Long-Range Interactions, Voltage Sensitivity, and Ion Conduction in S4 Segments of Excitable Channels

H. Richard Leuchtag
Department of Biology, Texas Southern University, Houston, Texas, USA

ABSTRACT Forces acting on the S4 segments of the channel, the voltage-sensing structures, are analyzed. The conformational change in the Na channel is modeled as a helix–coil transition in the four S4 segments, coupled to the membrane voltage by electrical forces. In the model, repulsions betweeen like charges make the S4 segment unstable, but field-dependent forces hold it in an α-helix configuration at resting potential. At threshold depolarization, the S4 helices cooperatively expand into random coils, breaking the hydrogen bonds connecting adjacent loops of the α helices. Exposed electron pairs left on the carbonyl oxygens constitute sites at which cations can bind selectively. The first hydrogen bond to break is at the channel exterior, then the second breaks, and so on in a zipper-like motion along the entire segment. The Na^+ ions hop from one site to the next until all H bonds are broken and all sites are filled with ions. This completes the pathway over which the permeant ions move through the channel, driven by the electrochemical potential difference across the membrane. This microscopic mechanism is consistent with the thermodynamic explanation of ion-channel gating previously formulated as the ferroelectric-superionic transition hypothesis.

INTRODUCTION

The problem of cellular excitability is now focused on the question of the mechanism by which ion channels open and close under voltage control (reviewed in Hille, 1992; Catterall, 1988; Barchi, 1988; Jan and Jan, 1989). The strong electric field across the membrane of a viable cell, of order 10^5 V/cm, must strongly affect the conformation of a transmembrane protein molecule. The configuration of the majority of Na channels at resting potential, the closed configuration, can be considered a metastable state, as it yields under a relatively small depolarization to the open state. Because the α helix is a major membrane-spanning component of the channel (Noda et al., 1984), it is worthwhile to consider its mechanics. This applies particularly to the S4 segment, which, because of its distribution of positive charges, has been identified as the voltage sensor. Of special interest is the mutual Coulomb repulsion of these charges, which may be considered long-range interactions, in the sense that they extend over large distances compared with interatomic distances. We will be concerned here primarily with the Na channel, although the similarities between excitable cation channels, based on their common evolutionary ancestry (Strong et al., 1993), will allow us to consider data from other channels as well. This paper proposes a new molecular model in which the closed–open conformational transition of the channel is modeled as a helix–coil transition in its four S4 transmembrane segments. A partial presentation of this work has been made (Leuchtag, 1993).

Onsager (1967) suggested an ion channel of protein helices with polar groups to provide local solvation for the ion. Holland (1973) postulated that a channel consists of a bundle of α-helical strands with axes perpendicular to the membrane, and that the oxygen and nitrogen atoms of the helices form chains of electronegative centers, along which cations hop. Holland explained the spontaneous closing of the Na channel by energy dissipation in the interaction of the moving ions with the helices. A number of molecular channel models postulate a water-filled pore (Hille, 1992); these necessarily leave aspects of gating and selectivity unexplained (Leuchtag, 1992). A sodium-channel model that shares with the present model the concept of a helix–coil transition in the S4 segments but retains the concept of ion conduction through a file of water molecules (based on an analogy to gramicidin A) is that of Benndorf (1989).

Sodium-channel architecture

The primary structure of the major polypeptide constituting the Na channel is known, and many features of the secondary and tertiary structures have emerged (Noda et al., 1984; Guy and Seetharamulu, 1986; Catterall, 1988; Stühmer et al., 1989). The peptide consists of four highly conserved domains with six membrane-spanning segments each. Although the 3_{10} helix structure has also been suggested, the secondary structure of the segments is mostly α helical (Noda et al., 1984; Kosower, 1985; Guy and Seetharamulu, 1986). This was demonstrated by circular dichroism spectroscopy, which gives estimates of 64 and 68%, respectively, for the α-helix content of Na channels from rat brain and eel electroplax (Elmer et al., 1985; Miller et al., 1983). The α helix is stabilized by hydrogen bonds between the N-H of the ith residue and the C=O of the $i + 4$th residue. The H bonds are roughly parallel to each other and normal to the membrane plane (Cantor and Schimmel, 1980; Wada, 1962).

The major emphasis here will be the role of the S4 helix, which has been identified as the voltage-sensing substructure of the Na and related cation channels. The S4 helix has an

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Address reprint requests to Dr. H. Richard Leuchtag, Department of Biology, Texas Southern University, Houston, TX 77004.

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unusual structure in that generally every third residue is positively charged, either arginine or lysine, with nonpolar residues between them. Another important feature of the S4 helix is a proline residue that is frequently present a few residues from the cytoplasmic end. Because proline introduces a kink in the peptide, the S4 helix of a potassium channel has been considered as made up of two parts, S4 and S45, which are bent at an angle to each other (Durell and Guy, 1992). This notation will not be used here, and the term S4 will refer to the entire transmembrane segment.

The primary structures of the S4 helices from eel electroplax membranes show 5–8 positively charged residues, arg or lys (Noda et al., 1984). Because the Na and Ca channels have four repeated domains, they contain four S4 segments. The tetrameric K channel also has four S4 segments in its quaternary structure (Durell and Guy, 1992). The four domains are probably arranged in a fourfold symmetry about the central axis (Noda et al., 1986), but with the S4 segments closest to the axis (Guy and Conti, 1990). One possible structural motif for the S4s is the four-α-helical bundle (Weber and Salemme, 1980; Regan et al., 1990), but with the four helices directed parallel instead of in two parallel-antiparallel pairs.

Conformational transition in the S4 helix

That the positive charges are the basis for the voltage-sensing property has been demonstrated in rat sodium channels (Stühmer et al., 1989) and in two voltage-gated potassium channels (Fayemi et al., 1992; Logothetis et al., 1992). Clearly, the S4 segment is the structure that responds to the electric field; if a transition from a metastable to a stable state is to be found in the channel, it is likely to be located here.

