A COMPARISON OF THE SUBUNITS OF THE ACETYLCHOLINE RECEPTOR FROM ELECTRIC EEL AND *TORPEDO CALIFORNICA*

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1. Introduction

The nicotinic acetylcholine receptor (AChR) from the electric organs of eel and *Torpedo* species appears to be an oligomeric protein. The number of subunits and the relative sizes of subunits in purified AChR vary in reports from several investigators and may vary with species [1]. There has been good agreement that in *T. californica* there are 4 subunits, the smallest one having mol. wt ~40 000 [1,2–6]. Recent reports on eel receptor have described 2 [7] or 3 [3,8] subunits, the smallest one having mol. wt 40 000 [3] or 43 000 [7,8]. The similarity of size in the smallest subunit of eel and *Torpedo* raises the possibility that they may be identical. There are two pieces of supporting evidence. One is that in both cases the smallest subunit contains the recognition site for acetylcholine. This is based on the finding that 4-(*N*-maleimido)-benzyltri[^{3}H]methylammonium iodide ([^{3}H]MBTA) covalently bound to it [3,7] and this binding was blocked by α-toxin [7]. The other is that the immunological crossreactivity between AChR from eel and *Torpedo* was due only to the smallest subunit [9].

Here we compare the relative sizes of subunits of AChR from eel and *Torpedo* on SDS–gels, and their peptide fingerprints, particularly those of the smallest subunit. We report that the smallest subunits differ in molecular weight and much of their structure, but share one peptide in common.

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2. Materials and methods

2.1. *Purification of AChR*

AChR was purified from the electric organs of *T. californica* and eel by the method in [6]. Its protein content was determined by the Lowry procedure [10].

2.2. *Sodium dodecyl sulfate (SDS)*

SDS-gel electrophoresis was run according to a modification [6] of the Laemmli procedure [11].

2.3. *Isolation of AChR subunits*

AChR subunits were separated by analytical SDS–gel electrophoresis in tubes, as in [6]. SDS was removed from samples by dialyzing against buffer solution and water. Each of the recovered subunits was re-electrophoresed in the presence of SDS in order to check purity.

2.4. *Microbore chromatography*

Microbore chromatography combined with fluorescent assay with *o*-phthalaldehyde was carried out as in [6], by a modification of the method in [12]. All analyses were performed with 8 stepwise buffers.

2.5. *Preparation of samples for peptide fingerprints*

Samples were prepared as detailed in [6]. The protein was oxidized by performic acid to cleave disulfide bonds. The fragmentation of oxidized protein into low molecular weight peptides was done by trypsine.

3. Results and discussion

3.1. *Separation of subunits*

SDS–gel electrophoresis of AChR purified from *Torpedo* showed 4 subunits of mol. wt 68 000, 57 000, 47 000 and 38 000. The AChR from eel had 2 subunits of mol. wt 48 000 and 39 000. The molecular weights of most of these bands agree with those in [1,2–8]. The 2 subunits from eel AChR had molecular weights very close to the 2 smallest subunits from *Torpedo*. This raised the possibility that AChR from different organisms had the same subunits.

A mixture of the AChR from *Torpedo* and from eel was then run on SDS–gel electrophoresis. As fig.1 shows, the 4 *Torpedo* and 2 eel subunits were readily separated in spite of the small difference in molecular weight. Consequently, none of the eel subunits is identical with any of the *Torpedo* subunits.

3.2. *Peptide fingerprints*

These were examined by high-pressure liquid chromatography following cleavage with trypsin, employed because of the selectivity of its cleavage. A control analysis using the tryptic digestion but without AChR was used as a background against which all peptide fingerprints were compared. There was a large peak in the baseline at ~65 min and a large broad peak after 660 min. These observations imply that contaminants were present in the buffer solutions. Several small contaminant peaks, corresponding to elution time of amino acids in analysis of a calibration mixture of amino acid, appeared within the first 100 min in control analysis. The peak at ~160 min is probably of ammonia, because ammonia appeared at ~160 min in the analysis of a calibration mixture of amino acids. Other small peaks are of unknown origin. These were not observed in baseline analysis, i.e., without injection of samples. The origin of these contaminants was described in [6].

