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Complete amino acid sequence of fetal bovine serum acetylcholinesterase and its comparison in various regions with other cholinesterases

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The complete amino acid sequence of a mammalian acetylcholinesterase from fetal bovine serum (FBS AChE) is presented. This enzyme has a high degree of sequence identity with other cholinesterases, liver carboxyesterases, esterase-6, lysophospholipase, and thyroglobulin. The locations of 191 amino acids in 10 regions of the FBS enzyme were compared with corresponding sequences of *Torpedo*, human, and *Drosophila* AChEs and human serum butyrylcholinesterase (BChE). In one region there is a marked difference in both the number of amino acids and their sequence between mammalian AChE and other AChEs and the human serum BChE. The amino acid sequence of FBS AChE showed overall homologies of 90% with human AChE, 60% with *T. californica* AChE, 50% with human serum BChE, and 39% with *Drosophila* AChE in these regions.

Acetylcholinesterase; Amino acid sequence; Sequence homology; Serine esterase

1. INTRODUCTION

Fetal bovine serum (FBS) contains relatively abundant amounts (0.3 mg/liter) of true acetylcholinesterase (EC 3.1.1.7; AChE) and thus is a good source for obtaining large quantities of purified mammalian AChE with relative ease [1,2]. We have attempted in the past four years to (a) develop a procedure to obtain large amounts of a mammalian AChE in purified form, (b) elucidate its primary structure, and (c) explore the possibility of using this enzyme as a pretreatment drug for protection against highly toxic organophosphate anti-ChEs [3,4]. For determination of chemical structure and for demonstrating in vivo efficacy of AChE to sequester organophosphate anti-ChEs before they reach physiological targets, large quantities of purified enzyme are required. Using procainamide Sepharose 4B gel as an affinity ligand, we have developed a simplified batch extraction procedure for purification of large quantities (up to 100 mg) of purified FBS AChE [5].

The use of AChE as an in vivo scavenger for organophosphate anti-ChEs requires that it be (a) immunocompatible, (b) physiologically compatible with the species of animal, (c) not degraded or cleared rapidly (long half life), and (d) readily available in sufficient quantities, since slightly more than one mole of enzyme will be needed to sequester one mole of organophosphate.

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Primary structures of the catalytic subunit of AChE isolated from *Torpedo californica, Torpedo marmorata* and *Drosophila melanogaster*, and butyrylcholinesterase (EC 3.1.1.8, BChE) isolated from human serum have recently been reported [6–11], as have partial sequences of AChEs isolated from human erythrocytes [12,13], human brain [14], and bovine brain [15]. Pharmacological studies and enzyme kinetic studies with these enzymes, including multiple molecular forms, have demonstrated that they possess similar catalytic properties. The similarities and any differences in their catalytic functions should be reflected in the corresponding similarities and differences in the structural domains of their catalytic subunit(s). The esteratic regions of several cholinesterases share a high degree of amino acid sequence homology [6], while some other regions are markedly dissimilar.

In this report we present the complete amino acid sequence of fetal bovine serum AChE, the first mammalian AChE to be sequenced. We have also compared the amino acid sequences of several regions in the catalytic subunit of FBS AChE with reported sequences in the corresponding regions of other cholinesterases.

2. MATERIALS AND METHODS

Acetylcholinesterase from fetal bovine serum (FBS) was purified as previously described [5]. The specific activity of purified enzyme was 400 U/nmol, and 1 mg of enzyme contained 14 nmol of active site protein. Cyanogen bromide-cleaved peptides of the catalytic subunit of FBS AChE (approximately 200 nmol) were obtained by labeling the active site serine with [^{3}H]diisopropylphosphofluoridate (DFP), followed by denaturation with 7 M guanidine-HCl, reduction with

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dithiothreitol, and alkylation with [^{14}C]*N*-methylmaleimide to label the reduced cysteine residues. The sample was exhaustively dialyzed against 10 mM NH_4HCO_3, lyophilized, resuspended (10 mg/ml) in 80% formic acid, and digested with cyanogen bromide. The cleaved peptides were separated first on a Sephadex G-100 (superfine) column (1.2 × 50 cm) in 1.0% HOAC, followed by HPLC on μBondapack C_4 and/or C_{18} columns using TFA/acetonitrile or phosphate/acetonitrile solvent gradients. The amino acid sequences of purified peptides were determined using a gas phase peptide/protein sequencer (ABI model 477A). PTH derivatives were identified on-line with HPLC (ABI model 120A).

