Hypothesis

Revised assignments for the β-, γ- and δ-subunits of the acetylcholine receptor structural model

Edward M. Kosower

Department of Chemistry, Tel-Aviv University, Ramat-Aviv, Tel-Aviv 69978, Israel and *Department of Chemistry, State University of New York, Stony Brook, NY 11794, USA*

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Revised assignments for the bilayer helices of the β-, γ- and δ-subunits of the acetylcholine receptor are presented. A new feature of the model is extensive charge matching between the polar groups of the ion channel elements of different subunits.

Acetylcholine receptor *Ion channel element* *Structural model* *Single group rotation theory* *Amino acid sequence*

1. INTRODUCTION

Single group rotation (SGR) theory [1] led to the design of a model binding site for acetylcholine (ACh) and to a prediction for an acetylcholine receptor (AChR) ion channel sequence: lys(1)...glu(5)...lys(8)...glu(12).. (polar side chains lining one side of an α-helix). The amino acid sequences for all of the AChR subunits ($\alpha_2\beta\gamma\delta$) have been elucidated [2–6]. Remarkably, the predicted sequence was located in the α-subunit (α373–α391). In the form of a helix, the segment was amphipathic and thus suitable as a membrane-spanning portion of an ion channel (ion channel element) [7]. SGR theory was used to choose ion channel elements in the other subunits [8], and to construct plausible models for the ACh-binding site [9,10] and the exobilayer portion of the α-subunit [11].

Structural models for a complex protein molecule like the AChR can guide the experimenter into asking new questions and therefore can stimulate new types of experiments in the search for the actual structure. Models are valuable in many other ways: (i) as an aid in the design of labeling and blocking agents; (ii) for insight into potential mechanisms such as how ACh is bound and how the AChR ion channel might function; (iii) for the design of immunogenic peptides as probes of antibody specificity; (iv) for the selection of interesting points for genetic manipulation; and (v) for ideas about the complexity of biological receptors. Since it is likely that most of the 2333 amino acids in the AChR serve some purpose, models should be modified to reflect new information or sharper perceptions about the receptor.

New information on (i) the location of phosphorylated serines [12–15] (R. Huganir and P. Greengard, personal communication) and (ii) the inclusion of amides as potential channel-active groups (as in alamethacin [16–18] or the serine and aspartate bacterial chemoreceptors [19]) brings us to revise our original assignments for the ion channel elements of the β-, γ- and δ-subunits.

2. THEORY AND DISCUSSION

Serines in the acetylcholine receptor are phosphorylated by a specific cytoplasmic kinase and dephosphorylated by a specific phosphatase. The target sequence is normally arg$^+$-X-ser (table 2.5 of [20]), and the active serines have been identified tentatively as γ-ser354 and δ-ser361 (R. Huganir and P. Greengard, personal communication). In

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order to place the serines on the cytoplasmic side of the membrane of the cell, another hydrophobic transmembrane helix was added to those previously chosen for the β-, γ- and δ-subunits (essentially the same sequence had been selected as a hydrophobic membrane segment by others [4–6]).

The use of one amide as a polar group in the channel elements for the γ- and δ-subunits led to very attractive choices as ion channel element sequences. The amphipathic nature of the channel elements was improved and a striking new feature, charge-matching, appeared for most of the interactions between the 5 subunits (see below). The β-subunit ion channel element was also extended with an amide group.

