Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 508–512, January 1986
Neurobiology

Molecular model of the action potential sodium channel

(protein structure/sequence analysis/conformation theory/membrane protein/membrane channel)

H. ROBERT GUY* AND P. SEETHARAMULU†

Laboratory of Mathematical Biology, National Cancer Institute, National Institutes of Health, Building 10, Room 4B 56, Bethesda, MD 20892

Communicated by Charles F. Stevens, September 9, 1985

ABSTRACT **Secondary and tertiary structural models of sodium channel transmembrane segments were developed from its recently determined primary sequence in *Electrophorus electricus*. The model has four homologous domains, and each domain has eight homologous transmembrane segments, S1 through S8. Each domain contains three relatively apolar segments (S1, S2, and S3) and two very apolar segments (S5 and S8), all postulated to be transmembrane α-helices. S4 segments have positively charged residues, mainly arginines, at every third residue. The model channel lining is formed by four S4 transmembrane α-helices and four negatively charged S7 segments. S7 segments are postulated to be short, partially transmembrane amphipathic α-helices in three domains and a β-strand in the last domain. S7 segments are preceded by short apolar segments (S6) postulated to be α-helices in three domains and a β-strand in the last domain. Positively charged side chains of S4 form salt bridges with negatively charged side chains on S7 and near the ends of S1 and S3. Putative extracellular segments that contain 5 of the 10 potential N-glycosylation sites link S5 to S6. Channel activation may involve a 'helical screw' mechanism in which S4 helices rotate around their axes as they move toward the extracellular surface.**

Noda *et al.* (1) have deduced the *Electrophorus electricus* sodium channel primary structure from its cDNA sequence. Sodium channels purified from *Electrophorus* appear to consist of a single glyco-polypeptide of molecular weight 260,000–300,000, 29% of which is estimated to be carbohydrate (2). These findings are consistent with the 1820 residue sequence. Noda *et al.* (1) identified four homologous domains within the sequence and six segments within each domain: the first three segments (S1, S2, and S3) are relatively apolar but contain a few charged residues, the fourth segment (S4) contains positively charged side chains, predominately arginine, at every third position and noncharged side chains at all other positions, the last two segments (S5 and S8‡) are very apolar and contain no charged residues. They appear to favor a folding scheme in which S1 and S2 segments of each domain are transmembrane α-helices that form the channel lining and S5 and S8 segments of each domain also traverse the membrane as α-helices. S3, S4, and NH_2- and COOH-terminal segments, and negatively charged segments linking homologous domains are in the cytoplasm. Segments located between S5 and S8 (that contain 5 of 10 potential N-glycosylation sites [Asn-Xaa-(Ser or Thr) where Xaa is any residue except Pro] are on the extracellular surface.

There are two possible problems with this folding pattern. (*i*) There are few charged groups on transmembrane segments, and the electric field on the cytoplasmic surface may not be large enough to allow movement of even highly charged nontransmembrane segments to account for the large dipole change that occurs during channel activation. (*ii*) The first two relatively apolar segments may not be sufficiently polar to form the channel lining.

Models presented here were developed to avoid these problems and to provide a more detailed molecular model of the channel structure. The favored model has the same general folding pattern as postulated by Noda *et al.* (1) except that each domain has four additional transmembrane segments S3, S4, S6, and S7 (see Fig. 1). The postulated activation gating mechanism involves a screw-like motion of S4 helices.

METHODS

The sequence was analyzed with a method that predicts which portions of α-helices and β-structures are exposed to water, buried inside protein, or exposed to lipid (3, 4). The four domains will be referred to here as A, B, C, and D. This analysis made the following predictions for each domain. No portions of the two very apolar putative transmembrane α-helices (see S5 and S8 in Fig. 1) should be exposed to water. One face of putative helices S1, S2, and S3 is more polar than the other, but central segments of these helices should not be exposed to water. The positively charged polar face of putative helix S4 spirals around the helix. Central portions of S6 should be completely buried. Portions of negatively charged S7 should be exposed to water.

