Eur. J. Biochem. *162*, 191 – 197 (1987)

A peptidase activity exhibited by human serum pseudocholinesterase

Rathanam BOOPATHY and Aiylam S. BALASUBRAMANIAN

Neurochemistry Laboratory, Department of Neurological Sciences, Christian Medical College and Hospital, Vellore

(Received July 15/October 3, 1986) – EJB 86 0757

The identity of a peptidase activity with human serum pseudocholinesterase (PsChE) purified to apparent homogeneity was demonstrated by (a) co-elution of both peptidase and PsChE activities from procainamide-Sepharose and concanavalin-A – Sepharose affinity chromatographic columns; (b) comigration on polyacrylamide gel electrophoresis; (c) co-elution on Sephadex G-200 gel filtration and (d) coprecipitation at different dilutions of an antibody raised against purified PsChE. The purified enzyme showed a single protein band on gel electrophoresis under non-denaturing conditions. SDS gel electrophoresis under reducing conditions, followed by silver staining, also gave a single protein band ($M_r \approx 90000$). Peptidase activity using different peptides showed the release of C-terminal amino acids. Blocking the carboxy terminal by an amide or ester group did not prevent the hydrolysis of peptides. There was no evidence for release of N-terminal amino acids.

Potent anionic or esteratic site inhibitors of PsChE, such as eserine sulphate, neostigmine, procainamide, ethopropazine, imipramine, diisopropylfluorophosphate, tetra-isopropylpyrophosphoramide and phenyl boronic acid, did not inhibit the peptidase activity. An anionic site inhibitor (neostigmine or eserine) in combination with an esteratic site inhibitor (diisopropylfluorophosphate) also did not inhibit the peptidase. However, the choline esters (acetylcholine, butyrylcholine, propionylcholine, benzoylcholine and succinylcholine) markedly inhibited the peptidase activity in parallel to PsChE. Choline alone or in combination with acetate, butyrate, propionate, benzoate or succinate did not significantly inhibit the peptidase activity. It appeared that inhibitor compounds which bind to both the anionic and esteratic sites simultaneously (like the substrate analogues choline esters) could inhibit the peptidase activity possibly through conformational changes affecting a peptidase domain.

Human serum pseudocholinesterase (PsChE) is a tetramer with a subunit M_r of 90000 [1]. It has four identical subunits, two monomers covalently linked through a single disulfide bond into a dimer and two such dimers hydrophobically linked into a tetramer [2]. It is a less complex protein than acetylcholinesterase (AChE) from several sources [3 – 6]. However, there are several features common to PsChE and AChE. PsChE exhibits a serotonin-sensitive aryl acylamidase activity similar to AChE from diverse sources [7 – 10]. It bears a limited homology in its active-site amino acid sequence to AChE and some serine proteases [11]. Based on several lines of evidence it has been speculated that the cholinesterases may have more than one function [12, 13].

The hydrolysis of substance P by purified human serum PsChE was demonstrated by Lockridge [14] and Masson [15]. The cleavage products from substance P by the action of PsChE indicated the involvement of a dipeptidyl peptidase and a deamidase-like activity [14]. Nausch and Heymann [16] showed that the dipeptidyl peptidase activity was a contaminant present in the purified PsChE and suggested that the deamidase-like activity might be associated with the serum PsChE.

In the present work we have made a systematic study to establish the identity of a peptidase activity with human serum PsChE purified to apparent homogeneity. We also describe the nature of this peptidase and its inhibition characteristics in comparison to PsChE.

MATERIALS AND METHODS

Materials

Various amino acids, peptides, *o*-phthalaldehyde, butyrylthiocholine iodide, procainamide HCl, ethopropazine, imipramine, tetra-isopropylpyrophosphoramide, phenyl boronic acid, choline and choline esters were obtained from Sigma Chemical Co. (St Louis, USA). Sephadex G-200 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals and solvents were of high grade purity or procured as described earlier [17]. Ethyl esters of peptides were prepared by bubbling dry HCl gas through the peptides (2 mg) in 0.5 ml absolute ethanol for 45 min [18]. *N*-Acetyl-[Met^5]enkephalin was prepared by acetylation of [Met^5]enkephalin with acetic anhydride in $NaHCO_3$ solution under nitrogen according to George and Balasubramanian [19]. Diisopropylfluorophosphate (Dip-F) was a kind gift from Dr K. S. Krishnan, Tata Institute of Fundamental Research, Bombay.

Correspondence to A. S. Balasubramanian, Neurochemistry Laboratory, Christian Medical College and Hospital Vellore, India 632 004

Abbreviations. PsChE, pseudocholinesterase; AChE, acetylcholinesterase; Dip-F, diisopropylfluorophosphate; SDS, sodium dodecyl sulfate.