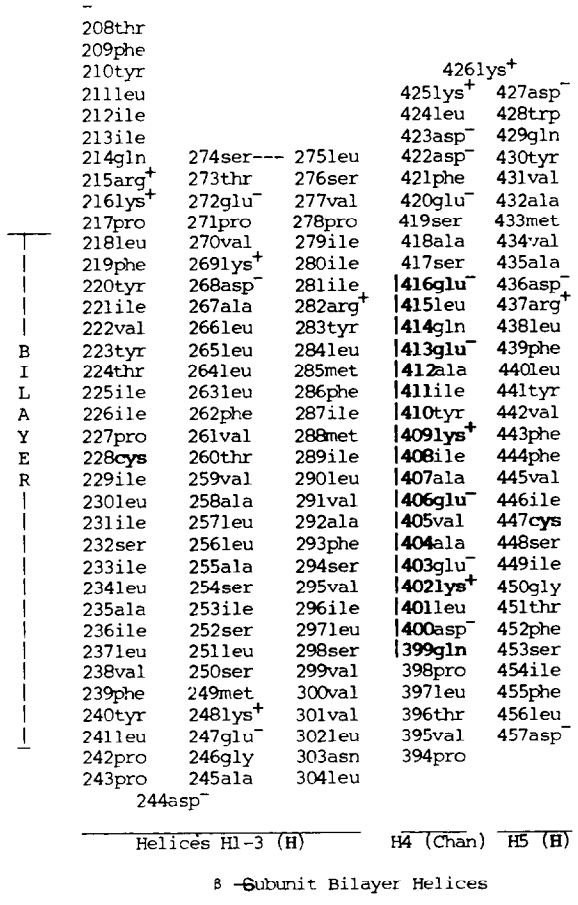


Fig.1. Partial tertiary structure of the bilayer portion of the β-subunit of AChR, the acetylcholine receptor. Channel elements are indicated by vertical line (|) in front of the component amino acids. The amino acids with polar side chains lining one side of an α-helix are shown in boldface. Cysteines are also shown in boldface.

The revised assignments for the ion channel element and 4 hydrophobic helices for each subunit (β-, γ- and δ-) are shown in figs.1–3.

The choices for the channel elements are made on functional grounds. The word functional implies a reasonable mechanism for motion of ions through a membrane ion channel. This will be explained in a full article. The validity of the current choices is strongly supported by the considerable genetic homology of the β-, γ- and δ-channel elements with the 5α-channel element, using the sequence homology constructed by the Numa group [4,21] and slightly modified by Guy [22]. In addition, the channel elements have amphiphilic character, as required if they are located within the bilayer.

The revised set of channel elements is assembled in fig.4. The helices are numbered within given subunits; 4α is the fourth bilayer helix found within the amino acid sequence for the α-subunit. Examination of the channel element set reveals a new feature of receptor structure, that of charge-

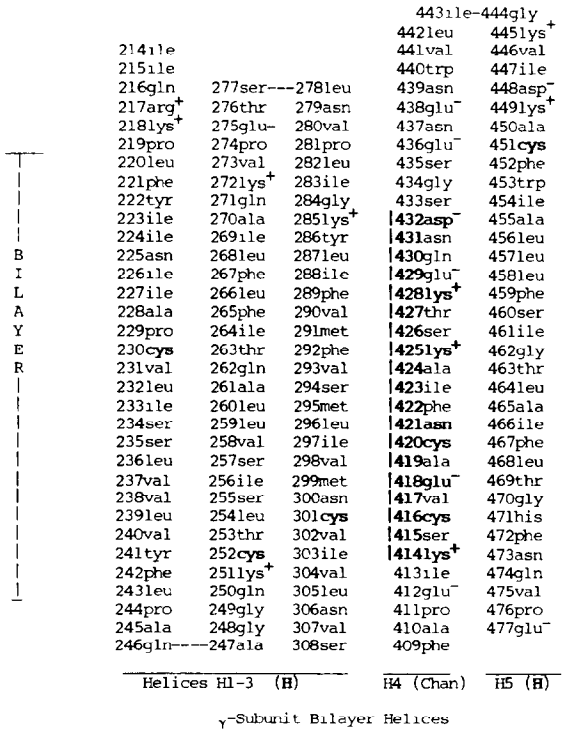


Fig.2. Partial tertiary structure of the bilayer portion of the γ-subunit of AChR, the acetylcholine receptor. See legend to fig.1.

