A STRUCTURAL MODEL OF THE ACETYLCHOLINE RECEPTOR CHANNEL BASED ON PARTITION ENERGY AND HELIX PACKING CALCULATIONS

H. ROBERT GUY
Laboratory of Biophysics, National Institute of Neurological, Communicative Diseases, and Stroke, and Laboratory of Mathematical Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

ABSTRACT A structural model of the transmembrane portion of the acetylcholine receptor was developed from sequences of all its subunits by using transfer energy calculations to locate transmembrane α-helices and to calculate which helical side chains should be in contact with water inside the channel, with portions of other transmembrane helices, or with lipid hydrocarbon chains. "Knobs-into-holes" side chain packing calculations were used with other factors to stack the transmembrane α-helices together. In the model each subunit has the following structures in order along the sequence from the NH_2 terminus: a large extracellular domain of undetermined structure, a short apolar α-helix that lies on the extracellular lipid surface of the membrane; three apolar transmembrane α-helices (I, II, and III), a cytoplasmic domain of undetermined structure, an amphipathic transmembrane α-helix (L) that forms the channel lining, a short extracellular α-helix, another apolar transmembrane α-helix (IV), and a small cytoplasmic domain formed by the COOH-terminal end of the chain. Three concentric layers form the pore. A bundle of five amphipathic L helices forms the channel lining. This bundle is surrounded by a bundle of 10 alternating II and III helices. Helices I and IV cover portions of the outer surface of the bundle formed by helices II and III. Positions of disulfide bridges are predicted and a mechanism for opening and closing conformational changes is proposed that requires tilting transmembrane helices and possibly a thiol-disulfide interchange reaction.

INTRODUCTION

More is known about nicotinic acetylcholine receptors (AChR) from the electric organ of *Torpedo* fish than any other membrane channel protein of the nervous system. Protein sequences of the four *Torpedo californica* AChR subunits have been determined by Noda et al., (1983) (see Table I). The α-sequence differs only by five or six residues from sequences obtained by Sumikawa et al., (1982) and Devillers-Thiery et al., (1983) from *Torpedo marmorata* and the γ-subunit sequence differs by only one residue from that obtained from *Torpedo californica* by Claudio et al., (1983). The four subunits are highly homologous and probably evolved from the same protein.

Kistler et al. (1982) have postulated a nonspecific structure for the AChR. In their model, a large water-filled pore is formed between five subunits. When viewed from the extracellular phase, the subunits are arranged in the clockwise order $\alpha,\beta,\alpha,\gamma,\delta$. Each subunit has an extracellular domain that extends up to 55 Å above the membrane, a transmembrane domain that contains α-helices oriented perpendicular to the membrane surface, and a cytoplasmic domain that extends 15 Å below the membrane surface. The goal of this paper is to develop a specific model of the transmembrane domain based on subunit sequences. The subunit arrangement proposed by Kistler et al. (1982) was used; however, an arrangement proposed by Wise et al. (1981) in which the positions of the β- and γ-subunits are switched appears to work as well.

Many integral membrane proteins possess very apolar segments that are long enough to span a lipid bilayer in an α-helical conformation. Each AChR subunit contains four such segments. The nomenclature of Devillers-Thiery et al. (1983) was used to label these segments helices I, II, III, and IV in Table I. By minimizing secondary energies in the scheme of Jernigan et al. (1980), we have confirmed the strong stability of these helices for all subunits. Three of the groups (Noda et al., 1983, Devillers-Thiery et al., 1983, Claudio et al., 1983), who determined sequences of one or more subunit, postulated that transmembrane domains are formed by these helices, that extracellular domains are formed by segments preceding helix I, and that the cytoplasmic domain is formed by the segments between helices III and IV. These models are based on the assumption that the environment to which all transmembrane helices are exposed is substantially more apolar than the environments

Correspondence should be addressed to Dr. Guy, The Laboratory of Mathematical Biology, National Cancer Institute, Bldg. 10, Room 4B-50, Bethesda, MD 20205.

TABLE I
SEQUENCES OF AChR SUBUNITS

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  :                :                :                :                :                :
α SEHETRLVANLL-EN-YN-KVIRPVEHHTHFVDITVGLQLIQLISVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNAD
β SVMEDTLLSVLF-ET-YNPKV-RPAQTVGDKVTVRVGLTLTNLLILNEKIEEMTTNVFLNLAWTDYRLQWDPAAYEGIKDLRIPSSDVWQPDIVLMNNND
γ ENEEGRLIEKLL-GD-YDKRII-PAKTLDHIIDVTLKLTLTNLISLNEKEEALTTNVWIEIQWNDYRLSWNTSEYEGIDLVRIPSELLWLPDVVLENNVD
δ VNEEERLINDLLIVNKYNKHV-RPVKHNNEVVNIALSLTLSNLISLKETDETLTSNVWMDHAWYDHRLTWNASEYSDISILRLPPELVWIPDIVLQNNND
                 :                :                :                :
α GDFAIVHMTKLLLDYTGKIMWTPPAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVS--ISPES-----DRP------DLSTFMESGEWVMKDYRGW
β GSFEITLHVNVLVQHTGAVSWQPSAIYRSSCTIKVMYFPFDWQNCTMVFKSYTYDTSEVTLQ-HA--LDAKGEREVKEIVINKDAFTENGQWSIEHKPSR
γ GQFEVAYYANVLVYNDGSMYWLPPAIYRSTCPIAVTYFPFDWQNCSLVFRSQTYNAHEVNLQLSAE--EGE---AVEWIHIDPEDFTENGEWTIRHRPAK
δ GQYHVAYFCNVLVRPNGYVTWLPPAIFRSSCPINVLYFPFDWQNCSLKFTALNYDANEITMDLMTDTIDGK-DYPIEWIIIDPEAFTENGEWEIIHKPAK
                 :                :                :                :
α KH--WVYYTCCPD-TPYLDITYHFIMQRIPLYFVVNVIIPCLLFSFLTGLVFYLPTDSG-EKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLF
β KN--W---RSD-DPS-YEDVTFYLIIQRKPLFYIVYTIIPCILISILAILVFYLPPDAG-EKMSLSISALLAVTVFLLLLADKVPETSLSVPIIIRYLMF
γ KNYNWQL-TKD-D-TDFQEIIFFLIIQRKPLFYIINIIAPCVLISSLVVLVYFLPAQAGGQKCTLSISVLLAQTIFLFLIAQKVPETSLNVPLIGKYLIF
δ KN---IYPDKFPNGTNYQDVTFYLIIRRKPLFYVINFITPCVLISFLASLAFYLPAESG-EKMSTAISVLLAQAVFLLLTSQRLPETALAVPLIGKYLMF
               (--OS Helix--)(Helix Iα)  (--Helix Iβ--)     (-------Helix II--------)       (-------
                 :                :                :                :
α TMIFVISSIIITVVVINTHHRSPSTHTMPQWVRKIFIDTIPNVMFFS-------------------------TMKRASKEKQENKIFADDI-DISD-
β IMILVAFSVILSVVVLNLHHRSPNTHTMPNWIRQIFIETLPPFLWIQ-----RPVTTPSPD----------SKPTIISRANDEYFIRK-PAGDFVCPVDN
γ VMFVSMLIVMNCVIVLNVSLRTPNTHSLSEKIKHLFLGFLPKYLGMQLEPSEETPEKPQP--------RRRSSFGIMIKAE-EYILKK-PRSELMFEEQK
δ IMSLVTGVIVNCGIVLNFHFRTPSTHVLSTRVKQIFLEKLPRILHMS-----RADESEQPDWQNDLKLRRSSSVGYISKAQ-EYFNIK-SRSELMFEKQS
  Helix III------------)(-------------------------------------------------Cytoplasmic Domain---------
                 :                :                :                :
α -----ISGKQV---TGEVIFQ------TPLIK-NPDVKSAIEGVKYIAEHMKSDEESSNAAEEWKYVAMVIDHILLCVFMLICIIGTVSVFAGRLIELSQE
β ARVAVQPERL---FSEMKWHLNG--LTQPVTLPQDLKEAVEAIKYIAEQLESASEFDDLKKDWQYVAMVADRLFLYVFFVICSIGTFSIFLDASHNVPPD
γ DRHGLK--RVNKMTSDIDIGTTVDLYKDLANFAPEIKSCVEACNFIAKSTKEQNDSGSENENWVLIGKVIDKACFWIALLLFSIGTLAIFLTGHFNQVPE
δ ERHGLVP-RV---TPRIGFGNNNENIAASDQLHDEIKSGIDSTNYIVKQIKEKNAYDEEVGNWNLVGQTIDRLSMFIITPVMVLGTIFIFVMGNFNHPPA
  -------------------------------)(------Helix L------)   (---Helix C---)(-------Helix IV---------)
α G
β NPFA
γ FPFPGDPRKYVP
δ KPFEGDPFDYSSDHPRCA
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Sequences from Noda et al. (1983). Homology alignment was made to minimize insertions and deletions.