Peptide fingerprints from total AChR of *Torpedo* and eel were compared. There were very few similarities, an observation which was not surprising in view of the fact that we had shown [6] that all 4 subunits of *Torpedo* AChR had different fingerprints. We therefore attempted to compare the smallest subunit from each, in view of the evidence, (reviewed above) favoring their identity. The problem was made difficult by the very small amounts of eel receptor

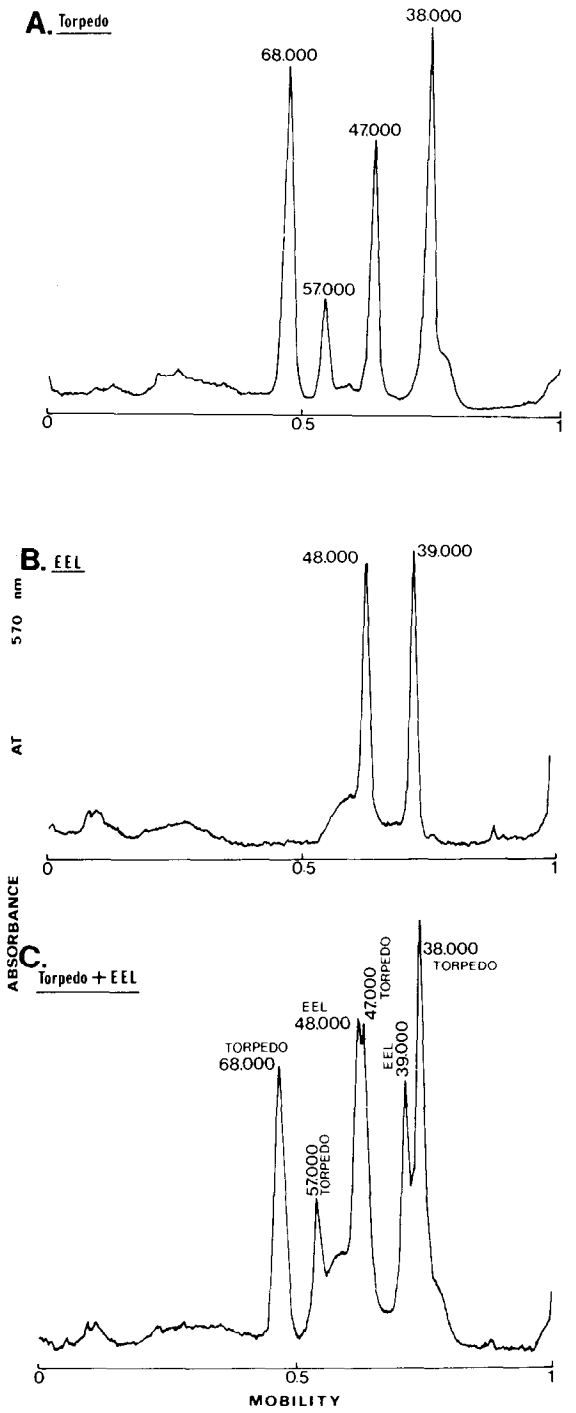

Fig.1. Electrophoresis of purified AChR in SDS–gels, stained for protein: (A) *Torpedo*; (B) eel; (C) *Torpedo* + eel.

available, caused by the small content of it in eel electroplax.

The subunits were separated electrophoretically and the smallest subunits were successfully isolated. In the case of *Torpedo*, a significant contaminant was present which was also present (but to a lesser extent) in the whole receptor (fig.1). We believe it is a breakdown product of the smallest *Torpedo* subunit.

Figure 2 shows the fingerprints of this subunit from *Torpedo* and eel; there is very little detail in the eel fingerprint because of the small amount applied. However, on the basis of this limited data, there appears to be little in common between the two, especially when it is noted that the peak at about 410 min may be due to arginine, released in all tryptic digests, and that at 160 min is ammonia. Yet there appears to be one major peak, labeled X, present in both digests. That this is indeed the same peak was shown by mixing the smallest subunit from *Torpedo* with total receptor from eel, and showing that the X peak was eluted as a single peak. The X peak was not seen in the fingerprint from the larger subunit of eel and from the 3 largest subunits of *Torpedo*.

We conclude that although the smallest subunits of eel and *Torpedo* receptor differ in molecular weight and most of their peptide composition, they have 1 peptide in common, as judged by the single criterion of elution time in a single system. It is naturally tempting to speculate that the common peptide contains the acetylcholine binding site and is also the antigenic determinant.