The voltage sensing of the S4 segment must clearly be an instrumental component of the conformational transition that opens and closes the channel. The working assumption here is that the transition of the S4 segments to a great extent *is* that conformational transition. The stabilizing hydrogen bonds connecting every fourth residue of an α helix can be weakened by deuterium substitution or by heating, which leads to a thermal unfolding in which the α helix structure becomes disordered with the breaking of H bonds to assume a random-coil conformation (Rohl et al., 1992; Scholtz et al., 1992). Can such a helix–coil transition be the conformational transition of voltage sensing in the S4 segments?

The voltage sensing in the S4 segment must be the result of a changing balance between competing forces, including: (1) the long-range electrostatic repulsion of the positive charges at every third residue, which tends to destabilize the α helix; (2) dipole–dipole forces within the helix, which are repulsive at short range and attractive at long range; (3) the interaction between the external field and the channel dipoles; (4) an electrostrictive force compressing the membrane, including the segments comprising the channel, which tends to stabilize it; (5) a piezoelectric force, which is linear in the field, and therefore may be stabilizing or destabilizing, depending on the direction of the field; (6) surface forces at the boundaries between the channel helices and the aqueous media, due to the differences between the dielectric constant of protein (~4) and water (~80), and the high mobility of ionic species in water, leading to the formation of an ionic shielding layer at the interface; and (7) interactions with the other three S4 segments and the surrounding segments. The segment will maintain its α-helical configuration as long as the stabilizing forces prevail; otherwise it will expand into a random coil.

Dipole moments in the α helix

The present molecular model is consistent with an earlier thermodynamic model in which the nonequilibrium resting state of the channel is ferroelectric and the equilibrium open state is paraelectric and a superionic conductor (Leuchtag, 1991a,b; Bystrov and Lakhno, 1992; Shirane et al., 1993). The ferroelectric-superionic transition hypothesis implies that the channel protein undergoes a reversible transition; that the more ordered configuration corresponds to the closed channel, and the less ordered to the open channel; and that the ordered state has a net dipole moment, which is lost in the disordered state (Leuchtag, 1991a,b).

Because ferroelectrics are defined by their reorientable dipole moment, we must look for a dipole moment in the channel. We find it in the α helices, which can be described as one-dimensional crystals because of the ordered orientation of their structural units. These units, the amino-acid residues, each have a dipole moment of ~4.4 D. The dipole moments of the amide planes are aligned approximately parallel to the helix axis (Cantor and Schimmel, 1980; Wada, 1962, 1976).

That hydrogen bonds can contribute to a ferroelectric state is shown by a class of ferroelectric crystals, represented by potassium dihydrogen phosphate (KH_2PO_4, KDP), that has as the basis of its polarization an array of hydrogen bonds. Triglycine sulfate (TGS) is another hydrogen-bonded order-disorder ferroelectric. For both KDP and TGS, the H bonds are symmetrical, of the type -OH $\cdots$ O- (Lines and Glass, 1977). In this they differ from the H bonds in the α helix, which are asymmetrical, of the type NH $\cdots$ O=.

The sodium-channel polypeptide threads repeatedly through the membrane, with both its amino and carboxy terminals inside the cell. Thus segments S1, S3, and S5 of all four domains have their amino terminals inside, whereas segments S2, S4, and S6 of each have their amino terminals outside. The dipole moment of an α helix is directed from the carboxy to the amino terminal (Cantor and Schimmel, 1980; Wada, 1962). Thus the odd-numbered segments have their dipole moments directed inward and the even ones, outward.

The voltage sensor S4 thus has its dipole moment directed outward, negative inside and positive outside. Therefore its dipole moment is in stable equilibrium at rest potential, when the inside potential is negative, but unstable when the polarization is reversed. While this is true for all even-numbered segments, the S4 segments are already in a meta-

stable state because of the mutual repulsions of the positive charges. The property of electrostriction, present in all substances (Lines and Glass, 1977), will produce a compressive strain, stabilizing the segments at rest potential. Therefore a small depolarization from rest potential can trigger the breaking of the H bonds holding the S4 segments in the α-helix conformation.

Clearly, a helix–coil transition would disrupt the dipole moment of the S4 segment. The moments of the amide planes would now be oriented randomly or at least would be less ordered than in the α helix, where they are nearly parallel to the axis. Thus the basis of the electric polarization of the S4 helices would be eliminated. Assuming the net change in polarization of the rest of the channel is relatively small, a helix–coil transition in the S4 segments can account for a ferroelectric-paraelectric transition in the channel. Consistent with this earlier hypothesis (Leuchtag, 1988), the helix–coil transition is an order–disorder transition (Poland and Scheraga, 1966).

Instability in the S4 segment

Because the end residues are least well secured by H bonds, the transition from α helix to random coil can nucleate most easily at the end of a helix. (The ends of the α helices at the membrane surfaces are not free, but are connected to other segments by linking polypeptide loops, which are presumably sufficiently flexible to adapt to the helix–coil transition.) The instability of an α helix will be greatly increased by the mutual Coulomb repulsion of a distribution of like charges in its side chains. Thus the existence of positively charged side chains at every third residue makes the S4 helix unstable and vulnerable to a transition to a random coil. The charged residues most likely to be repelled with sufficient force to break the H bonds initially are the ones at either end, where the repulsions, adding vectorially, are greatest.

The S4 helix is a substructure of 21–22 residues in each of the four domains of the major peptide, 1820 residues long, of a sodium channel (Noda et al, 1984). Roughly, it can be considered a cylindrical region in a protein environment bounded by parallel planar aqueous media, because at the atomic scale the lipid phase is relatively distant and the curvature of the membrane is negligible. To keep the calculations simple, the kink produced by the proline residues in segments IS4, IIS4 and IIIS4 will be ignored. The S4 segment contains 5–8 point charges arranged in a helical array.

