HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY Research Article
Hacettepe J. Biol. & Chem., 2009, 37 (4) 331-336

Purification and Characterization of Van Lake Fish (*Chalcalburnus tarichii* P.1811) Liver and Brain Acethylcholinesterase

Esrin Akman[1], Vedat Turkoglu[1*] and Ismail Celik[2]

[1]Yuzuncu Yıl University, Sciences and Letters Faculty, Department of Chemistry, Van, Turkey

[2]Yuzuncu Yıl University, Science and Letters Faculty, Department of Biology, Van, Turkey

Article Info

Article history:

Received
September 29, 2009

Received in revised form
December 15, 2009

Accepted
December 19, 2009

Available online
December 31, 2009

Key Words

Purification,
Characterization,
Acethylcholinesterase,
Chalcalburnus Tarichii,
Fish

Abstract

In this study, acethylcholinesterase was purified from liver and brain of Van Lake fish (*Chalcalburnus Tarichii* P.1811) by using affinity chromatography technique. The purification folds were determined as 142 and 344 for brain and liver, respectively. The purity of enzyme was controlled with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The optimal pH was determined as 7.5-8.5 for brain and liver, respectively. On the other hand, the optimal temperature was determined as 35°C for both liver and brain. Also, the highest activities were observed in concentration of 0.2 M $(NH_4)_2SO_4$ as ionic strength for both liver and brain.

INTRODUCTION

Vertebrates have two types of Cholinesterases (ChEs): acetylcholinesterase (AChE) and butyrylcholinesterase or pseudocholinesterase (BChE), which differs from their substrate specificity. On the one hand, AChE hydrolyzes acetylcholine faster than the other choline esters and is less active with butyrylcholine. In contrast, BChE is highly efficient at hydrolyzing both butyrylcholine and acetylcholine [1]. Acethylcholinesterase is mainly found in brain, nerve cells (especially end plates), in muscle and erythrocytes. In fishes, acethyl-cholinesterase was first isolated by extraction from the electric organ of *Torpedo marmorata* in 1938, following the discovery of an extraordinary concentration of the enzyme in the tissue [2]. AChE is predominant in brain and muscle tissues, whereas BChE presents mostly in the liver and plasma [3]. Later, such enzymes were found in blood. Both serum and red cells hydrolyze choline esters far more rapidly than other esters at low substrate concentrations. The red cell type has been called pseudo acethylcholinesterase [4]. In addition, several studies have reported atypical ChEs in teleost marine fishes [5-8]. The main role of AChE is to catalyze the hydrolysis of acetylcholine into choline and acetic acid at cholinergic synaptic sites [9].

* Correspondence to: Vedat Türkoğlu,

Yuzuncu Yil University,Department of Chemistry, Letters and Science Faculty, 65080 Van, Turkey

Tel: +90432 225 1707/2226 Fax: +90432 225 1188
E-mail: vturkoglu_62@yahoo.com

The aim of the present study is to purify and characterize AchE enzyme from liver and brain of Van lake fish by affinity chromatography, and compare the results with other living species. Characterization of AChE is important because their activity level varies greatly across organs within and individual, as well as across species. Specifically, in this study we characterized AChE purified from brain and liver by the specific and different properties such as optimum pH, temperature and ionic strength.

MATERIALS AND METHODS

Chemicals

The chemicals used in the present study are: Sepharose 4B, EDCI (1-ethyl-3-(3-dimethyl-amino propyl) carbodiimide hydrochloride), Sephadex G-25, decamethonium bromide, standart bovine serum albumin, coomassie brillant blue G-250, coomassie brillant blue R-250, N,N,N',N'-tetramethylethylene diamine (TEMED), trichloroacetic acid (TCA), sodium dodesilsulfate (SDS), dialysis membranes, sodium hydroxide (Sigma Chem. Co.), sodium carbonate, sodium bicarbonate, trihydroxymethyl aminomethane (Tris), sodium chloride, cyanide bromide (CNBr), sodium citratedehydrate, hydrochloric acid, acetic acid, 2-mercaptoethanol, propanol, methanol (Merck.). The fish's liver and brain samples were obtained from Van lake fishes (*Chalcalburnus Tarichii* P.1811) Van, Turkey.

