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AMINO ACID SEQUENCE HOMOLOGY BETWEEN "α" SUBUNITS FROM TORPEDO AND ELECTROPHORUS ACETYLCHOLINE RECEPTOR

Bianca M. Conti-Tronconi*, Michael W. Hunkapiller**,
Jon M. Lindstrom[§] and Michael A. Raftery*

Church Laboratory of Chemical Biology
Division of Chemistry & Chemical Engineering*
and Division of Biology**
California Institute of Technology
Pasadena, California 91125

[§]The Salk Institute
P.O. Box 85800
La Jolla, California 92037

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SUMMARY: The amino terminal amino acid sequence of the 41,000 dalton subunit of Electrophorus electricus acetylcholine receptor has been determined for 35 cycles by automated sequencing procedures. Comparison of the unique polypeptide sequence obtained for this molecule with that of the major subunit of Torpedo californica acetylcholine receptor reveals extensive primary structural homology between the two proteins.

INTRODUCTION

The acetylcholine receptor (AcChR) has been purified from species of electric ray (Torpedo and Narcine) and has been shown to be a complex of four related peptides of M_r 40, 50, 60 and 65K commonly referred to as α, β, γ, and δ, respectively (reviewed in 1 and 2). The AcChR has also been purified from the electric organ of Electrophorus electricus (reviewed in 2). When the purification is carried out under conditions that limit proteolysis this Electrophorus AcChR comprises four polypeptides, whose M_r (41, 50, 55 and 64K dalton) are in the same range as those of Torpedo AcChR (3). Immunological cross-reactivity between the subunits of similar

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molecular weight of Torpedo, Narcine and Electrophorus has been observed (13, 14, 3, 16) using either policlonal (13, 14, 3) or monoclonal (3, 16) antibodies.

The definitive elucidation of Torpedo AcChR subunit structure and subunit interrelationships stemmed from the demonstration by amino terminal acid sequencing that four peptides of M_r 40, 50, 60 and 65K are closely related, exhibiting extensive primary structural homology and forming a pentameric, pseudosymmetric structure (4). In the present study, the amino terminal amino acid sequence of the first 35 amino acids of the smallest (41K) M_r subunit ("α_1") of Electrophorus AcChR was determined, and a comparison with the 40K subunit ("α") of Torpedo is made, as a preliminary part of a study designed to elucidate the subunit structure of Electrophorus AcChR and to determine the extent of its relationship with Torpedo AcChR.

MATERIALS AND METHODS

Preparation of AcChR:

Purified solubilized-AcChR preparations were obtained from Electrophorus electricus electric organ as described in (3) using Naja naja siamensis α-neurotoxin complexed to Sepharose 6B (Pharmacia) as an affinity resin. The specific activity of the preparations used ranged between 4.3 and 7.1 nmole/mg of protein.

Purification of the 41K dalton subunit:

The purified AcChR, in 10 mM phosphate buffer, pH 7.4, containing 0.2% cholate, 0.5 M NaCl and 0.1% glycerol, was made 1.5% in sodium dodecyl sulfate (SDS) and incubated at room temperature for 5 minutes to achieve complete dissociation of the subunits. The denatured AcChR was dialyzed 1-2 hours at 4° C in Spectra/Por dialysis tubing with a 25K dalton cutoff versus 31 mM Tris-HCl buffer, pH 6.8, containing 1.5% SDS. The dialyzed sample was made 5% in glycerol, 2.5% in mercaptoethanol, 0.002% in bromphenol blue and 0.2% in sodium thioglycollate.

The sample was loaded on a slab gel, prepared according to Laemmlie (5), containing 0.1% SDS and 9.75% polyacrylamide in the running gel, 4% in the stacking gel. Torpedo AcChR and the BioRad low molecular weight protein standards were used as m.w. markers. The dimensions of the slabs were: 0.1 x 9 (length) x 13 (width) cm for the running gel, 0.1 x 1.5 x 13 cm for the stacking gel. The gels were run overnight at 5 mA/gel; stained for 2 hours in 0.25% Coomassie brilliant blue, 50% methanol, 7.5% acetic acid; destained overnight in 20% methanol, 7.5% acetic acid; and incubated in several changes of distilled water at 4° C (4-8 hours total soaking). Gel strips containing stained bands were cut away from the remainder of the gels and stored frozen at -20° C.

