Structure of Human Serum Cholinesterase
Oksana Lockridge

Summary
Human cholinesterase has recently been sequenced and cloned. It is a glycoprotein of 4 identical subunits, each subunit containing 9 carbohydrate chains and 3-5 disulfide bonds. Protein folding is likely to be very similar in human cholinesterase and Torpedo acetylcholinesterase. The cholinesterases have no significant sequence homology with the serine proteases and seem to belong to a separate serine esterase family.

Introduction
There are generally considered to be two types of cholinesterase: acetylcholinesterase (EC 3.1.1.7) which has the function of terminating nerve impulse transmission at cholinergic synapses, and cholinesterase or butyrylcholinesterase (EC 3.1.1.8) which has no known function. The focus of this review is on the latter enzyme which we will refer to as cholinesterase.

Cholinesterase is studied because of its clinical usefulness in predicting prolonged apnea (cessation of breathing) in response to the muscle relaxant succinylcholine. People with genetic variants of cholinesterase have an abnormal response to succinylcholine, being unable to breathe for hours after receiving a dose of succinylcholine that produces 3–10 min of apnea in the majority of the population. It is not yet known what structural alterations are present in the atypical variant or in any other genetic variant of cholinesterase. A second clinical use of cholinesterase is in the diagnosis of poisoning by organophosphate esters and carbamates of the type used in insecticides, in certain therapeutic drugs, and in nerve gas. Activity testing of the cholinesterase in serum is the most sensitive method for diagnosing exposure. These agents irreversibly inactivate both cholinesterase and acetylcholinesterase, though cholinesterase is generally inactivated more rapidly than acetylcholinesterase in red blood cells.

The purpose of this review is to summarize current knowledge regarding the structure of human cholinesterase and to compare it to the structure of acetylcholinesterase. Recent advances include determination of the amino acid sequence of human serum cholinesterase, isolation of a human cholinesterase cDNA clone and isolation of cDNA clones for Torpedo and Drosophila acetylcholinesterase.

Subunit Organization of Human Cholinesterase
The majority of the cholinesterase in human serum is a soluble, globular G4 form, having no glycolipid anchor and no collagen tail. It is a tetramer of four identical subunits. There are four active sites per molecule. The tetramer has a molecular weight of 340,000. Carbohydrates account for 23.9% of this weight. Figure 1 is a schematic diagram showing the four subunits connected by two interchain disulfide bonds. The interchain disulfide bonds involve cysteine 571 near the carboxyl terminus of each subunit. The interchain disulfide bonds are not required for the tetrameric organization of the subunits; the molecule is a tetramer even when the interchain disulfide bonds are reduced, or reduced and alkylated, or removed altogether by proteolysis.

Strong non-covalent bonds hold the 4 subunits together. The tetramer cannot be dissociated into active subunits. To generate subunits having cholinesterase activity one must use cleaving agents such as trypsin. The resulting subunits have apparent molecular weights that are indistinguishable from the molecular weight of intact subunits, though it can be shown that a small peptide has been cleaved off.

Structure of the Subunit
The complete amino-acid sequence of human serum cholinesterase was determined by Edman degradation of overlapping peptides. Later, cDNA clones were isolated. The nucleotide sequence of the cDNA confirmed that the amino-acid sequence was correct in every detail. Figure 2 is a schematic diagram of 2 subunits joined by a disulfide bond. There are 574 amino acids in each subunit. The cholinesterase subunits are identical. There are nine asparagine-linked carbohydrate chains in each subunit at Asn 17, 57, 106, 241, 256, 341, 455, 481, and 486. The molecular weight of the subunit is 85,534 of which 65,092 Da are contributed by amino acids and the remainder by carbohydrates. Each subunit contains three internal disulfide bonds between Cys 65–Cys 92, Cys 252–Cys 263, and Cys 400–Cys 519. There is one additional disulfide bond at Cys 571 linking two identical subunits via an interchain disulfide. No free sulfhydryls have been detected in cholinesterase. However, Cys 66 could be a free sulfhydryl that is inaccessible to reaction with iodoacetamide because of steric block by the adjacent disulfide bond. It is also possible that Cys 66 is not free but is bound to a small molecule.