to which protein segments in the extracellular and cytoplasmic domains are exposed. Calculations presented here suggest that this assumption may not be valid for the AChR channel. Here an alternative model is proposed in which there is a fifth transmembrane α-helix between helices III and IV (see segment labeled helix L in Table I). This helix has a very polar face that could be in contact with water inside the channel.

RESULTS

Identification of Transmembrane α-Helices

To analyze stability of a membrane channel structure formed by α helices it is useful to consider side-chain interactions with three categories of atoms: (*a*) water molecules inside the channel and polar side chains extending into the water, (*b*) very apolar hydrocarbon atoms of the lipid bilayer, and (*c*) intermediately polar protein atoms that are buried in the proteins structure between these two phases. When trying to identify protein segments that could form transmembrane α-helices, precise calculation of energies involved in these interactions is impossible. A crude calculation can be made, however, by approximating the energy required to move side chains from water into a solvent with either a polarity similar to that of protein interiors or a polarity similar to the hydrocarbon lipid phase. A theory was developed to calculate these energies from the difference between a term proportional to side chain contact areas (Richards and Richmond, 1978) and a term proportional to the polarity of atoms comprising the side chains. The polarity scale was developed from data on solubilities of amino acids in ethanol and methanol (Tanford, 1962; Nozaki and Tanford, 1971; Gekko, 1981), from partitioning of amino acids between water and octanol (Yunger and Cramer, 1981; Pliska et al., 1981), from radial distribution of residues in soluble proteins (Prabhakaran and Ponnuswamy, 1980), and from partitioning of side-chain analogs into organic solvents (Rekker, 1977) and air (Wolfenden et al., 1981). Surface areas and polarity scale values of each side chain are multiplied by proportionality constants related to solvent polarity. Polarity of the protein environment was approximated from radial distributions of residues in proteins (Prabhakaran and Ponnuswamy, 1980) and that of the lipid environment from partition coefficients of organic molecules into oil (Rekker, 1977). Results of this analysis indicate that only large alkyl side chains, Val, Ile, and Leu, should favor the lipid phase over the protein environment; these side chains are denoted by black circles in the figures. Most other uncharged side chains favor the protein phase; those with no hydrogen bond forming atoms are shaded in the figures and those that can form hydrogen bonds are white. Exceptions are Asn and Gln, which, together with the charged side chains Asp, Glu, Lys, and Arg, favor the water phase. The negatively charged Asp and Glu residues are colored red and positively charged Lys and Arg are blue. Pro also favors water because it disrupts α-helices and β-sheets thus exposing polar backbone atoms; Pro is stippled in the figures.

The program assumes the helix axes to be parallel to a water-protein interface formed between water inside the channel and the channel lining and to the protein-lipid interface formed between the outermost helices and the lipid phase. In this kind of structure, many side chains will be partially exposed to two phases. To compensate for this effect, transfer energies are calculated as a function of distance of the first side chain carbon from either the water-protein or the protein-lipid interface. Polar moieties of long side chains are assumed to be nearer than their β-carbons to the more polar phase. This assumption is consistent with their radial distribution in soluble proteins. Thus, the most energetically favorable location for Arg, Lys, Tyr, and Trp is near the water-protein interface where their large apolar side chain component can be buried in the protein phase and their polar component can be exposed to water. A detailed analysis of these equations and relationships will be presented elsewhere.

Portions of transmembrane α-helices that should be exposed to water, protein, or lipid are predicted in a similar way to a calculation of how a log that is weighted on one side would orient at a water-air interface and how deeply the log would sink into the water. The sequence is analyzed in seven-residue–long segments that are assumed to be α-helices. When viewed from the end, these helices have seven side chains uniformly distributed around their axis. The helix axis is positioned in the plane of a water-protein interface. The energy required to move each side chain from water to the position it has relative to the interface is calculated and these energies are summed. The helix is rotated slightly about its axes, and the calculation is repeated. Once the most energetically favorable orientation is found, the helix is oriented this way, and the energies required to displace the axis different distances from the interface are calculated until the most favorable displacement is determined. Calculations of helix orientation and displacement are repeated until optimal values are determined for both parameters. These values and the energy, ΔF, required to move the helix from water to the environment predicted by these values are assigned to the central residue of the sequence. The procedure is repeated for all seven-residue–long segments; i.e., segments 1–7, 2–8, 3–9, etc. The same procedure is used to calculate the energy required to completely remove each segment from water and position it in the most favorable way at a protein-lipid interface. Portions of putative α-helices that are exposed to water, protein, or lipid can be calculated from axis displacement and helix orientation values. If the energy required to move the segment from water to the protein-lipid interface is less than that required to position it at a water-protein interface, then the segment should be buried in protein and possibly exposed to lipid; otherwise, it should be partially exposed to water.

Results of this program for the AChR δ subunit are shown in Fig. 1. Similar results are found for homologous portions of other subunits. The four segments postulated to be apolar transmembrane α-helices are shaded and characterized by very negative ΔF values for the protein-lipid interface. Although some of these helices possess a slightly more polar face, calculations indicate that energies required to move all segments of these helices to a protein-lipid interface is less than that required to place them at a water-protein interface. Analysis of bacteriorhodopsin with this procedure indicates its transmembrane α-helices to be significantly more polar than the four apolar segments of the AChR even though helices of bacteriorhodopsin are not exposed to bulk water. These findings suggest that the four apolar AChR helices have no contact with water and that other protein segments form the water-exposed channel lining.

