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A Molecular Model for the Exobilayer Portion of the α-Subunit of the Acetylcholine Receptor with Binding Sites for Acetylcholine and Non-competitive Antagonists

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ABSTRACT A molecular model for the exobilayer portion of the α-subunit of the acetylcholine receptor is presented. Binding sites for an acetylcholine and non-competitive antagonists are indicated.

We recently presented structural assignments (1,2) for 24 transmembrane segments of the acetylcholine receptor (AChR)**, a membrane complex with two α- and one each of β-, γ- and δ-subunits.(3,4) The AChR ion channel and an acetylcholine binding site at the mouth of the ion channel are a central feature of the model (5). Our molecular model for all helices of the AChR in the bilayer accounted for 656 (28%) (6) of the 2333 amino acids in the receptor (7-12). The subunit order, α-, β-, α-, γ- and δ- around a central "pit", is favored (12), but not settled.(18)

We now present a molecular model for the exobilayer portion of the α-subunit of the AChR. Two binding sites, one for acetylcholine (ACh) and one for a non-competitive antagonist, are designated in the exobilayer region, for a total of 3 in our model.

2. THEORY AND DISCUSSION

A model for the exobilayer portion of the receptor helps us to (1) formulate labeling experiments designed to probe AChR structure (2) develop explanations for particular chemical effects (e.g., disulfide reduction) and

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** AChR, acetylcholine receptor; ACh, acetylcholine; glu, glutamate; asp, aspartate; lys, lysine; DTT, dithiothreitol; SGR theory, single group rotation theory

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physiological responses (such as differences between agonists and local anesthetics) (3) open the way to theories for the operation of structurally complex receptors and ion channels (4) generalize the approach to receptor structure analysis gained from our study of the AChR bilayer helices. (1,2,5,6)

The criteria used for the design of the exobilayer model are as follows: (1) **Two binding sites.** In addition to the ACh binding site near the mouth of the ion channel in the bilayer, two binding sites should appear, one for ACh near a reducible disulfide (cf.(3)), and another for noncompetitive blockers close to the δ-subunit (14). A total of three ligand sites are also implied by monoclonal antibody binding studies. (15,16) Activation of the ion channel depends upon two molecules of ACh. (17) The binding site near the disulfide may be blocked without eliminating ion channel function. (18,19)

(2) **55Å Extension from bilayer.** Electron microscopy indicates that the structural elements of the exobilayer portion of the acetylcholine receptor extend approximately 55Å from the bilayer. (12)

(3) **High antiparallel β-sheet content.** Raman spectroscopy indicates that antiparallel β-sheet is the most abundant form of polypeptide (34%) present in the AChR (20, 21). Most of the α-helical content of the receptor has been accounted for the bilayer α-helices.

(4) **Lysine-glutamate (aspartate) binding site** The model for the bilayer binding site for ACh contained this combination of positively and negatively charged groups. The use of the (+,-) pair in the successful formulation of a model for binding and gating suggested that the same choice be made for the exobilayer sites.

(5) **One reactive thiol (SH) group per α-subunit.** Dithiothreitol (DTT) could produce two reactive SH groups per disulfide reduced. However, only one is found, carefully executed affinity alkylations with bromoacetylcholine revealing one reactive thiol per α-subunit. (22) The model should thus include the possibility of forming one unreactive SH group per disulfide reduced.