Preparation of the Tissues Homogenate

The liver and brain were dissected and put in Petri dishes. After washing the tissues with physiological saline (0.9% NaCl), the liver and brain samples were cut into parts with blender. The ground liver and brain were homogenized for 5 min in solution containing 0.836 g $Na_2HPO_4.2H_2O$ and 8.25 g glucose (1:5 w/v) using a glass-porcelain homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000 g for 30 min. After membrane and intact cells were removed, the pH of supernatant was adjusted at 8.0 by adding sodium phosphate (25 mM) and solid phosphate salts for column application.

Preparation of affinity gel

20 mL of Sepharose 4B and 20 ml of water were combined and activated with 4 gr of CNBr. The mixture was titrated to pH 11 in an ice bath by stirring with a magnet, and maintained at pH 11 for 8-10 minutes. The reaction was stopped by filtering the gel on a Buchner funnel and washed with cold 0.1 M $NaHCO_3$ buffer (pH 10). 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride, using saturated EDCI solution in the same buffer, was coupled to Sepharose 4B. The reaction was completed by stirring with a magnet for overnight in the fridge. In order to remove excess of 1-ethyl-3-(3-dimethyl-aminopropyl) carbadiimide hydrochloride from the Sepharose 4B-EDCI gel, the mixture was washed with abundant water. The affinity gel was suspended in buffer A (50 mM sodium phosphate, pH: 8) then, packed in a column (1.3 x 60 cm) and equilibrated with the same buffer. The flow rates for washing and equilibration were adjusted by peristaltic pump 20 ml/h.

Purification of AchE by affinity chromatography

Supernatant samples obtained previously were loaded on Sepharose 4B-EDCI affinity column and the flow rate was adjusted to 20 ml/h. The column was washed with buffer B (0.4 NaCl, 50 mM sodium phosphate pH 8) until the A_{280} nm of effluent was £ 0.01. The AChE was eluted with buffer C (0.1 M NaCl, 50 mM sodium phosphate pH 8, containing 10 mM decamethonium). The enzyme activity was measured in final fractions and the activity showing tubes were collected together. Then, the enzyme solution was dialyzed in buffer A for 3-4 hours with changing the buffer twice [10-14].

AchE activity determination

For the colorimetric assay, according to Ellman's method, reaction mixtures were made up in 50 mM Tris, pH 7.4 containing 1 mM DTNB and acetylthiocholine iodide at a final concentration of 0.8 mM. The reaction was performed at 25°C and monitored at 412 nm [15].

Total protein determination

Quantitative protein determination was measured spectrophotometrically at 595 nm according to Bradford's method by using bovine serum albumin as a standard [16].

Optimal pH determination

For the optimal pH determination, the enzyme activity was measured in 1M Tris-HCl and phosphate buffers within the pH of 6.0 to 11.0. In brief, 50 ml of enzyme sample (specific activity 11.2 EU/mg protein, concentration 0.034 mg protein/ml) was added to incubation mixture containing 1 mM DTNB and acetylthiocholine iodide at a final concentration of 0.8 mM. The reaction was performed at 25°C.

The effect of temperature on AchE activity

Enzyme activity was measured between 10 and 60°C at optimal pH for this purpose. The enzyme activity was measured as follows: 50 ml of enzyme sample (specific activity 11.2 EU/mg protein, concentration 0.034 mg protein/ml) was added to the incubation mixture containing 1 mM DTNB and acetylthiocholine iodide at a final concentration of 0.8 mM.