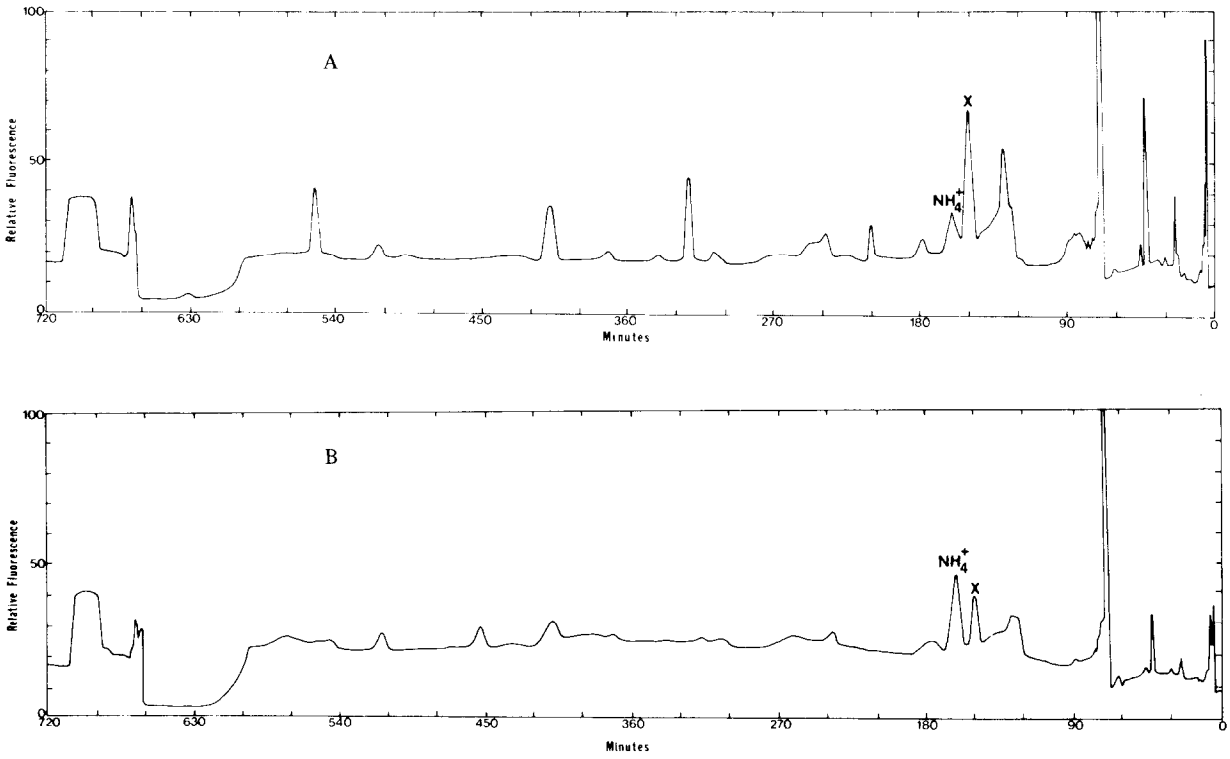

Fig.2. Peptide fingerprints of tryptic digest: (A) *Torpedo* 38 000 mol. wt subunit; (B) eel 39 000 mol. wt subunit. Eight stepwise buffers, 0.2 M in sodium, were used to elute the peptides. Their pH values were: 3.25 (citrate); 4.15 (citrate); 4.60 (citrate); 5.00 (citrate); 5.45 (citrate); 6.25 (citrate); 7.20 (phosphate); 9.50 (borate). The schedule of buffers, expressed in minutes, was: 60; 60; 180; 120; 120; 60; 60; 60. The peaks eluted within the first 60 min are probably amino acids. All current analyses were done using the same batch of buffer solutions, because the elution times of peaks were only fully reproducible when the same buffer batch solution was used.

Acknowledgements

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References

[1] Claudio, T. and Raftery, M. A. (1977) Arch. Biochem. Biophys. 181, 484–489.
[2] Lindstrom, J., Einarson, B. and Merlie, J. (1978) Proc. Natl. Acad. Sci. USA 75, 769–773.
[3] Karlin, A., Weill, C. L., McNamee, M. G. and Valderrama, R. (1975) Cold Spring Harbor Symp. Quant. Biol. 40, 203–210.
[4] Hucho, F., Layer, P., Kiefer, H. R. and Bandini, G. (1976) Proc. Natl. Acad. Sci. USA 73, 2624–2628.
[5] Chang, H. W. and Bock, E. (1977) Biochemistry 16, 4513–4519.
[6] Sumikawa, K. and O'Brien, R. D. (1979) submitted.
[7] Meunier, J. C., Scalock, R., Olsen, R. and Changeux, J.-P. (1974) Eur. J. Biochem. 45, 371–394.
[8] Teichberg, V. I., Sobel, A. and Changeux, J.-P. (1977) Nature 267, 540–542.
[9] Valderrama, R., Weill, C. L., McNamee, M. and Karlin, A. (1976) Ann. NY Acad. Sci. 274, 108–115.
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Laemmli, U. K. (1970) Nature 227, 680–685.
[12] Benson, J. R. (1976) Anal. Biochem. 71, 459–470.