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Amino Acid Sequence of the Active Site of Human Serum Cholinesterase from Usual, Atypical, and Atypical–Silent Genotypes

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Active-site tryptic peptides were isolated from three genetic types of human serum cholinesterase. The active-site peptide was identified by labeling the active-site serine with [^3H]diisopropylfluorophosphate. Peptides were purified by high-performance liquid chromatography. Amino acid composition and sequence analysis showed that the peptide from the usual genotype contained 29 residues with the sequence Ser-Val-Thr-Leu-Phe-Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser-Val-Ser-Leu-His-Leu-Leu-Ser-Pro-Gly-Ser-His-Ser-Leu-Phe-Thr-Arg. The active-site serine was the eighth residue from the N- terminal. The peptide containing the active-site serine from the atypical genotype contained 22 residues with the sequence Ser-Val-Thr-Leu-Phe-Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser-Val-Ser-Leu-His-Leu-Leu-Ser-Pro-Gly. The peptide from the atypical-silent genotype contained eight residues with the sequence Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser. Thus, the sequences of the atypical and atypical–silent active-site peptides were identical to the corresponding portions of the usual peptide.

KEY WORDS: cholinesterase; active-site sequence.

INTRODUCTION

Human serum cholinesterase (EC 3.1.1.8; acylcholine acyl hydrolase) is also known as pseudocholinesterase, butyrylcholinesterase, and nonspecific cho-

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linesterase. The genetic variants of human serum cholinesterase were discovered after the drug, succinylcholine, gained widespread use as a muscle relaxant. It was observed that 1 in approximately 2000 patients was unable to breathe for prolonged periods, even hours, after receiving a normal dose of succinylcholine (Kalow and Gunn, 1959). Kalow provided an explanation for this abnormal response by showing that it occurred in persons having an "atypical" form of cholinesterase (Kalow and Gunn, 1957) characterized by a reduced affinity for succinylcholine (Hersh *et al.*, 1974). Later, additional genetic variants were identified. One of these was the "silent" variant which had zero or nearly zero activity (Liddell *et al.*, 1962).

The properties of the atypical cholinesterase variant could be explained by a single amino acid alteration at the anionic site (Kalow and Davies, 1958; Muensch *et al.*, 1978; Lockridge and La Du, 1978). However, to date this has not been established and no amino acid sequence information for any portion of the atypical variant has been published. For the usual cholinesterase the total amino acid sequence information published to date is an 11-residue fragment from the active site (Yamato *et al.*, 1983). Yamato *et al.* suggested that the single amino acid alteration in atypical cholinesterase was a substitution from Glu to His within their 11-residue active-site peptide. The basis for their suggestion was the difference in electrophoretic mobility of the active site peptides obtained from the usual and atypical cholinesterases. We also found that the usual and atypical active-site peptides had different physical properties, since they eluted at different positions on HPLC[3] and had different solubilities. However, we disagree with Yamato's interpretation that the difference is due to an amino acid substitution within the active-site peptide. We found that the usual cholinesterase yielded a 29-residue tryptic peptide, while the atypical and atypical–silent cholinesterases yielded shorter peptides that were not the result of cleavage by trypsin. The sequences of the atypical and atypical–silent active-site peptides were identical to the region around the active-site serine of the usual form of cholinesterase.

EXPERIMENTAL PROCEDURES

Blood Samples. The source of usual cholinesterase, genotype UU, was outdated human plasma, kindly provided by Dr. Harold Gallick of the Michigan Department of Public Health, Lansing. The source of atypical cholinesterase, genotype AA, was a single female donor with a family history

[3] Abbreviations used: DFP, diisopropylfluorophosphate; MP, monoisopropylphosphoryl; SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; HFBA, heptafluorobutyric acid; PTH, phenylthiohydantoin; A, alanine; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; L, leucine; P, proline; R, arginine; S, serine; T, threonine; V, valine.

