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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

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Abstract. A functional model for the S4/IV α-helix of the action potential sodium channel is described by means of a thermodynamic approach. The model is based on a phase transition between the α-helix and an ion conducting channel-helix which is similar to the well established helix-coil transition in solution. The right hand channel-helix is a peptide chain with an alternating sequence of torsional angles $(\phi_1, \psi_1) = (87°, 315°)$ and $(\phi_2, \psi_2) = (22°, 107°)$ which yields a helix of 13.5 Å per turn. The axial dipole moments of the peptide bonds of this chain of L-amino acids nearly cancel each other out in a similar way to those in the gramicidin A channel, which is formed by alternating D- and L-amino acids. The helix, which does not contain any H-bonds, is stabilized by a helical file of water molecules which includes the permeating ion(s). This file turns around the channel-helix to form a relatively stable "double helix" structure which corresponds to the open channel. Since every third side chain in the S4/IV helix carries a positive charge their environments must be polarized. These polarized regions form a left hand screening-helix around the α-helix with 27 Å per turn. If all H-bonds of the α-helix are broken and the internal α-carbon atom is considered as fixed, the outer ten residues leave the membrane while the internal ten residues form the channel-helix. In this configuration every positively charged side chain matches nearly exactly every second polarized region of the screening-helix leaving the three regions in-between exposed to the water file containing the ion(s). This further stabilizes the channel and agrees nicely with the idea of cationic selectivity. An analysis of the energetics of the α-helix-channel-helix transition showed that the voltage-independent part of the free energy per helix residue could well be close to 0 kcal/mol and thus be in the range where a transition could occur. Two voltage-dependent contributions were included: the break down of the considerable dipole of the α-helix and the outward shift of the positive charges of the side chains upon channel-helix formation. Taking into account the fact that the formation of an α-helix is a highly cooperative process the degree of voltage dependence of the probability of formation of a channel-helix proved to be in the same range as experimental values for the open probability of modified Na channels whose inactivation had been removed. With regard to gating currents, the model predicts that 2.74 positive charges are moved in an outward direction. Consequences of the model for other experimental findings are discussed.

Key words: Na channel S4/IV α-helix, phase transition, channel-helix, ion permeation, dipole moments of peptide bonds, molecular model

Introduction

Ionic channels in excitable and non-excitable membranes have been the subject of intensive research work for many decades and there is now considerable information about the functional properties of the various channel types. However, to date there is no unifying theory which answers the two basic questions: How does the channel open and close (gating) and how is the permeation pathway designed. This indicates that in comparison with many other proteins, such as enzymes for example, there is a great deficiency in knowledge about molecular structure and function. This arises primarily from the necessity to study membrane channels in their membrane environment where X-ray and electron diffraction techniques are limited at present. The successful cloning and sequencing of the sodium channel (Noda et al. 1984) and acetylcholine receptor subunits (Noda et al. 1983a, b) was

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probably an important step in gaining understanding of the function of ionic channels and led to a series of proposals for possible secondary and tertiary structures (Noda et al. 1984, 1983a, b; Guy 1984; Greenblatt et al. 1985; Guy and Seetharamulu 1986; Guy and Hucho 1987). All these models suggest that the channel proteins traverse the membrane several times, mostly as α-helices of about twenty amino acids. The connecting parts between the α-helices are thought to be more or less randomly coiled in the cytoplasm and extracellular space. The channel is thought to be formed by contributions from the side chains of several α-helices and gating is assumed to be exerted by a conformational change.

The α-subunit as the major part of the Na channel protein consists of 1820 amino acids and contains four homologous domains (I–IV) (Noda et al. 1984). Each domain has three moderately apolar transmembrane α-helices (S1, S2, S3) and two very apolar segments S5 and S6 [S8 in Guy and Seetharamulu (1986)]. The S4 segments show the unique property of having positively charged residues in every third position which are separated by apolar amino acids. Four (Noda et al. 1984) or eight (Greenblatt et al. 1985; Guy and Seetharamulu 1986) transmembrane α-helices per domain are assumed and this yields 16 and 32 such α-helices per α-subunit, respectively. According to the model of Guy and Seetharamulu (1986), four S4 and four negatively charged S7 segments are thought to form the channel lining. Activation is explained in this model by a helical screw mechanism of S4 helices similar to that proposed in the sliding helix model by Catterall (1986a). In general, gating is explained by the concerted action of a great part of the protein and the permeation pathway is assumed to be lined preferentially by negatively charged side chains of the pore forming amino acids. Such a lining could make the channel selective for cations.

On the other hand, permeation of cations is also possible through pores of comparatively simple structure, of which the gramicidin A molecule is the best studied (Hladky et al. 1974; McLaughlin and Eisenberg 1975; Läuger et al. 1981; Kim and Clementi 1985). This compound is a pentadecapeptide with alternating D- and L-amino acids which forms head-to-head dimers between its *N*-formyl ends in lipid bilayers (Urry 1971; Urry et al. 1971). Because of the hydrophobicity of the side chains this dimer is able to fold in the membrane as a β-helix with a central hole that is long enough to span the membrane. The peptide is stabilized by H-bonds between carbonyl and imine groups of the backbone which are almost parallel to the channel axis and cause almost complete cancellation of the dipole moments of the peptide bonds. Sung and Jordan (1987a, b) calculated that the remaining small net dipole moment for the gramicidin A monomer of $\mu = -1.7$ Debye (D) is sufficient to cause the pronounced cationic selectivity of this channel.

Up to now there has been no suggestion of how one might reconcile both concepts of ion permeation through channels embedded in membranes: The low molecular gramicidin A channel has a central pore which is lined by negatively charged carbonyls, is free of side chains, and has a very small net dipole moment owing to the alternating sequence of D- and L-amino acids. In contrast, macromolecular biological channels, composed of only L-amino acids, cannot form a comparable permeation structure. Their transmembrane α-helices have a large dipole moment along their helical axis of about 20×3.95 D $= 79.0$ D with $\mu = 3.95$ D per peptide bond (Sung and Jordan 1987a, b). Therefore, only polar and charged side chains of the α-helices are considered to form the channel walls. With regard to the amino acid side chains, there is one link between the two channel concepts: Kennedy et al. (1977) described a synthetic channel of only L-amino acids with the polar side chains of serine arrayed in its interior. However, a necessary requirement was the presence of a glycine in every fourth position. Between these amino acids they incorporated hydrophobic leucine residues. Such a molecule can form $\beta^{12}_{6,6}$-helical ion channels with cationic selectivity. However, no sequence of this type has been found in the proteins of the α-subunit of the Na channel (Noda et al. 1984) or in the subunits of the acetylcholine receptor (Noda et al. 1983a, b) and there is no alternative structure for an oligopeptide consisting only of L-amino acids which could form such a pore. Nevertheless, it is very attractive to think that in large channel proteins only a very small part of them may need to undergo a conformational change in order to configure the open channel.

In this paper, a model has been developed for Na channel gating and permeation. It is based on the physically well established helix-coil transition (Schellman 1955, 1958; Zimm and Bragg 1959; Gibbs and DiMarzio 1959; Wada 1976) to describe the gating process for the formation of a channel lining which has a net dipole moment near 0 D to allow for ion permeation. In addition, rough quantification of the energetics of such a channel forming mechanism is presented for the S4 segment of domain IV (S4/IV) of the Na channel protein.

Methods

All steric relations within peptide structures were studied in a mechanical model of a peptide chain which was composed of the parts illustrated in Figs. 2 and 3. It was built using piacryl (for the planar peptide bonds) and steel wire. All dimensions were taken from

Dickerson and Geis (1969). Angles and distances were measured with special measuring devices with an error of $\pm 3°$ and ± 1 mm. The latter value was less than 5% of the smallest measured distance. Since only regular structures were considered, most of the data could be averaged to improve their accuracy.

