hydrogen bonds but this dipolar effect is due to the backbone partial charges and applies to any helical polypeptide section in a protein. This energy drop may explain why even strongly hydrophobic α-helical sections of proteins so often span the complete low dielectric constant region of both membranes and globular proteins to terminate at the surface.

When a gated channel switches in response to a change in the voltage across the membrane, gating charge transfer occurs. The charge transfer, probably due to the combination of charged particle translation and electric dipole rotation, is currently estimated (Keynes 1983) to be the equivalent of the transfer across the membrane of $14|e|$ for the sodium channel in the squid giant axon and $22|e|$

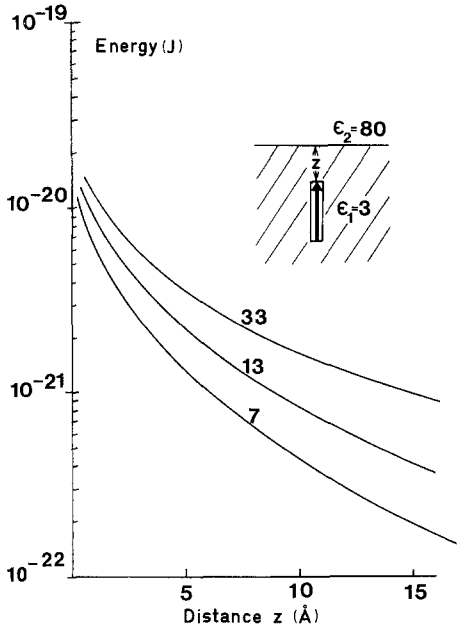

Fig. 2. The calculated negative electrostatic energy of an α-helix oriented perpendicular to a plane interface between material of relative dielectric constant $\varepsilon_1 = 3$ in which the helix is immersed and a second material of relative dielectric constant $\varepsilon_2 = 80$, as a function of the distance Z between the end of the helix and the interface. The calculation is performed using the method of images on helices of 7, 13, and 33 residues. The energy arises from the interaction of the electric dipoles of the helix and the charges induced at the interface by these dipole moments. The most negative energy is obtained when Z is small and is approximated given by $-1 * 10^{-20}$ J, independent of the length of the helix

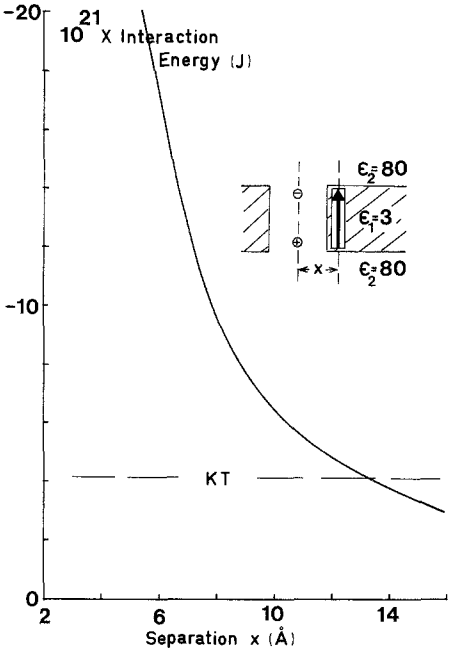

Fig. 3. This figure shows the calculated negative interaction energy between an α-helix of 33 residues embedded perpendicularly across a parallel sided slab of material with relative dielectric constant $\varepsilon_1 = 3$ and charges of $-5|e|$ and $5|e|$ situated on an axis parallel to that of the helix, separated by a distance of 48 Å and disposed symmetrically with respect to the helix as sketched in the figure. The dielectric slab is surrounded by a material of relative dielectric constant $\varepsilon_2 = 80$ and the charges are supposed to be embedded in material of the same high dielectric constant. The calculation is again performed by the method of images using the first two image helices

for the frog node. The gating charge transfer changes the electric dipole moment of the channel and hence the electrostatic forces in the plane of the membrane acting on the protein helices in its vicinity. To first order these forces depend on the total dipole moment of the channel and not on the detailed distribution of the charges and so for simplicity I will assume an electrically neutral channel which, in the resting state, has a total charge of $-5|e|$ located near its outer end and a total charge of $+5|e|$ located near its inner end as sketched in Fig. 3a. When the channel is activated I assume that the positions of the two charges are interchanged leading to a total equivalent charge transfer of $10|e|$. A calculation that treats the channel as a uniformly polarized cylinder with the same equivalent gating charge transfer yields similar results. Assuming that the dipole moment of a neighbouring parallel α-helix is directed outward, the electrostatic interaction of the helix and the dipole moment of the pore will result in a force attracting the helix radially inward toward the channel axis when it is resting, and a radially outward force tending to repel the helix when the gating charge transfer occurs.