Trypsin-digested peptides of FBS-AChE were obtained by digesting denatured, reduced, and alkylated enzyme before lyophilization, as described above, with 1% CPTK trypsin (w/w) in 50 mM NH_4HCO_3, pH 8.2, for 16–20 h. The peptides were separated on a Sephadex G-75 column (1.2 × 50 cm) followed by HPLC on μBondapack C_4 and C_{18} columns using TFA/acetonitrile solvent gradients.

The *Torpedo californica* AChE sequence is from Schumacher et al. [7]. The *Torpedo marmorata* sequence used here for comparison was reported by Bon et al. [15]. The amino acid sequence of human serum BChE was obtained from published reports by Lockridge et al. [10]. The sequence of AChE from *Drosophila melanogaster* was done by Hall and Spierer [9]. The partial amino acid sequences of human AChEs shown here were determined by Soreq and Prody (human brain) [14], Chhajlani et al. (human erythrocyte) [13], and Haas and Rosenberry (human erythrocyte) [12].

3. RESULTS AND DISCUSSION

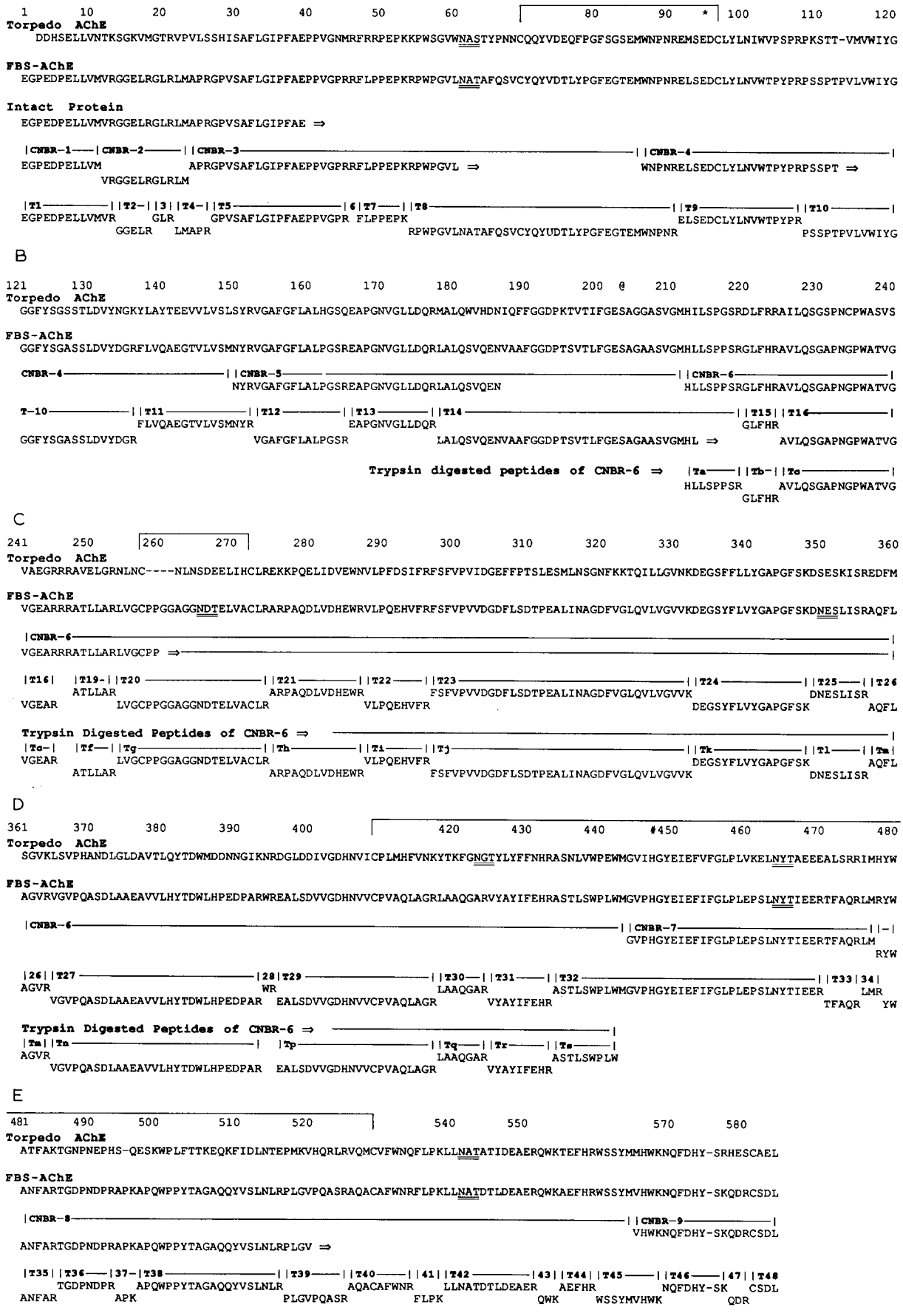
Fig. 1 describes the complete amino acid sequence of the catalytic subunit of FBS AChE, which consists of 583 amino acids. For comparison, the amino acid sequence of *Torpedo californica* AChE is shown, as well as the strategy of the sequence determination. Gapping is introduced in these sequences to maximize amino acid sequence homology: at amino acid 113 in *Torpedo* AChE (to accommodate one extra amino acid in FBS AChE), at 258–261 in *Torpedo* (to accommodate four extra amino acids in FBS AChE), at 495 in *Torpedo* (to accommodate one extra amino acid in FBS), and at 575 (to accommodate one additional amino acid in human serum BChE, sequence not shown here). The calculated molecular mass for the FBS enzyme is 64 238, and there are five apparent N-linked glycosylation sites (underlined in the figure). The amino acid sequence of the amino-terminus region was obtained by subjecting the intact protein to direct sequence analysis. The analysis of nearly all of the trypsin-digested peptides and the partial analysis of CNBr-cleaved peptides were performed several times. Most of the overlaps could be obtained by these analyses. One CNBr fragment consisted of 232 amino acids. The overlaps in this region were obtained by digesting the peptide with trypsin, separating the fragments, and analyzing each of these tryptic peptides. The rest of the overlaps were determined by comparison of the amino acid sequence of this AChE with the *Torpedo* AChE sequence.