Enzymes. Pseudocholinesterase or butyrylcholinesterase or cholinesterase or acylcholine acylhydrolase (EC 3.1.1.8); acetylcholinesterase or acetylcholine acetylhydrolase or true cholinesterase (EC 3.1.1.7).

Preparation of affinity media

Concanavalin-A – Sepharose and lysine-Sepharose were prepared by coupling concanavalin A or lysine to cyanogen-bromide-activated Sepharose 4B [20] according to methods described earlier [21, 22]. Procainamide was coupled to Sepharose through a ten-carbon spacer arm [10, 17]. Zn^{2+} and Co^{2+}-chelated iminodiacetic-acid – Sepharose was prepared as described by Porath et al. [23].

Purification of the PsChE

PsChE was purified by the procedure described earlier [17] involving the use of a procainamide-Sepharose affinity column twice. The enzyme eluted from the first procainamide-Sepharose chromatography with NaCl, after dialysis it was rechromatographed and eluted with 0.05 M procainamide in 20 mM potassium phosphate buffer, pH 7.0. The active fractions were pooled and used in further studies.

Chromatography on other affinity gels

The purified enzyme (1 ml) was applied on to a concanavalin-A – Sepharose column (4 × 0.5 cm) pre-equilibrated with 20 mM sodium acetate buffer, pH 5.0, washed with the same buffer and eluted with 0.5 M methyl α-glucoside in the equilibrating buffer. The lysine-Sepharose column (4 × 0.5 cm), equilibrated with 20 mM potassium phosphate buffer pH 7.0, was washed with the same buffer and eluted with 0.6 M NaCl in the equilibrating buffer. In the case of Zn^{2+} and Co^{2+}-chelated Sepharose columns (4 × 0.5 cm) the equilibration and washing were done with 20 mM potassium phosphate buffer, pH 7.0, and elution with 10 mM imidazole in the equilibrating buffer.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of the purified enzyme (8 μg) was carried out according to Davis [24] in Tris/glycine buffer, pH 8.3, in tubes with 7% acrylamide. After electrophoresis the gels were either stained by Coomassie brilliant blue G or assayed for enzyme activities as follows. The gels were cut into slices of 2.0 mm thickness, extracted in 0.4 ml 20 mM potassium phosphate buffer pH 7.0 overnight, dialysed against the same buffer to remove Tris/glycine and assayed for PsChE and peptidase activity using suitable aliquots. SDS gel electrophoresis of the purified enzyme under reducing and non-reducing conditions [17] was carried out in 10% acrylamide slab gels according to Laemmli [25], which were stained with silver nitrate in an alkaline medium in the presence of formaldehyde [26]. Standard marker proteins used were immunoglobulin (M_r 150000), bovine serum albumin (M_r 68000), ovalbumin (M_r 45000) and myoglobin (M_r 17800).

Sephadex G-200 gel filtration

The concentrated purified enzyme (0.5 ml) was applied on a Sephadex G-200 gel filtration column (35 × 0.7 cm) pre-equilibrated with 20 mM potassium phosphate buffer, pH 7.0, containing 0.5 M NaCl. The enzyme was eluted by the same buffer at a flow rate of 2.7 ml/h and fractions of 1 ml were collected. Each fraction, after dialysis against the phosphate buffer, was assayed for both enzyme activities.

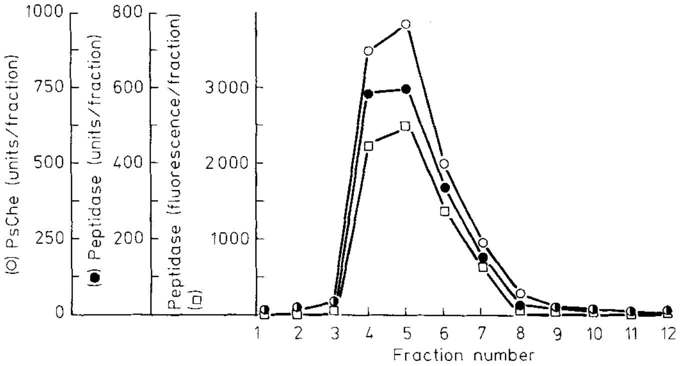

Fig. 1. *Affinity chromatographic elution profile of PsChE (○——○) and peptidase activity from the procainamide-Sepharose column by 0.05 M procainamide in buffer.* The peptidase activity was measured by the *o*-phthalaldehyde fluorimetric method using Phe-Leu (●——●) and [Leu⁵]enkephalin (□——□) as substrate. Details of the chromatography are described in Materials and Methods

Immunological studies

Anti-PsChE serum was raised in a rabbit and purified by DEAE-cellulose chromatography as described earlier [10]. The purified antibody was free of any detectable PsChE or peptidase activity. Immunoprecipitation was done by cross-reacting different dilutions (0.03 – 0.5 mg) of antibody with the purified enzyme (0.004 mg) in 0.4 ml 20 mM potassium phosphate buffer, pH 7.2, followed by incubation at 4°C overnight. After centrifugation of the mixture at 10000 × *g* for 30 min the PsChE and peptidase activities were determined in the supernatant.