If additional transmembrane segments exist, they may well be amphipathic α-helices; i.e., helices that are polar on one face and apolar on the opposite face. Possible amphipathic α-helices are easily identified with the present procedure by locating segments with relatively constant orientation and displacement values. A number of strands that could form amphipathic α-helices can be identified in the NH_2-terminal segment that precedes helix I; however, Devillers-Thiery et al. (1983) have made a compelling argument that all portions of this segment should be extracellular. The segment labeled helix L in Table I is between helices III and IV and corresponds to a favorable amphipathic α-helix. Fig. 2 shows how helix L of each subunit would appear if it were split down the apolar side and spread flat. Most of the side chains in the center of the diagrams are negatively charged Asp(D) or Glu(E) or positively charged Lys(K). These should of course be exposed to water. Dots indicate positions of closest contact

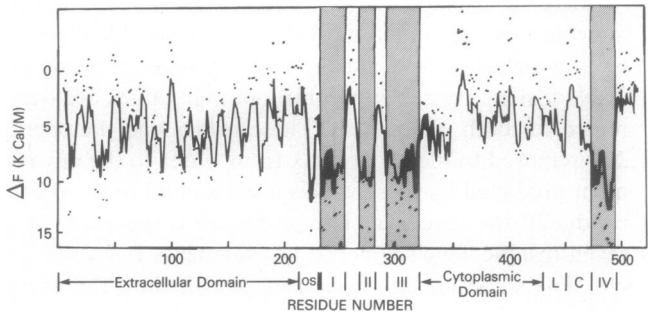

FIGURE 1 Transfer energies predicted for presumed helical segments of the δ-subunit. Values are calculated for each residue by assuming it is in the center of a seven-residue-long α-helix as described in the text. ΔF is the energy required to move each seven residue segment from water to its optimal displacement and orientation from either a water-protein (—) or protein-lipid (···) interface. If the dots are more negative than the solid line, the segment is probably buried in protein and possibly exposed to lipid; otherwise, it should be partially exposed to water. Shaded regions correspond to the four strands postulated to be apolar transmembrane helices. Positions of postulated α-helices and domains are indicated below the abscissa.

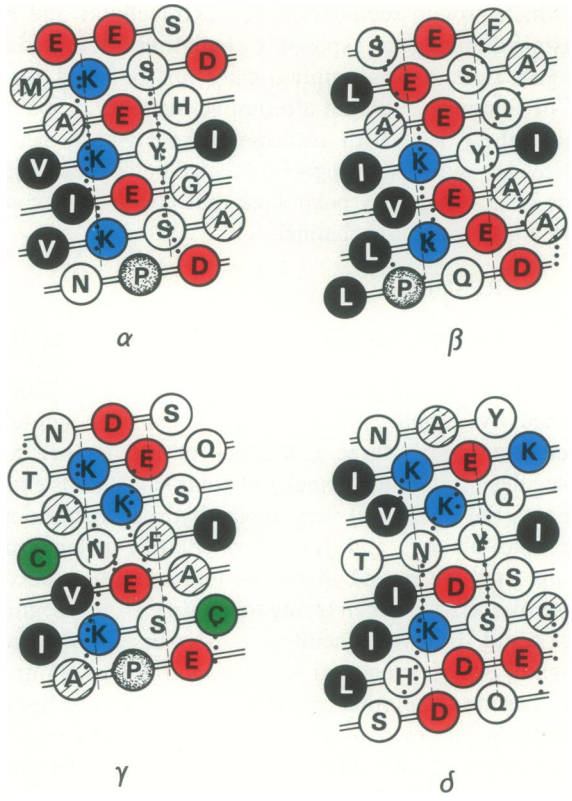

FIGURE 2 Radial projection of helix L. This shows how the L helices would appear if they were split down the apolar side and spread flat. Dots (···) represent points of closest contact between adjacent helices predicted by transfer energy calculations. Dashed lines (---) represent planes of closest contact between adjacent helices predicted by helix side chains packing analysis for the "open" conformation. Lipid-preferring side chains, Leu (L), Val (V), Ile (I) are indicated by a black circle with white letters; protein-preferring side chains that do not form hydrogen bonds, Phe (F), Met (M), Gly (G), Ala (A) by shaded circles; noncharged side chains that form hydrogen bonds, Thr(T), Ser (S), His (H), Try (Y), Trp (W), Gln (Q), and Asn (N) by white; negatively charged side chains, Glu (E) and Asp (D) by red; positively charged side chains, Lys (K) and Arg (R) by blue; Cys (C) by green; and Pro (P) by stippled circles. This coding scheme is used in all figures.

to adjacent parallel helices predicted by the program. Dashed lines are positions of closest contact predicted later by helix side-chain packing analysis for the open-channel conformation. Agreement between these predictions is excellent for α- and β-subunits and well within the approximations and uncertainty of the transfer energy calculations for γ and δ. Thus, the ratio of water-preferring to apolar and protein-preferring side chains on the L-helices is the value expected if five of these helices stack side by side to form the channel lining.

Stacking of Transmembrane Helices To Form the Channel

Once possible transmembrane helices are identified, a structural model can be constructed. An attempt has been made to find the most simple, energetically stable structure

by imposing the following constraints. (*a*) All transmembrane strands are α-helices. (*b*) Homologous regions of all the subunits have the same secondary structure and fold in the same general way. This is usually true for homologous proteins. (*c*) Homologous regions of all subunits are exposed to the same solvent environment. This is usually true for homologous proteins and requires that subunits be arranged around the channel with approximate fivefold symmetry. (*d*) Helices are positioned so their apolar portions begin and end near membrane surfaces. (*e*) The maximum number of disulfide bridges are formed between Cys side chains within a subunit. There is no evidence that disulfide bridges form between subunits within monomers. (*f*) Side chains of adjacent helices will pack between each other in a manner found in other proteins. (*g*) Residues preceding prolines are not in α-helical conformations. Ends of helices were identified by positions of proline residues and calculations indicating which segments should be exposed to water.

Two closely related subunit structures were found that satisfied these constraints. Both subunit conformations can be used to construct a channel that has three concentric layers of protein: an inner cylindrical bundle of five L-helices, a cylindrical bundle of 10 alternating II- and III-helices, and a outer layer containing 10 I- and IV-helices with lipid between helices of different subunits. A schematic representation of the central two bundles is shown in Fig. 3 for both subunit conformations. When all subunits are in one conformation, the pore through the bundle of L-helices is small, when they are in the other conformation the pore is large. Transitions between these structures are suggested as a mechanism for channels to open and close. Suggestions are made later about conformations for helices I and IV and how they could influence channel gating.

Dunker and Zaleske (1977) have analyzed side-chain–packing arrangements for large bundles of helices that could form membrane channels. They used a "knobs-into-holes" analysis first proposed by Crick (1953) for α-helix interactions in fibrous proteins. They concluded that helices that stack side by side to form cylindrical bundles will be tilted relative to the axis of the cylinder so that every seventh side chain of one helix fits between those of the adjacent helix in regions of close contact between helices. The size of the pore through the bundle depends upon how much the helices are tilted. Much of their analysis was used to develop the models presented here with small modifications to treat the special case in which a bundle of 10 helices surrounds a bundle of five helices.

The channel model was developed from the center outward. Space-filled CPK and "stick and ball" Nicholson molecular models were used to construct a bundle of five L-helices arranged in the order proposed by Kistler et al. (1982). The helices stacked together very well when tilted ~8° relative to the channel axis (see Fig. 4 *a* and the center of the structures in Fig. 5 for representations of this packing arrangement). The channel lining primarily contains charged side chains but is electrically neutral through the central region because the number of positively charged Lys side chains is the same as the sum of negatively charged Glu and Asp side chains. All charged groups can be positioned so that they form at least two salt bridges. There are clusters of negatively charged side chains just past the postulated COOH-terminal ends of the L-helix. These carboxyl groups should be near the extracel-

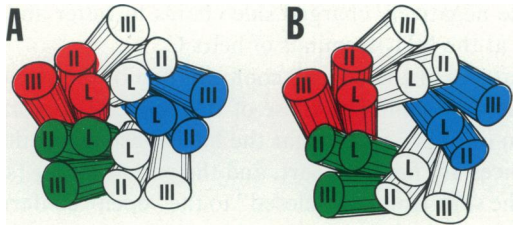

FIGURE 3 Schematic representation of the bundle of five L-helices surrounded by the bundle of 10 alternating II- and III-helices when all subunits are either in the "open" (*A*) or "closed " (*B*) conformation. Cylinders represent α-helices. The α subunits are white, β is blue, γ is green, and σ is red.