Consider the following idealized model of the long-range forces in the S4 helix. Six equal positive charges are arranged at points located on a helix of radius R, equal to the distance of the centers of the guanidinium charges from the helical axis. Taking the backbone diameter of the α helix as 6 Å (Cantor and Schimmel, 1980), and allowing 5 Å for the distance from the helix to the charge center of an arginine or lysine sidechain (Lehninger, 1982), we use $R = 8$ Å. The charges are assumed equidistant along the axial (z) and radial (θ) coordinates, and embedded in a continuous protein medium of dielectric constant $\epsilon = 4$. The magnitude of the charges is $e = 1.60 \times 10^{-19}$ C. Because the residues are $d = 1.5$ Å apart along the z axis (Wada, 1962), the axial separation, $A = 3d$, of the charges, is 4.5 Å. The angular separation of the charges is three times the angular displacement of the residues, 100° to the right in a right-handed helix (Wada, 1962). Therefore the charges are separated by +300° or $-60° = -\pi/3$. The location of the nth charge from origin on the axis (counting $+n$ outward and $-n$ inward) will be $(R, \theta_0 - n\pi/3, z_0 + na)$ (See Fig. 1). The protein medium will be assumed to be bounded by two parallel planes normal to the z-axis, with aqueous electrolytes as the bounding media.

To simplify the calculations, the effects of boundary charges and external fields will be neglected initially. The potential energy of the outermost charge, 1, due to another charge, j, is (Jackson, 1962)

$$U_{1j} = K/r_{1j} \quad (j = 2, \ldots, 6) \tag{1}$$

where $K = e^2/(4\pi\,\epsilon_0\epsilon) = 5.76 \times 10^{-29}$ J m. From Fig. 1B it can be seen that $r_{12}^2 = A^2 + (2R \sin\theta/2)^2$; more generally,

$$r_{1j} = \{(j-1)^2A^2 + 4R^2\sin^2[(j-1)\theta/2]\}^{1/2} \tag{2}$$

The potential energy due to the long-range forces exerted by charges 2–6 on charge 1 is the sum of the interactions of Eq. 1,

$$U_1 = \frac{K}{2R}\sum_{j=2}^{6}\left[\frac{(j-1)^2A^2}{4R^2} + \sin^2\frac{(j-1)\theta}{2}\right]^{-1/2}. \tag{3}$$

For the α helix, with $\theta = -60° = -\pi/3$ and $A/2R = 0.28125$, $U_1 = 1.75 \times 10^{-19}$ J = 105 kJ/mol. Because the two ends are equivalent, $U_6 = U_1$. Similar calculations for the other charges, using for the long-range interaction energy

$$U_i = \frac{K}{2R}\sum_{j\neq i}\left[\frac{(j-i)^2A^2}{4R^2} + \sin^2\frac{(j-i)^2\theta}{2}\right]^{-1/2} \qquad (i, j = 1, \ldots, 6) \tag{4}$$

show that $U_5 = U_2 = 128$ kJ/mol and $U_4 = U_3 = 134$ kJ/mol. The fact that electrolyte counterions at the membrane boundary were not included in this model contributes to the high values obtained. In the absence of boundary effects and external fields, the forces stabilizing the helix are essentially opposed only by the intrahelix hydrogen bonds. Because the outermost charged residue is held by only one hydrogen bond, and the energy required to break a hydrogen bond is ~20 kJ/mol (Jeffrey and Saenger, 1991), the residue is highly unstable. It therefore seems that an S4 segment with six positive charges cannot exist freely in equilibrium as an α helix in the assumed configuration.

Now let us remove one of the unrealistic assumptions of the previous calculations, by restoring the boundaries. The electrolyte counterions outside the parallel boundary planes will rearrange to form an induced surface charge distribution. Although the calculation of this distribution is beyond the scope of this paper, its effect will be discussed semiquantitatively. Consider the channel to be enclosed by a Gaussian pillbox, of radius large enough that no electric lines of force