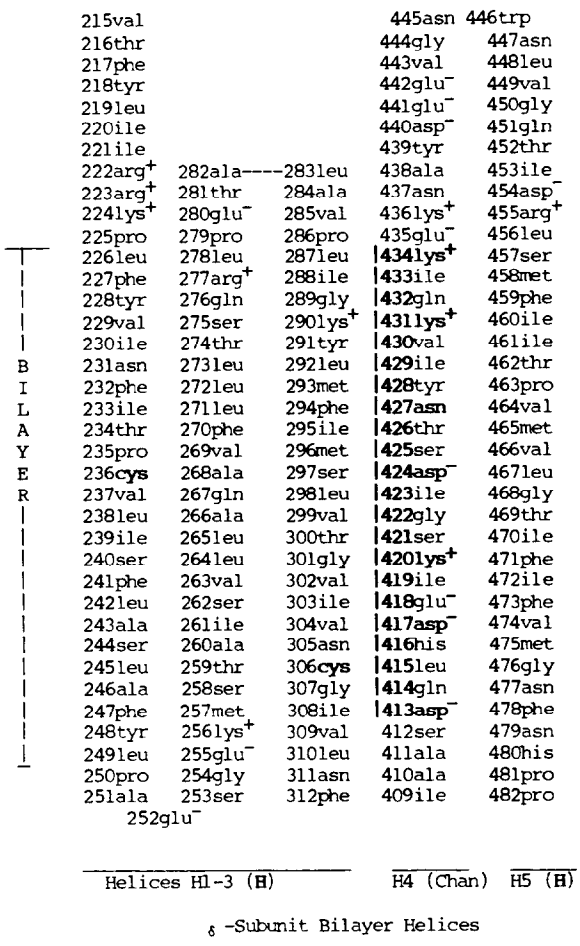
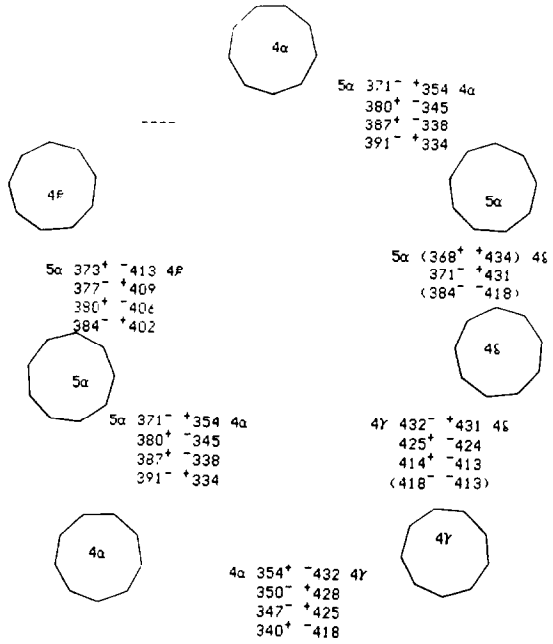


Fig.3. Partial tertiary structure of the bilayer portion of the δ-subunit of the AChR, the acetylcholine receptor. See legend to fig.1.

matching between the polar groups along the same sides of adjacent helices. Matching is good between the 5α and 4β, 4α and 4γ, 4γ and 4δ-channel elements, and is consistent with the subunit order favored in our previous papers [9,10]. The charge-matches are listed in scheme 1; there are no special interactions between 4α and 4β, and a net of one unfavorable interaction between 5α and 4δ.

The extensive genetic homology among subunits [6] and among species ranging from *Torpedo* to human [21] gives rise to the idea that the exobilayer model for the α-subunit [11] can be applied to the exobilayer portions of the other subunits. The AChR structural model developed thus far is thus expanded to include >80% of the 2333 amino