Enzyme assay and protein estimation

PsChE activity was assayed with butyrylthiocholine iodide as substrate according to Ellman et al. [27]. 1 unit of PsChE activity was defined as the absorbance change of 1/min at 412 nm under standard assay conditions. The peptidase activity was assayed in 50 mM potassium phosphate buffer, pH 7.2, using 400 μM of either Phe-Leu or [Leu^5]enkephalin as substrate and 0.1 ml enzyme (4 μg) in a total volume of 0.2 ml reaction mixture. After incubation at 37°C for different time periods of up to 6 h the reaction mixture was heated in a boiling water bath for 1 min and the peptidase activity was measured by the released amino acids as determined by the *o*-phthalaldehyde fluorimetric method [28]. Briefly, to 0.05 – 0.1 ml reaction mixture, 0.3 ml *o*-phthalaldehyde reagent (2 mg *o*-phthalaldehyde/0.2 ml ethanol mixed with 2 μl 2-mercaptoethanol/0.2 ml ethanol and made up to 12 ml in 50 mM borate buffer, pH 9.5) was added in a final volume of 0.4 ml. Fluorescence was measured in a Hitachi spectrofluorimeter (model 204-A) using an excitation wavelength of 345 nm and an emission wavelength of 455 nm. The amount of the substrate (for Phe-Leu) hydrolysed was determined from a standard curve obtained with known concentrations of an equimolar mixture of phenylalanine and leucine. 1 unit of peptidase activity is defined as 1 nmol each of phenylalanine and leucine liberated/h.

Protein was determined according to Lowry et al. [29] with crystalline bovine serum albumin as standard or by absorbance at 280 nm (for column effluents).

Paper chromatography

One dimensional paper chromatography (descending) on Whatman no. 1 paper was done using the solvent system

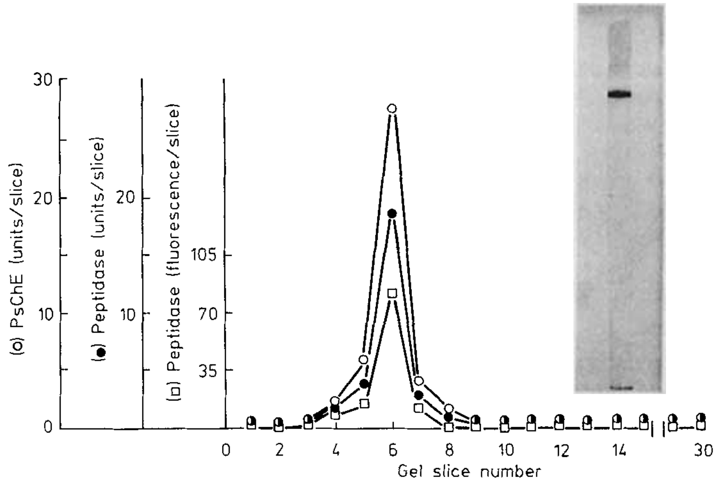

Fig. 2. *Polyacrylamide gel electrophoresis of the purified PsChE and the activity profile of the enzyme extracted from gel slices.* Enzyme activities were measured using the substrate butyrylthiocholine (○——○) for PsChE and Phe-Leu (●——●) and [Leu⁵]enkephalin (□——□) for the peptidase. Details of the electrophoresis and assay procedure are given in Materials and Methods