The helix-coil transition of polypeptides in solution

Before applying the theory of helix-coil phase transitions in polypeptide chains to ionic channels, some aspects, which are important for the following, are reviewed in brief. Several years after the discovery of the α-helix as one of the most important protein structures (Pauling and Corey 1951; Pauling et al. 1951) Doty and co-workers (Doty et al. 1954; Doty and Yang 1956; Doty et al. 1956) observed that polypeptide chains in solution can be reversibly converted from the α-helix to a randomly coiled chain by small changes in temperature or solvent composition. The sharpness of such transitions has been explained by cooperativity (Zimm and Bragg 1959; Gibbs and DiMarzio 1959), i.e. the formation of the first helical turn is difficult because of the large entropy decrease, but, once formed, this first turn is a nucleus to which further H-bonds can be added more easily. A small change in the free energy, ΔF, for adding another residue to the helix can therefore cause a considerable change in the helix content. According to Schellman (1955), the free energy of unfolding, ΔF, of an α-helix of polyglycine in water can be expressed as a linear function of the residue number

$$\Delta F_{\mathrm{unf}} = n' \Delta F_{\mathrm{res}} + C \tag{1}$$

where n' is the number of residues in the helix, ΔF_{res} is the free energy change of a helix residue when it is transformed to the random configuration, and C is a correction factor which is defined as

$$C = -4\Delta H_{\mathrm{res}} + T\Delta S_{\mathrm{res}} + RT\ln 2\,, \tag{2}$$

where $-4\Delta H_{\mathrm{res}}$ arises from the four unformed H-bonds, $T\Delta S_{\mathrm{res}}$ from the looseness at the ends of the helix and $RT\ln 2$ from the two possibilities of a right hand and left hand helix. R is the gas constant and T the absolute temperature. Using the fundamental relation $\Delta F_{\mathrm{res}} = \Delta H_{\mathrm{res}} - T\Delta S_{\mathrm{res}}$ one obtains

$$\Delta F_{\mathrm{unf}} = (n'-4)\,\Delta F_{\mathrm{res}} - 3\,T\Delta S_{\mathrm{res}} + RT\ln 2\,. \tag{3}$$

Since a helix is stable only if $\Delta F_{\mathrm{res}} > 0$, it must exceed a critical length which is estimated by the author to be of the order of 15 to 20 amino acids, i.e. either there is an α-helix of at least this length or the chain is randomly coiled. Thus, the very simple Eq. (3) describes nucleation characteristics.

Schellman (1958) extended the discussion of the helix-coil transition to include the effect of fluctuations. For chains of 20 amino acids, which are short and therefore form only one helical segment at a time, the distribution of helical lengths as function of $\Delta F_{\mathrm{res}}/RT$ (Figs. 1–4 in his report) illustrates that for $\Delta F_{\mathrm{res}}/RT = 0$ more than 80% of the chains are randomly coiled while at increased values the percentage of long helices increases. At $\Delta F_{\mathrm{res}}/RT = 0.4$ the distribution peaks at the completely folded form. The breadths of the peaks represent the fluctuations, i.e. the occasional fraying off of a few residues from either end.

In contrast to Schellman's approach, most data about helix-coil transitions have been obtained by the use of dielectric dispersion techniques from synthetic peptides such as poly-(γ-benzyl-L-glutamate) dissolved in a mixture of a helicogenic solvent, such as dihalogenoethane, and a strong organic acid such as dichloracetic acid (for review see Wada 1976). In such a system Schwarz and Seelig (1968) studied the influence of electric field on the helix-coil transition and showed that an electric field of $E = 2 \times 10^5$ V/cm parallel to the helical chain fragments causes the helix content to increase from 20% to 80%. Field strengths of just this order are effective across biomembranes and therefore suggest an influence on transmembrane α-helix segments. However, it should be noted that results obtained in such synthetic systems need not be valid for the system polypeptide chain-water, since one of the most essential properties, the temperature dependence of the helical content, is opposite in these two systems (Schellman 1955; Zimm and Bragg 1959). In conclusion, the next sections are based on the terminology of Schellman (1955, 1958) because they describe the formation of ionic channels by the transition of a transmembrane α-helix to a stretched peptide chain in an at least partly aqueous environment.

The channel structure

Before discussing whether the transition of a single transmembrane α-helical segment, which is surrounded by other α-helices of the channel protein, to a non-α-helical structure is possible or not, a specific structure for the non-α-helical transmembrane chain is proposed. This is because an estimation of the free energy of the transition does, of course, depend on the conformation of the chain after the transition. Therefore, in this section it is assumed that such a transition is indeed possible.

From studies in gramicidin A it is known that this channel is filled with a file of six to nine water molecules and sometimes with one or two ions (Kim and Clementi 1985; Hladky et al. 1979). Hence, it is intriguing to speculate that biological channels may work in a similar way, i.e. by the formation of a file consisting of both water molecules and ion(s) which

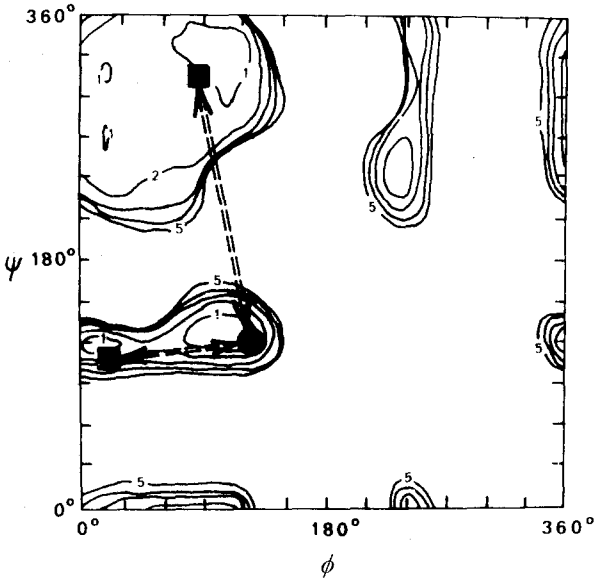

Fig. 1. Contour map of the conformational energy of poly-L-alanine (Brant et al. 1967) containing the position of the regular α-helix [*circle*, obained from the Ramachandran-ϕ, ψ-diagram in (Dickerson and Geis 1969)] and both pairs of angles of internal rotation (*squares*) measured for the channel-helix, (87°, 315°) and (22°, 107°). Isoenergy lines differ by 1 kcal/mol; the outer contours mark an energy of +5 kcal/mol. Both helices are similar in energy. For calculations an increase in energy upon the transition from α-helix to channel-helix of 1 ... 3 kcal/mol per residue is assumed

are coordinated by the backbone of a special transmembrane peptide chain. However, transmembrane α-helices contain many hydrophobic amino acids, which obviously must not be present in a "watery" pore. Instead, a structure for the transmembrane peptide chain is required that (*i*) leaves enough space for a transmembrane single-file transport of water molecules and ions, (*ii*) has a net dipole moment close to 0 D, (*iii*) avoids contact of hydrophobic side chains with water molecules and ions, and (*iv*) is stabilized long enough to cause channel open times in the range of milliseconds. If there is a tight packing of α-helices within the transmembrane part of the protein, space for a pore can be provided in a reversible way either by an overall conformational change or by stretching a transmembrane substructure such as, for example, a transmembrane α-helix. If a transmembrane α-helix were stretched completely it would leave a hole of about 3/5 of the space the helix with its side chains had occupied. (The volume of the side chains is assumed to be homogeneously distributed along the helix.) This is more than would be needed for a single-file transport of water molecules and ions even if some arrangement of the surrounding parts of the protein molecule were allowed. In conclusion, the necessary volume of the channel can be provided by a considerable stretch of a single α-helix after all H-bridges have been broken.