The proteins were recovered from the gel strips by electroelution. The gel was chopped in 0.5 mm cubes and incubated overnight a room temperature in 2% SDS, 0.4 M Tris-acetate buffer, pH 8. 10 ul of 10% dithiothreitol was added, and the proteins were electroeluted at 80 volts, 4° C, for 3-4 days using 0.1% SDS, 0.05 M Tris-acetate buffer. pH 7.8, in the apparatus described in (6). The efficiency of the elution was monitored by following the parallel elution of a ^{125}I-labelled subunit of Torpedo AcChR. The eluted samples were desalted at 150 volts, 4° C, for 24 hours as described in (6) using 0.05% SDS, 0.05 M ammonium bicarbonate.

The purity and the integrity of the eluted and desalted samples were determined by SDS polyacrylamide gel electrophoresis (5) using small slabs of gel (0.1

x 5 x 8 cm). The gels were run at 8 mA (stacking) and 16 mA (running) for 1 1/2 hours, and the protein bands were visualized by silver staining (7).

Amino terminal amino acid sequence analysis:

The purified subunit samples were lyophilized, dissolved in 30 µl of distilled water, and submitted to amino terminal sequence analysis by automated Edman degradation in either a spinning cup (8) or a gas phase (9) sequenator. Phenylthiohydantoin (PTH) amino acids were identified by high performance liquid chromatography (HPLC) on an IBM Cyano column. Details of identification of PTH amino acids and standard chromatograms have been described in (10).

RESULTS AND DISCUSSION

In the case of Torpedo AcChR, amounts of AcChR subunits sufficient for determination of their amino terminal amino acid sequences (4) have been isolated by preparative gel electrophoresis. Using this approach, it was possible to show that Torpedo AcChR is composed of four homologous polypeptides of M_r 40, 50, 60 and 65K in a stoichiometric ratio of 2:1:1:1, respectively (4, 11). The same stoichiometry was also obtained by separation of the polypeptides on polyacrylamide gels, extraction of the individual polypeptides from the gels and estimation of the relative amounts of polypeptides present (12).

The AcChR has also been purified from other sources (reviewed in 2). In the case of Electrophorus AcChR, it has not been possible to reach the degree of sophistication achieved for Torpedo AcChR because of difficulties in purifying the large amounts of intact AcChR necessary for reconstitution and structural studies, due to the low content of AcChR and the high content of proteases in this tissue. However, it is possible to isolate amounts of Electrophorus AcChR sufficient for microsequence analysis. In Figure 1, an SDS gel of purified AcChR's from Torpedo and Electrophorus is shown. For the latter AcChR, four major polypeptides whose M_r are in the same range as that of the four Torpedo subunits are present. The slight difference in M_r between the subunits of the two species could be due either to different degrees of glycosylation or even to mild proteolysis. In this respect, a degradation product(s) of M_r 38K is consistently present with Electrophorus AcChR despite extensive efforts to limit proteolysis (3). Such products increase with aging of preparations or with the heating of samples prior to electrophoresis, with concomitant decrease of the staining intensities of the higher M_r subunits.