Periodicity of negative charges on S7B should favor formation of an amphipathic α-helix whereas distribution of negatively charged residues at every other position on S7D should favor a β-strand (see Fig. 2*C* and *D*). A helical conformation is made unlikely in S6D and S7D by five prolines that are not found in other homologous segments. Long apolar segments S1, S2, S3, S5, and S8 were given α-helical conformations because long apolar sequences appear to form α-helices in other membrane proteins. Positively charged residues at every third position suggests that S4 could form a 3_{10}-helix; however, α-helices were used, because long 3_{10}-helices have never been observed, and because positively charged residues on the apolar side of these helices form salt bridges with negative groups on helices S1 and S3 in the final model. S4C is the only segment assigned a helical conformation that contains a proline anywhere other than the NH_2 termini where it is highly favorable. Assignments of α-helix termination positions were made tentatively on the basis of residues that favor end positions (proline and negatively charged residues at the NH_2 termini, positively charged residues at the COOH termini) and by constructing molecular models.

Interactions among transmembrane segments were examined carefully by constructing Nicholson molecular models of alternative conformations and by using computer graphics.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed.
†Present address: School of Mathematics and Computer/Information Sciences, University of Hyderabad, Hyderabad 500 134, India.
‡S8 is the nomenclature used in this paper. Noda *et al.* (1) called this segment S6.

Tertiary structures (see Fig. 2) were developed to satisfy the following criteria: (*i*) Transmembrane segments that are adjacent to each other in the sequence and have short connecting segments were usually packed next to each other. Antiparallel helix interactions were favored because of dipole interactions between helices (5, 6). Most adjacent α-helices cross each other at an angle of about 20° as predicted by "knobs into holes" (7, 8) or "3–4 ridges into grooves" (9) helix packing (see Fig. 2*A*). (*ii*) There are several homologous positions that have the same or similar residues in all domains. These conserved residues of one helix were packed next to complementary residues that are conserved in another helix. (*iii*) Preference was given to structures with more disulfide bridges, salt bridges, hydrogen bonds, and apolar interactions. (*iv*) Helices were arranged so that the four domains could form a channel with similar backbone structures of homologous regions and with minimal surface exposed to lipid. (*v*) Domains were not allowed to overlap or intertwine. (*vi*) Helices were packed so that most charged side chains could be at least partially exposed to water and so that most apolar side chains were buried or exposed to lipid. (*vii*) An attempt was made to place most potential N-glycosylation sites on the extracellular surface. (*viii*) Models in which NH_2- and COOH-terminal segments and segments connecting homologous domains in the cytoplasm were favored. It is not likely that these segments cross the membrane during insertion because there is no signal peptide on the NH_2-terminus, and these large segments contain some very polar regions but no long extremely apolar regions. This constraint requires that segments within each homologous domain span the membrane an even number of times and that all domains have the same orientation in the membrane.

MODEL DESCRIPTION

Structurally Homologous Folding Scheme. Fig. 1 shows the postulated secondary structure of homologous domains and segments that may extend through the membrane for a simple model in which conformations of the four homologous domains are similar and no transmembrane segments are located outside these domains. The model is similar to that suggested by Noda *et al.* (1) in that NH_2- and COOH-terminal segments and segments linking homologous domains are in the cytoplasm, in that segments linking S5 and S6 that in domains A and C contain five potential N-glycosylation sites are on the extracellular surface, and in that S1, S2, S5, and S8 are transmembrane α-helices. It differs in that S3 and S4 form transmembrane helical hairpins instead of being on the cytoplasmic surface, and S6 and S7 form shorter partially transmembrane hairpins instead of being on the extracellular surface. All homologous transmembrane segments are assumed to have the same secondary structure except S6D and S7D that were given β- instead of α-conformations.

There are several reasons to suspect that S3, S4, S6, and S7 cross the membrane. (*i*) S1 and S2 are not good candidates for channel lining α-helices because their central regions are very apolar and calculations indicate that these regions should not be exposed to water (see Fig. 1). (*ii*) The calculated dipole shift during activation of squid sodium channels indicate that the equivalent of approximately six charges cross the membrane (10). It is difficult to account for this dipole change with transmembrane segments that contain few charges and/or with charged segments on the membrane surfaces. Armstrong (10) has proposed a sodium channel activation gating mechanism in which strings of negative charges move relative to strings of positive charges. S7 and S4 may correspond to these hypothetical charged strings. (*iii*) Conservation of arginines for all domains at three positions on S4 suggests they are crucial to the channel's function and/or structure. (*iv*) The channel is cation selective. If S4 segments cross the membrane and form part of the channel lining, their positive charges must be at least neutralized by negative charges. Apolar S1 and S3 contain negatively charged side chains that can interact with some positively charged side chains on the apolar side of S4 helices. Remaining positive charges can be neutralized by negative charges on S7. Linings that contain almost equal numbers of positively and negatively charged side chains should be electrostatically stable and have been proposed for acetylcholine receptor channels (2, 11, 12) and colicin channels (13, 14). S7 segments were selected as negative channel lining segments because they and the apolar S6 segments that precede them can form hairpin structures that in the tertiary model are the proper length to cover apolar portions on other helices. Also, it is easy to see how S6 and S7 could be inserted into the membrane since they are located between putative glycosylated extracellular segments that must cross the membrane during insertion and S8. (*v*) Each domain has five apolar segments. If the rationale described earlier that there are an even number of transmembrane segments in each domain is correct then either one of these apolar segments does not cross the membrane as suggested by Noda *et al.* (1) or an odd number of additional segments in each domain cross the membrane as proposed here.