The FBS-AChE sequence shares an overall sequence identity of 90% with mouse AChE (personal communication, P. Taylor) and 60% with *T. californica* AChE [7]. In the small disulfide loop located between amino acids 257 and 272, there are four additional residues in the FBS and murine enzymes. FBS AChE also has a relatively high degree of identity (52%) with butyrylcholinesterase from human serum [10] and human fetal tissue BChE [14], but somewhat less identity (36%) with *Drosophila* AChE [9], rabbit and rat liver carboxyesterases [16] (33% and 35%, respectively), *Drosophila* esterase-6 [17] (29%), bovine thyroglobulin [18] (32%), and rat lysophospholipase [19] (32%).

The amino terminus sequences of several ChEs were compared by Bon et al. [15]. Fig. 2 updates their data for the first 12 amino acids with the addition of the FBS AChE sequence for this region. The amino acid sequences of multiple molecular forms of enzyme from the same source appear to be identical, although little homology appears among the ChEs isolated from various other species. The sequences of AChEs from the two species of *Torpedo* differ at position 5 (His/Asp, FBS AChE numbering) and at 12 other locations in the molecule (not shown). In the sequences compared here, *T. marmorata* differs from *T. californica* at positions 171, 199, and 517 (M vs V, L vs I, I vs M, respectively, FBS AChE numbering system).

Since multiple molecular forms of AChE isolated from various species possess similar catalytic properties, the active site serine-containing region (Ser-203) might be expected to have structural similarities. This was shown to be true by MacPhee-Quigley et al. [6], who compared the amino acid sequences of *Torpedo* AChE with human serum BChE in this region, and Soreq and Prody [14], who added human brain AChE to the comparison. Similarities are now further revealed with our elucidation of the amino acid sequence of mammalian AChE from fetal bovine serum, as shown in Fig. 3C. There is complete identity among 21 amino acids between bovine and human AChEs, and matching of 17 of 21 with human serum BChE, 16 of 21 with *Torpedo* AChE and 12 of 21 with *Drosophila* AChE The sequence PXXXTXFGESAGXXSV is common t

Fig. 1. Amino acid sequence of FBS AChE and strategy of sequence determination. The amino acid sequence was determined by automated Edman degradation of (i) denatured, reduced, and alkylated intact enzyme (39 amino acid residues from amino terminus end), (ii) complete sequence of 4 CNBr-cleaved peptides (1,2,7, and 9), (iii) partial sequence of the remaining 5 CNBr-cleaved peptides, (iv) complete sequence of 44 tryptic peptides, and (v) partial sequence of one tryptic peptide (T14). CNBr peptide 6 was further digested with trypsin, the peptides were fractionated and sequenced (Ta-Ts). Cysteine residues were identified by ^{14}C label. The disulfide loops shown are for *Torpedo* AChE. The active site serine (@) was identified by ^{3}H-labeling. The asterisk over Asp-95 and the pound sign over His-447 denote residues which, along with Ser-203, may be involved in charge relay. The presumed N-linked glycosylation sites in *Torpedo* and FBS AChE are underlined. Gaps have been introduced into the *Torpedo* sequence to accommodate additional amino acids in the FBS sequence; gapping has been introduced into both sequences at position 575 to accommodate an extra residue in human serum BChE (not shown here) [10].