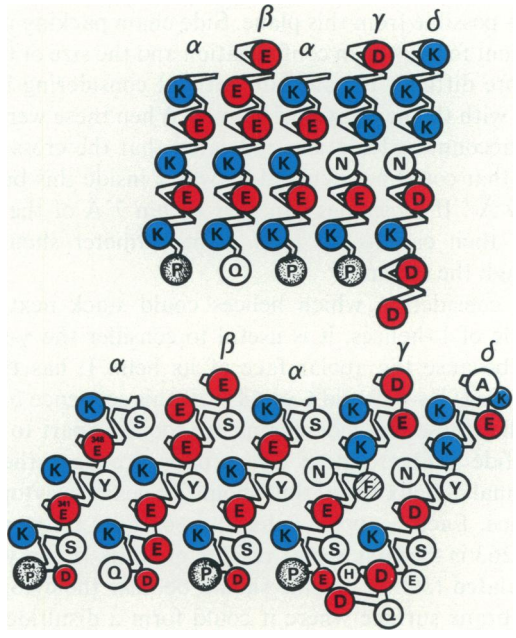

FIGURE 4 Representation of channel lining. This figure shows how the lining of the channel would appear if the bundles of L-helices were separated between the α- and δ-subunits and the cylinder they had formed were spread flat with the interior polar side up. All subunits are either in the "closed" conformation *top* or the "open" conformation *bottom*. Only those side chains that can have contact with water inside the channel are shown. Note the relative positions of blue positively charged Lys (*K*) side chains and red negatively charged Asp (*D*) and Glu (*E*) side chains, and that helices of the "open" conformation are tilted more, spaced farther apart, and oriented differently. Charged side chains on the "back" side of L-helices are shown in *bottom* panel.

lular entrance of the narrow region of the channel and should make the channel cation selective. There are also some unbalanced negative charges on the NH_2-terminal regions of the β- and δ-helices that should be near the cytoplasmic entrance of the channel and could contribute to the selectivity.

Helix packing calculations predict that the cross section area inside the channel that can be occupied by water should be ~70 Å^2; however, the pore size appears small because the side chains are long and extend into the channel. Organic cations 6–7 Å in diameter that have been shown to pass through ACh-activated channels of frog sartorius muscle (Dwyer et al. 1980, Maeno et al., 1977) cannot pass through the channel. Most water molecules fit between charged side chains and should be tightly bound. It was concluded that if this structure exists, it must be a closed or low-conducting conformation.

The pore size can be made larger by tilting the helices more. Helices illustrated in Fig. 4 *b* tilt 20° relative to the cylinder axis and are spaced farther apart than those in Fig. 4 *a*. Three columns of side chains are shown because the helix was rotated one-fourteenth of a turn about its axis to allow side chains to fit between each other. Side chains lie almost directly on the plane connecting axes of adjacent helices, and thus the helices must be spaced farther apart than in the closed conformation where side chains were as far as possible from this plane. Side chain packing is not as efficient for this open conformation and the size of the pore is more difficult to calculate without considering interactions with the next layer of helices. When these were taken into account, calculations indicated that the cross section area that could be occupied by water inside this bundle is ~260 Å^2. If molecules can pass within 7 Å of the helical axes, then cations up to 8 Å in diameter should pass through the channel.

In considering which helices could stack next to the bundle of L-helices, it is useful to consider the γ-subunit first because the apolar face of its helix L has two Cys residues (Cys-439 and Cys-443). If this sequence forms an α-helix, these Cys side chains are too far apart to form a disulfide bridge. These Cys groups are near the NH_2-terminal of helix L and thus should be near the cytoplasmic surface. Each γ-apolar helix also contains a Cys residue. Cys-263 is the first apolar residue of Helix II and with the postulated folding scheme should be near the cytoplamsic membrane surface where it could form a disulfide bridge with Cys-439. Cys-312 is eight residues from the last apolar residue of helix III and is the best candidate to form a disulfide bridge with Cys-443 because each should be about the same distance from the cytoplasmic membrane surface. These disulfide bridges can form if helices L and II are parallel to each other and antiparallel to helix III. Physical constraints prevent more than 10 helices coming into contact with the bundle of five L-helices that are presumed to form the channel lining. Thus, models were constructed of 10 helix bundles formed from helices II and III of all the subunits.

Two stacking arrangements were calculated to be most favorable, one in which the helices are tilted ~16° and a larger one in which they are tilted ~33°. When possible stacking arrangements of these large bundles around the central bundle of L-helices were analyzed, some interesting observations were made. The smaller bundle of 10 helices was almost the perfect size to surround the "closed" conformation bundle of L-helices and the large bundle of 10 helices was near the optimal size to surround the postulated "open" conformation bundle of L-helices. In addition, the bundles could be stacked together so that side chains from one bundle could stack between those of the adjacent bundle in a manner predicted by the helix side-chain packing analysis.

The helix packing arrangement for the two bundles in the "closed" conformation is illustrated in Fig. 5. Each layer is two turns of an α-helix thick. The most important aspect of this structure is that, given the severe constraints imposed by side-chain packing factors, an arrangement can be made from the AChR subunits wherein (*a*) Cys side chains can form disulfide bridges (note locations in the γ-subunits in the third and bottom layer of green circles labeled *C*), (*b*) most side chains are able to form hydrogen bonds with each other (note locations between the bundles of white circles *S,T,N,Q,H,* and *Y*), and (*c*) most of the charged side chains can be in contact with water and form salt bridges (note locations on channel lining of positively charged Lys, *K* in blue circles, and negatively charged Glu and Asp, *E* and *D* in red circles). The segment labeled helix *C* in Table I connects helix L to helix IV. A horizontal representation of this helix is shown in the top layer in Fig. 5. This helix passes over the top of the COOH-terminus of helix II of the adjacent subunit. The NH_2-termini of these helices have a number of negatively charged side chains that surround the extracellular entrance of the transmembrane portion of the channel. If the model is correct, these negative groups are probably responsible for the cation selectivity of the channel. Residues of the different subunits in the very polar transition region between helix L and C are not very homologous. The break between the helices was made at a different position for the α-subunit to expose negatively charged side chains to water and to put them at the NH_2-terminus of helix C.