The Active Site
When cholinesterase is exposed to diisopropyl fluorophosphate, the enzyme loses all activity, and a single amino acid becomes alkylated. The alkylated amino acid is the active site serine located 198 amino acids from the N-terminal. To date, only Ser 198 is known to belong to the active site. Other amino acids that participate in catalysis or substrate binding are specu-
REVIEW ARTICLES

Disulfide bonds are between Cys 65-92, 252-263, and 400-519. The nine carbohydrate chains (CHO) are attached to asparagine. The active site is serine 198. The carboxyl terminus is at residue 574. Carbohydrates contribute 23.9% to the molecular weight, which is 85534 per glycosylated subunit.

The amino-acid sequence of human cholinesterase. The configuration shown is similar to that in ref. 18 for Torpedo californica acetylcholinesterase. Carbohydrate chains are indicated by CHO; the active site serine 198 by OH; the potential free sulfhydryl, Cys 66, by SH. Asp 105, His 423, or His 438 may be part of the catalytic triad, Asp-His-Ser, of the serine proteases. Trypsin and chymotrypsin may be in the hydrophobic-binding pocket.

The anionic site is thought to be a negatively charged amino acid, probably aspartic or glutamic acid, which binds the positively charged choline portion of the ester substrate. Atypical cholinesterase is normal in every respect except in its binding affinity for positively charged substrates. This suggests that atypical cholinesterase probably contains a single amino acid mutation and that the location of this mutation is at the anionic site.

One approach to identifying amino acids in the active site is to compare the amino acid sequences of the one cholinesterase and three acetylcholinesterases which have been sequenced to date, and to assume that residues involved in catalysis must be conserved. This approach identifies His 423 and His 438 as candidates for His of the catalytic triad. Out of the six conserved aspartic acids, only Asp 91 is located in a position similar to Asp 105 of the catalytic triad of chymotrypsin. On this basis, Asp 91 is a candidate for belonging to the catalytic triad of cholinesterase.

Figure 3 shows a folding scheme for cholinesterase derived from a figure by MacPhee-Quigley et al.19 for Torpedo acetylcholinesterase. The spatial relationships suggested by Figure 3 are speculative. The locations of Ser 198, Asp 91, His 423, and His 438, are indicated.

The Cholinesterase Gene

The cDNA for human cholinesterase was isolated independently by two laboratories. Both laboratories used oligonucleotide probes to isolate cholinesterase clones. Prody et al. found one clone in fetal brain and an overlapping clone in fetal liver. McTiernan et al. isolated overlapping clones from a newborn brain. The nucleotide sequences from the two laboratories were nearly identical. The difference in developmental age of the tissues from which the mRNA was prepared, and the difference in tissue of origin, did not affect the nucleotide sequence. Furthermore, the coding sequence of the clones corresponded exactly to the known amino-acid sequence of adult serum cholinesterase. It can be concluded that cholinesterase in human embryonic and...
adult tissues has the same amino-acid sequence.

The amino-acid sequence deduced from the brain cDNA and from the liver cDNA exactly matched the amino acid sequence of the cholinesterase in serum. This leads to the conclusion that the cholinesterase in three different human tissues, brain, liver, and serum is identical.

The origin of the cholinesterase in serum is considered to be the liver. Recent supporting evidence for this is the demonstration that liver transplantation altered the genetic identity of the patient's serum cholinesterase from a typical to usual cholinesterase. The fact that Prody et al. isolated a cDNA clone from a liver cDNA library confirms the presence of cholinesterase mRNA in liver and supports the likelihood that the cholinesterase in serum is made by liver.