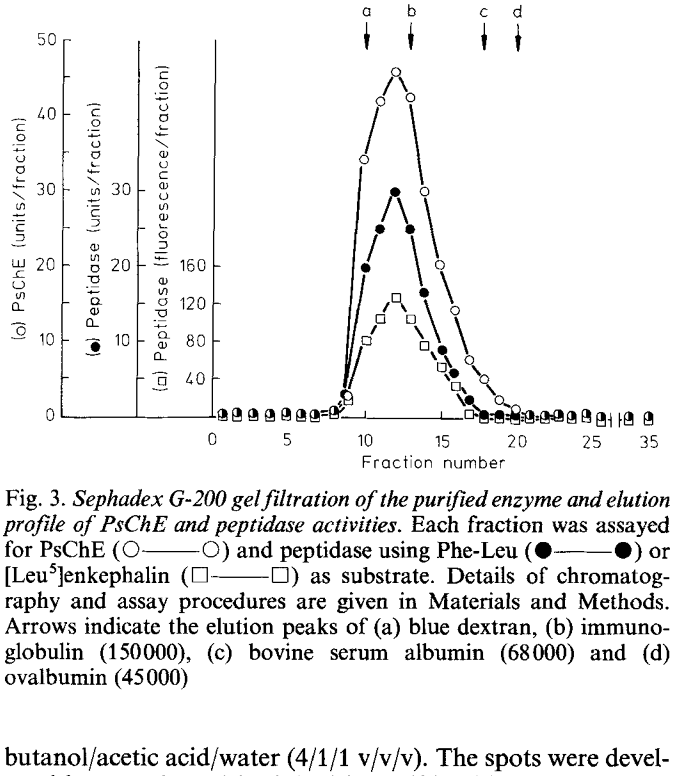

Fig. 3. *Sephadex G-200 gel filtration of the purified enzyme and elution profile of PsChE and peptidase activities.* Each fraction was assayed for PsChE (○——○) and peptidase using Phe-Leu (●——●) or [Leu^5]enkephalin (□——□) as substrate. Details of chromatography and assay procedures are given in Materials and Methods. Arrows indicate the elution peaks of (a) blue dextran, (b) immunoglobulin (150000), (c) bovine serum albumin (68000) and (d) ovalbumin (45000)

Fig. 4. *SDS/polyacrylamide gel electrophoresis of the purified enzyme (8 μg) under reducing (A) and non-reducing conditions (B).* The protein bands were stained with silver nitrate. Details of electrophoresis and staining procedures are given in Materials and Methods. Arrows ► and ● respectively indicate the dimeric and monomeric forms of the enzyme

butanol/acetic acid/water (4/1/1 v/v/v). The spots were developed by spraying with ninhydrin (0.4% w/v) in acetone and warming the paper at 70 °C for 5 min.

RESULTS

Binding and co-elution of PsChE and peptidase activity in various affinity column chromatographic procedures

The elution profiles of PsChE and peptidase activities of the purified enzyme from the procainamide-Sepharose affinity column chromatographic procedure is shown in Fig. 1. Both enzyme activities were bound 100% to the column and co-eluted and the ratios of the two activities were similar in all the active fractions.

In order to verify the identical behaviour of the PsChE and peptidase activities the purified enzyme was passed through different types of affinity columns. Both the enzyme activities were completely bound to a concanavalin-A – Sepharose column, indicating the glycoprotein nature of the enzyme, and were co-eluted more than 90% with methyl α-glucoside in buffer. Both enzymes bound to a lysine-Sepharose column and were completely elutable with NaCl. Zn^{2+} and Co^{2+} metal chelate columns did not bind both enzyme activities.

Comigration on polyacrylamide gel electrophoresis

Fig. 2 shows the gel electrophoresis pattern of the purified enzyme under non-denaturing conditions and the profile of the enzyme activities in the gel slices. A single protein band, which corresponded to both PsChE and peptidase activities, was observed indicating comigration of both activities.

Sephadex G-200 gel filtration

The Sephadex G-200 gel filtration pattern of the purified enzyme is shown in Fig. 3. The elution profile showed that

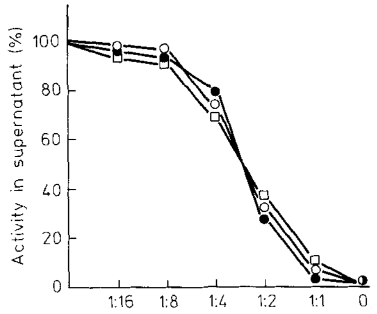

Fig. 5. *Immunoprecipitation of PsChE (○——○) and peptidase activities at different dilutions of the antibody raised against purified PsChE.* Peptidase was assayed using Phe-Leu (●——●) or [Leu[5]]-enkephalin (□——□) as substrate. Details of the experiment are given under Materials and Methods

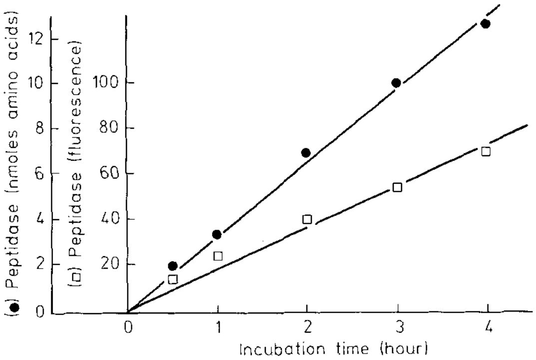

Fig. 6. *The degradation of Phe-Leu (●——●) and [Leu[5]]enkephalin (□——□) by the peptidase activity of PsChE at different time intervals of incubation.* The amino acids released from the peptides were monitored by the *o*-phthalaldehyde fluorimetric method. Details of the assay procedures are given in Materials and Methods

both PsChE and peptidase activities co-eluted as a single peak from the column. From the elution pattern of marker proteins the relative molecular mass of the native enzyme was calculated to be approximately 170000 corresponding to the dimeric form of PsChE. The ratio of PsChE to peptidase activity remained approximately constant in all the eluted fractions.