Features of the "open" conformation of the two central bundles are similar to those of the "closed" conformation shown in Fig. 5 except that the helices are more tilted, the L-helices are farther apart, and the pore is larger (see Fig. 3). The shift from the "closed" to the "open" conformation is accomplished by altering the packing both within and between the subunits. Helix III rotates clockwise (viewed from the top) one-seventh of a turn, shifting downward relative to helix II of the same subunit by two residues, and shifting upward relative to helix II of the adjacent subunit

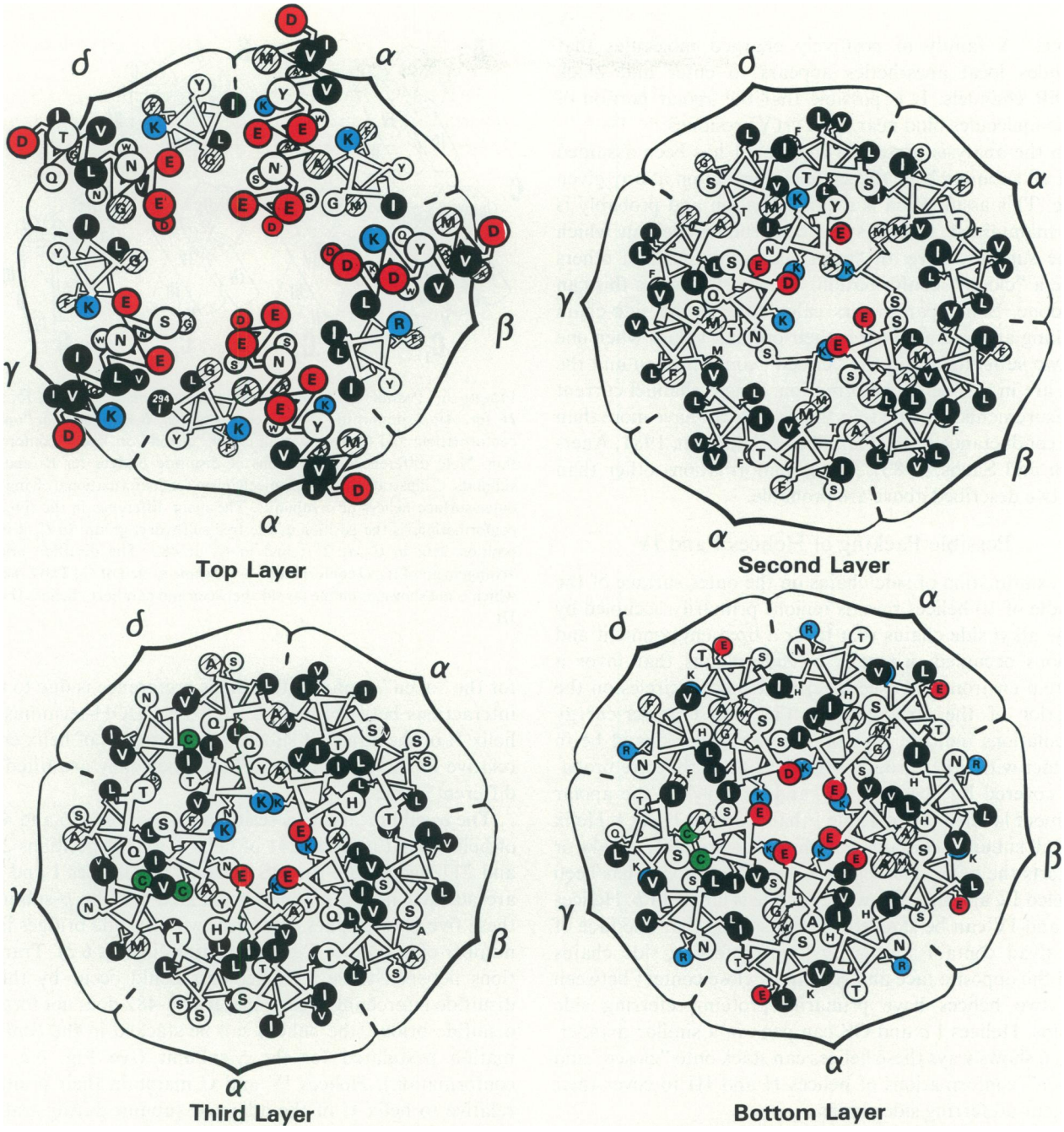

FIGURE 5 Detailed view from the top of bundle of five L-helices surrounded by the bundle of 10 alternating II- and III-helices at different levels. The top level is the most extracellular and shows C-helices lying horizontally between NH_2-termini of type-III helices. Other more intracellular levels show the bundle of five L-helices surrounded by the bundle of 10 helices of alternating type II and III. The break between L- and C-helices occurs earlier in the sequence for the α-subunit. Helices of each level have seven residues. Helical axes are tilted so that backbone structure rotates 10° about the channel axis for each of seven residues. Note positions of disulfide bridges (green, *C*) and noncharged polar side chains (*S,T,N,Q,H,Y*) in the γ-subunits and of charged side chains (blue, *K;* red, *E* and D) that line the channel.

by three residues. The rotation allows side chains to move out of each others' way during the shift, and the shift within the subunit allows the ends of helices II and III to remain near the membrane surfaces when the subunits tilt. Helix L shifts downward relative to helix II of the same subunit by about one residue and rotates slightly as described earlier. Relative positions of side chains that form disulfide bridges, hydrogen bonds, and salt bridges are at least as favorable as those of the "closed" conformation. The only aspect that appears less favorable is the packing arrangement between helices in the bundle of L-helices. There are some empty spaces between these

helices. A family of positively charged molecules that includes local anesthetics appears to enter and block AChR channels. It is possible that the apolar portion of these molecules bind near the Tyr(Y) residues.

In the analysis presented thus far it has been assumed that all subunits have the same conformation at any given time. This assumption is a simplification and probably is not maintained. It is easy to construct models in which some subunits have an "open" conformation and others have a "closed" conformation. There are 2^5 ways this can be done. Some parameters calculated in the side-chain packing analysis are nearer their optimal values when one or two subunits are in the "closed" conformation and the rest are in a "open" conformation. Single channel current measurements indicate that the AChR can have more than one conductance state (Hamill and Sakmann, 1981; Auerbach and Sachs, 1983). Thus, conformations other than the two described above are probable.

Possible Packing of Helices I and IV

An examination of side chains on the outer surface of the bundle of 10 helices reveals regions primarily occupied by large alkyl side chains that favor a lipid environment and regions occupied primarily by side chains that favor a protein environment (see black and shaded circles on the exterior of the structures in Fig. 5). Transfer-energy calculations indicate that the latter regions should be in contact with other protein segments; thus, they are probably covered by helices I, IV, and possibly by the apolar segment labeled *OS* in Table I that precedes Helix I. Helix I of all subunits contains a proline that probably breaks or distorts the helix. The segment above the proline has been labeled I *a* and that below the proline is labeled I *b*. Helices I *b* and IV can be stacked side by side so that one face of the dyad contains primarily lipid-preferring side chains and the opposite face and regions of close contact between the two helices have primarily protein-preferring side chains. Helices I *a* and OS can pack in a similar manner. Fig. 6 shows ways these helices can stack onto "closed" and "open" conformations of helices II and III to cover their protein-preferring side chains.

All subunits have a Cys-241 in the region between helix I *a* and I *b*. In the β-subunit, I*b* can be stacked next to helix IV so that Cys-241 forms a disulfide bridge with Cys-482 (see Fig. 6 *a*). Helix IV of the γ-subunit does not have a Cys at this position, but does have one eight residues nearer the NH_2-terminus. The γ-helices I and IV can be stacked to form a disulfide bridge between Cys-241 and Cys-474; however, the conformation must be different than that of disulfide bridged β-helices (see Fig 6 *b*). If both disulfide bridges are formed, the β- and γ-subunits may be constrained to different conformations. When Nicholson models of the structures were constructed, the conformation shown for the β-subunit appeared to be best for the "closed" conformation and that shown for γ appeared best