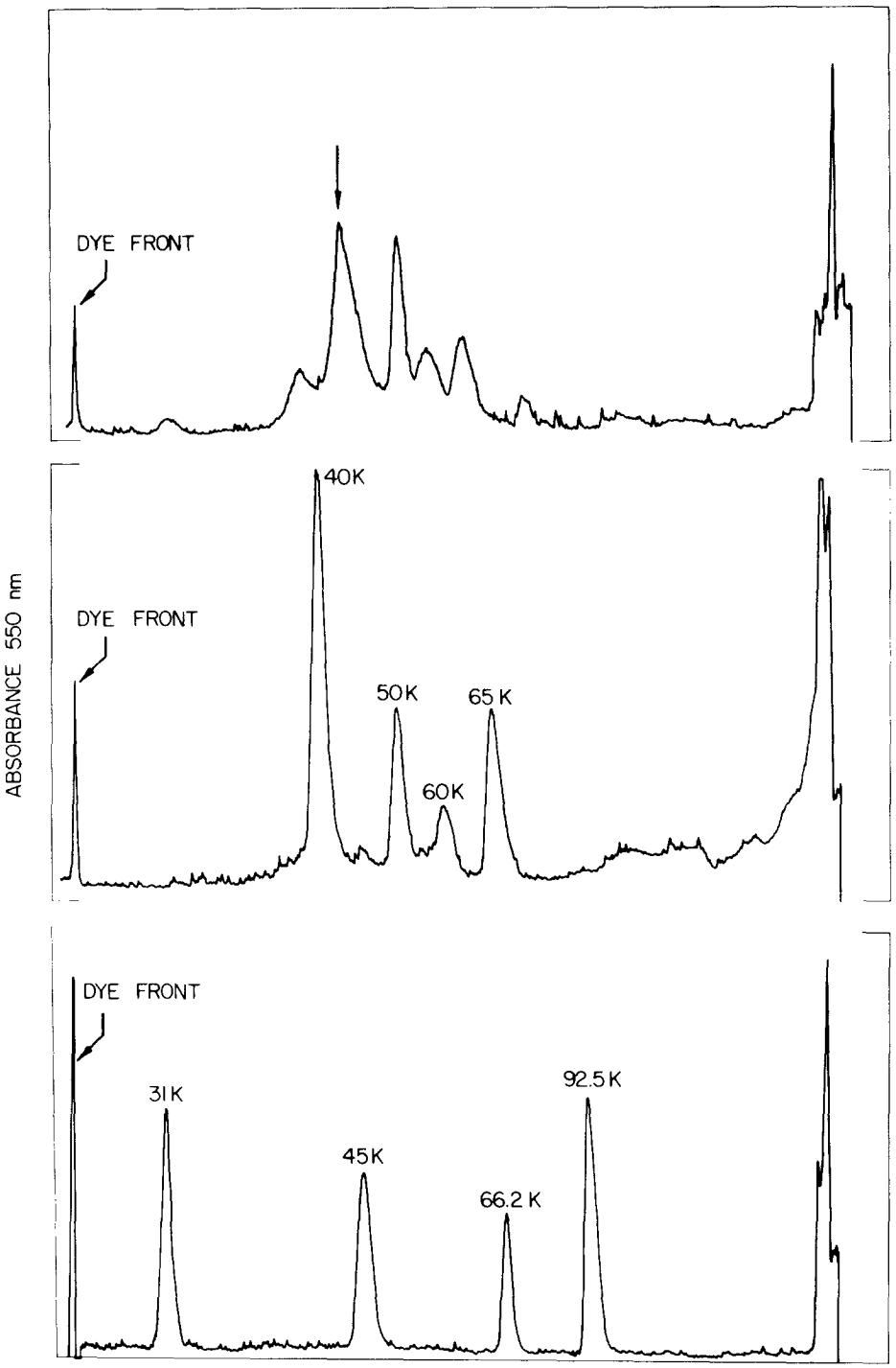

FIGURE 1: SDS gel profiles of Electrophorus AcChR (top), Torpedo AcChR (center) and standard proteins (bottom). The arrow indicates the subunit isolated for the sequence studies reported here.