As noted above, α-helices have considerable dipole moments of 3.6 ... 3.95 D times the amino acid number (Schwarz and Selig 1968; Wada 1976). Their side chains are directed to the outside. On the other hand, dipole moments along the chain of β-sheets cancel each other out; however, the side chains are directed to both sides of the sheet and therefore do not fulfil condition (iii). A compromise with similar low conformational energy to that in the α-helix was needed and found by adjusting the model peptide chain to a helix with 13.5 Å per turn and equal torsional angles at every second residue. The two pairs of torsional angles were found to be $(\phi_1, \psi_1) = (87°, 315°)$ and $(\phi_2, \psi_2) = (22°, 107°)$ and the axial dipole moments per peptide bond were +2.24 D and −2.19 D, respectively. The conformation energy was estimated by comparison of the torsional angles with a contour map of the conformational energy (which is a Ramachandran-ϕ, ψ-diagram (Ramakrishnan and Ramachandran 1965) containing iso-energy levels) of poly-L-alanine as reported by Brant et al. (1967). Figure 1 shows the values of the α-helix and both pairs of values of the torsional angles of the proposed helix. The figure illustrates that all three energies are close to each other. The resulting three-dimensional structure over a "transmembrane" distance of 28.7 Å is illustrated in Fig. 3. In the following it will be called the channel-helix, although it is not stabilized, as are other protein structures, by H-bonds. However, if the groove along its axis is packed by a helical file of water molecules which interact with the ten carbonyl oxygens and ten imine hydrogens and taking into account the fact that an overall cylindrical shape is given by the surrounding α-helices, the stabilization of the channel-helix (condition (*iv*)) by the water molecule file itself is possible. The side chains of the residues are directed to the outside of the channel-helix and thus (*iii*) is satisfied: there is no direct influence of the side chains on the aqueous pathway.

The structural picture of the double helix composed of the channel-helix and a water plus ion strand is still not complete because nothing is known about the outside of the "water-helix" which is directed to the side chains of the surrounding α-helices. This question is closely related to another question that asks which of the transmembrane α-helices are the most likely to undergo a transition to a channel-helix. The positively charged S4 segments were thought by several investigators to play a decisive role in Na channel gating (Guy and Seetharamulu 1986; Catterall 1986a, b). These S4 helices are most appropriate for at least two reasons. First, the enormous concentration of positive charge makes them react more sensitively in the electric field than any other helices. Second, obeying the plus-minus charge compensation principle in proteins

(Wada and Nakamura 1981; Wada et al. 1985), the positive charges of the corresponding side chains are screened by counter ions and partial atomic charges to a high degree. If the α-helix transforms to a channel-helix, some surplus negative charge remains for a certain time within the channel wall and this agrees well with the idea of a cationic channel. Figure 2 shows the detailed structure of an α-helix of 20 residues in a similar representation to that used by Dickerson and Geis (1969). An α-helix with seven positively charged residues was chosen because it is present in the S4 segment of domain IV of the Na channel protein in *Electrophorus electricus* (Noda et al. 1984). This S4/IV helix was used for all the following considerations because its unique regularity facilitated the analysis. The fact that every third residue in this helix is positively charged causes a left hand helix of the screening polarized regions, indicated by the hemispheres, which turns around the α-helix. This kind of helix with 27 Å per turn will be denoted in the following as the screening-helix.

Figure 3 illustrates the channel-helix surrounded by the screening-helix which has been reproduced from the previous figure. The bottom α-carbon atom has been taken as vertically fixed (therefore it need not be positively charged) and the ten top residues of the α-helix are thought to form a random coil above the channel-helix and are not illustrated. Since every polarized region is turned by 60° around the axis of the left hand screening-helix with a distance in the axial direction of 4.5 Å, the positive charges at every third α-carbon atom of the right hand channel-helix must be close to every second polarized region after 240° at an axial distance of 9 Å. Indeed, the channel-helix which was derived solely by other criteria, excellently matches this structure. This state must therefore be energetically favourable and the channel-helix gets further stabilized as demanded in (*iv*).

A schematic top view of the channel-helix is illustrated in Fig. 4. The lines are the projections of the distances of adjacent α-carbon atoms to the plane down the channel axis. Their values were measured in the mechanical model to be 2.9 Å for the even and 1.6 Å for the odd numbered peptide bonds. Since the first and the seventh positively charged side chains in the α-helix are in identical positions, the first and the fourth side chains of the channel-helix were also assumed to be in the same position in this top view. The resulting pattern is a polygon curve which perfectly matches a curve obtained by connecting in an alternating way every second and fourth corner of a 13-gon (triscaidecagon). It can be seen that the α-carbon atoms carrying the side chains with the positive charges no. 2 and 3 (counted from below) are not exactly positioned at 240° and 120°, respectively. However, this small deviation is certainly negligible because in reality the charges are positioned at the terminals of the side chains of Arg or Lys and are thus surely mobile enough to be focused in the center of the preformed polarized region.

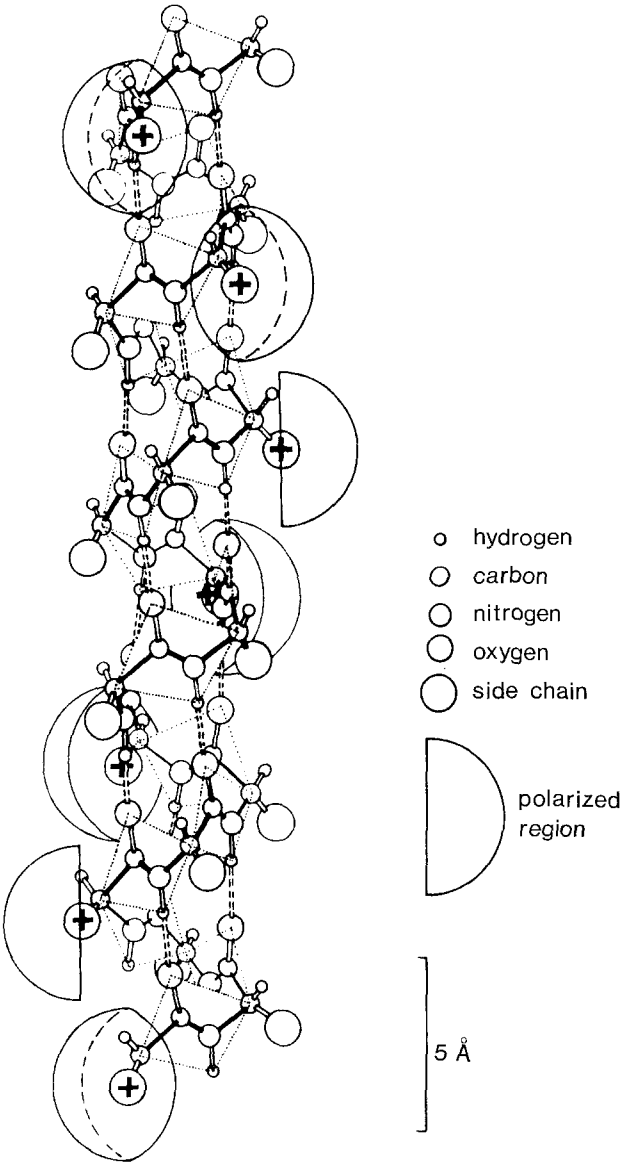

Fig. 2. Model of regular α-helix of 20 amino acids. The planar peptide bonds were drawn in the same way as in Dickerson and Geis (1969). The positive charges on every third side chain induce regions of polarization around them (indicated by hemispheres) which form a left hand "screening-helix" with 27 Å per turn