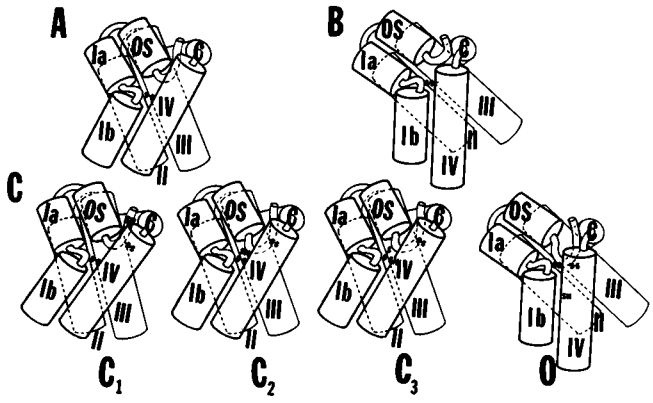

FIGURE 6 Possible conformation of outer layer helices *OS*, *Ia*, *Ib*, and *IV* for, *A*), β-subunit in "closed" conformation; *B*, γ-subunit in "open" conformation; and *C*, α-subunit in either "closed" or "open" conformation. Note differences in positions of disulfide bridges for β- and γ-subunits. *C* illustrates a possible mechanism for conformational change of outer surface helices of α-subunits. The main difference in the three *C* conformations is the position of the free sulfhydryl group. In C_1 it is at position 210; in C_2 at 211; and in C_3 at 482. The disulfide bridge arrangement of the *O* conformation is the same as that of C_3. The L-helix, which is not shown, is on the far side between and parallel to helices II and III.

for the "open" conformation. This preference is due to the interactions between helix C and the COOH-terminus of helix II of the adjacent subunit and positions of helix ends relative to the membrane surface when they are tilted at different angles.

The α-subunit has Cys residues at position 476 and 482 of helix IV, at position 241 of helix I, and at positions 210 and 211 just before the OS helix. When helices I and IV are stacked in the manner postulated for the β-subunit, these five Cys residues can form two disulfide bridges in a number of ways (see C-conformations in Fig. 6 *c*). Transitions between these arrangements could occur by thiol-disulfide interchange reactions. If Cys-482 does not form a disulfide bridge, the helices can be stacked in the conformation postulated for the γ-subunit (see Fig. 6 *c*, O-conformation). Helices IV and C maintain their position relative to helix II of the adjacent subunit during transitions between "closed" and "open" conformations. It is thus possible that the β-subunit is always in the "closed" conformation, that the γ-subunit is always in the "open" conformation, and that opening and closing of the channel involves thiol-disulfide interchange interactions within the α-subunit. The δ-subunit has no Cys residues on helix IV and no speculation will be made about whether or not it can have only one conformation.

An alternative model is possible using a similar mechanism. In this model, helices I and OS are on the opposite side of helix IV and maintain their position relative to helix II of the adjacent subunit when the change between "open" and "closed" conformations occurs. The β-subunit is in the "open" conformation, and the γ-subunit is in the

"closed" conformation. The "closed" conformation of the α-subunit has only one disulfide arrangement in which Cys-482 is a free sulfhydryl, whereas the "open" conformation has a number of possible disulfide arrangements.

COMPARISION TO MODELS OF COLICIN E1 AND δ-HEMOLYSIN

It is difficult to test calculations for membrane proteins because no structures have been experimentally determined. Analysis of bacteriorhodopsin sequence with the transfer energy method yields results consistent with present knowledge of its structure. Also, I have previously used the transfer energy method together with helix side-chain packing and other factors to develop models of large water-filled channels formed by colicin E1 (Guy, 1983) and δ-hemolysin.[1] Both of these proteins are secreted by bacteria and lyse cells. In the model, a pore forms between two colicin E1 monomers. The COOH-terminal one-third of each monomer has four amphipathic transmembrane α-helices that form the channel lining and two apolar transmembrane α-helices that pack between some of the amphipathic α-helices and the lipid bilayer. The δ-hemolysin model has 12 monomers. Each monomer is a 26-residue amphipathic α-helix. These helices stack in an antiparallel manner to form a 29 Å-diam pore. Models of the transmembrane regions of the AChR, colicin E1, and δ-hemolysin have the following features in common. (*a*) All the transmembrane segments are α-helices, except for a portion of one strand on colicin E1 that was assigned a 3_{10}-helix conformation and the segment on helix I of the AChR that is broken by proline. (*b*) The lining of the channel is virtually a matrix of alternating positively charged amine groups (from Lys) and negatively charged carboxyl groups (from Asp and Glu). The number of Lys side chains is exactly the same as the number of Asp and Glu side chains for colicin E1 and δ-hemolysin and is only slightly less for the AChR. Each Lys is positioned so that it can form at least two salt bridges with Glu or Asp. Lys residues tend to be positioned near regions of close contact between helices in a manner that buries much of the hydrocarbon portion of their side chains, whereas most Glu and Asp side chains are located so they will be maximally exposed to water. There appears to be a strong selection for Lys over Arg, because no Arg side chains are found in any of the channels. There are also a few amide groups from Asn and Gln in the lining. (*c*) Almost all side chains in contact with the central portion of the hydrocarbon phase are large alkyl (Leu, Ile, or Val) side chains. (*d*) Gly, Ala, Ser, and Thr, are frequently located near regions of close contact between adjacent helices. Hydroxyl groups of Ser and Thr can usually form H-bonds. (*e*) In AChR and colicin E1 models, aromatic side chains and Met tend to be buried in the protein, where they are not exposed to water and only partially, if at all, exposed to lipid. In δ-lysin they are on the lipid exposed surface of the protein but very near the surface of the membrane where the environment is more polar. (*f*) Most helices stack in a manner predicted by helix side-chain packing analysis. The models differ primarily in the ratio of apolar to amphipathic α-helices. This probably occurs because colicin E1 and δ-hemolysin must be soluble when secreted.

DISCUSSION

The models presented here are preliminary. The principal purpose has been to develop testable hypotheses. Several categories of experiments are possible.

(*a*) Labeling experiments can be conducted to determine which protein segments are exposed to the extracellular phase, to the apolar lipid phase, and to the cytoplasmic phase. Another approach is to make antibodies to specific protein segments, and then determine whether these antibodies bind to the AChR. These experiments could determine on which side of the membrane the COOH-terminus is (the present model predicts the cytoplasmic side, whereas other models predict the extracellular side), and whether segments forming the L- and C-helices in the present model are inaccessible to large reagents or exposed to the cytoplasmic phase as suggested by other models.

(*b*) The present model predicts that disulfide bridges connect the γ-helix L to the γ-II and γ-III helices. Analysis of disulfide sites should determine whether helix L stacks next to helices II and III. The thiol-disulfide interchange mechanism that was postulated to regulate conformational changes of the α-subunit is highly speculative. Moore and Raftery (1979) observed that exposure of *Torpedo* AChR to mercurials prior to addition of agonist prevents conversion to the desensitized conformation and that addition of mercurials when agonist is present prevents conversion back to the resting conformation when the agonist is removed. They suggested that a thiol-disulfide interchange reaction may be involved. The thiol-disulfide interchange hypothesis can be tested by determining the location of α subunit sulfhydryl groups that react with mercurials in the presence and absence of agonists and the locations of disulfide bridges when the free sulfhydryl groups are covalently bound to reagents. Determination of disulfide position in the β- and γ-subunits should also be informative in analyzing packing of helices I and IV. Reagents that react with sulfhydryl groups have an number of electrophysiologically measurable effects on AChR. Identification of side chains involved in these reactions and models of how these reactions should affect channel properties could make the electrophysiological experiments easier to interpret.

(*c*) A category of drugs and toxins has been postulated to enter and block the transmembrane portion of the AChR channel. Oswald and Changeux (1981) have covalently

[1]Guy, H. R., unpublished results.

bound some of these agents to the AChR and determined that all of the subunits are labeled, with larger amounts of label on the δ-subunit. The most probable locations in this model are in the vicinity of Tyr-445 and/or between the L-helices. This hypothesis can be tested by determining the location in the sequence where these agents bind.