of succinylcholine apnea. From her activity of 0.53 μmol benzoylcholine hydrolyzed/min/ml of serum (Kalow and Lindsay, 1955), her dibucaine number of 24 (Kalow and Genest, 1957), and her fluoride number of 37 (Harris and Whittaker, 1961), it was concluded that she had the AA genotype. Her AA genotype was confirmed by genotyping three generations of family members. Our AA donor underwent plasmaphoresis over a period of 2 years, until 3.5 liters of plasma had accumulated. Plasma was stored at −20°C. The source of atypical–silent cholinesterase, genotype AS, was a single male donor who experienced 3hr of apnea after receiving 120 mg of succinylcholine. He had the characteristic dibucaine number (DN 18), fluoride number (FN 24), and activity (0.25 μmol benzoylcholine/min/ml serum) of the atypical phenotype. Genotyping of three generations of family members revealed that he was heterozygous for atypical–silent cholinesterase. Nine and four-tenths liters of his plasma was collected by plasmaphoresis over a period of 3 years.

Enzyme Purification. The usual cholinesterase was purified by ion-exchange chromatography at *p*H 4 on Whatman DE52, followed by ion-exchange chromatography at *p*H 7, and finally by affinity chromatography on procainamide Sepharose 4B (Lockridge and La Du, 1978). The second ion-exchange step could be omitted when the purification on the *p*H 4 ion-exchange column was 1200-fold or greater. This degree of purification was not always achieved, and therefore the second ion-exchange step was included. The procainamide step yielded pure enzyme only if the applied enzyme was already at least 1200-fold pure. A total of 100 liters of plasma was used. Seven and one-half liters of plasma was processed at one time, with a yield of 8 to 12 mg of cholinesterase. On SDS gel electrophoresis the purified usual cholinesterase had a major band with a molecular weight of 90,000. The only other band visible with either silver staining or Coomassie blue staining was a faint dimer band with a molecular weight of 180,000. The atypical and atypical–silent cholinesterases were purified by similar strategies.

Labeling the Active Site. Three to 64 mg of cholinesterase was treated with 2 equivalents of [^{3}H]DFP (5.2 Ci/mmol). This amount of DFP brought the enzyme activity to zero. The reaction mixture was incubated for 4 days or more to achieve "aging." During aging the diisopropylphosphate derivative is catalytically converted to the more stable monoisopropylphosphate derivative (Berends *et al.*, 1959). It proved advantageous to have the monoisopropyl-phosphate derivative because this gave a higher yield of active-site tryptic peptide, and it gave a single radioactive peptide.

Reduction and Alkylation with Iodoacetamide. The aged MP-cholinesterase was concentrated to 1 to 5 mg/ml and placed inside a glass screw-cap tube. Solid guanidine-HCl (Schwartz–Mann Co., ultrapure, or Pierce Co.,

sequanal grade) was added to a final concentration of 6 M, the *p*H adjusted to 8.1 with 1 M unneutralized Trizma base, and EDTA added to 4 mM. A mininert valve (Pierce Co.) was screwed on the tube and nitrogen bubbled into the solution for 1 hr. A freshly prepared solution of 0.7 M dithiothreitol was injected through the valve to a final concentration of 4 mM dithiothreitol. Nitrogen was bubbled into the solution for an additional 3-hr period. A freshly prepared solution of 0.7 M iodoacetamide in water was injected through the valve to give a 10% excess of iodoacetamide over total dithiothreitol and protein sulfhydryls. The glass tube was wrapped in aluminum foil to exclude light and the reaction allowed to proceed for 1 hr while continuing to bubble nitrogen. The reaction was terminated by dialysis which was begun immediately. It was important to maintain oxygen-free conditions throughout the reduction and alkylation procedure to avoid problems later during digestion with trypsin. One sample was alkylated with vinylpyridine (Tarr, 1985) rather than iodoacetamide, but this was not helpful because it made the protein precipitate during digestion. Iodoacetic acid could be used in place of iodoacetamide with equally good results.