The permeation process through such a channel, lined by a channel helix with its ten carbonyl and ten imine groups as well as three negatively charged regions in the surroundings as negative copies of the three positively charged side chains, can be thought to function in a similar manner as in the well understood

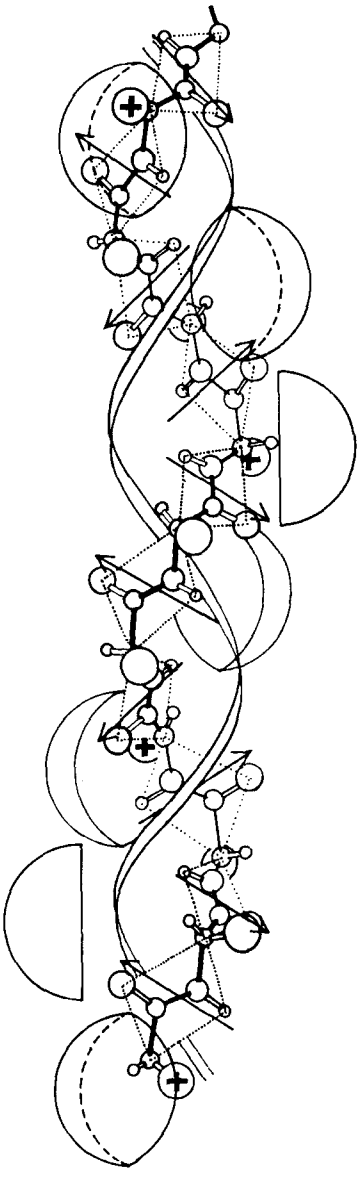

Fig. 3. Model of the channel-helix composed of the first ten residues of the α-helix (from below) in Fig. 2 with the bottom α-carbon atom fixed in its vertical position. The positively charged side chains no. 2, 3 and 4 are now close to the polarized regions no. 3, 5, and 7 (all counted from below) of the screening-helix in Fig. 2 and stabilize the structure. The *spiral line* symbolizes the helical strand of water molecules and ion(s) in the groove of the channel-helix which is also thought to stabilize the peptide structure. The *arrows* illustrate the direction of the dipole moments of each peptide bond. Their axial components approximately cancel each other out. The *symbols* are the same as for the previous figure

gramicidin A channel. The dipole moments of the peptide bonds cancel each other out in the axial direction, however net dipole moments for the whole channel of one or more Debye are easily imagined to derive from small conformational changes which could thus determine the special channel properties.

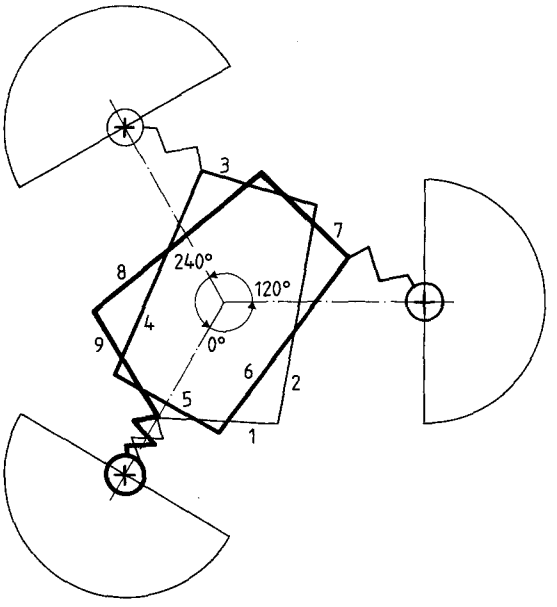

Fig. 4. View of the channel-helix down the axis. The corners are the α-carbon atoms, the *short* (1.6 Å) and long (2.9 Å) *lines* are the distances between two adjacent atoms. The *numbers* denote the peptide bonds as counted from below in Fig. 3. The first and fourth positively charged side chains coincide. The origins of the second and third side chains deviate by 0.55 Å from the ideal position at 240° and 120° respectively, but the side chains of Arg and Lys are probably flexible enough to allow the two charges to occupy the energetically optimal positions with respect to the polarized regions

Energetics of the transition between α-helix and channel-helix

The thermodynamic considerations that follow are based on these assumptions:

1. The transmembrane α-helix which performs a transition to the channel-helix is considered as a thermodynamically independent molecule which is embedded in a group of stable α-helices and has a length of 20 amino acids (28.7 Å).

2. The α-helix is considered to be positioned perpendicular to the membrane.

3. Since negative intracellular voltages stabilize the Na channel, the peptide bond dipoles must be oriented in the electric field in such a way that the *N*-terminal end of the helix must be directed to the inside. This is opposite to all previous suggestions (Noda et al. 1984; Guy and Seetharamulu 1986).

4. The inner α-carbon atom of the α-helix is considered as fixed in its vertical position (as already assumed above). Thus, the transition to the channel-helix means that the outer ten amino acids leave the membrane to be randomly coiled extracellularly. This assumption was included to allow one to explain the existence of outwardly directed

gating currents (Armstrong and Bezanilla 1974, 1977) by moving the positively charged side chains of the S4 helix in an outward direction.

The energetic treatment of the transition between the α-helix and the channel-helix, which is termed the helix-channel transition in what follows, was carried out in a similar way to that described above for the helix-coil transition in aqueous solution. Contributions of free energy from the following sources were included:

The intrahelical hydrogen bonds of the α-helix backbone. For the helix backbone the free energy could be estimated as done by Schellman (1955, 1958) for polyglycine since water also interacts with those residues of the channel-helix which remain within the membrane. Accordingly, the energy of dissociation of one H-bond, ΔH_{res}, was set to 1.5 kcal/mol, the entropy ΔS_{res} was 4.3 e.u. This is approximately valid for the ten external polyglycine residues. For the internal ones which remain within the protein the entropy value is certainly too large. Therefore, it was somewhat arbitrarily set at half the above value to be 2.1 e.u. Thus, the free energy for each of the ten outer residues was $\Delta F_{res,o} = 0.24$ kcal/mol, that of the ten inner residues $\Delta F_{res,i} = 0.87$ kcal/mol. Since on the average the breaking of one H-bond leads to the extrusion of half a residue into the extracellular space the dissociation of one H-bond must have a free energy of $\Delta F_{res} = (\Delta F_{res,i} + \Delta F_{res,o})/2 = 0.55$ kcal/mol. In terms of energy and entropy, ΔF_{res} is now

$$\Delta F_{res} = \Delta H_{res} - (3/4)\, T\, \Delta S_{res}\,. \tag{4}$$

Equation (1) can now be written as

$$\Delta F_{unf} = n'(\Delta H_{res} - (3/4)\, T\, \Delta S_{res}) + C\,, \tag{5}$$

where n' is the number of residues per helix. The constant C is assumed to be slightly modified because only one end of the α-helix is assumed to be "loose". Therefore

$$C = -4\Delta H_{res} + (1/2)\, T\, \Delta S_{res}\,. \tag{6}$$

Inserting (4) and (6) in (5) yields

$$\Delta F_{unf} = (n' - 4)\, \Delta F_{res} - (5/2)\, T\, \Delta S_{res}. \tag{7}$$

Equation (7) is similar to (3). The term $RT \ln 2$ could be ignored because the probability for the formation of a left hand α-helix approximates zero. With $n' = 20$ one obtains $\Delta F_{unf} = +5.72$ kcal/mol. Thus, the backbone alone could not unfold (Schellman 1955).

