Partial tertiary structure assignments for the β-, γ- and δ-subunits of the acetylcholine receptor on the basis of the hydrophobicity of amino acid sequences and channel location using single group rotation theory

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Four transmembrane segments from each of the β-, γ- and δ-protein subunits of the acetylcholine receptor (AChR) [Nature (1983) 301, 251–255], [Proc. Natl. Acad. Sci. USA (1983) 80, 1111–1115] have been selected on the basis of single group rotation (SGR) theory [Symp. Structure and Dynamics of Nucleic Acids and Proteins (Sept. 1982) abst. pp.52–53], [Biochem. Biophys. Res. Commun. (1983) 111, 1022–1029] and the hydrophobicity of amino acid sequences. One helix from each subunit is assigned to the AChR ion channel. Criteria for the selection of ion channel elements are outlined.

Acetylcholine receptor *Subunit β,γ,δ* *Tertiary structure* *Amino acid hydrophobicity* *Ion channel location* *Single group rotation*

1. INTRODUCTION

In [1] we presented a structural assignment for 6 transmembrane segments of the α-protein subunit of the acetylcholine receptor (AChR) which consists of 5 subunits, two α- and one each of β-, γ- and δ-. Complete amino acid sequences of the β- (469 residues) [2], γ- (489 residues) [3], and δ- (501 residues) [2] subunits have been reported using DNA cloning techniques based on the partial N-terminal sequences [4]. Although the order of the amino acids of all subunits is now known, the functional portions (relationship to the acetylcholine binding site and the ion channel) or even the portions which span the membrane are not readily identifiable. We now apply the principles used to identify the transmembrane segments of the α-subunit to the remaining subunits of the receptors. Criteria for the choice of ion channel elements are outlined.

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2. THEORY AND DISCUSSION

Two types of search procedures (selection of hydrophobic sequences and choice of ion channel elements) are used to identify transmembrane segments in the amino acid sequence of the AChR subunits.

Hydrophobic segments are identified by their high content of hydrophobic amino acids. Our approach was to assume a 24-amino acid transmembrane helix and to look for segments with a high fraction of hydrophobic amino acids. (These are readily selected by color coding all hydrophobic amino acids [Leu, Ile, Val, Phe, Tyr, Trp, Met] in the same way.) All of the hydrophobic subunits we selected have at least 16/24 hydrophobic units, and some have as many as 20/24. In each of the β-, γ- and δ-subunits, only 3 segments were selected as hydrophobic transmembrane bilayer segments (fig.1–3). In the α-subunit, 4 hydrophobic segments were found [1]. A search procedure (SOAP) [6] which makes a running average of the hydrophobicity of 7 amino acid sections of the

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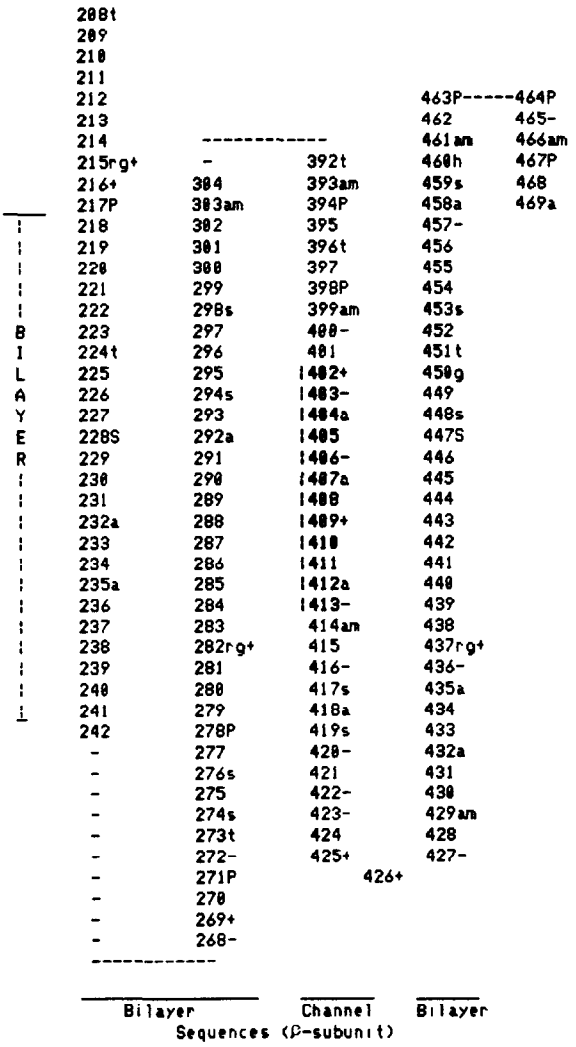

Fig.1. Partial tertiary structure of bilayer portion of the β-subunit of AChR, the acetylcholine receptor: +, Lys; rg+, Arg; −, Glu or Asp; S, Cys; s, Ser; t, Thr; h, His; g, Gly; P, Pro; a, Ala; am, Gln or Asn; all hydrophobic residues (Leu, Ile, Val, Phe, Tyr, Trp, Met) unmarked; Asn, 303, 461, 466; Asp, 268, 400, 422, 423, 427, 436, 457, 465.