Trypsin Digestion. The dialyzed protein in 0.1 M Tris–Cl, *p*H 8.1, was digested with trypsin/L-1-tosylamido-2-phenylethyl chloromethyl ketone (Worthington Co.) at 37°C for 4 hr. The amount of trypsin was 2% of the weight of cholinesterase. A single addition of trypsin was sufficient to achieve complete digestion. In contrast to the report of Muensch *et al.* (1978) we did not need to digest longer than 4 hr or make multiple additions of trypsin, nor did the sugar chains of cholinesterase present a problem. We believe that these differences are due to the fact that we achieved complete reduction of disulfides and alkylation of reduced sulfhydryls with our strict anaerobic conditions. This made the protein available to trypsin digestion. Cholinesterase with partly intact disulfide bridges could resemble native cholinesterase, which is known to be extremely resistant to trypsin (Lockridge and La Du, 1982), probably because of its 24% carbohydrate content (Haupt *et al.*, 1966).

HPLC to Purify MP-Tryptic Peptide. A Varian HPLC, Model 5060, was used with UV and fluorescence detectors. The MP-tryptic peptide was not fluorescent but it had to be separated from a fluorescent contaminating peptide. Fluorescence excitation passed through an interference filter which had a maximum of 220 nm. Emission passed through a Corning 7-60 glass band filter which included wavelengths 300 to 400 nm, with a maximum at 352 nm. MP-peptide from the usual genotype, UU, was purified as follows: (1) HPLC on a Synchropak RP-P column (Synchrom Co., Linden, Ind.) eluted with a gradient of 0.1% TFA versus acetonitrile containing 0.075% TFA as in Fig. 1A—65% of the applied counts were recovered; (2) HPLC on a