SDS gel electrophoresis

The SDS gel electrophoretic pattern of the purified enzyme under reducing and non-reducing conditions is shown in Fig. 4. Under non-reducing conditions protein bands corresponding to both the monomeric and dimeric forms of enzyme appeared, whereas under reducing condition only the monomeric form ($M_r \approx 90000$) was seen.

Immunoprecipitation studies

Immunoprecipitation of the purified enzyme showed that both the peptidase and PsChE activities could be co-precipitated by the purified antibody. The ratio of peptidase to PsChE activity precipitated at different dilutions of the antibody was constant (Fig. 5).

Table 1. *Release of amino acids from various peptides by the peptidase associated with PsChE*
Each substance at a concentration of 400 µM was incubated with 4 µg enzyme in a total volume of 0.2 ml potassium phosphate buffer, pH 7.2, for 4 h at 37°C. Liberated amino acids were identified by paper chromatography as described in Materials and Methods

Peptide substrate used	Amino acids released
Phe-Leu	Phe, Leu
[Leu[5]]Enkephalin (Tyr-Gly-Gly-Phe-Leu)	Leu, Phe
[Met[5]]Enkephalin (Tyr-Gly-Gly-Phe-Met)	Met, Phe
Tyr-Gly-Gly	not detectable
N-Acetyl-[Met[5]]enkephalin	Met, Phe
Phe-LeuNH$_2$	Phe, Leu
Phe-Leu ethyl ester	Phe, Leu
[Leu[5]]Enkephalin ethyl ester	Leu, Phe
N-Acetyl-[Met[5]]enkephalin ethyl ester	Met, Phe
Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH$_2$)	Met, Leu
Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)	Arg, Phe
Angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu)	Leu
Tyr-bradykinin (Tyr-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)	Arg, Phe

Characterization of the peptidase associated with PsChE

The peptidase activity in the purified enzyme was labile to freezing and thawing, as was PsChE (100% loss of peptidase and 82% loss of PcChE after two freezing and thawing). The activity was linear up to 4 h for hydrolysis of either Phe-Leu or [Leu[5]]enkephalin (Fig. 6). A Lineweaver-Burk plot for hydrolysis of Phe-Leu and [Leu[5]]enkephalin showed K_m values of 303 µM and 211 µM respectively. The peptidase activity was inhibited 55–65% by 1 mM Cd^{2+}, Co^{2+} or Zn^{2+}. EDTA inhibited the peptidase 50–75% in the concentration range of 0.1–1 mM (data not shown). The inhibitory effect of EDTA was verified by paper chromatography of the reaction mixtures using different peptides as substrate.

The products released by the peptidase from various peptide substrates, as identified by paper chromatography, are given in Table 1. From both [Leu[5]]enkephalin and [Met[5]]enkephalin only two amino acids from the carboxy terminal, namely Leu and Phe or Met and Phe, were released. This was also verified in separate experiments by subjecting the reaction mixtures, incubated for periods from 30 min to 4 h to paper chromatography. Tyr-Gly-Gly was a poor substrate and did not show any release of amino acids. Blocking the N-terminal by an acetyl group, as in *N*-acetyl-[Met[5]]enkephalin, also gave the two C-terminal amino acids released. Surprisingly blocking the C-terminal amino acid by an amide or ester group did not prevent the release of the amino acids as in Phe-LeuNH$_2$, Phe-Leu ethyl ester, [Leu[5]]enkephalin ethyl ester or *N*-acetyl-[Met[5]]enkephalin ethyl ester.

Use of other peptide substrates like substance P, bradykinin, angiotensin I and Tyr-bradykinin also resulted in the release of C-terminal amino acids (Table 1). Gly, His and Pro appeared to be unfavourable amino acids to be released.