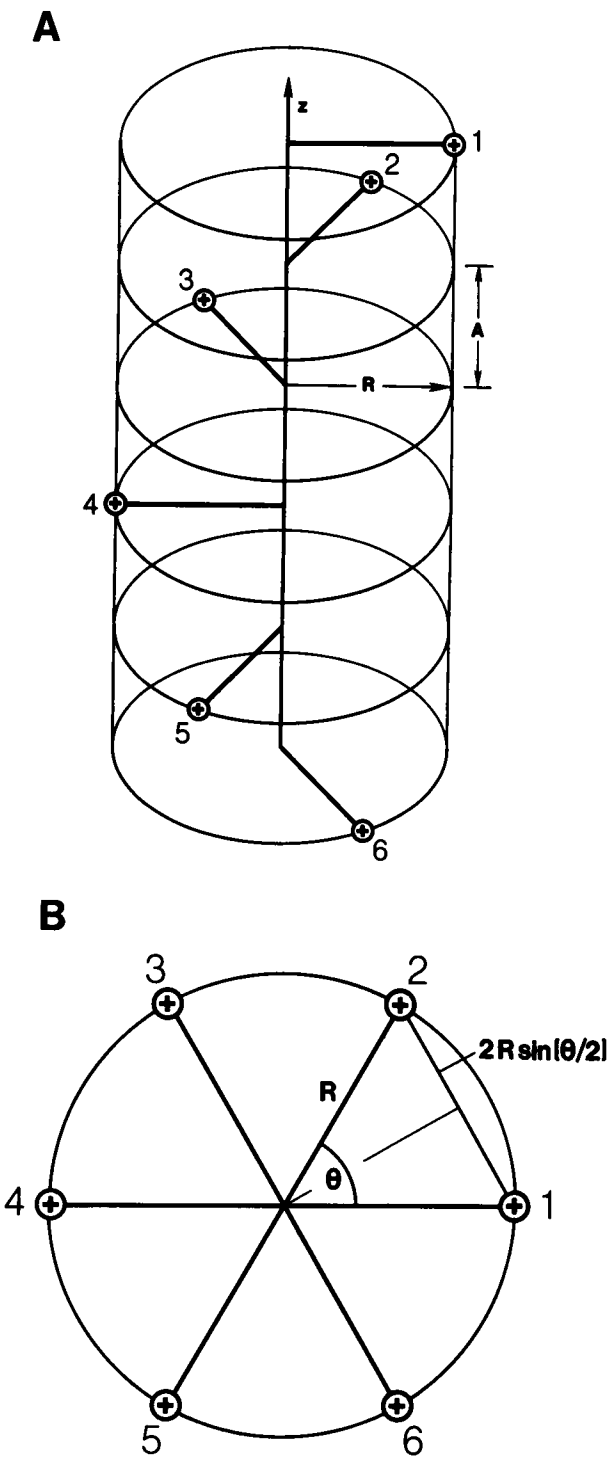


FIGURE 1 Simplified model of an S4 segment with six positive charges equally spaced at every third residue along an α helix. (*A*) Because every third residue advances 300° = −60°, this subset of a right-handed α helix forms a left-handed helix. (*B*) End view of the helix from the extracellular side, showing the planar component of the distance between adjacent charges.

cross the cylindrical surface. Let the planar ends of the pillbox be several Debye lengths into the solution, so that the field is also zero there. Because, by Gauss's law, the net charge enclosed in the box at equilibrium is zero, a surface charge σ of $-6e$ equal and opposite to the enclosed charge must be distributed between the two surfaces S_I and S_{II}:

$$\int_{S_I} \sigma\, da + \int_{S_{II}} \sigma\, da = -6e \qquad (5)$$

If the ionic solutions are equal, the α helix is located symmetrically within the membrane, and the charges are symmetric about the midplane in the helix, then the field across the midplane will be zero and, consequently, the induced charges will be equally divided, $-3e$ outside and $-3e$ inside. Because the assumptions of equal solutions and zero field do not hold in biological membranes, the surface charges will generally be divided unequally.

This model is still inadequate, however, because the other segments of the channel have not been considered. Because the Na, Ca, and tetrameric K channels have four S4 helices, they carry a charge of $+24e$ or, more generally, $+pe$. (Although the actual numbers of charged residues are not all 6, but 5, 5, 6, and 8 for IS4 to IVS4, these fortuitously also add to 24.) Furthermore, both negative and positive charges exist in the outer segments. Even though the channel, embedded in the phospholipid bilayer, is a hydrophobic environment, so that local electroneutrality does not necessarily prevail, these charges must be accounted for within the Gaussian pillbox. As before, shielding charges will be induced in the aqueous media; see Fig. 2. If the outer charges add algebraically to $-ne$, Eq. 5 becomes

$$\int_{S_I} \sigma\, da + \int_{S_{II}} \sigma\, da = (n - p)e \qquad (6)$$

The value of n depends on the placement of the residues in the structural model, because charges on residues located in the aqueous phases do not contribute to n. This calculation shows that the repulsive energies previously calculated will be reduced somewhat by the induced ionic charges at the membrane boundaries. Nevertheless, the conclusion that the cluster of S4 helices is unstable in the absence of an external field (such as that due to the resting potential) still stands.

Channel activation

Because of the resting potential, the intracellular surface charge will be more negative and the external charge less negative. The additional surface charge density induced by potential difference V across the membrane can be estimated by $\sigma_V = CV$, where membrane capacitance per unit area $C \approx 1\ \mu F/cm^2$. At a resting voltage of −70 mV, $\sigma_V \approx 7 \times 10^{-4}\ C/m^2$. If the effective area of the channel is about 5000 $Å^2$, the average induced charge on the resting channel is about 1/5 of an electronic charge, negative inside and positive outside.

Consider residue charge 1, at the outer extremity of one of the α helices. A negative induced surface charge at the outer (extracellular) surface will attract it, adding to the tension in the H bonds holding it because of the repulsion of the