Other Folding Schemes. Assumptions that all homologous domains have similar folding patterns and that all transmembrane segments are contained within homologous domains may not be valid. The sodium channel is highly glycosylated on the extracellular side of the membrane. Seven of ten potential N-glycosylation sites (positions 205, 278, 288, 317, 591, 1160, and 1174) are on the extracellular surface in Fig. 1. Two of three potential sites associated with the B domain are on the cytoplasmic side; one is just before S5B (position 690) and the other is just after S8B (position 797). Alternative folding patterns for the B domain alone or for all domains that place these sites on the outside are conceivable. These alternatives require additional transmembrane segments and in some cases require that S5 and S8 each form β-hairpins.

Tertiary Models. Tertiary models were developed to test the feasibility of general folding schemes and to make more precise predictions about the sodium channel's structure and gating mechanisms. Fig. 2 shows a channel tertiary structure that appears to satisfy best the criteria described in the methods. This model is highly tentative because of the nonquantitative nature of the methods. S1, S2, S3, and S4 are postulated to form a bundle of antiparallel α-helices. The helix backbone structure of hemerythrin (15) was used to develop the computer graphics model of these helices. Most side chains in the center of this bundle near the cytoplasmic surface are conserved in the four domains and are the same as or similar to side chains in the center of the bundle of four helices in hemerythrin. S6-S7 hairpins were placed between S1 and S4 bundles so that S4 and S7 segments form the channel lining. The backbone of a β-hairpin in cobra toxin was used for S6D and S7D. S5 and S8 were placed on the outside of the structure because they are very apolar. All adjacent α-helices are antiparallel if helices directly across from each other in bundles of four helices are not counted.

Channel activation may involve movement of positively charged S4s toward the extracellular surface and/or movement of negatively charged S7s toward the cytoplasm. One way this could be done that is consistent with "3–4 ridges into grooves" packing (9) is for S4 helices to rotate about their axes about 60° as they move 4.5 Å toward the outside. This screw-like movement places each S4 side chain of the open conformation in the same position as the side chain that preceded it by three residues in the closed conformation and essentially moves one positive charge across the membrane for each S4 segment. Interactions of positively charged arginine side chains of S4 with carboxyl groups of S7 shown

