THE MOLECULAR WEIGHT AND SUBUNIT STRUCTURE OF ACETYLCHOLINESTERASE PREPARATIONS FROM THE ELECTRIC ORGAN OF THE ELECTRIC EEL

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SUMMARY: Acetylcholinesterase (AChE) preparations obtained from electric organ tissue of Electrophorus electricus subsequent to tryptic digestion and/or autolysis had sedimentation coefficients of about 11 S and molecular weights of 320,000-350,000. These values did not significantly decrease upon prolonged autolysis. The major polypeptide in 11 S AChE had molecular weights of 82,000 ± 6,000 and 59,000 ± 4,000. The ratios of these two components was a function of the degree of autolysis of the tissue from which the enzyme was purified. In less autolysed tissue the principle component was the 82,000 one, while after prolonged autolysis the 59,000 component predominated. The appearance of the 59,000 component was accompanied by the appearance of polypeptides of ~ 25,000. It is proposed that autolysis and/or proteolysis cleave the 82,000 polypeptide chain of native AChE into fragments of 59,000 and ~ 25,000 which are retained in the quaternary structure of the enzyme unless it is denatured. Possible models for the quaternary structure and arrangement of disulfide bridges in 11 S AChE are discussed.

Acetylcholinesterase (AChE) can be obtained from electric organ tissue of the electric eel, either by prolonged autolysis or by controlled tryptic digestion, in a molecular form with a sedimentation coefficient of about 11 S (1,2). This form is apparently a degradation product of the large asymmetric "native" forms of the enzyme present in fresh tissue (3,4). The molecular weight of 11 S AChE preparations obtained in several laboratories has been reported to be 230,000-260,000 (5-7), and values of 64,000 (6) and 42,000 (7) were reported for the molecular weight of the subunits of such preparations.

We earlier reported a molecular weight of ~ 335,000 (4) for preparations of 11 S AChE purified from fresh or partially autolysed electric organ tissue by affinity chromatography (2), and also presented evidence that the major polypeptide component, which contains the active site of the enzyme, has a molecular weight of about 80,000 (2,8,9). We also observed a minor active-site-containing polypeptide of molecular weight about 60,000 as well as traces of polypeptide chains of much lower molecular weight. In the present study the molecular properties of an 11 S AChE preparation purified from extensively autolysed tissue are examined, and it is shown that the relative amounts of the major polypeptide components of electric eel

AChE are dependent on the history of the tissue preparation. The 80,000 polypeptide is the major component in enzyme purified from fresh tissue, while the lower molecular weight components are present in increasing amounts in tissue which has undergone prolonged autolysis. However, conversion of the 80,000 subunit to smaller polypeptide components is not accompanied by a significant reduction in the molecular weight of the enzyme. On the basis of these data the quaternary structure of 11 S AChE and the discrepancies between the results obtained by different groups will be discussed.

EXPERIMENTAL

AChE Preparations. Purified 11 S AChE was obtained either following tryptic digestion of fresh tissue, partially autolysed tissue and extensively autolysed tissue, or from partially autolysed tissue without tryptic digestion. The tissue was autolysed by storage under toluene at 4^0 for prolonged periods as described by Rothenberg and Nachmansohn (13). The various preparations were purified by affinity chromatography as described previously (2). The preparations obtained by tryptic digestion of fresh tissue and partially autolysed tissue (i.e. tissue which had been stored under toluene at 4^0 for periods ranging from several weeks to 2 years) correspond to AChE A and AChE B respectively in Ref. 2. The preparation obtained by tryptic digestion of extensively autolysed tissue (which had been stored under toluene at 4^0 for about 4 years) was purified by the same procedure employed for purification of AChE B, and will be called AChE B'. The preparation obtained from partially autolysed tissue without tryptic digestion corresponds to AChE C in Ref. 2. "Native" forms of AChE (i.e. 18 S + 14 S AChE) were also purified by affinity chromatography as described previously (9).

Labelling of AChE with ^{3}H-diisopropylfluorophosphate (^{3}H-DFP; 0.9 Ci/mmol in propylene glycol, New England Nuclear, Boston) and treatment with pyridine 2-aldoxime methiodide (2-PAM) were performed as described earlier (8).

Enzyme assays and sucrose gradient centrifugation were performed as previously described (2).

Equilibrium sedimentation experiments, by the high speed meniscus depletion method of Yphantis, were performed as before (4).