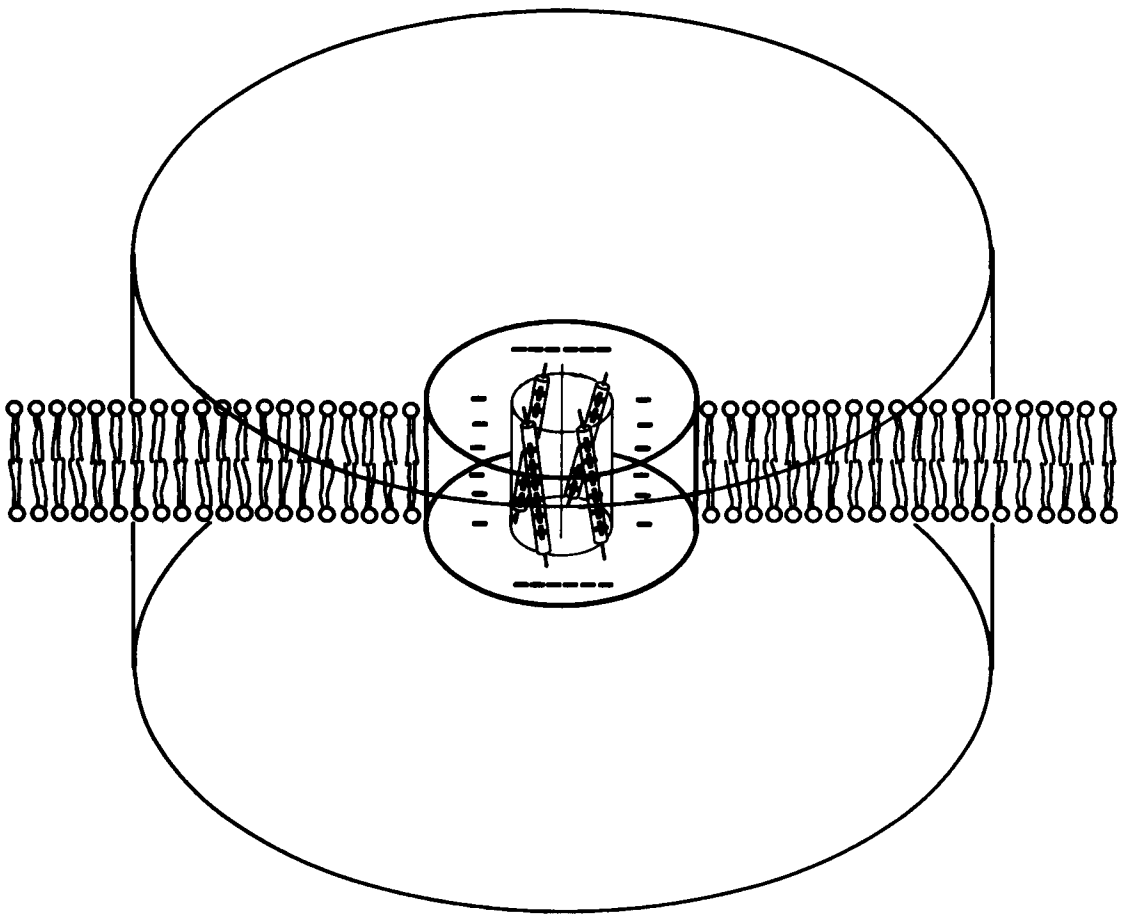


FIGURE 2 Model of a sodium channel enclosed in a pillbox-shaped region of integration. The 24 positive charges of the four S4 segments are neutralized by negatively charged residues in surrounding segments and induced ionic charges at the boundaries of the aqueous media. The net charge enclosed by the Gaussian pillbox is zero.

other positive charges, and so increasing its instability. However, inward repulsion due to the positive surface charge present at rest potential contributes to its stability. So does the electrostrictive force on the membrane, which compresses the α helix. Suppose that these forces (and presumably others, such as field–dipole interactions and piezoelectricity) are sufficient to maintain the S4 segment in its α-helix configuration. Now let the membrane be depolarized by the approach of an action potential or by experimental manipulation. The outer surface charge becomes less positive (and the inner one less negative), and the electrostrictive and other stabilizing forces decrease. These effects combine to increase the instability of charge 1; for an appropriate balance of forces, the H bond holding it will break at threshold.

The removal of charge 1 (and probably its neutralization by counter-ions in the solvent) greatly increases the stress on the H bonds holding charge 2 in place and this, together with changes in other forces, causes them to break, and so on for the entire segment, which opens into a random-coil configuration. A helix–coil transition in one of the S4 segments (presumably the one with the largest number, 8, of positive charges) will probably trigger like transitions in the other three.

The conformational transition of the channel

The present analysis suggests that at rest potential, the four S4 segments are in a compressed, α-helical, state. They are maintained in that state by forces that depend on the electric field, and, hence, the transmembrane voltage. As the voltage is decreased by a depolarization, these stabilizing forces become less than the destabilizing electrostatic repulsions between the positive residues and thermal motions, and the S4 helices expand to become random coils. The helix–coil transformation has been described as remarkably sharp, comparable to a phase transition (Zimm and Bragg, 1959; Lifson and Roig, 1961; Poland and Scheraga, 1970). Structural data (Noda et al., 1986; Durell and Guy, 1992) suggest that the outer helices of the ion channel define a roughly cylindrical sheath within which the four active S4 helices are contained. Because of constraint forces from this sheath and from the end loops, the coil configuration will not actually be random, but defined by the rest of the channel molecule.

Thus, in the present hypothesis, the voltage-dependent conformational change of channel opening is a helix–coil transformation in the S4 segments caused by long-range electrostatic repulsions that is induced by the removal of the

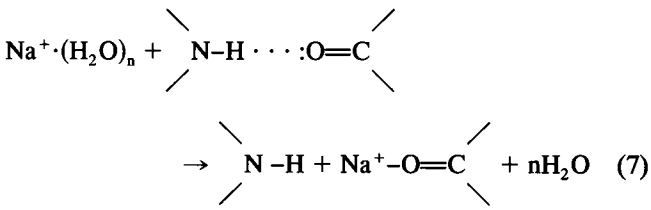
stabilizing influence of the high electric field present in the resting channel. The resting conformation can be equated with an α helix and the depolarized one with a random (more precisely, pseudorandom) coil. Channel closing would require the slower, endergonic process of rebuilding of the α helix. Therefore the channel would be in a mixed state for a considerable time while closing, during which the S4 segments would contain stretches of both α helix and random coil. In this state, the channel would be neither conducting nor excitable; it is inactivated. This is consistent with the observations that inactivation time constants are much larger than activation time constants (Hodgkin and Huxley, 1952). Hyperpolarization of a resting membrane would further compress the S4 segments, increasing the number of channels with S4s in the α-helical state, and making that state more stable. This is consistent with the results of experiments on prehyperpolarizations in axons (Hodgkin and Huxley, 1952).