It may be possible to refine the model by conventional energy minimization techniques and to compare stabilities of alternative conformations. The first step is to approximate the atomic coordinates for each conformation. This is made much easier by predictions of the helix analysis. Once refined coordinates of all groups within the channel are available, it may be possible to analyze potential drug-binding sites using computer graphics and energy calculations.

The hypothesis that channel opening and closing is due to tilting of subunits may be difficult to test in the absence of improved structural data. The tilt direction and increase in the degree of tilt when the channel opens are the same as determined by Unwin and Zampighi (1980) for gap junction channels. Gap junction channels could have a structure similar to the AChR except that they are hexamers and would have a bundle of six parallel α-helices surrounded by a bundle of 12 antiparallel α-helices. The bundle of 12 antiparallel α-helices of the δ-hemolysin model has excellent side-chain packing. A conformational change of the AChR transmembrane domain is supported by the voltage dependence of channel-closing rates (Magleby and Stevens, 1972) and other experiments implying changes in the lipid exposed regions of the AChR (Davis et al., 1983). The gating mechanism postulated here suggests that the pore could have a number of sizes. It is important to determine whether kinetics of transitions between different channel conductance states (Auerbach and Sachs, 1983; Hamill and Sakmann, 1981) are consistent with this model.

Complete sequences of AChR from other organisms should be determined soon. If the model is correct, these sequences should have homologous segments that can be used to construct a model using the same general backbone structure proposed here. If correct, the approach used here could also be applied to sequences of related membrane channel proteins.

Severe constraints were used in developing the model presented here. The most important aspect of the specific model is that all these constraints were satisfied with a model that has near optimal pairing of Cys residues, oppositely charged residues, and hydrogen-bonding residues. Also the model has the features of a negatively charged selectivity filter, proper channel size, and a possible gating mechanism that must be part of any detailed model of the AChR. The most remarkable feature of the structure in which a bundle of 10 helices surrounds a bundle of five helices is that helix side-chain packing requirements can be satisfied simultaneously for all helices both within and between the bundles. This structure should be very stable and is relatively independent of which side chains are on the helices as long as complementary side chains are next to each other in the final structure. Because the structure would be stable for other protein sequences, it may be common to other pentameric membrane channel proteins composed of transmembrane α-helices.

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DISCUSSION

Session Chairman: V. Adrian Parsegian *Scribes*: Glenn S. Edwards and Stephen Slatin

FINKELSTEIN: This is a question that deals with model building of these channels in general. As I understood it, you are placing a very high premium on putting charged amino acids in the lining of the channel to account for conductance. Given the example of the gramicidin channel, which has a substantial conductance without charged groups lining the pore, can having the carbonyls or whatever part of the peptide backbone lining the channel account for the conductance?

GUY: It is hard to build such a model due to the difference between the β helix in gramicidin and the α helix. Gramicidin has a unique alternating D-L-D-L-D-L structure with all the side chains on the outside that prevents it from forming either an α helix or a straight β sheet and makes it spiral to form a pore with water in contact with those carbonyls. There are no side chains shielding those carbonyl groups as there would be in an α helix. The β structure may be a little more flexible than the α helix so the amide groups can orient into the channel and provide the polar environment. The program described in my paper does put some premium on charged side chains but their existence is not a requirement. Amide groups could lie in the channel.

FINKELSTEIN: In your model do you have salt bridges between the groups that are not lining the channel?

GUY: Almost every charged group in the final models that I have built can form a salt bridge somewhere. Does that answer your question?

FINKELSTEIN: The point is that you have a lot of them. How do you decide where to put a particular helix? Should it be placed so that its polar residues are lining the channel lumen or should it be even further away, say, almost in the bilayer region? The fact that a helix has a free carboxyl group does not preclude its being in the hydrocarbon region if on another α helix there is an amino group with which it could form a salt bridge.

GUY: The program does not recognize what side chains adjacent helices might have. For example, with bacteriorhodopsin, where the putative transmembrane α helices do have some charged groups, these could be balanced with lysines or arginines to form salt bridges. It is not clear that actually occurs or that it provides a lot of energy. The program would not recognize it except to predict that it is going to cost you more energy if you bury the charged groups between helices. The program gives just a first approximation. You have to look at the specific sequences and see how they might best fit together.

EISENMAN: I'd like to raise two points regarding ion ligands. First, the only situation that I know of where a monovalent binding site structure is known is for the K or Tl site in *Subtilisin novo* (cf., Fig. 3 in Hol, W. G. H. 1971. The 3-dimensional structure of *Subtilisin novo*. Proefschrift. Royal Univ. Groningen, Drukkerij van Denderen Groningen, The Netherlands). Two of the ligands come from the side chain carboxyl of aspartate 197 in the vicinity; five carbonyl ligands come from the backbone of two different nonadjacent regions of the protein, and one additional oxygen comes from a water molecule at 3.2 Å separation. (There is also a water at 6.2 Å.) So the site is actually made up of a side-chain carbonyl from the "outside" of the "helix" molecule plus backbone carbonyls from the "inside," as well as a water molecule. This is interesting because the proposed models for the ACh channel all put the ion path outside the helix (in contrast to gramicidin, where it is inside) and they have not used the backbone carbonyls.

My second point is that whenever prolines are involved, a nearby carbonyl group is freed for cation binding, because proline has no proton to donate to an intramolecular H-bond.

GUY: I treat prolines as polar precisely because of that.

KOSOWER: Your overall scheme is quite clever but has a major defect in that it relies on transfer energies which are not accurately known and are unlikely to be constant for particular side chains in the same protein because of the variation in local environments. These difficulties have been spelled out in detail by Kyte and Doolittle (Kyte, J. and R. F. Doolittle. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157:105–132).

GUY: This comment addresses the validity of how I do my partition energies. I cannot go into all the details because it is fairly complex, but I base my partition energies in the protein phase on the statistical distributions of residues in proteins of known structure as a function of their distance from the surface. I have run the program on myoglobin, for example, which is a soluble protein that has a lot of α helices in it. The program locates every α helix in myoglobin and every one looks like an amphipathic helix. It orients every helix in the right way. I hope to run this program on many soluble proteins to get a statistical idea of how well it predicts and orients helices.

I have also run the program on bacteriorhodopsin. The resulting prediction on protein/protein and protein/lipid contact agrees with Engelman's neutron diffraction data. We do not really know that

structure in detail so you can't be sure. That is a terrible limitation in dealing with membrane proteins; we don't have known structures to test the methods on.

KOSOWER: Where are the binding sites for the agonists or antagonists in your model?

GUY: The binding sites are somewhere in the first half of the sequence, probably close to those cysteines at one of the agonist-binding sites described in your paper at this meeting. The site seems to be the one you say is not important for opening the channel.

KOSOWER: There are assumptions made about the occurrence of disulfide bonds that should have experimental support. How are the locations of the disulfide bonds to be determined?

GUY: I know good membrane protein chemists who believe these experiments are quite feasible and intend to perform them. I don't want to tell them how to do their experiments.

KOSOWER: The driving force for the conformational change from closed-to-open is not clear. The time scale for the change may well be slower than the ~500 μs found for channel opening by Lester et al. (Lester, H. A., M. E. Krouse, N. M. Nass, N. H. Wassermann, and B. F. Erlanger. 1980. A covalently bound photoisomerizable agonist. *J. Gen. Physiol.* 75:207–232). Is there any evidence for large scale conformational change in the receptor?