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Laemmli's procedure was carried out in 4% and 10% acrylamide concentrations for running and stacking gel, respectively, to control the enzyme purity, SDS was added into the gel solution at 10%. The gel was stabilized in the solution containing 50% propanol + 10% TCA + 40% distilled water for 30 min. The staining was made for about 2 hours in the solution of 0.1% Coomassie Brillant Blue R-250 + 50% methanol + 10% acetic acid. Finally the washing was carried out in the solution of 50% methanol + 10% acetic acid + 40% distilled water until protein bands were cleared [17].

RESULTS AND DISCUSSION

In this study, the acethylcholinesterase was first purified from Van Lake fish's (*Chalcalburnus Tarichii* P.1811) liver and brain. The purification steps comprise preparation of supernatant and Sepharose 4B-EDCI affinity chromatography. Sepharose 4B was chosen as a matrix because of better flow properties than other matrixes. At first, free hydroxyl groups of the matrix were activated with CNBr [18]. Table 1 shows a purification of liver supernatant characterized with a specific activity of 233.52 EU/mg proteins, with a yield of 49% and a purification coefficient of 142. Table 2 shows also a purification of brain supernatant characterized with a specific activity of 367.92 EU/mg proteins, with a yield of 49% and a purification coefficient of 344. The AChE from liver and brain supernatant was purified by using the affinity gel with elution buffer of

Table 1. Purification of liver homogenate from fish's liver.

Purification steps	T volume (ml)	T protein (mg)	T activity (EU)	S activity (EU/mg)	Yield (%)	Purification factor
Homogenate	300	50.6	83.1	1.64	100	0
Affinity column	50	0.017	3.97	233.52	49	142

Table 2. Purification of liver homogenate from fish's brain.

Purification steps	T volume (ml)	T protein (mg)	T activity (EU)	S activity (EU/mg)	Yield (%)	Purification factor
Homogenate	300	55	59	1.07	100	0
Affinity column	50	0.0318	11.7	367.92	49	344

(0.1 M NaCl, 10 mM sodium phosphate pH: 8.0 containing 10 mM decamethonium). The elutes were plotted by arraying out protein determination at 280 nm and AChE activity for tissues homogenate (Figure 1 and 2). Specific activities for tissues were calculated by using supernatant and purified enzyme solution. For the purification of supernatant AChE by affinity chromatography, the column (1.3x60 cm) was eluted by buffer C at pH 8. It was buffer at 20 ml/h flow rate for fraction volumes of 6 ml.

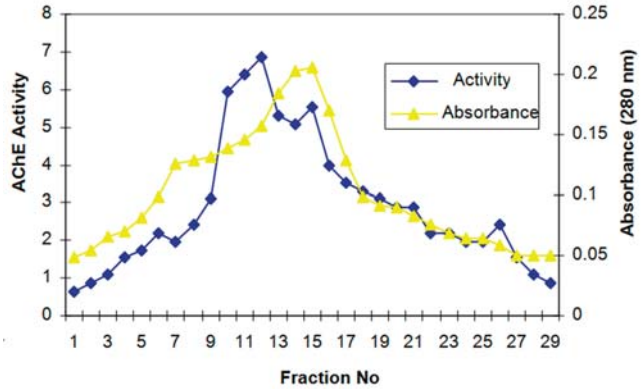

Figure 1. Purification of liver homogenate AChE by affinity chromatography the columns (1.3 x 60 cm) eluted by buffer C at pH 8. It was buffer at 20 ml/h flow rate for fraction volumes of 6 ml.