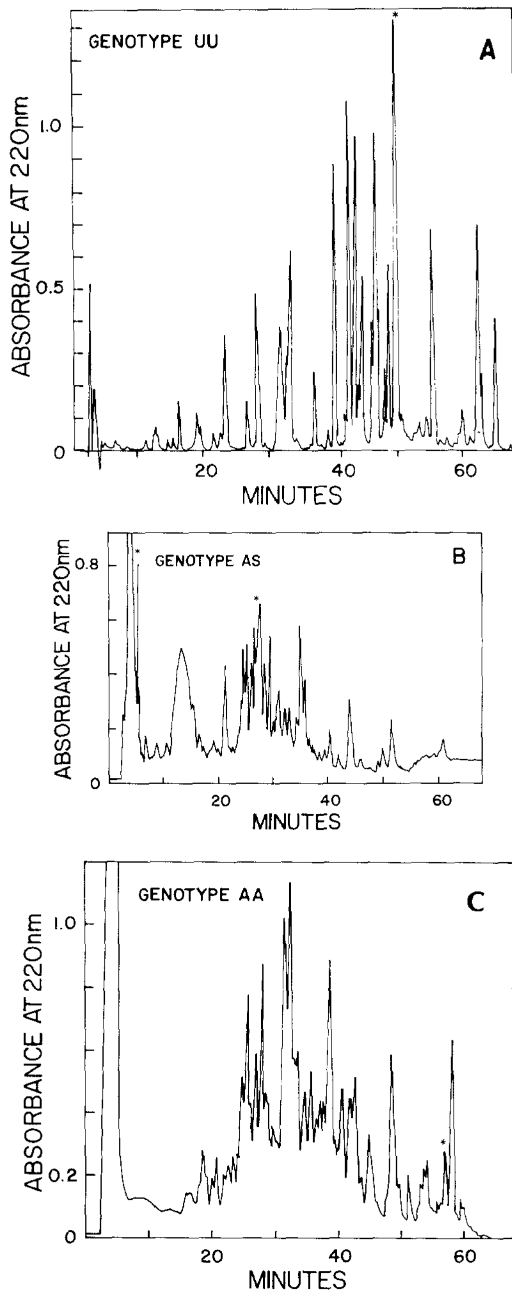

Fig. 1. Isolation of the DFP-labeled tryptic peptide from cholinesterases of various genotypes. A 1.0-ml portion (5 to 10 nmol) of the 4-hr tryptic digest of the [^{3}H]DFP-labeled, *S*-carboxyamidomethylated cholinesterase was applied to the Synchropak RP-P column (0.41 × 25 cm) equilibrated with solvent A (0.1% TFA in water). Peptides were eluted with a linear gradient increasing in solvent B (acetonitrile containing 0.075% TFA) at a gradient rate of 0.75%/min. The flow rate was 1 ml/min. Radioactivity is indicated by asterisks. (A) Genotype UU; (B) genotype AS; (C) genotype AA. Protein sylfhydryls in C were alkylated with vinylpyridine, and the protein was digested with trypsin and *S. aureus* protease.

Synchropak RP-P column eluted with a gradient of 10 mM Na/K phosphate, *p*H 7.4, versus methanol—62% of the applied counts were recovered; and (3) HPLC on a Synchropak RP-P column eluted with a gradient of 0.1% HFBA versus acetonitrile—97% of the applied counts were recovered. It was essential to use a neutral *p*H buffer to achieve separation from a fluorescent peptide. The third HPLC step was for desalting and also for additional purification. The overall yield was 39%.

The MP-peptide from the atypical–silent genotype (Fig. 1B) was purified with the same three solvent systems as the usual cholinesterase peptide. The recoveries of applied counts were 34, 48, and 93%, with an overall yield of 15%. The first HPLC step actually yielded 82% of the applied counts but most of these eluted at the solvent front and only 34% eluted with the MP-peptide at 27 min.

Conditions for purifying the atypical, AA, MP-peptide were changed because this peptide was found to have different solubility properties. It eluted in a broad peak over 30 min when 10 mM Na/K phosphate, *p*H 7.4, was used in a gradient with acetonitrile and, furthermore, could not be redissolved in TFA after it had been dried in the presence of a large amount of phosphate salts. In contrast, the usual MP-tryptic peptide eluted in a sharp peak with this same solvent system and was easily dissolved in TFA. The major change in HPLC conditions was the use of 0.1 M hexafluoroacetone-ammonia, *p*H 7.1, in place of 10 mM Na/K phosphate, *p*H 7.4. Hexafluoroacetone (Aldrich Co.) has the advantages that it is volatile and transparent at 220 nm (Tarr and Crabb, 1983) but has the disadvantages that it is toxic and expensive. The atypical (AA) MP-peptide was purified as follows: (1) HPLC on a Synchropak RP-P column eluted with a gradient of 0.1% TFA versus acetonitrile containing 0.075% TFA—recovery of the applied counts was 18%; (2) HPLC on a μbondapak CN column (Waters Co.) eluted with 0.1% HFBA versus acetonitrile:1-propanol (3:1)—recovery of the applied counts was 62%; and (3) HPLC on a μbondapak CN column eluted with 0.1 M hexafluoroacetone–ammonia, *p*H 7.1, versus acetonitrile:1-propanol (3:1)—recovery of the applied counts was 55%. The overall yield was 6%. The low yield in step 1 was due to the use of vinylpyridine to alkylate sulfhydryls. The vinylpyridine-derivatized protein precipitated during digestion, although it was in solution in 0.1 M Tris–Cl, *p*H 8.1, at the start of digestion.

Amino Acid Analysis. Two commercial laboratories measured the amino acid composition of peptides. They were the AAA Labs, Mercer Island, Wash., under the direction of Dr. Lowell Ericsson, and the University of Michigan Sequencing Facility, Ann Arbor, under the direction of Dr. George Tarr. Salt-free peptides were hydrolyzed for 24 hr at 110°C in HCl. Amino acids were quantitated by ninhydrin at the AAA Labs or by derivatization

with phenylisothiocyanate at the University of Michigan. The latter method was developed by Tarr (1985) and is now marketed by the Waters Co. as the "pico-tag" method. The pico-tag method was used for analyses where the amount of sample was less than 1 nmol.

Manual Sequencing. Salt-free peptides were sequenced by Edman degradation using the manual batch method of Tarr (1982). PTH-amino acids were identified by HPLC by the method of Black and Coon (1982), in which one analysis cycle is completed every 16 min.