A
Torpedo AChE
FBS-AChE
Intact Protein
B
Torpedo AChE
FBS-AChE
Trypsin digested peptides of CNBR-6 ⇒
C
Torpedo AChE
FBS-AChE
Trypsin Digested Peptides of CNBR-6 ⇒
D
Torpedo AChE
FBS-AChE
Trypsin Digested Peptides of CNBR-6 ⇒
E
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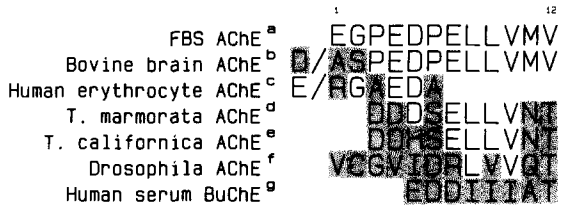

Fig. 2. Comparison of the first 12 amino acids of the N-terminal sequence of the catalytic subunit of FBS AChE with cholinesterases from other species and sources. Numbering corresponds to the amino acid sequence of FBS AChE; non-matching amino acids are shaded; the identity of the first amino acids in (b) and (c) has not been resolved. [a]FBS AChE sequence reported here; [b]sequence from bovine brain AChE obtained by Bon et al. [15]; [c]sequence of human erythrocyte AChE from Haas and Rosenberry [12]; [d]sequence determined by Bon et al. for *T. marmorata* AChE [15]; [e]sequence reported for *T. californica* by MacPhee-Quigley et al. [6] and Schumacher et al. [7]; [f]sequence for *Drosophila* AChE by Hall and Spierer [9]; and [g]sequence of human serum BChE by Lockridge et al. [10].

all 5 cholinesterases. It should be pointed out that the folding pattern of this active site domain may be pivotal in conferring on these molecules the specificity of catalytic function. Using polyclonal and monoclonal antibodies it has recently been shown that this region is located in a pocket-like conformation [20].

Soreq and Prody [14] have reported partial amino acid sequences, deduced from a cDNA clone of fetal human brain AChE, which represent 4 regions of the molecule which are potential candidates for catalytic sites. We have compared the amino acid sequence of FBS AChE with the sequences of five other ChEs in the same regions (Fig. 3A–C,G). Additionally, Soreq and Prody have suggested residue combinations for the charge relay system [14]. Asp-170 (Fig. 3B, Asp-175 in the FBS AChE numbering scheme) is central to all their combinations. The sequence in this region is highly conserved, and there is complete homology between human and bovine AChEs. However, based on results of the binding of inhibitory monoclonal antibody AE-2 to FBS AChE, elucidation of its epitope, location, and the sequence homology in the region surrounding Asp-95 [21], we feel that this amino acid, Asp-95, is a better candidate than Asp-170. Fig. 3G shows the amino acid sequence following His-432 and around His-447, both of which are conserved in all ChEs, and which are suggested by Soreq and Prody to be involved (His-423 and His-438 in their numbering scheme). We believe that the higher degree of sequence homology surrounding His-447 makes this amino acid a more likely candidate for involvement than His-432. Finally, the sequence presented in Fig. 3A includes Arg-152, which is proposed by Soreq and Prody as an alternate to histidine residues (Fig. 3G) in the charge relay system. Our proposal is that since both His-447 and Asp-95 are located in disulfide loops [6] and since Asp-95 is located next to Cys-96, which may affect its net charge, His-447 and Asp-95 are the most likely candidates for involvement with Ser-203 in the charge relay system.