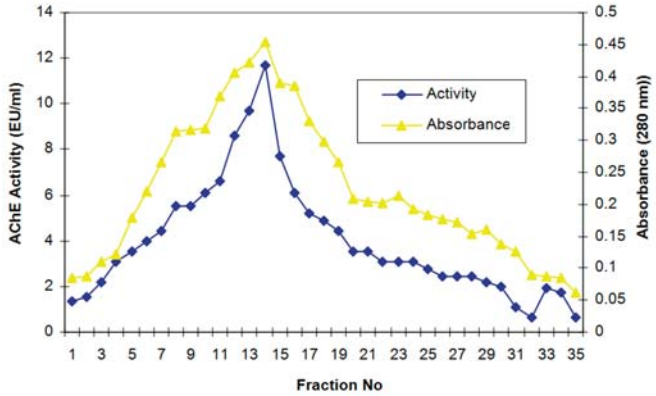

Figure 2. Purification of brain homogenate AChE by affinity chromatography the columns (1.3 x 60 cm) eluted by buffer C at pH 8. It was buffer at 20 ml/h flow rate for fraction volumes of 6 ml.

Optimal pH for liver and brain AChE has been determined as 7.5-8.5 using 1 M Tris-HCl and was showed in Figure 3 and 4 respectively. The plot temperature for liver and brain, AChE enzyme activity was measured at 35°C and was shown in Figure 5 and 6 respectively. The highest activity was seen in concentration of 0.2 M $(NH_4)_2SO_4$ as ionic strength and was shown in Figure 7 and 8. The purification of AChE was controlled with SDS-polyacrylamide gel electrophoresis (Figure 9). So far, no studies on the examining the enzyme characterization in liver and brain of the fish have been made. Therefore, we could not have the chance to compare our results with the previous results. But, our results deal with characterization of enzyme was in accordance with previous studies

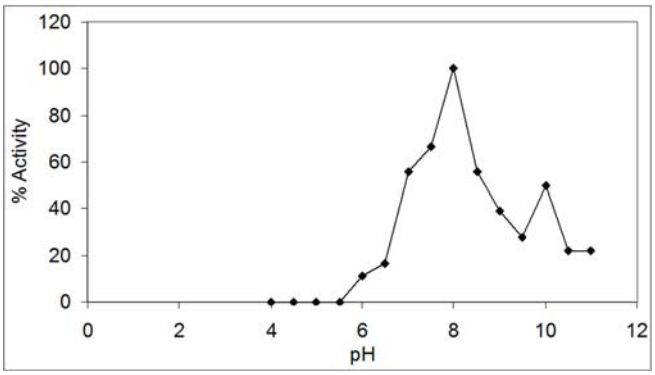

Figure 3. Optimal pH for liver AChE.

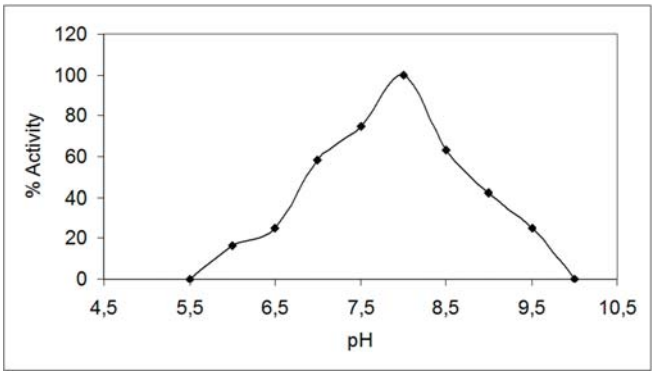

Figure 4. Optimal pH for brain AChE.

[19,20] although different species, tissue and the setting of studies are different. In addition, it is difficult to compare data from different laboratories regarding the enzyme characterization because of high variability in analyzing enzyme *in vitro* and *in vivo* due to inconsistent factors like species tissue differences. Our observations led us to conclude that AchE purified from the various tissues of Van Lake fish's characterizations such as the optimal pH, ionic strength and temperature is in accordance with the other living species.