RESULTS

HPLC traces of tryptic digests of cholinesterase are shown in Fig. 1, where the MP-tryptic peptide is indicated by radioactive counts. In Fig. 1A the MP-tryptic peptide from the usual genotype eluted at 49 min. A single radioactive peak was obtained. In Fig. 1B the MP-tryptic peptide from the atypical–silent genotype eluted at 26 min. A second radioactive peak eluted at the solvent front. This second radioactive peak was not studied because it was thought to be monoisopropylphosphate released from the preparation during 2 years of storage at −7°C, or a very short peptide. In Fig. 1C the MP-tryptic peptide from the atypical, AA, genotype eluted at 54–59 min.

The MP-tryptic peptides were purified by two additional HPLC steps. Table I shows the amino acid composition analysis of the usual (UU), atypical

Table I. Amino Acid Composition of DFP-Labeled Tryptic Peptides Isolated from Usual (UU), Atypical (AA), and Atypical–silent (AS) Variants of Cholinesterase

Amino acid	UU[a,b]	UU[c]	AA[c]	AS[c]
Ala	3.1 (3)	2.6 (3)	3.1 (3)	3.0 (3)
Arg	1.0 (1)	0.6 (1)	0 (0)	
Glu	1.2 (1)	1.6 (1)	0.1 (1)	0.9 (1)
Gly	3.2 (3)	3.6 (3)	3.0 (3)	2.8 (2)
His	2.1 (2)	1.4 (2)	0.7 (1)	
Leu	5.1 (5)	5.0 (5)	3.9 (4)	
Phe	2.1 (2)	2.0 (2)	1.3 (1)	
Pro	1.0 (1)	1.0 (1)	1.7 (1)	
Ser	7.0 (7)	7.2 (7)	3.8 (5)	1.7 (2)
Thr	2.3 (2)	1.4 (2)	0.7 (1)	
Val	2.2 (2)	1.9 (2)	2.1 (2)	
Total residues	29	29	22	8
nmol analyzed	5.0	0.25	0.58	0.45

[a]Values in parentheses were determined from sequence analysis.
[b]Method of detection was ninhydrin.
[c]Method of detection was "pico-tag."

(AA), and atypical–silent (AS) MP-tryptic peptides. The peptide from the usual cholinesterase contained 29 residues, atypical contained 22 residues, and atypical–silent contained 8 residues.

Manual sequence analysis of the usual peptide (Table II) yielded data for the first 19 residues. Half of each PTH-amino acid sample was counted, and the radioactive counts showed that DFP was attached to serine at the eighth position from the N terminal. The radioactive label was not cleaved off during Edman degradation to a significant extent. To obtain the complete sequence, the MP-tryptic peptide was subfragmented. The peptide was digested with chymotrypsin and the subfragments were separated on a C18 μbondapak (Waters Co.) column. Sequencing results are in Table III. The data in Table III provided important overlapping peptides which aligned the subfragments obtained in the following digestion with pepsin. The MP-tryptic peptide was subfragmented with pepsin and the subfragments were separated as shown in Fig. 2. Sequencing results are given in Table IV. Subfragment P6 was analyzed for amino acid composition (Table V) because it contained a particularly difficult sequence, Ser-His-Ser. The data established that the sequence for the usual genotype was Ser-Val-Thr-Leu-Phe-Gly-Glu-Ser-

Table II. Manual Sequence Analysis of the DFP-Labeled Tryptic Peptide Isolated from Usual Cholinesterase[a]

Edman cycle	Assigned amino acid	Yield of PTH amino acid (pmol)	cpm[b]
1	Ser	459	8,085
2	Val	1742	6,842
3	Thr	660	16,985
4	Leu	727	9,939
5	Phe	1828	7,300
6	Gly	200	6,176
7	Glu	440	10,566
8	Ser-[^{3}H]MP	—	568,188
9	Ala	329	150,204
10	Gly	806	65,133
11	Ala	317	27,830
12	Ala	181	10,419
13	Ser	—	19,927
14	Val	257	2,471
15	Ser	—	7,001
16	Leu	87	
17	His	148	
18	Leu	50	
19	Leu	49	

[a]This peptide was sequenced four times, using 27, 10, 8, and 8 nmol peptide.
[b]Radioactivity, expressed as counts per minute (cpm), was determined for each PTH-amino acid by liquid scintillation counting.