The side chains. The formation of a channel-helix in the way described would move ten side chains from the interior of the protein to the extracellular bulk solution. Using dynamic vapor pressure measurements, Wolfenden et al. (1981) determined hydration energies of isolated amino acid side chains and further showed that these values highly correlate with free energies of amino acids associated with their transfer from the interior to the surface of globular proteins. Therefore, the latter data (Table 1) which the authors calculated from a set of partition coefficients as reported by Chothia (1976) were used to quantitate the side chain effects. These values were applied to the outer half of the S4 helices of all four domains of the Na channel protein to obtain $\Delta F_{si,o}$ (in kcal/mol): I: −6.14; II: −7.97; III: −10.72; IV: −8.30. Furthermore, ten side chains of the surrounding helices are supposed to become exposed to a more or less aqueous environment within the channel. This value was estimated to be $\Delta F_{si,i} = 0 \ldots -6.37$ kcal/mol. The number is the tenfold average calculated for all amino acids except Lys and Arg which certainly are not positioned around the strongly positive α-helix.

Table 1. Free energy change, ΔF, for transfer of amino acid residues from the interior to the surface of proteins as reported by Wolfenden et al. (1981)

Amino acid	ΔF [kcal/mol]
Ala	−0.29
Arg	−2.71
Asn	−1.18
Asp	−1.02
Cys	0
Gln	−1.53
Glu	−0.90
Gly	−0.34
His	−0.94
Ile	+0.24
Leu	−0.12
Lys	−2.05
Met	−0.24
Phe	0
Pro	−0.90
Ser	−0.75
Thr	−0.71
Trp	−0.59
Tyr	−1.02
Val	+0.09

Insertion of polypeptide backbone into bulk water. From the outer ten residues not only the side chains are transferred to the surface of the protein. The "polyglycine" backbone also moves to the extracellular space for which an additional amount of free energy contributes to the reaction. As a rough estimate, the data in Table 1 for Asn and Gly as well as Gln and Ala were subtracted from each other to obtain (in kcal/mol) −0.84 and −1.24, respectively. Therefore, for the transfer of one Gly to the extracellular solution a free energy of 1 kcal/mol was assumed to obtain for ten residues $\Delta F_{back} = -10$ kcal/mol.

Repulsion of positive charges in the side chains. Schellman (1955) reported that one fraction of clupein with a total of 28 amino acids, of which about 20 are positively charged, cannot form α-helices because of the repulsive electrostatic forces. These forces were roughly estimated for the four S4 helices. The distance between neighbouring positive charges at the ends of the side chains of every third amino acid was estimated to be 6.5 Å and 12.0 Å in the α-helix and the channel-helix, respectively. Since there are only vague suggestions about dielectric constants within proteins, which scatter between 1 and 60, the simple application of Coulomb's law was not promising. Furthermore, it has been shown that the dielectric constant of a protein around a charge strongly depends on the distance from the charge (Warshel and Levitt 1976; Warshel 1978). Regarding this, Warshel et al. (1984) calculated the interaction energy for charged groups within proteins as a function of their distance. From Fig. 4 of their report a change in free energy per pair of positive charges of -2 kcal/mol was estimated for the helix-channel transition. This yields for the S4 segments of the four domains for ΔF_{rep} (kcal/mol): I: -6; II: -8; III: $-6 \ldots -10$ (difficult to evaluate because there is one Pro as helix breaker included); IV: -12.

Steric strain of the backbone. As already pointed out the steric strain of the ten channel-helix residues is small. From Fig. 1 a free energy of $+1 \ldots +3$ kcal/mol per residue was estimated. Thus, for the ten residues ΔF_{str} is $+10 \ldots +30$ kcal/mol.

Summing all energy contributions discussed above yields

$$\Delta F_{\text{add}} = \Delta F_{\text{si,e}} + \Delta F_{\text{si,i}} + \Delta F_{\text{back}} + \Delta F_{\text{rep}} + \Delta F_{\text{str}} \tag{8}$$

$$= \begin{Bmatrix} -6.14 \\ -7.97 \\ -10.72 \\ -8.30 \end{Bmatrix} - (0 \ldots 6.37) - 10 \begin{Bmatrix} -6 \\ -8 \\ -(6 \ldots 10) \\ -12 \end{Bmatrix}$$

$$+ (10 \ldots 30) \text{ kcal/mol} .$$

The two columns contain the values corresponding to the four domains (I top). Despite the fact that all these estimations are vague and certainly incomplete, two basic conclusions can be derived:

1. ΔF_{add} can be chosen such that it contains enough helix breaking energy to compensate for $\Delta F_{\text{unf}} = +5.72$ kcal/mol as determined above, i.e. the proposed helix-channel transition might be possible.
2. Among the four S4 segments those of domain III and IV contain more helix breaking energy than those of S4/I, II.

Although the side chains are very different and ionic repulsion takes place only between every third residue, for the following it was reasonable to use an averaged value of ΔF_{add} per residue

$$\Delta F_{\text{add,r}} = (1/n')\, \Delta F_{\text{add}} , \tag{9}$$

with n' as the number of residues. Such an assumption of equal $\Delta F_{\text{add,r}}$ values for all residues does not oversimplify the situation too much. On the other hand it considerably facilitates the employment of Schellman's (1958) theory in discussing the helix-channel transition including its voltage dependence.

The addition of $(n'-1)\, \Delta F_{\text{add,r}}$ to (7) leads to an expression describing the complete energetics of the helix-channel transition

$$\Delta F_{\text{unf}} = (n'-4)\, \Delta F_{\text{res}} - (5/2)\, T\, \Delta S_{\text{res}} + (n'-1)\, \Delta F_{\text{add,r}} . \tag{10}$$

One $\Delta F_{\text{add,r}}$ less than n' was taken because the innermost residue was assumed to be fixed. After rearranging one obtains

$$\Delta F_{\text{unf}} = (n'-4)\, (\Delta F_{\text{res}} + \Delta F_{\text{add,r}}) - (5/2)\, T\, \Delta S_{\text{res}} + 3\, \Delta F_{\text{add,r}} \tag{11}$$

The effect of membrane potential on the helix-channel transition

To include the influence of the membrane potential on ΔF_{unf}, two major energy contributions were considered:

1. The energy of the macrodipole α-helix in the electric field vanishes upon the transition to the channel-helix. This means that negative intracellular voltages stabilize the α-helix if it is oriented as proposed.
2. A negative intracellular voltage also stabilizes the α-helix by affecting the positively charged side chains. This is because the charges reach a minimum in potential energy in the α-helix configuration whereas on moving outwards upon the transition to the channel-helix their potential energy is increased.

Both contributions are quantitated now:

Assuming an axial dipole moment per peptide bond of $\mu_0 = 3.95$ D, an α-helix of n' residues has a dipole moment of $(n'-1)$ times 3.95 D whereas in the channel-helix all dipole moments, μ_1, approximately cancel each other out because of their alternating signs. The difference in dipole energy between an α-helix of n' residues and a channel-helix is therefore

$$\Delta F_{\text{dip},n'} = (\Delta V/d) \sum_{i=1}^{n'-1} [\mu_0 + \mu_1 (-1)^i] , \tag{12}$$

where ΔV and d are the membrane voltage and membrane thickness, respectively. Since energies stabilizing the α-helix are counted positively, the convention used herein is that the dipoles point toward their neg-

atively charged end and voltages are referred to the extracellular space. A constant field of $\Delta V/d$ is assumed along the transmembrane helix. The membrane thickness is equal to that of a complete α-helix of 20 residues, i.e. 28.7 Å. Since an α-helix of 20 residues contains 19 peptide bonds, which form only 16 H-bonds, four peptide bonds have to be oriented to form the first H-bond. This is important because it reflects nucleation characteristics of the helix forming process which will be discussed below.