We have therefore arranged strands of 17-18 amino acids (5Å-6Å) as a folded antiparallel β-sheet (designated as strands X1-X3, X4-X6 in Fig.1) so

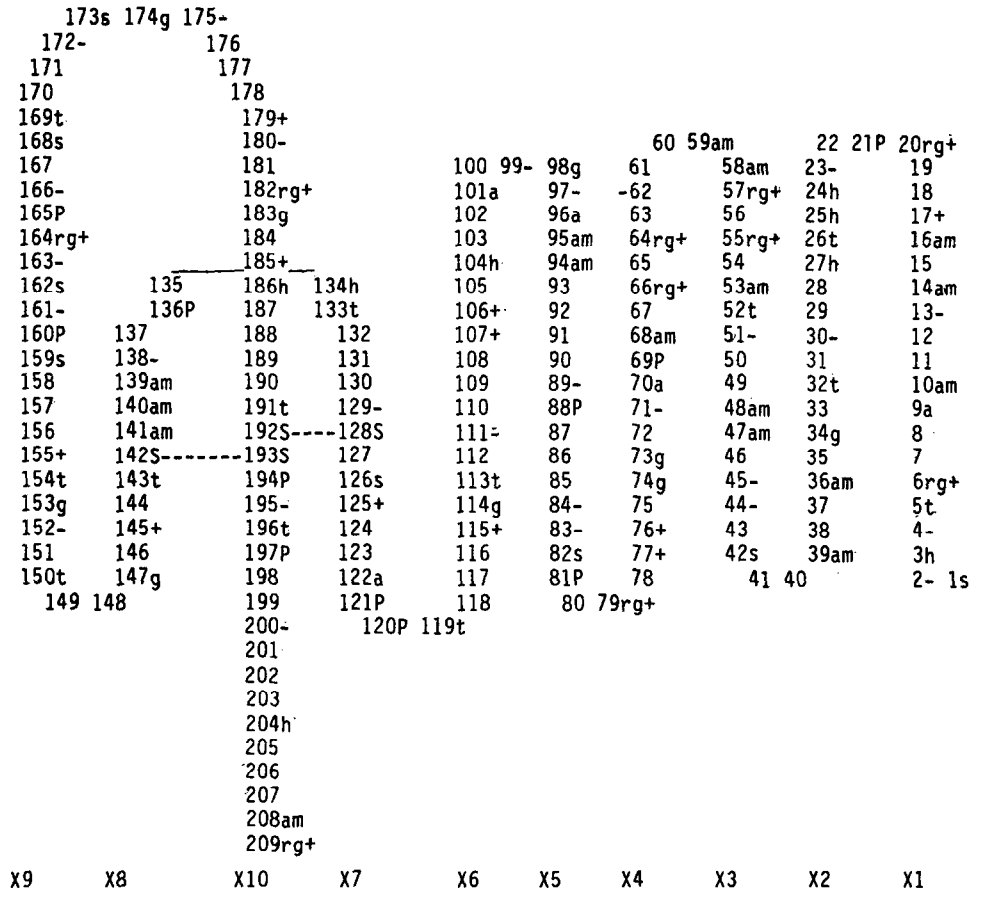


Fig.1 Partial tertiary structure of exobilayer portion of α-subunit of AChR, the acetylcholine receptor. [+,lys; rg+,arg; -,glu or asp; S,cys; s,ser; t,thr; h,his; g,gly; P,pro; a,ala; am,gln or asn; all hydrophobic residues [leu, ileu, val, phe, tyr, trp] unmarked (asp 30, 44, 62, 71, 83, 84, 89, 97, 99, 111, 138, 152, 166, 180, 195, 200; asn 10, 14, 16, 47, 53, 68, 94, 95, 141)

as to include half of a suitable binding site [lys and glu or asp]. (Designated as half-site A, Fig.2, upper portion) The design of the binding sites is based on single group rotation (SGR) theory. (23) The internal apposed chains (X1-X3 versus X4-X6) in the model are almost all compatible, including significant numbers of hydrophobic-hydrophobic interactions.