Polyacrylamide gel electrophoresis in the presence of SDS, with and without β-mercaptoethanol, was performed as described previously (2, 8). 12 cm gels were used for molecular weight calibration. ^{3}H-DFP-labelled AChE was electrophoresed similarly and the labelled peaks monitored as before (8).

RESULTS AND DISCUSSION

Three different preparations of AChE (A, B' and C), purified from electric organ tissue after different degrees of tryptic digestion and/or autolysis, were subjected to equilibrium sedimentation. The molecular weights obtained are shown in Fig. 1. All three preparations appear to be quite homogeneous, as can be deduced

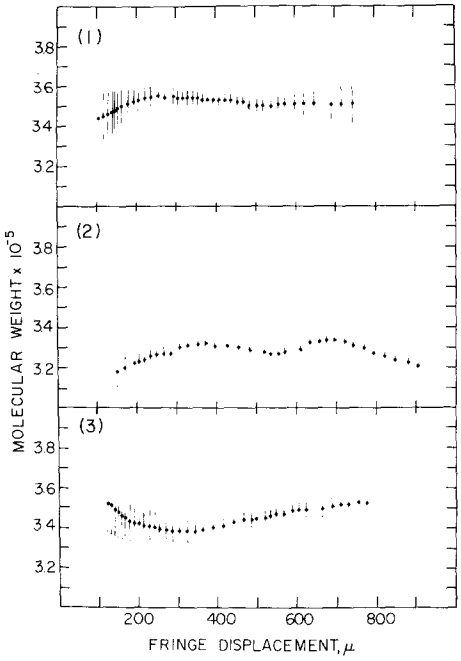

Figure 1. The weight-average molecular weight of various 11 S AChE preparations, plotted against fringe displacement as computed by a computer high-speed equilibrium ultracentrifugation program (4). (1) Acetylcholinesterase A; (2) acetylcholinesterase C; (3) acetylcholinesterase B'. All three preparations were centrifuged at 10,000 rpm, as described in the text.

from the small variation of the molecular weight plotted vs. fringe displacement (which is proportional to concentration) along the ultracentrifuge cell. The preparations have molecular weights in the range of 320,000-350,000, assuming a $\bar{v}$ of 0.723 (4). These values are all significantly higher than the molecular weight of 230,000-260,000 previously reported for AChE preparations with a sedimentation coefficient of about 11 S (5-7).

AChE-B', prepared from extensively autolysed tissue, displays the same specific activity and sedimentation coefficient as AChE-A, AChE-B and AChE-C.

The molecular weights of the polypeptide components of all four 11 S preparations were determined by performing polyacrylamide gel electrophoresis, in the presence of SDS and β-mercaptoethanol. They all contain two main polypeptide components with molecular weights of 82,000 ± 6,000 and 59,000 ± 4,000, respectively. However, whereas in the "native" forms of AChE (18 S + 14 S, Ref. 9) the 82,000 polypeptide is the major component and the 59,000 polypeptide is present only in small amounts, if at all (Fig. 2.1, Ref. 9), the relative amounts of the two polypeptides varies markedly in different 11 S acetylcholinesterase preparations (Fig. 2.2 and 2.3). In

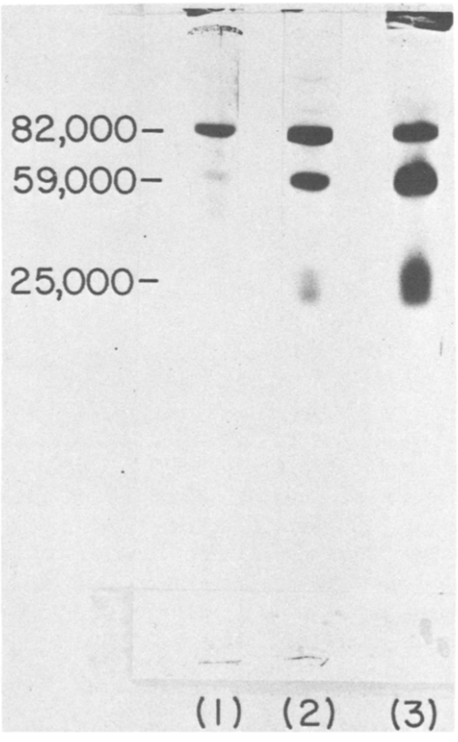

Figure 2. Acrylamide gel electrophoresis, in the presence of SDS and β-mercaptoethanol, of AChE preparations. 5% acrylamide gels were used, and stained with Coomassie blue. (1) "Native" AChE (14 S + 18 S); (2) AChE-B; (3) AChE-B'.