Table III. Manual Sequence Analysis of Chymotryptic Subfragments of the Usual DFP-Labeled Tryptic Peptide[a]

Edman cycle								
1	S 2073	G 2143	G 1907	A 1230	H 3415	L 1090	L 6127	S 6267
2	V 2615	E 2691	E 2067	S 1453	L 5738	S 1347	L 6780	L 21976
3	T 4063	S 453	S-^{3}H-DFP	V 728	L 5454	P 775	S 5364	F 7256
4	L 2167	A 71	A 987	S 102	S 297	G 176	P 1744	
5	F 1973	G 150	G 539			S 76	G 617	
6			A 549				S 389	
7			A 710				H 440	
8			S 30					
9			V 363					
10			S 148					
11			L 194					
No. of times sequenced	1	2	2	1	1	1	1	1

[a]The PTH-amino acid observed by HPLC analysis for each Edman degradation cycle is shown. The number that follows each residue is the yield in picomoles.

Ala-Gly-Ala-Ala-Ser-Val-Ser-Leu-His-Leu-Leu-Ser-Pro-Gly-Ser-His-Ser-Leu-Phe-Thr-Arg.

Sequence analysis (Table VI) of the MP-tryptic peptide isolated from the atypical–silent cholinesterase in Fig. 1B showed that the sequence was Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser. Table VI shows that DFP is attached to the serine at cycle 3.

The atypical, AA, MP-peptide was subfragmented by digestion with pepsin. The sequence results are given in Table VII. The atypical sequence was Ser-Val-Thr-Leu-Phe-Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser-Val-Ser-Leu-His-Leu-Leu-Ser-Pro-Gly.

DISCUSSION

We have isolated and sequenced the DFP-labeled tryptic peptides from three genetic types of human serum cholinesterase: the usual (UU), atypical (AA), and atypical–silent (AS). Their sequences are compared in Table VIII. The usual cholinesterase yielded a 29-residue peptide which is probably the complete tryptic peptide since it terminated in arginine. The 22 residues of atypical and 8 residues of atypical–silent exactly matched portions of the usual peptide. There were no amino acid substitutions. Of special note was the presence of glutamic acid in position 7 from the N terminal in all three sequences. This is of interest because Yamato *et al.* (1983) had suggested that atypical cholinesterase contained histidine in place of glutamic acid at this