A lysine(-125) and an aspartate(-195) suitable for the second half-binding site (half-site B) are located near the disulfide bond. The only reasonable combination of cysteine SH groups as disulfides (proximate binding site, appropriate geometry) is 128-cys-192-cys, 142-cys-193-cys. The overall

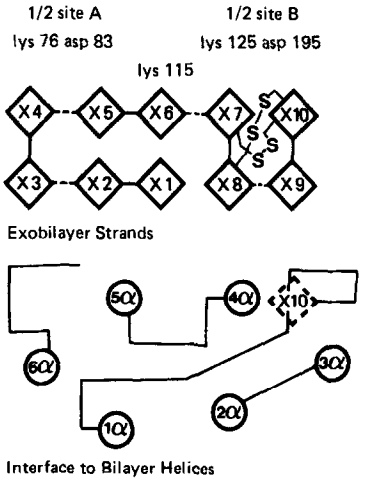


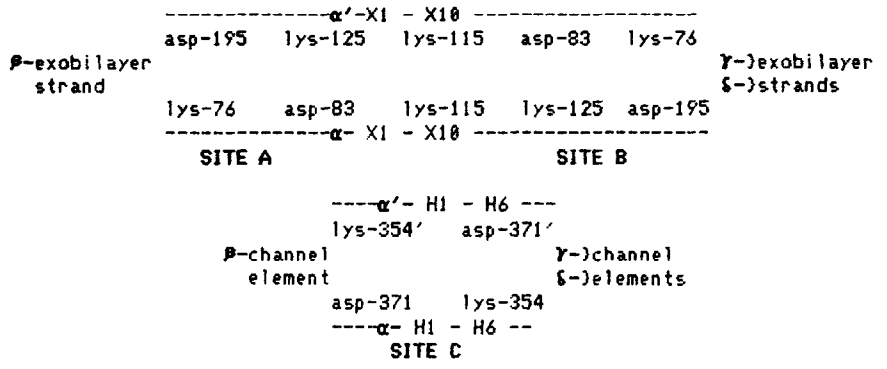
Fig.2 (a) Lower portion. The interface region between the bilayer helices and the exobilayer strands of the α-subunit. The strands are represented by parallelopipeds, the bilayer helices by circles. The strand order is shown in Fig.1. An example of a favorable interaction is that between amino acid side chains *360 val-361 ile-362 phe* (4α- to 5α- connection) and *207 met-206 ile-205* phe (1α- to X10 connection). (b) Upper portion. Two half-sites are indicated for the exobilayer strands. The other half of each site would be found on the apposite exobilayer portion of the α-subunit.(shown in Scheme) The disulfide sulfur easily accessible to external reagents is that from cys-193.

arrangement of strands X7-X10 bears a resemblance to that of the snake α-neurotoxins (24), possessing even a proline next to one disulfide, and likely to have an "open" β- pleated sheet structure. Given that the nearby binding site (lys-125, asp-195) has side chains projecting into the ion channel opening, the X7-X10 disulfide (128-cys-192-cys) would be hidden and not readily reducible, while 193-cys would carry the SH group accessible for labeling and the 142-cys SH group would be "hidden". (See Fig.2, upper portion)

The connection between the bilayer helices and the exobilayer strands is constructed so as to minimize the separation between the helices and the exobilayer polypeptide. The link is indicated schematically in the lower portion of Fig.2, with one favorable interaction among several noted in the caption.

The model for the exobilayer strands is consistent with the finding that only one disulfide per α-subunit is susceptible to reduction (25) and not inconsistent with the finding that the same subunit has seven antigenic determinants. (26) The model contains two different exobilayer binding sites. The first (A) is suitable for acetylcholine, the structure being similar to that

of the site (C) at the ion channel mouth. (5, 6). The second (B) must be larger (because it is bounded by the γ- and δ-subunits) and should be suitable for local anesthetic molecules. Affinity labeling with 5-azidotrimethoisoquin at this site links the label to the δ-subunit (14), whereas uv irradiation of AChR carrying chloropromazine leads to labeling of the α-, β- and δ-subunits along with less labeling on the γ-subunit. (27) Half-sites (A and B) are indicated in the upper portion of Fig.2. The exobilayer sites (A and B) and the bilayer site (C) are shown in the Scheme below. (X = strand, H = helix)



Scheme 1

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