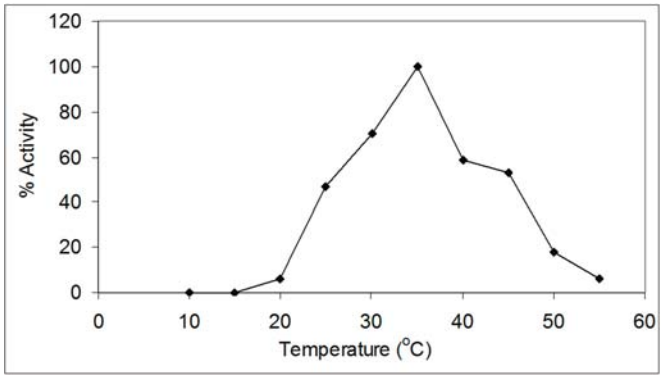

Figure 5. Optimal temperature for liver AChE.

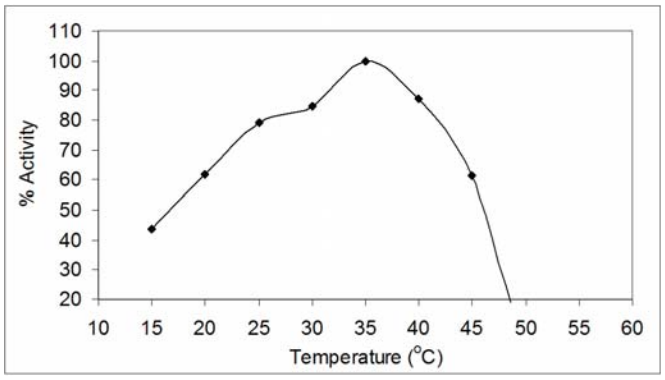

Figure 6. Optimal temperature for brain AChE.

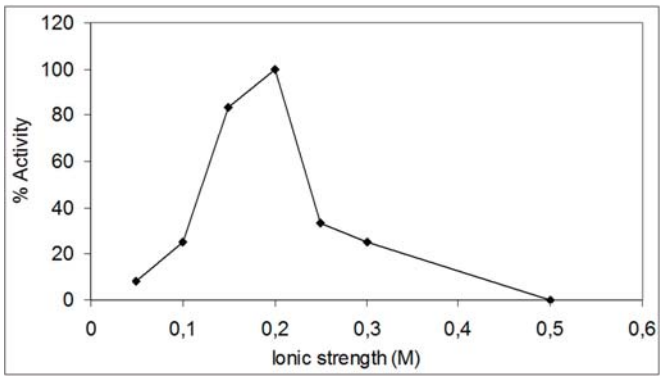

Figure 7. Activity-ionic strength for liver AChE.

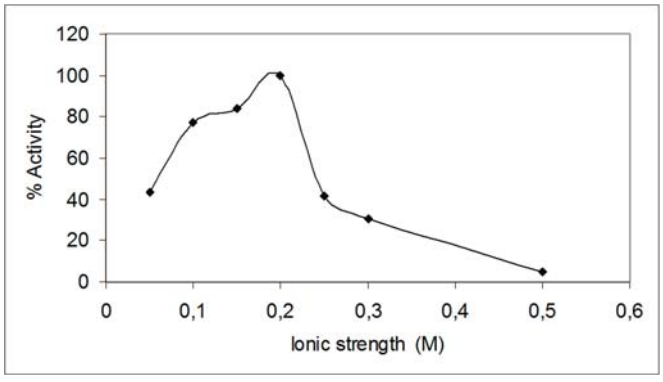

Figure 8. Activity-ionic strength for brain AChE.

Figure 9. SDS-polyacrylamide gel electrophoresis of AChE purified by affinity chromatography.

REFERENCES

1. Alpuche, G.L., Gold, B.G., Determination of esterase activity and characterization of cholinesterases in the reef fish Haemulon plumieri. Ecotoxicol. Environ. Safety. 71, 787-797, 2008.

2. Nachmansohhn, D., Chemical, molecular basis of nerve activity. Academic Press, New York, 137, 1959.

3. Habig, C., Di Giulio, R.T., Biochemical characteristics of cholinesterase in aquatic organisms. In: Mineau, P. (Ed.), cholinesterase-inhibiting insecticides. Their impact on wildlife and the environment. Elsevier, Amsterdam, 20–30, 1991.