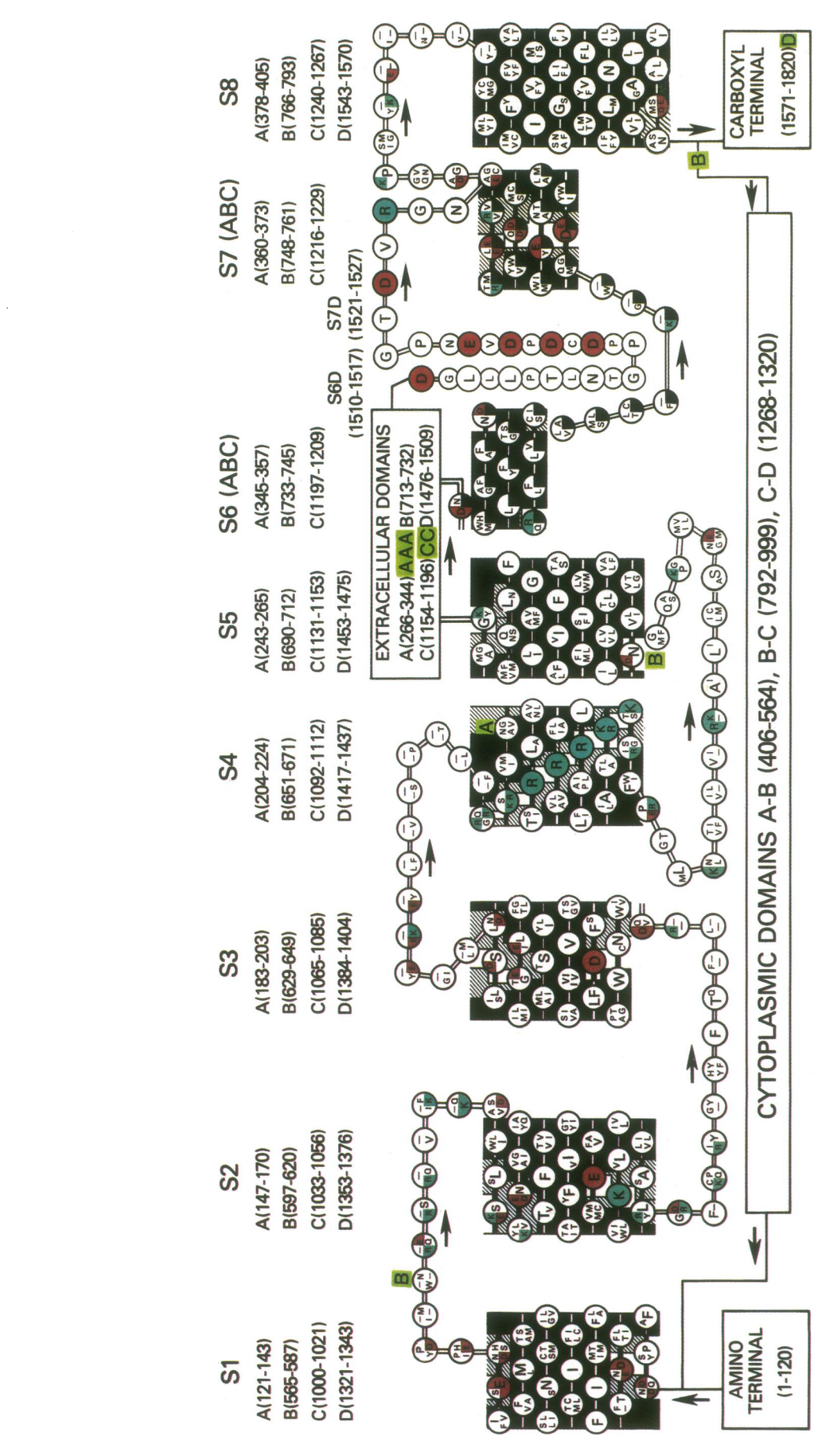

FIG. 1. General folding pattern of model in which homologous segments of all domains fold in the same general way, and all transmembrane segments are located within homologous domains. Circles show one letter code for residues of domain A in the upper left, B in upper right, C in lower left, and D in lower right. If the same residue occurs in adjacent quadrants a single letter is placed between quadrants. Positions in which a residue is completely conserved have only one letter. Transmembrane helices are represented by helix nets with more polar face in its center. Portions predicted to be buried in all domains have a black background, those exposed to water in all domains have a white background, and the remainder have hatched backgrounds. S6D and S7D are shown in extended β conformations. Residue numbers of cytoplasmic and extracellular domains are shown in boxes and those of transmembrane segments are above the segments. Potential N-glycosylation sites and domains with which they are associated are indicated by green. Positively charged residues are colored blue and negatively charged ones are red.

FIG. 2. Stereo computer graphics representation of postulated tertiary structure. (*A*) α Carbons of putative transmembrane α-helices and β-strands. Homologous segments have the same color; S1 olive green, S2 yellow, S3 turquoise, S4 royal blue, S5 and S8 grey/slate blue, S6 white, and S7 red. (*B*) Channel lining S4 (grey/slate blue) and S7 (white) segments viewed from the outside. Positively charged guanidium and amine groups are royal blue and negatively charged carboxyl groups are red. (*C*) S7B α-helix between S4B and S4A as seen from inside the channel. Color scheme is the same as *B* except the channel lining S4 (light aqua) and the positively charged guanidium and amine groups (dark aqua). (*D*) S6D and S7D β-hairpin between S4D and S4C. Color scheme is the same as *C*.

in Fig. 2*C* and *D* would appear virtually identical for open and closed conformations. Ridges formed by every third side chain in S4 can remain between ridges formed by every fourth side chain on adjacent α-helices throughout this movement. The cytoplasmic ends of S4 segments and/or segments linking S4 to S5 contain three more residues in the closed than in the open conformation. These residues may block the channel near the cytoplasm when it is closed. Negatively charged groups near COOH terminus of S7 may bind tetrodotoxin and saxitoxin and affect ion selectivity. Although one can envision other gating mechanisms that involve movements of S7 segments or twisting motions of the domains, the helical-screw gating mechanism, described above, conserves "ridges into grooves" packing, is consistent with hypotheses that the activation gate is near the cytoplasmic surface where it prevents entrance of local anesthetics when the channel is closed and that tetrodotoxin and saxitoxin bind to both open and closed channel conformations in the extracellular channel entrance (10, 16), and can explain sigmoidal activation kinetics if all S4 segments must move for the channel to open. The inactivation gate may involve cytoplasmic domains that are not modeled here.