Because the channel opening in this hypothesis is interpreted as an unfolding and lengthening of the positively charged S4 segments into random coils, we must ask whether this lengthening is inward into the cytoplasm or outward or both; i.e., where the stationary point of the motion is located. The most likely positions for this role are those of the proline residues, where the S4 segment is bent, and those of particularly bulky residues, such as phenylalanine or tryptophan, often found near the proline residues. (IVS4, with eight positive charges and no proline or bulky residues, may play a special role as trigger in channel opening, as mentioned above.) Because these positions can be seen from existing models (Noda et al., 1984; Salkoff et al., 1987) to be closer to the cytoplasmic side, the motion of the positive charges will be preponderantly outward. This implies an outward swelling of the membrane during activity, which has been observed to occur simultaneously with the action potential in experiments by Tasaki and Iwasa (1982). The motion of these charges will also contribute to the outward gating current (Armstrong and Bezanilla, 1973). However, a full discussion of the gating current in this model is beyond the scope of this paper.

The ion pathway

The unfolding of the S4 α helices into the extended coil form has several implications. a) This form is less bulky than the α helix, leaving room for the unhydrated Na^+ or Li^+ to pass through the "pore" created by the transition of a group of four α helices to mutually repelling random coils. b) The coils have charged donor sites at the amide carbonyl oxygens when the H bonds are broken. It is known, e.g., that lithium ions form specific interactions with amides. Li^+ binds strongly to the amide carbonyl group of formamide. The binding energies of the adducts are large (70–110 kcal/mol) and considerably higher than the hydration energy of Li^+ (50 kcal/mol) (Rao, 1973).

Unless the H bonds reform in a new configuration, the backbone carbonyl oxygens are left with a pair of outer-shell electrons, which can serve as a selective binding site for cations. Because the first H bond broken is the outermost one, adjacent to the aqueous solution, a sodium ion can shed its hydration shell and attach to this site by the ion-exchange reaction



As the second hydrogen bond breaks, the newly opened site becomes filled with an Na^+ that has jumped the energy barrier from the first site, while another Na^+ replaces it from the aqueous solution. Equation 7 is somewhat oversimplified in that the Na^+s can form chelation complexes with several carbonyl oxygens simultaneously, as well with amino nitrogens. The helix–coil transition can be thought of as a nucleation. The first H-bond that is broken nucleates the coil, facilitating the breaking of other H-bonds, zipper-like, so that the transition is cooperative. As H-bonds break farther in the channel, away from the Na^+ reservoir at the outer surface, Na^+s hop from site to site in the four S4 helices until all hydrogen bonds are broken and all sites filled. Now a continuous pathway is established across the channel, and Na^+, stripped of its waters of hydration and sequestered from the solvent, can flow between the extracellular and intracellular compartments by cooperative displacements of neighboring ions. This pathway can be thought of as a membrane-spanning molecular salt bridge (Honig and Hubbell, 1984). The ion flow is then determined by the difference in the electrochemical potential between the outer and inner electrolytes and the ion conductance of the pathway. The open state of the channel therefore exists, in the present hypothesis, when the four S4 segments are in the random-coil conformation, with all sites occupied by permeant ions. In the closed state, by contrast, the S4 segments are α helices, which repel Na^+ with energies of 0–240 kcal/mol (Van Duijnen and Thole, 1981).

The strength of ion binding to the site must depend on the ionic species and will in turn determine the jump probability. A divalent ion such as Ca^{2+} is expected to bind more strongly at a given site, and so have a lower probability of hopping. It will also repel nearby Na^+s more strongly. This not only implies a lower conductivity for Ca^{2+} in the sodium channel, but also implies the possibility of Ca^{2+} block of Na^+ conduction, as is found in the Na channel (Nilius, 1988; Chahine et al., 1992). Thus the specificity of binding at the sites can help account for the highly selective nature of ion conduction through ion channels. The thermally activated hopping of the ions from site to site here proposed for the Na channel is comparable to ion movement in superionic conductors, which is also highly selective (Owen, 1989; Leuchtag, 1991a, b).

The blocking of the Na channel by tetrodotoxin (TTX) and analogues may be explained in this model by the binding of

the active groups of the TTX molecule to stereospecific counterpart groups in the Na channel (Kao and Walker, 1982). If the TTX binds to the S4 segments in such a way as to stabilize the α-helix state, at least locally, it can prevent the helices from unfolding completely. This would inhibit the completion of the cooperative helix–coil transformation needed to form the transmembrane ion pathway that constitutes the open channel.

A possible means of testing this hypothesis may be the measurement of circular dichroism (CD) in a homogeneous population of channels in a membrane. Because one out of six segments is expected to undergo a helix–coil transition, a threshold depolarization should reduce the CD spectrum by about one-sixth. Watanabe (1987) has observed a change in optical activity in lobster nerve and suggested it may be related to molecular changes involved in the propagation of the action potential.

SUMMARY

The hypothesis proposed here is that the conformational transition in the channel is a cooperative helix–coil transition in the four S4 helices. The importance of the positive charges of the S4 segments to channel gating has been confirmed in site-specific mutagenesis experiments. The mutual repulsion of the positive charges in an S4 segment tends to destabilize its α-helix conformation, whereas the negative charges in the segments surrounding the bundle of four S4 segments tend to oppose this instability. It is here proposed that the balance of charges in the excitable ion channel is so adjusted that the S4 segments require an external force to exist in the α-helix conformation at physiological temperatures. This force is provided by the resting potential found in living excitable cells. The resting potential helps to stabilize the S4 helices by an interaction between the electric field and the amino-acid dipoles, by readjustment of the ionic layers in the bounding aqueous solutions, and by electrostriction and other forces.

In this way the channel's S4 segments stochastically only exist in their α-helix configuration at or near rest potential, or when hyperpolarized. In this configuration the dipole moments of the residues in the S4 helices are aligned and provide a net electric polarization to the channel. A depolarizing stimulus of sufficient magnitude triggers a transition in which the hydrogen bonds break and the α helix unfolds into a random coil. Because of the interactions between the dipoles, this unfolding is cooperative, both within the S4 segment and among the four segments of the axial S4 bundle. Consequently, the helix–coil transition is a rapid, all-or-nothing process. Although individual segments unfold stochastically, the voltage at which the majority of the channels in a membrane region have their S4 segments unfold is the threshold potential. Because the dipoles of amino-acid residues are randomly oriented in the coil conformation, the segment loses its net polarization. Thus the S4 segments are predominantly ferroelectric at rest potential and become paraelectric on threshold depolarization.