To evaluate the contribution of the charge shift associated with the helix-channel transition, the calculations were based on the following assumptions. An α-helix as illustrated in Fig. 2, which carries seven positive charges in every third side chain (the innermost is unimportant), is assumed to be fixed vertically at its most internal α-carbon atom. Since the channel-helix has only half the number of residues per length as the α-helix, it is assumed that releasing the first residue leads to a transfer of the first side chain in an outward direction by $1/19\ \Delta V$. The release of the second residue would also lead to a shift of only one side chain because the first one has already left the membrane. The release of the third residue would cause two side chains to shift by $1/19\ \Delta V$, etc. Figure 5 shows the plot of such shifts by $1/19\ \Delta V$ of the positively charged side chains as a function of the number of the actual residue which is released in series from the α-helix. To facilitate the following calculations, the discrete values were translated to analog ones by linear regression

$$y = 0.164\,x + 0.106\,, \tag{13}$$

where y is the charge shift per $1/19\ \Delta V$ and x is the number of the released residue. Again, the concept of nucleation must be included: The first 15 residues are released by breaking one H-bond whereas breaking the last H-bond releases the remaining four residues (apart from the twentieth which is regarded as fixed). This means that the formation of the first H-bond of an α-helix can only happen if four adjacent residues are positioned correctly. There are eight possibilities ($k=1-8$) for where in a channel-helix the first H-bond ($m=1$), and with it the first complete intramembrane turn of an α-helix, can form. The potential energy change, $\Delta F_{ch,m,k}$, due to the shift of charges in the side chains is according to (13)

$$\begin{aligned}\Delta F_{ch,m,k} &= (\Delta V\,e/19)\,[0.164\,(21-2k+20-2k+19-2k \\ &\qquad +18-2k)+4\,(0.106)] \\ &= (\Delta V\,e/19)\,[0.164\,(78-8k)+0.424]\,, \\ k&=1\ldots 8\,; \quad m=1\end{aligned} \tag{14}$$

where e is the elementary charge. The formation of a second ($m=2$) adjacent H-bond [only adjacent H-bonds are reasonable in short helices (Schellman 1958)] needs only the energy

$$\begin{aligned}\Delta F_{ch,m,k} &= (\Delta V\,e/19)\,[0.164\,(17-21\,k)+0.106] \\ k&=1\ldots 8\,; \quad m=2\,.\end{aligned} \tag{15}$$

A third ($m=3$) adjacent H-bond needs the energy

$$\begin{aligned}\Delta F_{ch,m,k} &= (\Delta V\,e/19)\,[0.164\,(16-2\,k)+0.106] \\ k&=1\ldots 7\,; \quad m=3\,.\end{aligned} \tag{16}$$

Now the number of locations for three adjacent H-bonds which are formed in a channel-helix is reduced by one. Continuing this kind of analysis, a general formula for the energy content of every possible number of H-bonds (m) in every possible position (k) in an α-helix of n' residues has been derived.

$$\begin{aligned}\Delta F_{ch,m,k} &= [\Delta V\,e/(n'-1)]\,0.164\,[4\,(n'+1)-6+(m-1) \\ &\qquad \cdot(2\,n'-4-m)/2-2\,(m+3)\,k]+0.106\,(m+3) \\ m&=1\ldots n'-4\,; \quad k=1\ldots \mathrm{Int}\,[(n'-m-2)/2]\,.\end{aligned} \tag{17}$$

This means that every α-helix configuration of the fluctuating system, α-helix-channel-helix, differs in its energy content, which is given by (11), (12), and (17) as

$$\begin{aligned}\Delta F_{\mathrm{sum,m,k}} &= m\,(\Delta F_{\mathrm{res}}+\Delta F_{\mathrm{add,r}})-(5/2)\,T\,\Delta S_{\mathrm{res}}+3\,\Delta F_{\mathrm{add,r}} \\ &\qquad +(3\,\mu_0+\mu_1)\,(\Delta V/d)+\Delta F_{\mathrm{dip,m}}+\Delta F_{ch,m,k}\end{aligned} \tag{18}$$

with m and k in the limits of (17). It should be stressed that all sources which contribute to the energy of the system enhance its nucleation properties in the sense that a far higher energy contribution is needed to form the first H-bond than for the succeeding ones. Following Schellman (1958), the probability for the formation of m adjacent H-bonds in position k is given by

$$P_{m,k} = [\exp\,(\Delta F_{\mathrm{sum,m,k}}/RT)]/Q\,, \tag{19}$$

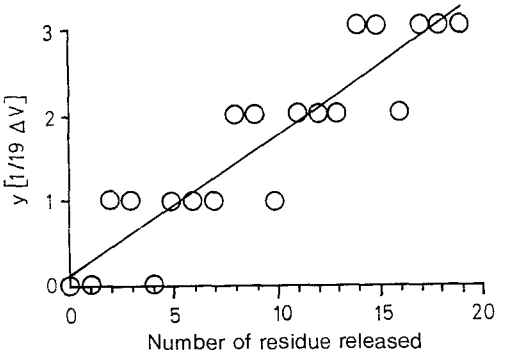

Fig. 5. Outward shift y of positively charged side chains in parts of 1/19 of the membrane voltage, ΔV, as a function of the number x of the released H-bond during the helix-channel transition. Data points were fitted by linear regression with $y=0.164\,x+0.106$. For further explanation see text

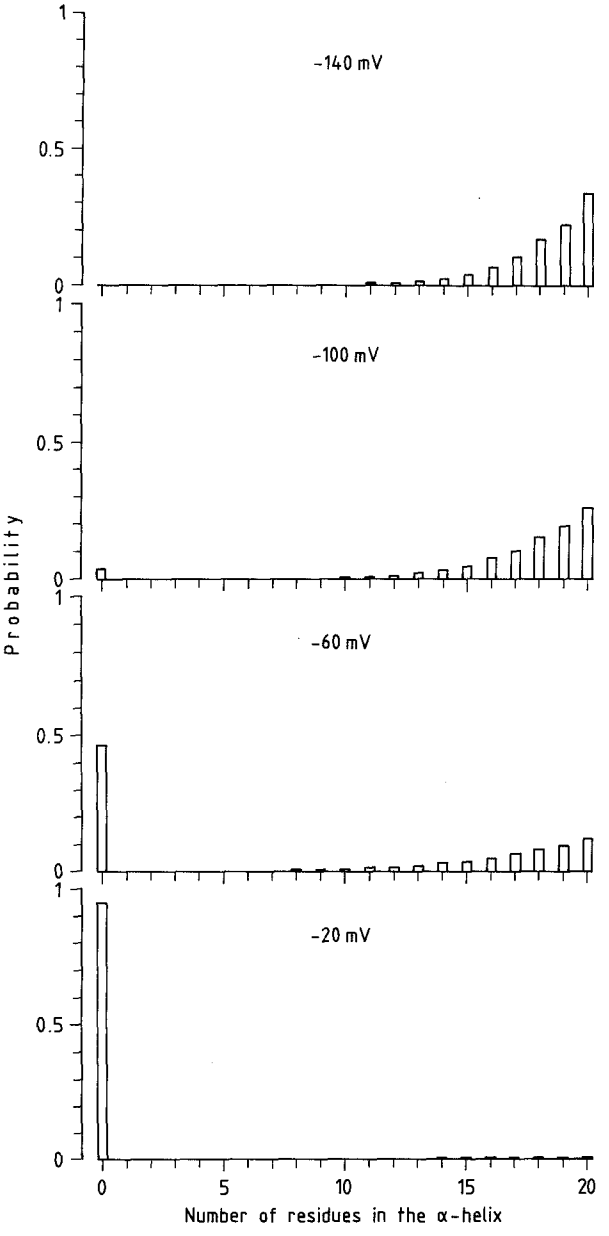

Fig. 6. Distribution of lengths of transmembrane α-helices of a peptide chain of 20 amino acids at four voltages. The probabilities were obtained from (19). The parameters used were: $\Delta F_{res} = +0.55$ kcal/mol; $\Delta F_{add,r} = -0.50$ kcal/mol; $T = 298$ K; $\Delta S_{res} = 4.3$ e.u.; $\mu_0 = 3.95$ D; $\mu_1 = 2.2$ D; $d = 28.7$ Å; $n' = 20$. At -140 mV the probability of forming an α-helix reaches unity although there is considerable fluctuation. At -100 mV α-helices are broken with a probability of 5% for forming a channel-helix. At -20 mV the probability of forming a channel-helix approaches unity

where R and T have their usual meanings and Q is

$$Q = 1 + \sum_{m=1}^{n'-4} \sum_{k=1}^{\mathrm{Int}[n'-m-2)/2]} [\exp(\Delta F_{sum,m,k}/RT)]. \qquad (20)$$

The factor 1 has been included for the unfolded state (channel-helix) as a reference state having a probability of $1/Q$. Int means the integer part of the term.