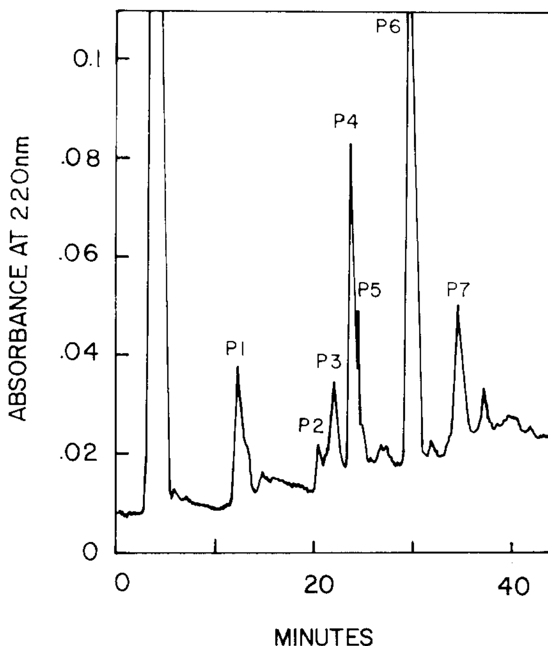

Fig. 2. HPLC of pepsin subfragments of the DFP-labeled tryptic peptide isolated from genotype UU cholinesterase. The MP-tryptic peptide, containing 26.9 nmol and 15,200,000 cpm, was dissolved in 10 μl of 88% formic acid and then diluted with 200 μl of water. Nineteen microliters of a freshly prepared pepsin solution, 1 mg/ml, was added. Digestion was for 3 hr, 45 min at room temperature. The entire sample was injected into a C18 Waters column equilibrated with solvent A (0.1% TFA in water). Peptides were eluted with a linear gradient increasing in solvent B (acetonitrile containing 0.075% TFA) at a gradient rate of 0.75%/min. The flow rate was 1 ml/min. Seventy-four percent of the applied counts were recovered.

Table IV. Manual Sequence Analysis of the Pepsin Subfragments of the Usual DFP-Labeled Tryptic Peptide[a]

Edman cycle	P3	P4	P2	P6	P1
1	Ser 1790	Phe 1075	Val 1376	His 694	Phe 1058
2	Val 2334	Gly 928	Ser 355	Leu 1024	Thr 253
3	Thr 1086	Glu 877	Leu 695	Leu 1081	Arg 372
4	Leu 2511	Ser-[^{3}H]DFP		Ser 571	
5		Ala 1724		Pro 772	
6		Gly 589		Gly 510	
7		Ala 725		Ser 203	
8		Ala 586		His 111	
9		Ser 247		Ser 140	
10				Leu 201	
No. of times sequenced	2	7	3	3	4

[a]The PTH-amino acid observed by HPLC analysis for each Edman degradation cycle is shown. The number that follows each residue is the yield in picomoles.

Table V. Amino Acid Composition of Pepsin Subfragment P6 of the Usual DFP-Labeled Tryptic Peptide

Amino acid	P6[a]
Gly	1.2 (1)[b]
His	1.7 (2)
Leu	3.0 (3)
Pro	1.1 (1)
Ser	3.5 (3)
Total residues	10
nmol analyzed	0.83

[a]Method of detection was "pico-tag."
[b]Values in parentheses were determined from sequence analysis.

position. The sequence obtained by Yamato *et al.* (1983) for the active site of usual human serum cholinesterase was Gly-Glu-Ser-Ala-Gly-Ala-Ser-Ala-Val-Ser-Leu. In our sequence the order of Ser and Ala in positions 7 and 8 is reversed.

The differences in the lengths of the peptides can probably be attributed to protease or peptidase contaminants in the AS and AA cholinesterase preparations. Contaminants are suspected because the AS and AA peptides were not the result of cleavage by trypsin since they did not terminate in Arg or Lys. The identity of the protease contaminant is unknown. Nausch and Heymann (1985) demonstrated that dipeptidylaminopeptidase IV can copurify with cholinesterase. Since this enzyme cleaves two amino acids at a time from the N terminal, other contaminants must be responsible for the missing amino acids. Five amino acids are presumed missing from the N terminal of AS. Seven and 16 amino acids are presumed missing from the C terminals.

Table VI. Manual Sequence Analysis of the DFP-Labeled Tryptic Peptide Isolated From Atypical–Silent Cholinesterase[a]

Edman cycle	Assigned amino acid	Yield of PTH amino acid (pmol)	cpm[b]
1	Gly	100	6,608
2	Glu	90	4,185
3	Ser-[^{3}H]MP		22,966
4	Ala	92	14,841
5	Gly	70	7,483
6	Ala	67	3,595
7	Ala	60	
8	Ser	224	

[a]This peptide was sequenced three times, using 122, 100, and 75 pmol peptide.
[b]Radioactivity, expressed as counts per minute (cpm), was determined for each PTH-amino acid by liquid scintillation counting.