Another conclusion from the cloning work is that there is only one gene or very few genes for cholinesterase. Southern blots suggest a minimum size for the cholinesterase gene of 17–20 kb. When blots of human genomic DNA were hybridized with human cholinesterase cDNA, no evidence of hybridization to a second gene, for example to the gene for acetylcholinesterase, was obtained. Therefore, the gene for human acetylcholinesterase may be less than 60% identical with the gene for human cholinesterase.

Human cholinesterase is on chromosome 3. This result was deduced from the linkage of genetic variants of cholinesterase with transferrin, and the known location of transferrin on the long arm of chromosome 3 in region 3q21–25. The linkage group includes transferrin, cholinesterase, ceruloplasmin, and α-2HS glycoprotein. This location was confirmed by Soreq et al. who showed that cholinesterase cDNA hybridized to chromosome 3. A second hybridization site was found by Soreq et al. on chromosome 16.

Comparison of Cholinesterase and Acetylcholinesterase

Acetylcholinesterase exists in several molecular forms. Fetal bovine serum acetylcholinesterase is a soluble, globular G4 tetramer similar to the structure in Figure 1. Other structural forms are: (1) human red blood cell acetylcholinesterase, which is a hydrophobic, globular G2 dimer with a glycolipid anchor at its carboxyl terminus; (2) human and bovine caudate nucleus acetylcholinesterase, which are hydrophobic, globular G4 tetramers with a hydrophobic anchor; and (3) the asymmetric, collagen-tailed acetylcholinesterase in mammalian muscle. All of these molecular forms are likely to be present in cholinesterase as well as acetylcholinesterase, though they have not been studied in detail in cholinesterase. The various structural forms occur in specific tissues.

Comparisons of amino acid sequences are possible only with acetylcholinesterase of fish and fly, as no mammalian acetylcholinesterase has yet been sequenced or cloned. Acetylcholinesterase from the electric organ of Torpedo fish has 575 amino acids per subunit, a number almost identical to the 574 amino acids of human cholinesterase. The two sequences contain 309 identical amino acids located in exactly the same positions. This sequence identity of 53.8% is between subunits derived from asymmetric acetylcholinesterase and subunits from soluble, globular G4 cholinesterase.

There are three internal disulfide bonds in Torpedo acetylcholinesterase and these are in precisely the same locations as in human cholinesterase. The three disulfide loops contain 27, 11, and 119 amino acids in both Torpedo and human enzymes. Torpedo acetylcholinesterase has an interchain disulfide near its carboxyl terminus at Cys 572, a location similar to the interchain disulfide of human cholinesterase at Cys 571. The active site serine is Ser 200 in Torpedo and Ser 198 in human enzymes.

Torpedo acetylcholinesterase has an interchain disulfide near its carboxyl terminus at Cys 572, a location similar to the interchain disulfide of human cholinesterase at Cys 571. The active site serine is Ser 200 in Torpedo and Ser 198 in human cholinesterase.

Their hydropathy profiles are highly similar. These results suggest that protein folding in Torpedo acetylcholinesterase and human cholinesterase is highly similar.

One significant difference in their structure is that Torpedo acetylcholinesterase has four asparagine-linked carbohydrate chains, while human cholinesterase has nine. Carbohydrate chains are located at the surface of a molecule and are not expected to affect protein folding, though they may affect antibody recognition and the net charge of the protein. Antibodies to acetylcholinesterase do not recognize cholinesterase, and vice versa, even when both antibodies are for purified human enzymes.

REFERENCES

REVIEW ARTICLES

Submit: existence of multiple 5'-untranslated regions. 


WHAT’S NEW/SCIENCE AND SOCIETY

BioEssays welcomes contributions for the Features columns ‘What’s New’ and ‘Science and Society’. Articles run in ‘What’s New’ describe recent technical advances in molecular and cellular biology of potentially widespread interest. ‘Science and Society’ features discuss the current ethical, social and legal issues raised by the new biology. Contributions from readers for either of these feature columns will be considered. Articles should be submitted to the Staff Editor, Dr Adam S. Wilkins, Cambridge University Press, Edinburgh Building, Shaftesbury Rd., Cambridge CB2 2RU, United Kingdom.