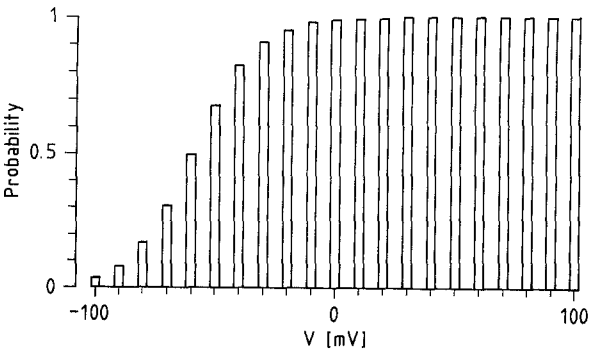

Fig. 7. Voltage dependence of the probability of forming a channel-helix. The data were calculated from the reciprocal of (20). Same parameters as in legend to Fig. 6. The sigmoidal envelope is similar to measured data for Na channels in which inactivation has been removed (Keller et al. 1986) and shows the usefulness of the theoretical approach used here

Equations (19) and (20) allow one to compare the predicted degree of voltage dependence of the helix-channel transition with the experimentally accessible open probability of the Na channels. Figure 6 illustrates distributions of lengths of the transmembrane α-helix at four voltages. n' has been plotted in the figure instead of m itself to emphasize the gap between the unfolded channel-helix and the region of existing α-helices. The parameters used, which are listed in the legend, are those that were derived in the text. $\Delta F_{add,r}$ was chosen to approximately compensate ΔF_{res}, which is a reasonable assumption as shown earlier. A value of -0.50 kcal/mol was used to obtain, at -100 mV, an open probability of about 5%, similar to that reported by Keller et al. (1986) in Na channels in planar lipid bilayers in which inactivation has been removed. At -140 mV the peptide chain is in a completely α-helical state, although the helix length does fluctuate. No unfolded peptide chain, i.e. no channel-helix, is present. This means that the model does not predict any open probability at this voltage. Less negative potentials cause a successive decrease and broadening of the peak of helices and an enormous increase of the unfolded form and thus of the open probability. The nucleation characteristic of the process is obvious: the longer helices form at the expense of the shorter ones; very short helices are absent. A close inspection of the amplitudes reveals an alternating tendency which originates from the orientation of the peptide bond dipoles. The relation between ΔF_{res} and $\Delta F_{add,r}$ has a strong influence on the energy range for the coexistence of α-helices and channel-helices. In conclusion, peptide chains generally exist in an unfolded form outside the membrane and folded as an α-helix or some other structure within the membrane. Only in special cases, where $\Delta F_{add,r}$ precisely balances ΔF_{res}, do transitions appear which cause special structures such as the channel-helix proposed here.

On the other hand, the degree of voltage dependence of the helix-channel transition, i.e. its steepness, does not depend on the relation between $\Delta F_{add,r}$ and ΔF_{res} but only on $\Delta F_{dip,m}$ and $\Delta F_{ch,m,k}$. Figure 7 shows a plot of the probability of the channel-helix, $1/Q$, as a function of the voltage ($\Delta F_{add,r} = -0.50$ kcal/mol). Although not as steep, the sigmoidal envelope is similar to that for the variation in the percentage of open time as a function of voltage in Na channels with removed inactivation, as illustrated in Fig. 4 of Keller et al. (1986). This similarity is especially encouraging because all of the assumptions which were included in the calculations were derived from experimental results or theoretical ideas which are well established in physical chemistry.

Discussion and concluding remarks

The two major results of this paper are:

(*i*) For a polypeptide chain containing only L-amino acids an ionic channel structure of helical shape with 13.5 Å per turn has been proposed. This peptide conformation is regular in the sense that the torsional angles alternate to cause a net dipole moment of the peptide bonds close to 0 D. A file of water molecules including one or more ions is thought to be positioned in the groove of the channel-helix to form a "double helix". Furthermore, the polarized regions around the channel-helix may also contribute to the lining of the channel.

(*ii*) A mechanism is proposed for how a transmembrane α-helix can be transformed to a channel-helix. For the case of the S4 segment of domain IV of the Na channel protein, it has been suggested that from the energetic point of view such an interconversion is possible in analogy to the helix-coil transition in solution. The degree of voltage dependence of the transition is of the same order as that of experimental results for Na channels.

The permeation process along the channel-helix

The structure described here as being responsible for the permeation process, including the channel-helix as its core, is probably the first proposal for how a short peptide chain of only L-amino acids can form the lining of a hydrophilic pathway through a membrane in which the dipole moments of the peptide bonds cancel each other out in the axial direction. The importance of the last property has been shown in studies of gramicidin A channels (Sung and Jordan 1987a, b). Small net dipole moments of 1.7 D, which are below the value of the dipole moment of a single peptide unit, proved to be sufficient to cause cationic selectivity. Furthermore, as with gramicidin A, the channel-helix suggests a lining formed predominantly by carbonyl and imine groups to coordinate with water molecules and ions. Polarized regions of the surrounding helices also contribute to the channel wall but do not form a comparable thread of negative and positive partial charges for coordination. The suggestion that the groove in the channel-helix is the important structure for ion and water permeation is further strengthened by another analogy to gramicidin A channels: Kim and Clementi (1985) showed that both ions and water molecules find the energetically most favourable path through the channel on a spiral between two strips of the chain balanced by the deep interaction well due to carbonyl oxygens and short range repulsive interactions due to the atoms in the chain. Basically, the situation in the channel-helix is similar, with the only difference being that water molecules and ions are coordinated by groups on the peptide chain which are far closer to each other. In conclusion, the channel-helix could be the first step in uniting the permeation concepts used for low molecular channels of known structure with those for high molecular biological channels.

It is probably too early to speculate about more detailed permeation mechanisms in Na channels as designed here. However, previous attempts to apply the reaction rate theory of Eyring (Glasstone et al. 1941) to ionic channels (Hille 1975; Hille and Schwarz 1978) could, a posteriori, find a new understanding at the molecular level. For example, the three binding sites assumed by Hille (1975) for the Na channel could correspond to the three polarized regions. On the other hand, the ten carbonyl oxygens in the channel-helix argue against the exclusive validity of barrier models since they include only two or three vacant sites. Therefore, the Nernst-Planck continuum theory (for review see Levitt 1986) should also be applicable in description of ion permeation along the channel-helix.

Energetics of the helix-channel transition

The section describing the energetics of the helix-channel transition of the Na channel S4/IV segment showed (*i*) that there is some evidence for its actual existence and (*ii*) that the degree of voltage dependence is of the same order as that found experimentally. However, many simplifying and probably inaccurate assumptions were included. Thus, it has to be stated clearly that none of the calculations is sufficient to prove the helix-channel transition. In particular, the list of contributions to $\Delta F_{add,r}$ is surely incomplete and the estimates for those contributions that are included

are probably poor. Furthermore, a homogenous distribution of these contributions has been assumed in order to facilitate the calculations and to provide the necessary basis for a thermodynamic approach. Concerning ΔF_{res} and $\Delta F_{add,r}$ the message of this report is simply that when these energies are similar a transition between α-helix and channel-helix can occur.