Table VII. Manual Sequence Analysis of the Pepsin Subfragments of the Atypical DFP-Labeled Tryptic Peptide[a]

Edman cycle				
1	Ser 507	Phe 1111	Val 848	His 192
2	Val 1536	Gly 668	Ser 264	Leu 340
3	Thr 502	Glu 693	Leu 419	Leu 355
4	Leu 938	Ser-[^{3}H]MP		Ser 45
5		Ala 488		Pro 244
6		Gly 448		Gly 196
7		Ala 335		
8		Ala 403		
9		Ser 191		
No. of times sequenced	1	2	2	2

[a]The PTH-amino acid observed by HPLC analysis for each Edman degradation cycle is shown. The number that follows each residue is the yield in picomoles.

As the complete tryptic peptide was not obtained from the atypical variant, one might argue that an amino acid substitution might be present in the missing seven residues. Our work does not rule out this possibility, but neither does it support it. Our results explain how DFP-labeled peptides from atypical and usual cholinesterases can have different solubilities, charges, and mobilities and still contain no amino acid substitutions. This leaves no reason for expecting the location of an amino acid alteration to be in the DFP-labeled peptide of atypical cholinesterase, as opposed to any other region in the 580 amino acid subunit. The mutation might be anywhere in the linear sequence, although in a three dimensional projection we expect it to be approximately 5 Å from the active-site serine. This expectation is based on the estimated distance between the anionic-site and the active-site serine (Wilson and Quan, 1958).

The atypical–silent cholinesterase molecule contains two genetically different subunits. It contains atypical subunits and silent subunits. It is likely that the DFP-labeled peptide from our AS sample originated from atypical

Table VIII. Active-Site Sequences for Usual, Atypical, and Atypical–Silent Human Cholinesterase Genotypes

Genotype	Sequence[a]
	* (above S of GESAG)
Usual	SVTLFGESAGAASVSLHLLSPGSHSLFTR
Atypical	SVTLFGESAGAASVSLHLLSPG
Atypical–silent	GESAGAAS

[a]The * denotes site of DFP binding.

subunits alone. Evidence for this conclusion comes from the stoichiometry of DFP binding which was calculated for our DFP-labeled, aged, cholinesterase preparations. Two AS samples bound 0.31 and 0.33 mol of DFP/mol of subunit. Two UU samples bound 0.80 and 0.76 mol of DFP/mol of subunit. The label had been aged so one would expect less than one equivalent bound per subunit. The significantly lower stoichiometry for DFP binding in the AS samples, compared to the UU samples, suggests that the silent subunits did not bind DFP. This is not surprising since silent cholinesterase from some donors has zero activity (Liddell *et al.*, 1962). Silent cholinesterase from other donors has been reported to have 1 to 3% of normal activity (Altland and Goedde, 1970; Rubinstein *et al.*, 1970) and therefore may be expected to bind DFP.

The single DFP-labeled peptide we observed is consistent with other evidence (Lockridge *et al.*, 1979) that the four subunits of cholinesterase are identical.

The active-site peptide is highly unusual in its high content of serine and threonine, which total 9 residues of 29. Since PTH-serine and PTH-threonine were obtained in a low yield from Edman degradation, the active-site peptide was difficult to sequence and had to be subfragmented to get the complete sequence.

The active-site tryptic peptide of acetylcholinesterase isolated from the electric organ of *Torpedo californica* is Thr-Val-Thr-Ile-Phe-Gly-Glu-Ser-Ala-Gly-Gly-Ala-Ser-Val-Gly-Met-His-Ile-Leu-Ser-Pro-Gly-Ser-Arg (MacPhee-Quigley *et al.*, 1985). There is a remarkable degree of homology with the human serum cholinesterase active-site peptide, as 17 of 24 residues are identical, and 3 are conservative substitutions. The active-site peptide from horse serum cholinesterase (Jansz *et al.*, 1959), Phe-Gly-Glu-Ser-Ala-Gly-(Ala-Ala-Ser), is identical to a portion of the peptide from human serum cholinesterase.

The results reported here provide the longest sequence information available to date for a cholinesterase of human origin. This should help to distinguish human butyrylcholinesterase from acetylcholinesterase when these are isolated as DNA clones and should, therefore, be useful to the cloning work already in progress in various laboratories.

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