Inhibition characteristics

Potent inhibitors of PsChE, like ethopropazine, imipramine, eserine sulphate, neostigmine, Dip-F, tetra-isopro-

Table 2. *Effect of PsChE inhibitors on peptidase activity*
Peptidase activity was assayed fluorimetrically as given in Materials and Methods using Phe-Leu (400 μM) as substrate for 4 h at 37 °C in potassium phosphate buffer, pH 7.2. In the control reaction mixture the inhibitors were added after arresting the reaction. The inhibition pattern was also confirmed qualitatively by paper chromatography (data not shown). Results are given as percentage of the control activity

Inhibitor	Concentration	Activity	
		PsChE	peptidase
	mM	%	
Eserine sulphate	0.02	36	124
	0.10	12	138
	2.00	0	141
Neostigmine	0.10	8	108
Procainamide	20.00	22	102
	100.00	7	91
Ethopropazine	0.10	0	92
Imipramine	0.10	0	86
Diisopropylfluorophosphate	0.02	0	132
	0.10	0	142
	2.00	0	152
Tetra-isopropylpyrophosphoramide	0.10	0	88
Phenyl boronic acid	0.02	29	100
	0.10	4	128
	2.00	0	128
Eserine sulphate + diisopropylfluorophosphate	0.10 0.10	0	129
Neostigmine + diisopropylfluorophosphate	0.10 0.10	0	163

pylpyrophosphoramide and phenyl boronic acid [30, 31] had no significant inhibitory effect on the peptidase activity (Table 2). Indeed there was an activation of the peptidase activity when eserine, Dip-F or phenyl boronic acid was used. The combined use of eserine or neostigmine with Dip-F also resulted in an activation of the peptidase activity (Table 2).

Fig. 7 shows the effect of choline and its esters on both PsChE and peptidase activities. While choline did not significantly inhibit the enzyme activities, the choline esters markedly inhibited both PsChE and peptidase activities. The peptidase inhibition by choline esters observed by the spectrofluorimetric assay was confirmed by paper chromatography also. For a 1-h incubation period of the reaction (which was necessary for measuring the peptidase activity) with various concentrations of the choline esters, there was a parallel inhibition of both PsChE and peptidase activities (Fig. 7). A greater inhibition of PsChE activity after 10 min of incubation instead of 1 h was observed at all concentrations of choline esters presumably because of the enhanced loss of the choline esters by hydrolysis during 1 h (Fig. 7). That the unhydrolysed choline esters were the effective inhibitors of PsChE and peptidase was also evident from the facts that (a) choline alone was ineffective as an inhibitor and (b) the acyl part of the choline ester (acetate, propionate, butyrate, benzoate or succinate) alone or with choline was not inhibitory to either PsChE or peptidase activity (data not shown).

DISCUSSION

The identity of the peptidase with the purified human serum PsChE is based on the following observations. (a) Both PsChE and peptidase co-eluted with constant ratios of activity in the procainamide-Sepharose affinity column chromatographic procedure and showed identical characteristics in other types of affinity chromatography. (b) Both the activities comigrated on gel electrophoresis under non-denaturing conditions and both the activities corresponded to the only protein band detectable on the gel. (c) Both activities showed similar elution profiles on Sephadex G-200 gel filtration. (d) Both enzyme activities were coprecipitated at different dilutions of the antibody raised against purified PsChE enzyme. (e) Procainamide, which is a specific reversible inhibitor of PsChE, inhibits PsChE more than 90% at 100 mM concentration but does not significantly inhibit the peptidase activity (Table 2). The fact that both peptidase and PsChE activities bind 100% to the procainamide-Sepharose column and that both are co-elutable with 0.05 M procainamide suggested that the peptidase must be associated with the PsChE enzyme.

The inhibition characteristics of the peptidases are of interest. Several potent inhibitors of PsChE did not inhibit the peptidase activity (Table 2). These inhibitors are known to act at either the anionic or esteratic site of PsChE [30, 31]. In fact a slight activation of the peptidase activity was observed with diisopropylfluorophosphate (Dip-F), eserine and phenyl boronic acid. It has been reported [30] that an aziridinium derivative, which alkylates the anionic site of AChE while inhibiting the hydrolysis of acetylcholine, can activate the hydrolysis of the very poor substrate indophenyl acetate. Apparently any inhibitor of PsChE which acted only at either the anionic or esteratic site of PsChE did not inhibit the peptidase. Use of an anionic-site inhibitor (eserine or neostigmine) together with an esteratic-site inhibitor (Dip-F) also did not inhibit the peptidase activity. On the other hand inhibitors of PsChE which occupied both the anionic and esteratic sites, like the substrate analogues acetylcholine, butyrylcholine, propionylcholine, benzoylcholine or succinylcholine, effectively inhibited the peptidase activity. Use of the acyl moiety of the choline esters (acetate, propionate, butyrate, benzoate or succinate) together with choline did not affect the peptidase. It was therefore evident that chemical entities which simultaneously occupied both esteratic and anionic sites and which were covalently linked to each other alone could bring about an inhibition of the peptidase activity. Under such conditions it is possible that a conformational alteration occurs resulting in the inhibition of a peptidase domain in the PsChE molecule. Evidence for alteration in conformational stability arising from structural modification of the active center in PsChE has been reported [32]. The tetrameric form of PsChE appears to be an asymmetric prolate ellipsoid [33] but the folded conformation of the enzyme is still unknown. Multistate denaturation of PsChE, caused by the possible unfolding and refolding of each domain independently at different concentrations of urea, is also known [32].