In conclusion, the ability of an α-helix to convert to a channel-helix would be determined only by this energy relation, independently of the degree of voltage dependence . This is an attractive idea since most modifiers of Na channel activation only shift the curve without affecting the steepness. This is the case for drugs in the chemically inhomogeneous class of lipid-soluble activators (Catterall 1980; Honerjäger 1982) which could act by inducing a significant change in $\Delta F_{add,r}$.

The degree of voltage dependence of the probability for formation of a channel-helix has been found to be of the same order as, though somewhat smaller than, the open probability in BTX modified Na channels (Keller et al. 1986). The discrepancy might be due to additional voltage-dependent mechanisms in or around the channel. For example, the orientation of permanent dipoles in the side chains can be expected to hinder the release of side chains from each other as required for the helix-channel transition.

The kinetics of the helix-channel transition

For the helix-coil transition in solution, kinetic data are still controversial. Dependent on the method used, relaxation times between 10^{-1} and 10^{-8} s have been determined (for review, see Wada 1976). Biological channels have open times which overlap at least partially with the lower half of this range while faster events are not accessible with present techniques. Nevertheless, the correspondence in time behaviour further strengthens the idea of a helix-channel transition. In fact, the situation within a membrane protein is certainly much more complex than that of an α-helix in solution. The channel-helix is thought to be stabilized by the helical file of water molecules plus ion(s) and by the arrangement of the positively charged side chains in the center of every second preformed polarized region. The three polarized regions in-between may further stabilize the file of water molecules (including the permeant ions) and thus the channel-helix. These stabilizing mechanisms will certainly increase the mean open time.

Two recent experimental findings concerning the mean open time of Na channels could be explained by the present model:

(*i*) In batrachotoxin modified Na channels (Keller et al. 1986), increased depolarizations cause an increase in the mean open time. This is consistent with the finding in inactivating Na channels in which the rate constant determining the transition from the open to the previous closed state decreased (Vandenberg and Horn 1984; Benndorf 1988). The model used here predicts an increase of the probability of unfolding to unity at strong depolarizations and thus of the mean open time.

(*ii*) The model suggests that increasing the probability of finding an ion within the channel, which certainly happens at increased bulk ion concentrations, should stabilize the channel-helix by coordinating its carbonyl groups and repriming the polarized regions. Indeed, increased concentrations of bulk Na ions have been shown to lengthen the mean open time of inactivating Na channels (Nilius and Marinov 1987). The interpretation of this experimental result by the model proposed here seems to be especially important because neither the classical Hodgkin-Huxley formalism (Hodgkin and Huxley 1952) nor the terminology of Markov state models (Vandenberg and Horn 1984; Horn and Vandenberg 1984) can easily explain such a current-dependent gating.

Inactivation of Na channels

Until now all considerations have been based on the assumption that Na channels do not inactivate, i.e. the model described only distributions at steady state. Experimentally, such modified Na channels can be obtained after enzymatic removal of inactivation by pronase, trypsin, chymotrypsin, and papain (Bezanilla and Armstrong 1977; Starkus and Shrager 1978; Quandt 1987; Gonoi and Hille 1987) at the inside of the membrane and by a variety of membrane permeant agents, such as *N*-bromoacetamide, applied from the outside (Patlak and Horn 1982). Thus, as suggested by many investigators, the inactivation machinery must be located on the inside of the channel. If the sodium channel activates by forming the channel-helix then inactivation must take place in the vicinity of its internal end. One can imagine that under the influence of the changing strong electric field at depolarization the protein conformation rearranges with the time course of macroscopic fast inactivation. A final step would then lead to a closure of the channel from the inside, e.g. by a folding of the adjacent internal peptide chain into the internal channel mouth. I now list several examples of how such an extended model might possibly explain some experimental findings.

1. Slow voltage ramps with depolarization rates slower than the relaxation time of the protein rearrangement could be expected to close the channel before or at the onset of the opening event. This would have the consequence of inactivation without opening.

2. Trypsin and papain are known to cleave peptide bonds only if the carbonyl group derives from Lys or Arg. The action of both enzymes could be simply the cleavage of a considerable intracellular portion of the protein and thus of the internally adjacent peptide chain thereby removing fast inactivation. Pronase as a mixture of proteolytic enzymes could act in a similar way.
3. After having passed the membrane, *N*-bromoacetamide and similar substances would prevent the adjacent peptide chain from folding into the internal channel mouth and thus leave the channel open.
4. Slow inactivation mechanisms as reported in many preparations (Schauf et al. 1976; Fox 1976; Rudy 1978; Collins et al. 1982; Benndorf and Nilius 1987) might also be caused by a complex series of small protein arrangements occurring after the α-helix has been unfolded. In particular, the relaxation of the polarized regions, which are thought to participate in channel lining, could be a suitable candidate for explaining the slowly changing availability of Na channels.
5. Na channels can occasionally open for extraordinarily long lasting bursts (Patlak and Ortiz 1985; Patlak et al. 1986). A similar property can be promoted artificially by the cardiotonic compound DPI 201-106 (Kohlhardt et al. 1986). Although it has been concluded that the Na channel protein can exist in more than one "mode" there has been no suggestion for a possible molecular basis. The helix-channel model offers as a possible explanation the idea that the free energy difference for inserting the internally adjacent peptide chain to inactivate the channel is reduced (by a conformational change or by the substance) such that a fluctuation of the peptide chain takes place.

Gating currents

The model can easily explain outwardly directed gating currents as first reported in the squid giant axon (Armstrong and Bezanilla 1974, 1977). The model implies that two sources contribute to gating currents: (*i*) the breakdown of the α-helix dipole which is equivalent to the transport of one unit charge to the outside (Wada and Nakamura 1981) and (*ii*) the shift of the charged side chains at the transition, which can be easily seen from Fig. 5 as a shift of 33/19 unit charges across the membrane. This means that 2.74 unit charges are shifted to the outside of the membrane and this is close to the corresponding experimental value of between 3 and 4 unit charges for the squid axon (Stimers et al. 1985) and in GH_3 cells (Vandenberg and Horn 1984). It also agrees fairly well with the 2.3 charges which are moved and not immobilized by inactivation as determined for sodium channels expressed in *Xenopus laevis* oocytes (Conti and Stühmer 1989).

The aim of this study was to devise a molecular mechanism for Na channel gating and permeation. The S4/IV helix of the channel protein was used as a model peptide chain to demonstrate the basic ideas. However, the ideas need not necessarily be limited to just this helix but could be of more general use in understanding the action of ionic channels in biological membranes. Two very recent reports further strengthen the concept of the isolated action of only one such helix: Firstly, Tosteson et al. (1989) prepared a 22-amino acid peptide with a sequence identical to that of the segment S4/IV of the Na channel. Inserted in bilayers, this peptide induces a voltage and time-dependent conductance with an apparent gating charge of 3. Secondly, the amino acid sequences of voltage gated *K* channels, which correspond to only one domain of the sodium channel, contain only one sequence like the S4/IV segment (Baumann et al. 1988). Nevertheless, one cannot rule out the possibility that more than one domain is involved in sodium channel activation. It also seems possible that the mechanism described is only part of a more complex process.

From the evolutionary point of view it is interesting to speculate about the significance of the presence of four homologous domains in the α-subunit of Na channels if, as postulated here, only one is necessary for the function of the channels. One might reasonably suppose that the number "four" results from two successive duplications of the genetic material during evolution. It seems possible that three of the four domains are redundant and that only one fulfils the conditions necessary to enable the α-helix to transform to the channel-helix by having a sufficiently small difference in free energy between both the states.

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