4. Nachmansohhn, D., Lededer, E., Chemical, Molecular Basis of Nevre Activity. Bull. Soc. Chim. Biol. 21, 797, 1939.

5. Sturm, A., da Silva de Assis, H.C., Hansen, P.D., Cholinesterases of marine teleost fish: enzymological characterization and potential use in the monitoring of neurotoxic contamination. Mar. Environ. Res. 47, 389–398, 1999,

6. Varo, I., Navarro, J.C., Amat, F., Guilhermino, L., Effect of dichlorvos on cholinesterase activity of the European sea bass (Dicentrarchus labrax). Pestic. Biochem. Physiol. 75, 61–72, 2003.

7. Rodriguez-Fuentes, G., Gold-Bouchot, G., Characterization of cholinesterase activity from different tissues of Nile tilapia (*Oreochromis niloticus*). Mar. Environ. Res. 58, 505–509, 2004.

8. Monteiro, M., Quintaneiro, C., Morgado, F., Soares, A.M.V.M., Guilhermino, L., Characterization of the cholinesterases presents in head tissues of the estuarine fish Pomatoschistus microps: application to biomonitoring. Ecotoxicol. Environ. Safety. 62, 341–347, 2005.

9. Walker, C.H., Thompson, H.M., Phylogenetic distribution of cholinesterases and related esterases. In: Mineau, P. (Ed.), Cholinesterase-inhibiting insecticides. Their impact on wildlife and the environment. Elsevier, Amsterdam, 3–19, 1991.

10. Brewer, G.J., Sing, C.F., An introduction to izoenzymes techniques. Academic Preaa Inc. 20, 1970.

11. Hoz, D.L.D., Raltson, J.S., Rush, R.S., Wolfe, A.D., A Simplified procedure for the purification of large quantities of fetal bovine serum acethylcholinesterase. Life Sciences. 39, 195-199, 1986.

12. Raltston, J.S., Rush, R.S., Doctor, P.B., Wolfe, A.D., Acethylcholinesterase from fetal bovine serum purification and characterisation of soluble G4 enzyme. J. Biol. Chem. 260, 4312-4318, 1985.

13. Main, A.R., Soucie, W.G., Buxton, L.I., Arinc, E., The purification of cholinesterase from horse serum. The Biochem. J. 143, 733-744, 1974.

14. Bruns, K., Casillas, E.D., Partial purification and characterization of on acethylcarintine hydrolase from bovine epididymal spermatozoa. Arch. Biochem. Biophys. 277, 1-7, 1990.

15. Ellman, G.L., Courtney, K.D., Andres, J.V., Featherstone, R.M., A new and rapid colorimetric determination of acethylcholinesterase activity. Biochem. Pharmacol. 7, 88-95, 1961.

16. Bradford, M.M., A Rapid and sensitive method for the quantation of microgram quantaties of protein utiliging the principle of protein-dye binding. Anal. Biochem. 72, 248, 1976.

17. Laemmli, N.K., Cleavage of structural proteins during in assembly of the heat of bacteropose T4. Nature. 227, 680, 1970.

18. Bell, J.E., Bell, E.T., Affinity chromatography. protein and enzymes. Prentice-Hall Inc., New Jersoy, 45-63, 1988.

19. Ulusu, N.N., Kus, M.S., Acan, L., Tezcan, E.F., Arapid method for the purification of glucose-6-phosphate dehydrogenase from bovine lens. Int. Biochem. Cel. Biol. 31, 787, 1999.

20. Aliriz, S., Turkoglu, V., Purification and Characterisation of Acethylcholinesterase from the Lake Van Fish (*Chalcalburnus Tarichii* Pallas, 1811). Prep. Biochem. Biotech. 33, 137-145, 2003.