11 S AChE derived from tissue which had undergone the most extensive autolysis, (AChE-B'), the relative amount of the 82,000 component is reduced and the amount of the 59,000 component is markedly increased (Fig. 2.3). The increase in the relative amount of the 59,000 component is accompanied by the appearance of polypeptides of lower molecular weight (~25,000).

Both the 82,000 and 59,000 components observed by staining for protein contain DFP-binding sites (Fig. 3). Again it can be seen that the relative amount of the 59,000 component, as revealed by DFP-binding, increases in the extensively autolysed 11 S AChE-B' (Fig. 3.3). We found that about 90% of the radioactive diisopropyl-

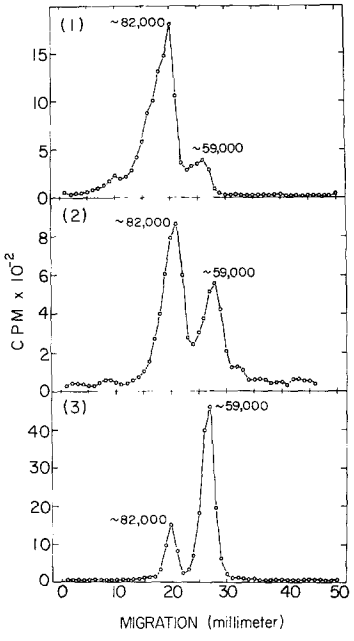

Figure 3. Acrylamide gel electrophoresis, in the presence of SDS and β-mercapto-ethanol, of ^{3}H-DFP-labelled AChE samples. The gels were sliced, radioactivity measured and the molecular weights calibrated as described under Methods. (1) "Native" AChE (14 S + 18 S); (2) AChE B; (3) AChE B'.

phosphoryl groups could be removed from both components with the specific acetyl-cholinesterase reactivator 2-PAM (14) under the conditions described by us (8). No radioactivity appeared to be associated with the faster-moving (~25,000) polypeptide components, indicating that these polypeptides do not contain active sites.

Polyacrylamide gel electrophoresis in the presence of SDS but in the absence of β-mercaptoethanol reveals that all the preparations of 11 S AChE display polypeptides of 168,000 ± 8,000 and 88,000 ± 4,000, both of which bind DFP. It thus seems plausible to assume that the 160,000 - 170,000 component is a dimeric structure in which two subunits are connected by intersubunit disulfide bonds (8). This possibility was also proposed by Froede and Wilson (15) on the basis of sucrose-gradient centri-

fugation experiments employing ^{14}C-DFP-labelled AChE treated either with guanidine alone or with guanidine and β-mercaptoethanol.

The sedimentation equilibrium and acrylamide gel electrophoresis data, as well as the results of the determinations of s values and specific activities of the various 11 S AChE preparations, can be best explained by assuming that autolysis can lead to cleavage of the 82,000 peptide chains present in the native enzyme molecule, at a limited number of susceptible sites. However, this cleavage does not lead to release of the 59,000 and ~25,000 molecular weight fragments produced and to disruption of the quaternary structure, unless the enzyme is denatured. Several groups have reported cases in which proteolytic cleavage per se does not change the overall mass and quaternary structure of proteins (16-19) and we have already suggested this possibility for AChE (2).

On the basis of the above, various models can be suggested for the quaternary structure of 11 S AChE. Since "native" (i.e. 18 S + 14 S) preparations of AChE and some 11 S preparations contain relatively small amounts of the 80,000 component on SDS electrophoresis in the absence of β-mercaptoethanol, it seems unlikely that a valid model will include subunits unlinked to any other subunit by disulfide bridges. We will consider, therefore, only models in which pairs of subunits are linked by disulfide bonds. Two simple models are shown in Fig. 4. In Model I the subunits are linked by disulfide bridges, as are the two parts of the polypeptide chain which are cleaved by proteolysis or autolysis. The polypeptides are arranged in parallel (i.e. with the same polarity from the NH_2-terminal to the COOH-terminal residue) as is the case in γ-globulin (20). This model does not seem very satisfactory, because whatever the percentage of subunits cleaved enzymically, one would not expect appearance of polypeptides of molecular weight 80,000 on acrylamide gels in the absence of β-mercaptoethanol, and this is not in fact the case (8). Another simple model, Model II, does not require intrasubunit disulfides and postulates an anti-parallel arrangement of polypeptide chains linked by disulfide bridges in each of which one cysteine residue is beyond the cleavage point. Antiparallel arrangement of polypeptide chains in multisubunit proteins has indeed been described in several cases (21). This model is more attractive since it can account for the appearance of a ~80,000 species even prior to reduction. Obviously, more complex models can account for the data assuming more disulfide bridges and/or more cleavage points. Moreover, 11 S AChE is itself a degradation product of "native" AChE (1,2), and the quaternary structure of the "native" forms of the enzyme must take into account inter-