DISCUSSION

Primary features of models presented here are that the sodium channel lining is formed by positively and negatively charged segments and that movement of the positively charged segments underlies voltage-dependent activation. It is possible that this concept is correct but that details of the folding scheme and/or tertiary models presented here are incorrect. Alternative folding schemes can be tested in a number of ways. (*i*) Several approaches could be used to determine which segments are on the extracellular and cytoplasmic surfaces; e.g., it may be possible to experimentally determine glycosylation and phosphorylation sites and/or to make antibodies to specific segments and determine to which side of the membrane they bind. (*ii*) Attempts will almost certainly be made to study sodium channels formed by injecting normal and modified mRNA into frog oocytes or other appropriate cells as has been done for acetylcholine receptors (17). Models are especially useful for site directed mutagenesis experiments because they suggest which residues are essential. It would be informative to determine how substitution of charged groups on S4 and S7 affect channel properties, whether deletions of three residues in S4 can essentially lock the S4 segments in an open conformation, and whether substitutions for negatively charged side chains near the COOH termini of S7 segments affect tetrodotoxin and saxitoxin binding and ion selectivity. (*iii*) It may be possible to covalently bind agents to the channel and determine which segments are involved. The present model would be supported if the local anesthetic binding site involves putative channel lining segments and if tetrodotoxin and saxitoxin bind near COOH termini of S7 segments.

Note Added in Proof. Greenblatt *et al.* (18) have proposed a model that has virtually the same transmembrane folding as shown in Fig. 1 except that S6D is an α-helix formed by residues 1484–1503. They have shown that all eight potential phosphorylation sites are in the cytoplasm in this scheme. The tertiary structure of their model differs from the one presented here in that S3 segments form the channel lining and S4 and S7 are buried in the transmembrane protein.

We thank Robert Jernigan and Gerald Ehrenstein for comments and discussions of the manuscript.

1. Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. & Numa, S. (1984) *Nature (London)* **312,** 121–127.
2. Miller, J. A., Agnew, W. S. & Levinson, S. R. (1983) *Biochemistry* **22,** 462–470.
3. Guy, H. R. (1984) *Biophys. J.* **45,** 249–261.
4. Guy, H. R. (1985) *Biophys. J.* **47,** 61–70.
5. Hol, W. G. J., Halie, L. M. & Sander, C. (1981) *Nature (London)* **294,** 532–536.
6. Wada, A. & Nakamura, H. (1981) *Nature (London)* **293,** 757–758.
7. Crick, F. H. C. (1953) *Acta Crystallogr.* **6,** 689–697.
8. Dunker, K. & Zaleske, D. J. (1977) *Biochem. J.* **163,** 45–57.
9. Chothia, C., Levitt, M. & Richardson, D. (1981) *J. Mol. Biol.* **145,** 215–250.
10. Armstrong, C. M. (1981) *Physiol. Rev.* **61,** 644–683.
11. Finer-Moore, J. & Stroud, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 155–159.
12. Criado, M., Hochschwender, S., Sarin, V., Fox, J. L. & Lindstrom, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2004–2008.
13. Guy, H. R. (1983) *Biophys. J.* **41,** 363a (abstr.).
14. Guy, H. R. (1984) *Biophys. J.* **45,** 123a (abstr.).
15. Stenkamp, R. E., Sieker, L. C. & Jensen, L. H. (1981) *Nature (London)* **291,** 263–264.
16. Hille, B. (1975) in *Membranes: A Series of Advances*, ed. Eisenman, G. (Dekker, New York), Vol. 3, pp. 255–323.
17. Mishina, M., Tobimatsu, T., Imoto, K., Tanaka, K., Fujita, Y., Fukuda, K., Kuraski, M., Takahashi, H., Morimoto, Y., Hirose, T., Inayama, S., Takahashi, T., Kuno, M. & Numa, S. (1985) *Nature (London)* **313,** 364–369.
18. Greenblatt, R. E., Blatt, Y. & Montal, M. (1986) *FEBS Lett.*, in press.