The peptidase activity in PsChE was able to release amino acids from the carboxy terminal of several peptides. There was no evidence for removal of N-terminal amino acids. Blocking of the C-terminal amino acid by an amide group or ester group did not prevent the release of the C-terminal amino acids. This would suggest that the enzyme has an esterase, amidase and peptidase activity. The reported aryl

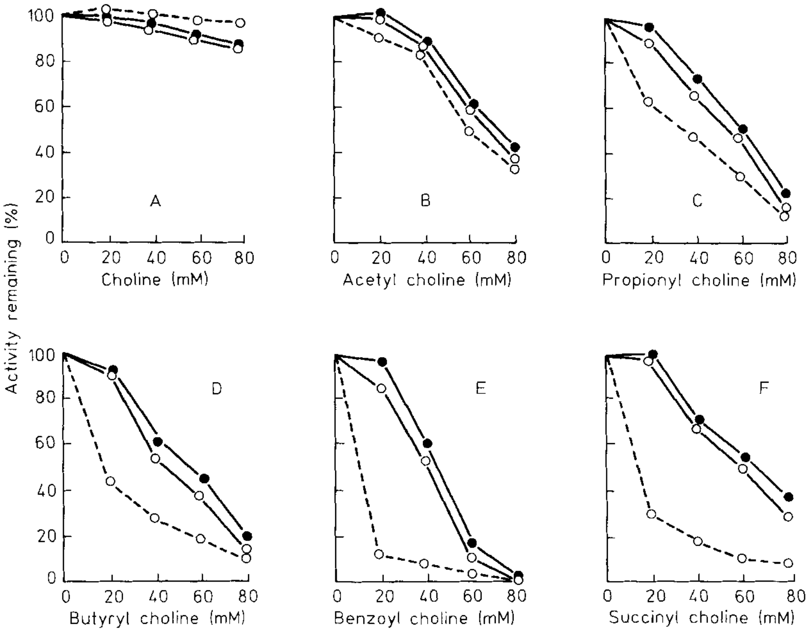

Fig. 7. *Inhibition of PsChE and peptidase activities by choline and choline esters at different concentrations.* Peptidase activity (●——●) was measured at 1 h of incubation in the presence of the inhibitors using Phe-Leu as substrate. PsChE activity was measured at 10 min of incubation in the presence of the inhibitor (○----○) or after 1 h of preincubation with the inhibitor (○——○)

acylamidase activity of PsChE is worth noting in this context [10, 17]. Lockridge found the release of N-terminal dipeptides from substance P by purified human serum PsChE [14]. Later work by Nausch and Heymann [17] showed that the dipeptidyl peptidase activity was a contaminant in the PsChE preparation. Dip-F inhibited this dipeptidyl peptidase activity [17]. The present peptidase activity of PsChE reported by us is not inhibited by Dip-F. Lockridge observed the release of the amide $-NH_2$ group of methionine from substance P by the purified PsChE but did not report the release of methionine and leucine from the C-terminal of substance P. This might be because of the use of EDTA by Lockridge in the reaction mixture [14]. In the present studies we have found that EDTA significantly inhibits the peptidase activity of PsChE with Phe-Leu, [Leu^5]enkephalin or substance P as substrate.

It is of interest that AChE from diverse sources has also been shown to have a peptidase activity [34]. The multiple types of reaction catalysed by AChE [9, 35, 36] may be attributed to the highly complex and possible multidomain nature of the AChE protein [37, 38]. Recent evidence for several mRNAs, each generating distinct monomer of AChE [39–41], suggests that such multiple monomers present in AChE can also provide the multiple catalytic sites of AChE. PsChE, being a less complex protein containing four identical monomers, may be considered to have a specific domain responsible for its peptidase activity.

This work was supported by a grant from the Department of Science and Technology, India. R.B. is a teacher fellow on deputation under University Grants Commission (India) Undergraduate Development Scheme.