The unfolding of the S4 helices into random coils breaks the hydrogen bonds within the α helix, thereby exposing negatively charged sites on the carbonyl oxygens of the polypeptide backbone. These sites are postulated to serve as binding sites for the permeant cations, primarily Na^+ and Li^+ in the case of the Na channel. As each hydrogen bond breaks, a site becomes available; that site is filled selectively by ions from the aqueous reservoir. The ion, stripped of its waters of hydration, binds to the outermost site and hops to the next available site if its energy is sufficient to overcome the barrier between the sites. The ion-filled sites of the completely transformed channel form a continuous ion-conducting pathway along the four S4 coils across the channel. This continuous pathway forms the open "pore," through which conduction is controlled by the electrochemical potential difference between the aqueous media. As in superionic conduction, ion permeability along the pathway depends on the binding force between the ion and the site. Thus the specificity of the binding sites determines the selectivity of the channel.

The refolding of the α helices requires the displacement of the cations and the cooperative formation of the hydrogen bonds. This process must therefore be much slower. Until the α helices are completed, the channel, although not conducting, will not be excitable; it is inactivated. TTX block is explained by a stereospecific binding of the toxin molecule to the Na channel that stabilizes the S4 segments in their α-helical conformations.

The helix–coil transition hypothesis of the conformational change in the voltage-sensing segments provides a unified physical framework for understanding the mechanism of channel excitability. It explains the macroscopic phenomena of channel gating in terms of established properties of α helices in the molecular structure of the channel.

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REFERENCES

Armstrong, C. M., and F. Bezanilla. 1973. Currents related to the movement of the gating particles of the sodium channels. *Nature (Lond.).* 242: 459–461.

Barchi, R. L. 1988. Probing the molecular structure of the voltage-dependent sodium channel. *Annu. Rev. Neurosci.* 11:455–495.

Benndorf, K. 1989. A reinterpretation of Na channel gating and permeation in terms of a phase transition between a transmembrane S4 α-helix and a channel helix. A theoretical study. *Eur. Biophys. J.* 17:257–271.

Bystrov, V. S., and V. D. Lakhno. 1992. Ferroactive models of ion channels in biomembranes. *In* Abstracts of the Polarons and Applications International Workshop, Pushchino Research Center, Russian Acad. Sci. 9.

Cantor, C. R., and P. R. Schimmel. 1980. Biophysical Chemistry. Part III. W. H. Freeman, San Francisco. 1041–1107.

Catterall, W. A. 1988. Structure and function of voltage-sensitive ion channels. *Science (Washington DC).* 242:50–61.

Chahine, M., L-Q. Chen, R. G. Kallen, R. L. Barchi, and R. Horn. 1992. Expressed Na channel clones differ in their sensitivity to external calcium concentration. *Biophys. J.* 62:37–40.

Durell, S. R., and H. R. Guy. 1992. Atomic scale structure and functional

models of voltage-gated potassium channels. *Biophys. J.* 62:238–247.

Elmer, L. W., B. J. O'Brien, T. J. Nutter, K. J. Angelides. 1985. Physicochemical characterization of the alpha-peptide of the sodium channel from rat brain. *Biochemistry.* 24:8128–8137.

Fayemi, B., S. D. Bisbas, S. Movahedi, C. Satler, K. L. Lindpaintner, B. Nadal-Ginard, and D. E. Logothetis. 1992. Differential contributions of individual S4 charges to voltage sensitivity of the delayed rectifier K channel, RCK1. *Biophys. J.* 61:A377.

Guy, H. R., and F. Conti. 1990. Pursuing the structure and function of voltage-gated channels. *Trends Neurosci.* 13:201–206.

Guy, H. R., and P. Seetharamulu. 1986. Molecular model of the action potential sodium channel. *Proc. Natl. Acad. Sci. USA.* 83:508–512.

Hille, B. 1992. Ionic Channels of Excitable Membranes, 2nd ed. Sinauer, Sunderland, MA.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Holland, B. W. 1973. Ion transport across cell membranes. *In* Cooperative Phenomena. H. Haken and M. Wagner, editors. Springer-Verlag, New York. 404–412.

Honig, B. H., and W. L. Hubbell. 1984. Stability of "salt bridges" in membrane proteins. *Proc. Natl. Acad. Sci. USA.* 81:5412–5416.

Jackson, J. D. 1962. Classical Electrodynamics. Wiley, New York.

Jan, L. Y., and Y. N. Jan. 1989. Voltage-sensitive ion channels. *Cell.* 56: 13–25.

Jeffrey, G. A., and W. Saenger. 1991. Hydrogen Bonding in Biological Structures. Springer, Berlin.

Kao, C. Y., and S. E. Walker. 1982. Active groups of saxitoxin and tetrodotoxin as deduced from actions of saxitoxin analogues on frog muscle and squid axon. *J. Physiol. (Lond.).* 323:619–637.

Kosower, E. M. 1985. A structural and dynamical model for the sodium channel of *Electrophorus electricus. FEBS Lett.* 182:234–242.

Lehninger, A. L. 1982. Principles of Biochemistry. Worth, New York.

Leuchtag, H. R. 1988. A proposed physical explanation of the activation of sodium channels. *Ferroelectrics.* 86:105–113.

Leuchtag, H. R. 1991a. Do sodium channels in biological membranes undergo ferroelectric-superionic transitions? *In* Proc. 1990 IEEE 7th Int. Symp. on Applications of Ferroelectrics. Piscataway, NJ, IEEE. 279–283.