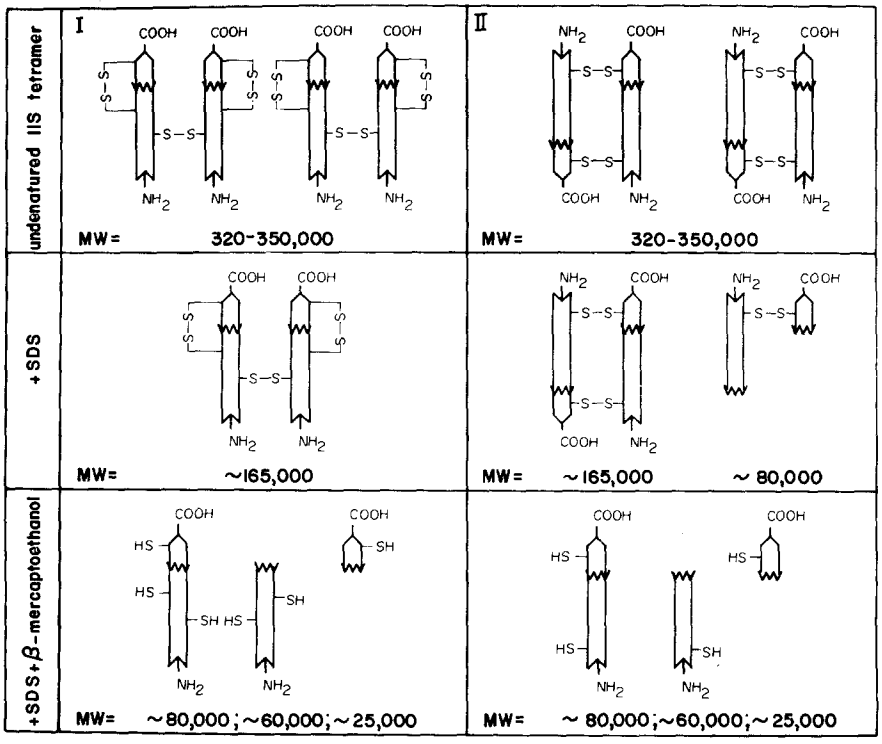

Figure 4. Schematic models for the subunit structure of 11 S AChE, together with the products to be expected under the conditions employed for SDS-acrylamide gel electrophoresis, both in the presence and absence of β-mercaptoethanol, subsequent to cleavage of either one or both polypeptides of the dimer. The site where a cleavage can occur is indicated by a serrated line. For discussion see text.

actions between larger numbers of subunits, and also the presumably specific mode of attachment of the "tail" of the native enzyme to the multisubunit head (3,4). However, any model should take into account the fact that reducing agents are necessary for complete dissociation of the AChE molecule into its subunits, and for release of fragments from the cleaved subunits, subsequent to proteolysis or autolysis.

Thus our results indicate that 11 S AChE is a tetramer of molecular weight 320,000-350,000, each subunit being of about 80,000. Our observation that the principal subunits present in electric eel AChE have molecular weights of about 80,000 and 60,000 is in agreement with recent results from other laboratories (10-12). Our results also indicate that polypeptides of about 60,000 and 25,000 appear gradually following autolysis or tryptic digestion, but that these polypeptide fragments, formed by cleavage of the 80,000 subunit, are not released from the molecule unless it is denatured. Similar conclusions have recently been reached by Chen et al. (22) on the

basis of acrylamide gel electrophoresis and exclusion chromatography in 6 M guanidine solutions.

Leuzinger et al. (6) reported earlier that 11 S AChE is a tetramer of 260,000, containing four 64,000 subunits. We cannot exclude the possibility that under certain conditions cleavage of the 80,000 polypeptide is followed by release of polypeptide fragments, even prior to denaturation. Such a process could result in formation of an active AChE tetramer of 230,000-260,000, as reported previously (5-7), with subunits of 64,000 or less (6,7). However, we did not observe the formation of such a species in our preparations.

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