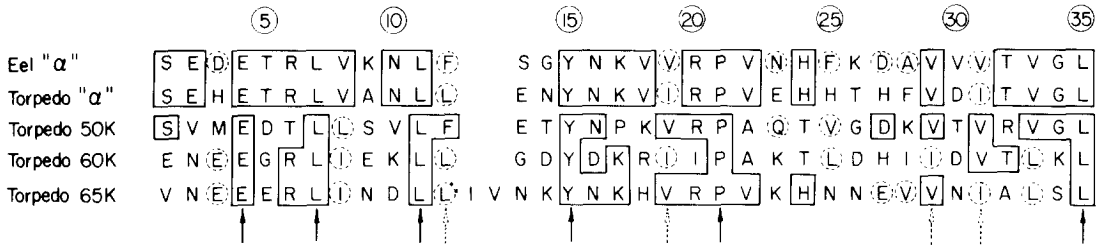

FIGURE 2: Comparison of the amino acid sequence of Electrophorus AcChR "α_1" subunit with the sequences of the four Torpedo subunits.

There are indications that at least some aspects of the structure of the AcChR are conserved throughout animal evolution (reviewed in 2). These include: (i) the dose response curve for the physiological effect of AcCh and the similar pharmacological profiles with drugs; (ii) the closely related physical properties of detergent solubilized preparations from different sources and similar morphology of membrane-bound or detergent-solubilized AcChR (see 2); (iii) the existence of immune cross-reactivity among AcChR preparations obtained from different sources (13, 3, 14-16). The extent of similarity between AcChR's from different sources can be defined precisely only by structural analysis. In this respect, the subunit of choice for an initial comparison is the lowest M_r subunit (41K in Electrophorus AcChR, 40K in Torpedo AcChR), since for both species it has been demonstrated to contain a cholinergic ligand binding site labelled by affinity reagents (reviewed in 1 and 2).

Amino terminal sequence analysis reveals that the primary sequence of Electrophorus "α_1" is identical in 24 of 35 positions to that of the "α" subunit from Torpedo (identity 69%). Comparison of the sequence data for the Electrophorus "α_1" subunit with those for the higher M_r subunits of Torpedo AcChR (Figure 2) also demonstrates a high degree of sequence homology (29 to 46% identity). The sequences at six invariant positions of Torpedo AcChR (residues 4, 7, 11, 15, 21 and 35) are conserved in the "α_1" subunit of Electrophorus, as are the sequences at five other positions (residues 6, 16, 17, 20 and 29) where three of the four Torpedo subunits have identical amino acid residues. Interestingly, Electrophorus "α_1" subunit has amino acid residues different from Torpedo "α" subunit but identical to those of one or two other of the other Torpedo subunits at four positions (residues

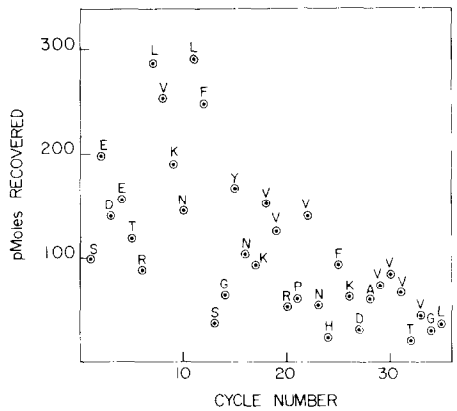

FIGURE 3: Yields of PTH amino acids identified in the amino terminal sequenator analysis of 320 pmole of Electrophorus AcChR "α" subunit. Aliquots (33%) from each sequenator cycle were analyzed by HPLC as previously described (4). Amounts of PTH amino acids were calculated by comparison of observed peak heights with those of a standard PTH amino acid mixture. The background PTH levels from the sequenator were subtracted from these values, and the resulting yields were normalized to 100% injection to give the values plotted in the figure. They are not corrected for different recovery levels of the various PTH amino acids.

12, 19, 27 and 31). Sequence identity between the "α" subunits of the two AcChR preparations represents a higher degree of homology (69% identity) than was found between the first 35 residues of the "α" subunit of Torpedo AcChR and the other three constituent polypeptides (4) where the degree of identity ranged between 37 and 43% (see Figure 3).

This high degree of amino acid sequence homology between Electrophorus and Torpedo AcChR demonstrates that this molecule is highly conserved and indicates the likelihood that molecular structure, binding site topography and the mechanism underlying physiological responses triggered by the AcChR are likely to be very similar among species.

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