REFERENCES

1. Lockridge, O. & LaDu, B. N. (1982) *J. Biol. Chem. 257*, 12012–12018.
2. Lockridge, O., Eckerson, H. W & LaDu, B. N. (1979) *J. Biol. Chem. 254*, 8324–8330.
3. Lwebuga-Mukasa, J. S., Lappi, S. & Taylor, P. (1976) *Biochemistry 15*, 1425–1434.
4. Rosenberry, T. L. & Richardson, J. M. (1977) *Biochemistry 16*, 3550–3558.
5. Carson, S., Bon, S., Vigny, M., Massoulie, J. & Fardeau, M. (1979) *FEBS Lett. 97*, 348–352.
6. Vigny, M., Bon, S., Massoulie, J. & Gisiger, V. (1979) *J. Neurochem. 33*, 559–565.
7. Fujimoto, D. (1976) *FEBS Lett. 71*, 121–123.
8. Oommen, A. & Balasubramanian, A. S. (1977) *Biochem. Pharmacol. 26*, 2163–2167.
9. George, S. T. & Balasubramanian, A. S. (1980) *Eur. J. Biochem. 111*, 511–524.
10. George, S. T. & Balasubramanian, A. S. (1981) *Eur. J. Biochem. 121*, 177–186.
11. MacPhee-Quigley, K., Taylor, P., Taylor, S. S. (1985) *J. Biol. Chem. 260*, 12185–12189.
12. Greenfield, S. (1984) *Trends Neurosci. 7*, 364–368.
13. Balasubramanian, A. S. (1984) *Trends Neurosci. 7*, 467–468.
14. Lockridge, O. (1982) *J. Neurochem. 39*, 106–110.
15. Chatonnet, A. & Masson, P. (1985) *FEBS Lett. 182*, 493–498.
16. Nausch, I. & Heymann, E. (1985) *Neurochem. 44*, 1354–1357.
17. Boopathy, R. & Balasubramanian, A. S. (1985) *Eur. J. Biochem. 151*, 351–360.
18. Greenstein, P. & Winitz, M. (1961) in *Chemistry of the amino acids* (Greenstein, P. & Winitz, M., eds) vol. 2, pp. 763–1295. Wiley, New York.
19. George, S. T. & Balasubramanian, A. S. (1983) *Biochem. J. 209*, 471–479.
20. Cuatrecasas, P. & Parikh, I. (1972) *Biochemistry 11*, 2291–2299.
21. Alam, T. & Balasubramanian, A. S. (1978) *Biochim. Biophys. Acta 524*, 373–384.
22. Chibber, B. A. K., Deutsch, D. G. & Mertz, E. T. (1974) *Methods Enzymol. 34*, 424–432.
23. Porath, J., Carlsson, J., Olsson, I. & Belfraze, G. (1975) *Nature (Lond.) 258*, 598–599.
24. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.
25. Laemmli, U. K. (1970) *Nature (Lond.) 227*, 680–685.
26. Merril, C. R., Goldman, D. & Van Keuren, M. L. (1983) *Methods Enzymol. 96*, 230–239.
27. Ellman, G. L., Courtney, D. K., Andres, V. J. & Featherstone, R. M. (1961) *Biochem. Pharmacol. 7*, 88–95.

28. Roth, M. (1971) *Anal. Chem. 43*, 880–882.
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
30. Main, A. R. (1976) in *Biology of cholinergic function* (Goldberg, A. M. & Hanin, I., eds) pp. 269–353, Raven Press, New York.
31. Garner, C. W., Little, G. H. & Pelley, J. W. (1984) *Biochim. Biophys. Acta 790*, 91–93.
32. Masson, P. & Goasdoue, J. L. (1986) *Biochim. Biophys. Acta 869*, 304–313.
33. Masson, P. (1979) *Biochim. Biophys. Acta 578*, 493–504.
34. Chubb, I. W., Ranieri, E., White, G. H. & Hodgson, A. J. (1983) *Neuroscience 10*, 1369–1377.
35. Naveh, M., Bernstein, Z., Segal, D. & Shalitin, Y. (1981) *FEBS Lett. 134*, 53–55.
36. Aberman, A., Segal, D., Shalitin, Y. & Gutman, A. L. (1984) *Biochim. Biophys. Acta 791*, 278–280.
37. Massoulie, J. & Bon, S. (1982) *Annu. Rev. Neurosci. 5*, 57–106.
38. Toutant, J. P., Massoulie, J. & Bon, S. (1985) *J. Neurochem. 44*, 580–592.
39. Sikorav, J., Grassi, J. & Bon, S. (1984) *Eur. J. Biochem. 145*, 519–524.
40. Schumacher, M., Camp, S., Maulet, Y., Newton, M., MacPhee-Quigley, K., Taylor, S. S., Friedmann, T. & Taylor, P. (1986) *Nature (Lond.) 319*, 407–409.
41. Soreg, H., Zevin-Sonkin, D. & Razon, N. (1984) *EMBO J. 3*, 1371–1375.