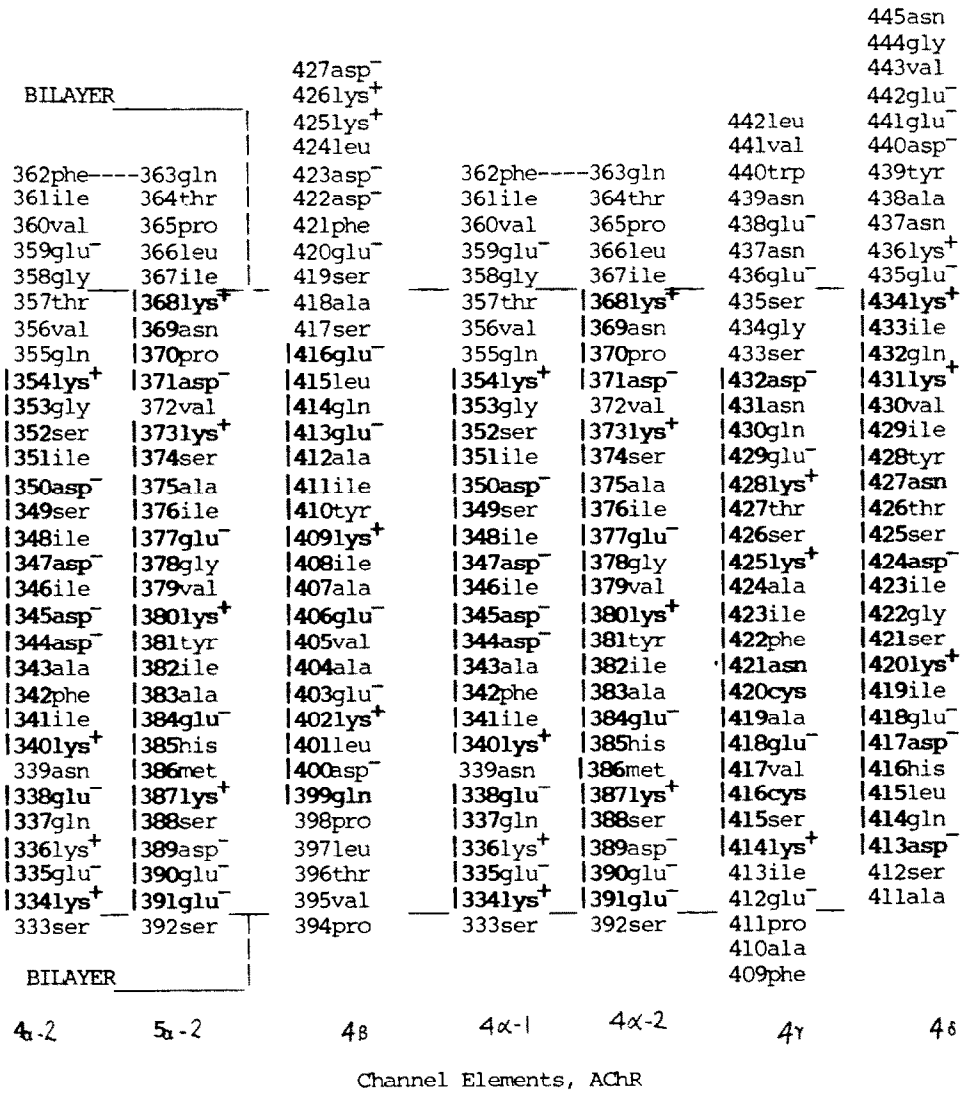
Scheme 1. Charge-matching between channel elements.

acids in the AChR (E.M. Kosower, in preparation).

Using the average transfer energies for the side chains of amino acids from a hydrophobic environment to water, Eisenberg [23–25] has formulated a way to evaluate a 'hydrophobic moment' and thus search for amphiphilic helices. As in the case of the Kyte-Doolittle SOAP procedure for selection of hydrophobic protein segments [26], a caveat is that the transfer energies for the side chains cannot be completely accurate. The Eisenberg procedure has been applied by Stroud [27–29] to the AChR, yielding a choice for the ion channel elements somewhat different from, but similar to those chosen on functional grounds.

Thermodynamic rules similar to those use by Eisenberg, and Kyte and Doolittle concerning the location of amino acid segments have been used by Guy [22] to formulate a model for the bilayer portion of the AChR. Although there is substantial similarity between the 'thermodynamic' model and our functional model, important differences exist especially with respect to the choices for the channel elements.

SGR theory could be useful for other receptor problems. We have applied it to the formulation of



Channel Elements, AChR

Fig.4. Channel elements of the AChR, the acetylcholine receptor.

a molecular theory of olfactory detection [30] and to the construction of a mechanism for the action of sodium channel opening toxins [31].

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