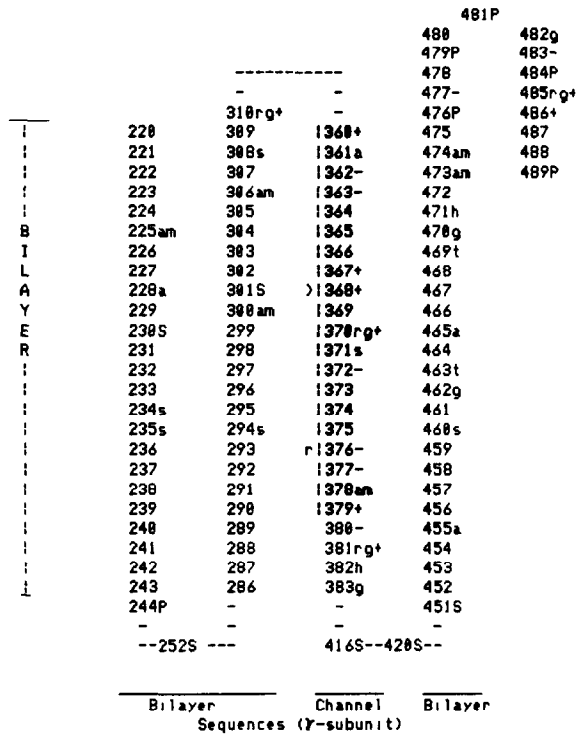

Fig.2. Partial tertiary structure of bilayer portion of γ-subunit of AChR, the acetylcholine receptor: +, Lys; rg+, Arg; −, Glu or Asp; S, Cys; s, Ser; t, Thr; h, His; g, Gly; P, Pro; a, Ala; am, Gln or Asn; all hydrophobic residues (Leu, Ile, Val, Phe, Tyr, Trp, Met) unmarked; channel-type sequence restarts; r, reverse charge order; Asn, 225, 300, 306, 473; Asp, 380, 483.

protein had led to the selection of 4 transmembrane bilayer segments of the γ-subunit [3]. One of the SOAP-selected transmembrane segments has only 12/24 hydrophobic residues, and was not included among our choices.

The choice of ion channel elements is based on the theory of single group rotation (SGR), outlined in [1,5]. Based on a model which proposed that a channel amino acid sequence might be Glu(1), Lys(5), Glu(8), Lys(12)..., a search is made for positively charged amino acids (Lys or Arg) and negatively charged amino acids (Glu or Asp). Pairs are marked, then a search is made for contiguous or non-contiguous pairs and for longer runs. At least 3 pairs (a 'triplet') qualify as a channel element. Applying this search procedure to the α-subunit identified one quintuplet and one non-contiguous triplet and thus, the location of the portion of the ion channel contributed by the subunit [1]. In each of the β-, γ- and δ-subunits, our search clearly identified only one transmembrane segment as meeting our criteria for channel elements (fig.1–3).

The channel elements have two features:

(1) An alternating sequence of positively and negatively charged groups thought to be appropriate for ion transport;

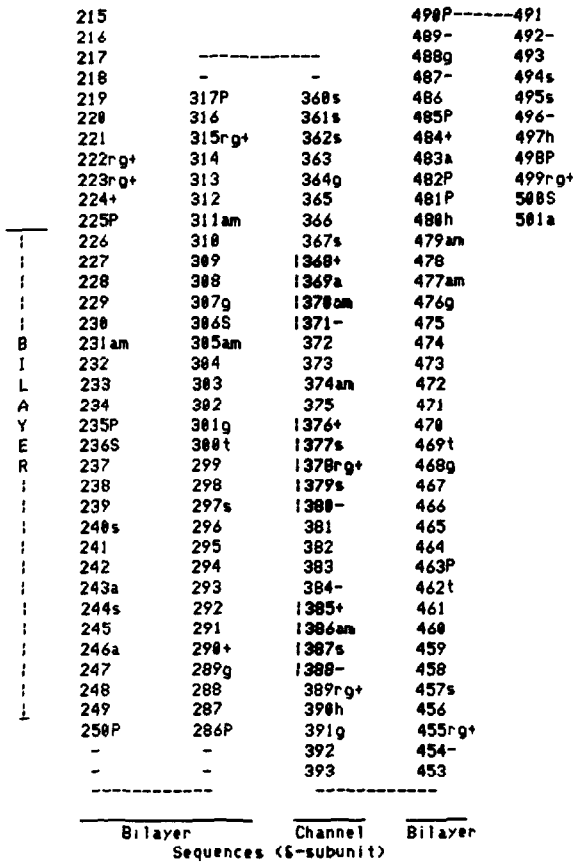

Fig.3. Partial tertiary structure of bilayer portion of δ-subunit of AChR, the acetylcholine receptor: +, Lys; rg+, Arg; −, Glu or Asp; S, Cys; s, Ser; t, Thr; h, His; g, Gly; P, Pro; a, Ala; am, Gln or Asn; all hydrophobic residues (Leu, Ile, Val, Phe, Tyr, Trp, Met) unmarked; Asn, 231, 305, 311, 374, 477, 479; Asp, 454, 489, 492, 496.

(2) A sufficient number of hydrophobic amino acids so that the hydrophilic channel α-helix would be compatible with adjacent hydrophobic α-helices.

Microscopic structure of this type would escape selection by running average procedures like SOAP [6].

A high proportion of the charged amino acids in channel elements of all 5 AChR subunits are Lys (21/21) and Glu (16/28), as the original SGR theory predicted [1,5].

Sequence homologies [2,4] and 'highly conserved regions' [2] have been noted in the sequences for the subunits. Although part of one 'conserved' hydrophobic segment (from 'c' [2]) appears in our selection, none of the transmembrane channel elements identified by SGR theory was previously 'recognized'. DNA sequence analyses require supplemental information (i.e., a theory about channel elements) for the identification of functional sites [7].

The matching of channel elements between helical segments and the construction of the AChR ion channel and an AChR binding site will be described in a separate communication (in preparation).

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