Leuchtag, H. R. 1991b. Mechanism of the voltage-induced conductance change in the Na channel. *In* Proc. 1991 IEEE Northeast Bioengineering Conf. Piscataway, NJ, IEEE. 169–174.

Leuchtag, H. R. 1992. Does the Na channel conduct ions through a water-filled pore or a condensed-state pathway? *Biophys. J.* 62:22–24.

Leuchtag, H. R. 1993. A helix–coil transition in the S4 segments as a model of the closed-open conformational change in the Na channel. *Biophys. J.* 64:A85.

Lifson, S., and A. Roig. 1961. On the theory of helix–coil transition in polypeptides. *J. Chem. Phys.* 34:1963–1974.

Lines, M. E., and A. M. Glass. 1977. Principles and Applications of Ferroelectrics and Related Materials. Clarendon, Oxford. 1–23, 59–173, 293–333.

Logothetis, D. E., S. D. Bisbas, B. Fayemi, K. L. Lindpaintner, and B. Nadal-Ginard. 1992. Structural basis underlying the differences in voltage-sensing properties of two voltage-gated potassium channels. *Biophys. J.* 61:A425.

Miller, J. A., W. S. Agnew, and S. R. Levinson. 1983. Principal glycopeptide of the tetrodotoxin/saxitoxin binding protein from *Electrophorus electricus*: isolation and partial chemical and physical characterization. *Biochemistry.* 22:462–470.

Nilius, B. 1988. Calcium block of guinea-pig heart sodium channels with and without modification by the piperazinylindole DPI201–106. *J. Physiol. (Lond.).* 399:537–558.

Noda, M., S. Shimizu, T. Tanabe, et al. 1984. Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. *Nature (Lond.).* 312:121–127.

Noda, M., T. Ikeda, T. Kayano, H. Suzuki, H. Takeshima, M. Kurasaki, H. Takahashi, and S. Numa. 1986. Existence of distinct sodium channel messenger RNAs in rat brain. *Nature (Lond.).* 320:188–192.

Onsager, L. 1967. Thermodynamics and some molecular aspects of biology. *In* The Neurosciences. G. C. Quarton, T. Melnechuk, and F. O. Schmitt, editors. Rockefeller University Press, New York. 75–79.

Owen, J. R. 1989. Fast ion conducting polymers. *In* Superionic Solids and Solid Electrolytes. Recent Trends. A. L. Laskar and S. Chandra, editors. Academic, Boston. 111–136.

Poland, D., and H. A. Scheraga. 1966. Phase transitions in one dimension and the helix–coil transition in polyamino acids. *J. Chem. Phys.* 45: 1456–1463.

Poland, D., and H. A. Scheraga. 1970. Theory of Helix–Coil Transitions in Biopolymers. Academic, New York.

Rao, C. N. R. 1973. Conformational studies on amides and related systems. *In* Conformation of Biological Molecules and Polymers. E. D. Bergmann and B. Pullman, editors. Israel Academy of Sciences and Humanities, Jerusalem. 107–119.

Regan, L., S. P. Ho, Z. Wasserman, and W. F. DeGrado. 1990. Helical proteins: de novo design. *In* Protein Folding. L. M. Gierasch and J. King, editors. American Association for the Advancement of Science, Washington, DC. 171–176.

Rohl, C. A., J. M. Scholtz, E. J. York, J. M. Stewart, and R. L. Baldwin. 1992. α-helix formation monitored by amide proton exchange and NMR. *Biophys. J.* 61:A345.

Salkoff, L., A. Butler, A. Wei, N. Scavarda, K. Giffen, C. Ifune, R. Goodman, and G. Mandel. 1987. Genomic organization and deduced amino acid sequence of a putative sodium channel gene in *Drosophila. Science (Washington DC).* 237:744–749.

Scholtz, J. M., E. J. York, J. M. Stewart, and R. L. Baldwin. 1992. Parameters of helix–coil transition theory for alanine-based peptides in water. *Biophys. J.* 61:A345.

Shirane, K., Tokimoto, T., Shinagawa, K., and Yamaguchi, Y. 1993. Ferroelectric diffused electrical bilayer model for membrane excitation. *Ferroelectrics.* 141:297–305.

Strong, M., K. G. Chandy, and G. A. Gutman. 1993. Molecular evolution of voltage-sensitive ion channel genes: on the origins of electrical excitability. *Mol. Biol. Evol.* 10:221–242.

Stühmer, W., F. Conti, H. Suzuki, X. Wang, M. Noda, N. Yahagi, H. Kubo, and S. Numa. 1989. Structural parts involved in activation and inactivation of the sodium channel. *Nature (Lond.).* 339:597–603.

Tasaki, I., and K. Iwasa. 1982. Rapid pressure changes and surface displacements in the squid giant axon associated with production of action potentials. *Jpn. J. Physiol.* 32:69–81.

Van Duijnen, P. Th., and B. T. Thole. 1981. The α-helix as an ion channel. An ab initio molecular orbital study. *Chem. Phys. Lett.* 83:129–133.

Wada, A. 1962. Chain regularity and dielectric properties of poly- α-amino acids in solution. *In* Polyamino Acids, Polypeptides, and Proteins. M. A. Stahman, editor. University of Wisconsin Press, Madison, WI. 131–146.

Wada, A. 1976. The α-helix as an electric macro-dipole. *Adv. Biophys.* 9:1–63.

Watanabe, A. 1987. Change in optical activity of a lobster nerve associated with excitation. *J. Physiol. (Lond.).* 389:223–253.

Weber, P. C., and F. R. Salemme. 1980. Structural and functional diversity in 4-α-helical proteins. *Nature (Lond.).* 287:82–84.

Zimm, B. H., and J. K. Bragg. 1959. Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys.* 31:526–535.