These forces can give rise to channel gating in the manner to be described below if they are of a sufficient magnitude and here I attempt an estimation of their size. To do so I make two extreme assumptions. The first is that the gating charge moves within a pore which has an effective dielectric constant of $\varepsilon_2 = 80$, as high as bulk water. It is in fact highly unlikely that all the charge is screened by water or indeed that the water molecules within a pore of diameter only 10 Å will so effectively screen any charge within it, particularly if the water is itself electrically ordered (Edmonds 1984), so that this represents an extreme lower bound to the size of the effect. An equally extreme upper bound is obtained by assuming that the gating charges move in an environment with an effective dielectric constant of $\varepsilon_1 = 3$, close to that of lipid. The lower bound for the energy U of interaction of the α-helix and the gating charges in the resting state is shown in Fig. 3 and the resultant inward force $-DU/DX$ is easily cal-

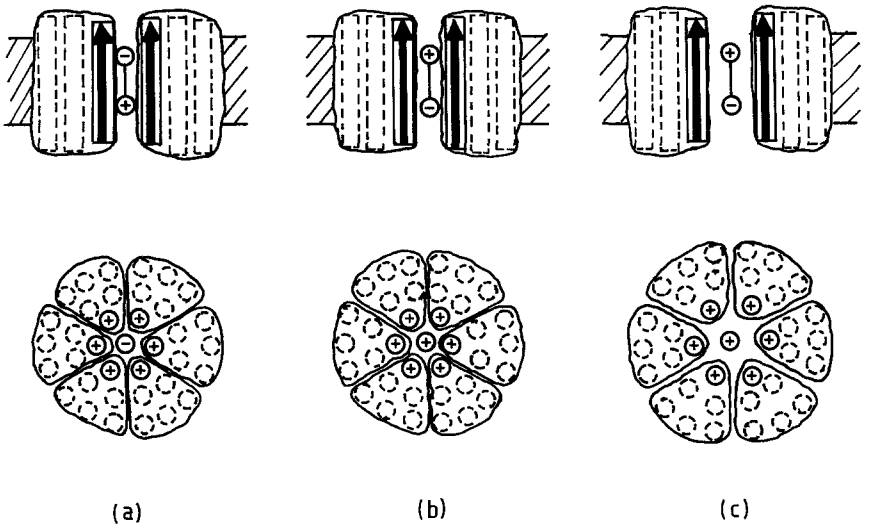

Fig. 4. **a** shows a pore with a dipolar structure represented by a pair of charges of equal magnitude but opposite sign surrounded in a barrel stave fashion by 6 identical proteins each with the nearest helical section of the protein to the pore having its electrical dipole moment directed outward. In this resting configuration the forces between the charged structure of the pore and the adjacent helices attract the protein inward towards the axis of the pore thus ensuring that the pore remains closed. **b** shows the proteins unchanged but the dipole moment of the pore reversed following the gating charge transfer perpendicularly across the membrane in response to a change in the voltage applied across the membrane. The forces on the neighbouring helices due to their interaction with the pore have now reversed in sign so as to point outward from the pore axis. **c** shows the surrounding proteins slightly displaced outward from the pore axis in response to the radial forces so that the pore diameter has reached some critical diameter allowing it to conduct ions

culated. The upper bound to the effects is obtained by multiplying the interaction energy displayed in Fig. 3 by a factor of $(\varepsilon_1 + \varepsilon_2)/2\varepsilon_1 = 13.83$. It is clear that despite the deficiencies of such simple calculations the energies, being of order 5 or 10 times KT for each α-helix, are probably of sufficient magnitude to provide a viable gating mechanism.

A much simplified picture of the new gating mechanism is displayed in Fig. 4. Figure 4a shows a channel with an electrical dipolar structure again represented by 2 charges of opposite sign and surrounded by 6 identical amphipathic proteins each with an outwardly directed α-helix closest to the pore axis. The interactions calculated above lead to forces on the helices acting in the plane of the membrane which attract the proteins toward the axis of the channel tending to close the channel. Because of the steep (approx. $1/X^4$) separation dependence of these radial forces the total force on the protein molecules is dominated by that on the nearest outward directed helix even if the protein contains equal numbers of helices directed inward and outward. Figure 4b shows the situation after the gating charge transfer has occured in response to a change in the voltage applied across the membrane. Now the forces between the channel core and the nearest helices are radially outward from the channel axis tending to enlarge the central pore size. Finally in Fig. 4c is displayed the situation when the proteins have moved slightly under the the influence of the forces opening the channel to some critical diameter that allows ion conduction through it. It is clear that the transition between the situations portrayed in Figs. 4b and c is electrically silent as far as gating charge transfer measurement is concerned and that it necessarily takes place after the majority of the gating charge transfer has occurred. High pressure experiments on the squid axon do suggest that the final rate limiting step in the conduction of sodium ions is electrically silent and has a sizeable activation volume (Conti et al. 1984).

Experiments involving deuteration (Schauf and Bullock 1979) also point to a final electrically silent step in the conduction process that follows the gating charge transfer. An alternative gating mechanism to that shown in Fig. 4 is channel opening due to protein rotation about axes parallel to the channel axis that would result from the equal and opposite forces acting between the pore and equidistant but oppositely directed pairs of helices in the protein blocks. This torque would reverse in sign when the gating charge transfers.

Discussion

A gating mechanism that depends on the electric dipole moment of helical proteins that span the

membrane has previously been described (Boheim et al. 1983) for the alamethicin pore but this relies on the flip-flop reversal of the helix across the membrane. In most biological membranes, including the two mentioned in the introduction, the helical sections that define the pore form part of large amphipathic proteins embedded in the membrane and helix flip-flop is not possible. The gating mechanism described here does not involve helix flip-flop and should be applicable to real channel structures. Indeed, at least for the voltage sensitive sodium channel, the gating charge transfer and the presence of helical protein sections in the close vicinity of the pore are both confirmed experimentally (Keynes 1983; Noda et al. 1984) so that the interaction energies described in this paper must exist. The question that remains concerns their magnitude in comparison with thermal energies and the calculations of this paper suggest that even making conservative assumptions they are of sufficient magnitude to provide a viable gating mechanism. Calculations of the likely kinetics of the process do reveal that it can give rise to the sigmoid (m^3) sodium current switch-on characteristic that is observed for the squid axon, and these calculations will be presented elsewhere.

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