GUY: I don't think that questions regarding precise kinetics can be answered without first having a very precise model of the entire structure and then doing extensive molecular dynamics simulations. That might take years of computer cpu time. Even then, I would not trust the results because of limitations in present molecular dynamics methods. Stroud and co-workers (Kistler et al., 1981) have listed several reasons to favor a large scale conformational change. As additional reasons, I mention in my text the voltage dependence of channel closing of Magleby and Stevens (1972) and the experiments of Davis et al. (1983).

KOSOWER: If one calculates the mass distribution in your model (what is outside the bilayer, what is in the bilayer, and what is inside the cell), it does not agree very well with what Stroud and his co-workers got from their electron micrographic analysis, reported at the last Biophysical Discussion. (Kistler, J., R. M. Stroud, Klymkowsky, R. A. LaLancette, and R. H. Fairclough. Structure and function of an acetylcholine receptor. *Biophys. J.* 37:371–383). Their model implied ~15% cytoplasmic material whereas yours has ~25%.

GUY: Those numbers are not precise. Stroud has developed a model that has the same protein distribution as mine. If he doesn't have any problem with his data then neither do I.

KOSOWER: Many of my own conclusions about amphipathic helices are in agreement with what Stroud has found, although some features are different. There is good agreement with the homology concerning the sequences for all the subunits found by Noda et al., which you modified in your text, particularly Table I.

Our models differ in that functional character is primary in my model. Hydrophobic chains were selected on the basis of a high content of apolar side chains in a 24-amino acid sequence, amphiphilic chains by single group rotation (SGR) theory. The homology of function is quite interesting: All hydrophobic chains except for the "unique" 2nd α-hydrophobic sequence, are homologous using the sequence alignment made by Noda et al. The first channel element of the α-subunit is homologous to the channel elements of the γ and δ subunits. The second channel element of the α subunit is homologous to the channel elements of the β subunit. This relationship is in accord with the evolutionary relationship Noda proposes for the genes encoding the AChR subunits.

Another difference between your model and mine is that in mine the mass distribution agrees with Stroud's results. (See references 1 and 2 in my article in this volume and also Kosower, E. M. 1983. A molecular model for the bilayer helices of the acetylcholine receptor. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 157:144–146; Kosower, E. M. 1983. A molecular model for the exobilayer portion of the α-subunit of the acetylcholine receptor with binding sites for acetylcholine and noncompetitive antagonists. *Biochem. Biophys. Res. Commun.* In press; Kosower, E. M. 1983. Selection of ion channel elements in the serine and aspartate methyl-accepting chemotaxis proteins of bacteria. *Biochem. Biophys. Res. Commun.* In press; and Kosower, E. M. 1983. A hypothesis for the mechanism of sodium channel opening by batrachotoxin and related toxins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 115:648–652.

GUY: In constructing your model I don't think you relied enough on homology. For example, in your model the α subunit crosses the membrane six times and others cross four times. To my knowledge, very different folding schemes like yours have not been observed experimentally for homologous proteins.

DURKIN: One of the nice features about the Guy model, of course, is that it generates a closed state and an opened state. Presumably what all electrophysiologists see is a transition between such states. I would like to compare the predicted change to known protein conformations. The best-characterized example is the oxy/deoxy hemoglobin transition, where the conformational change is very subtle. Virtually all side chain contacts within subunits are preserved; between subunits a mere handful of contacts change. In your model, helices tilt and virtually all the side chain contacts are disrupted. It is an enormous dislocation for a protein. Could you comment on that?

GUY: My model has various levels of opening and I'm not sure exactly which is the closed state or open state. I think I can make a model where everything within the subunit stays the same and you just tilt the subunits. If so, there is no more movement than what seems to occur in the gap junction, even though Lee Makowski's work suggests that the structure is β sheet and not α helix. Certain soluble proteins exhibit some movement of helices relative to each other that lead to a bigger conformational change somewhere else.

MIELKE: Raman spectroscopy results presented by Yager et al. at this meeting and CD spectroscopy data from our lab (Mielke and Wallace, in preparation) indicate that the secondary structure of the ACh receptor consists of a substantial portion of β sheet structure. How did you choose to model your proposed structure exclusively on α helix?

GUY: The structure is based primarily on Stroud's x-ray diffraction data where he postulates that there are transmembrane α helices which are oriented perpendicular to the surface of the membrane. There is no discrepancy with the secondary structure data provided that the β sheets are in extracellular domains. There is also a modeling problem: α helices are a lot easier to model than β sheets. The lengths of the apolar segments are appropriate for transmembrane α helices from what is known from other structures.

DANI: When the L helices tilt, does the channel remain a perpendicular cylinder or does the hole widen near the mouth?

GUY: I can't answer detailed questions like that yet. I'd need to perform much more refined calculations first.

DANI: When L helices tilt and open, apolar groups are exposed. Do the exposed apolar groups run the full length of the L helices or are they just exposed in a ring?

GUY: When I did that calculation I was trying to preserve the packing between the L-helices and the helices of the surrounding bundle. I think

now that probably the best thing to do is to disrupt that packing so that you no longer have the gaps between the L-helices. With this arrangement the only exposed apolar residues are a ring of tyrosines and one phenolalanine which may be the local anesthetic binding site.

MONTAL: What structures can you predict for each one of the subunits? Why five subunits for each channel?

GUY: I used five subunits because that is what all the biochemists say exist. The $\alpha_2\beta\gamma\delta$ stochiometry is dogma now. I don't think I could get anything published if I didn't use that.

MONTAL: What is the advantage of having five subunits?

GUY: If you want to build channels out of concentric bunches of α helices using knobs into holes or ridges into grooves (which I use now), a pentamer seems to be the only arrangement that works. In particular, a pentamer permits a bundle of helices to be surrounded by an integer-multiple bundle of helices.

MILLER: The size of the hole, as it appears in your closed structure, suggests a fairly strong prediction. Whereas the closed structure will not conduct normal cations, it ought to conduct protons, and you might expect to see a very high proton permeability for *Torpedo* electroplax vesicles. That is a very easy measurement to make. Is the hole size right for that?

GUY: I don't know what hole size you need to have proton conductance.

MILLER: Is it packed with water?

GUY: I suspect that hydrogens could go through if other portions of the protein don't block the entrance. There may not be any more water than is in the center of bacteriorhodopsin.

MILLER: What is the hole size?

GUY: It is not easy to answer that. The side chain positions are not clear and they're not regular.

SACHS: I'd like to try to relate the structure to the function of the channel. As you hyperpolarize you stabilize at least a doubly liganded state which is mostly open. Do the dipole moments of this structure favor that open configuration in what would be considered a hyperpolarizing field across the channel?

GUY: I could give a really fantastic 30 min answer to that. No, I haven't done that calculation. I'd have to take into consideration almost every model that was presented at the gating session last night. I can build nearly every single one of those models into the structure.

SACHS: There are some dipoles associated with those helical segments. The interaction with the field should be fairly straightforward to calculate.

MAKOWSKI: There maybe some virtue in attempting to build models of this sort, but when you do, it's very important to remember the large number of alternative models that are equally consistent with the data. Otherwise you will mislead people into believing you have a unique model.

GUY: How many alternate models will work depends on which level of refinement you're talking about. If you're talking about a model having five transmembrane α helices that form bundles, the model won't be very specific. At this level of detail, particularly if you don't deal with disulfide bridges, there are hundreds of ways of packing those α helices, hundreds of possible models. I am now modeling three conformations that have fivefold symmetry. If I were to let the backbone structure deviate from fivefold symmetry (and that could well occur), even if I kept all the other constraints, there could be 243 possible conformations. Even Auerbach and Sachs don't see that many. Present models represent a beginning point for more refined calculations.

MAKOWSKI: I think it's important in a model building study of this sort to state that explicitly.