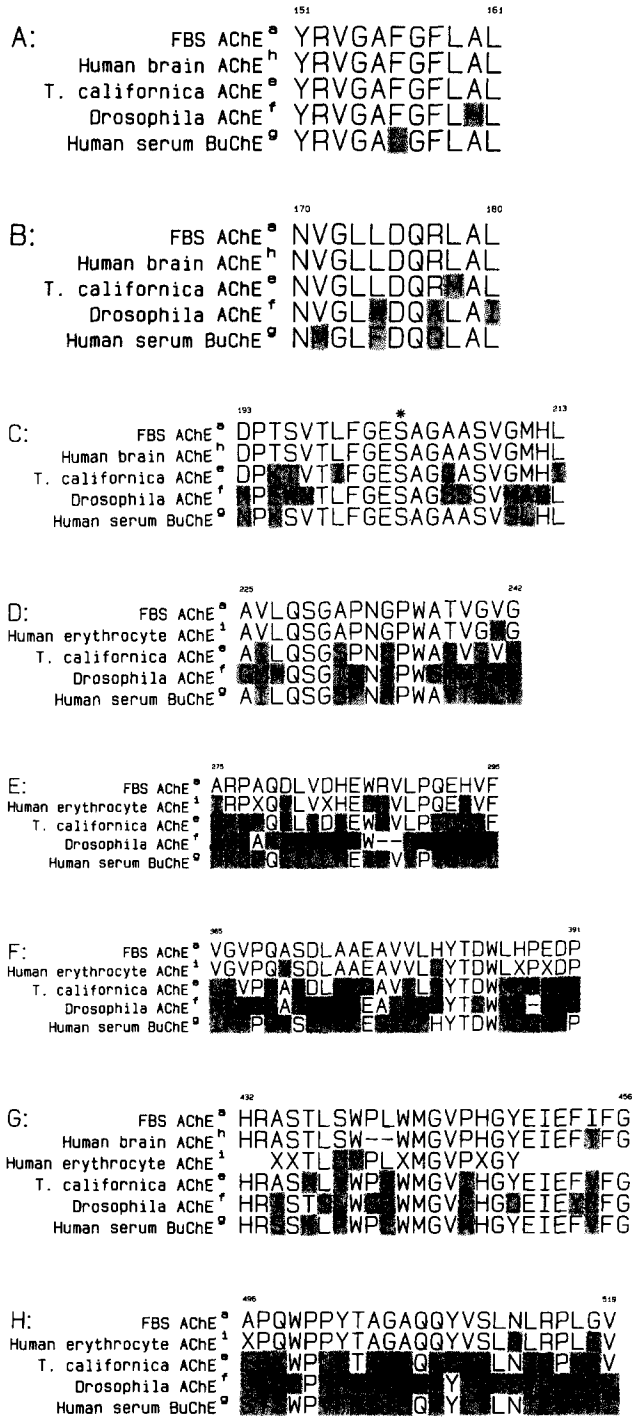

Fig. 3. Comparison of amino acid sequences in various regions of FBS AChE with other cholinesterases. Sources of sequences are as noted for Fig. 2; amino acids are numbered according to FBS AChE. (A) [h]sequence of human brain AChE, Arg-147 region as determined by Soreq and Prody [14]. (B) [h]Asp-170 region in [14]. (C) The gapping introduced in (h) between sites 6 and 7 by Soreq and Prody [14] to align this region with serine hydrolases has been eliminated; the active site serine is marked with an asterisk. (D) Sequence for human erythrocyte AChE (i) is reported by Chhajlani et al. [13]. (E,F) Xs denote unidentified amino acids; gapping is introduced into the *Drosophila* sequence for alignment in these regions. (G) [h]His-423 and His-438 regions are merged with gapping between amino acids 439 and 442 for alignment with FBS AChE.

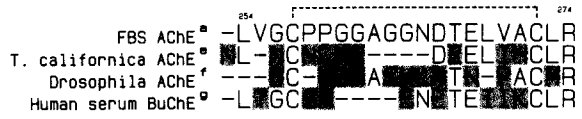

Fig. 4. The comparison of an amino acid sequence of FBS AChE with other cholinesterases in the region of a small disulfide loop (dashed line).

Chhajlani et al. [13] reported the amino acid sequences of 5 tryptic peptides of human erythrocyte AChE, shown in Fig. 3D-H. These authors observed little or no homology when comparing the sequences with *Torpedo* AChE and human serum BChE. However, when these sequences are compared to other mammalian AChEs, approximately 88% sequence identity is observed (85 of 97 amino acid residues).

Finally, the comparison of amino acid sequences of FBS AChE with *T. californica* and *Drosophila* AChEs and human serum BChE in the region of a small disulfide loop (amino acids 254-275, Fig. 4) shows that FBS AChE has four extra amino acids in the loop. The presence of these additional amino acids in the loop in mammalian AChEs would result in a different conformation in this region.

Among the sequences compared here, there is 90% amino acid sequence homology between FBS AChE and human AChEs, 60% with *T. californica* AChE, 39% with *Drosophila* AChE, and 50% with human serum BChE, values equivalent to those for the homologies of the catalytic subunits. In general we conclude that, not unexpectedly, mammalian AChEs appear to have structures more in common with each other than with *Torpedo* AChE or human serum BChE.

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