

# A NEW PURIFIED CHOLINESTERASE FOR SEPARATE DETECTION OF ORGANOPHOSPHATES AND CARBAMATES

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## INTRODUCTION

Among many xenobiotics entering aqueous media, anticholinesterase (antiChE) compounds notable for their high toxicity and selectivity are of particular hazard. Many compounds of these classes are used as pesticides, drugs, chemical warfare agents and many have thus become environmental contaminants. Currently, dozens of pesticides (organophosphorus compounds and carbamates) capable of polluting the aqueous media through the runoff from agricultural lands or as a result of chemical industry accidents are produced. The forthcoming destruction of chemical weapons, a major part of which constitutes antiChE compounds (sarin and soman) can also lead to water pollution. Although the stability of these substances in water is not high (e.g., when compared to organochlorine compounds), certain xenobiotics of these classes are highly toxic for humans, animals, and particularly for hydrobionts. At present, various types of purified commercial cholinesterases (ChE) are widely used to detect antiChE compounds. The detection is based on the property of xenobiotics to lower the activity of enzymes. However, this method is not universal because ChE has low sensitivity to many organophosphorus (OP) and carbamate pesticides (1,2).

It is known that the cholinesterase of fish's brain is the typical acetylcholinesterase (AChE) with the same substrate specificity and sensitivity to organophosphorus compounds as AChE from human erythrocytes (3,4). The fish's blood serum cholinesterase also has been identified as AChE (5,6). On the other hand the blood serum of only two freshwater fishes (blue bream - *Abramis ballerus* and roach - *Rutilus rutilus*) contains mainly butyrylcholinesterase (BuChE) with unusually high sensitivity to organophosphorus pesticides - dipterex and DDVP (7,8). This is of both scientific and practical interest.

## MATERIALS AND METHODS

In this work we studied the substrate specificity, kinetic behaviour and sensitivity of blue bream blood serum ChE for some OP and carbamate pesticides. The earlier unstudied blood serum of blue bream was used as a source of ChE. The fish were collected during autumn - winter period from the Volga pool of the Rybinsk Reservoir. The blood was taken away from fish tail vein and the fibrin clot was separated from the serum. After isolation and purification of blood serum by help of well - known methods (9,10), a stabilized lyophilized enzyme with activity of 5 - 10 units per mg of protein was obtained. The following Russian commercial purified lyophilized cholinesterases have been used for comparison: AChE (acetylcholine acetylhydrolase, EC 3.1.1.7) from the erythrocytes of human, BuChE (acylcholine acylhydrolase, EC 3.1.1.8) from the horse blood serum and propionylcholinesterases - PrChE (acylcholine acylhydrolase, EC 3.1.1.8) from the hen blood serum (PrChE-1) and from the squid (*Todarodes pacificus*) optic ganglion (PrChE-2). The kinetics of cholinesterase hydrolysis of different substrates was investigated at pH-7.5 and at 25°C. Acetylcholine iodide (ACh), propionylcholine iodide (PrCh), butyrylcholine iodide (BuCh), acetylthiocholine iodide (AThCh), propionylthiocholine iodide (PrThCh), butyrylthiocholine iodide (BuThCh) were used as substrates. The initial rate of enzyme hydrolysis was measured by the following methods. In the experiments with choline esters it was measured by the potentiometric titration (11) in 1mM of phosphate buffer and 100 mM of potassium chloride. In the experiments with thiocholine esters it was measured by the photometric method of Ellman (12) in the 20 mM of phosphate buffer containing 100 mM of potassium chloride and 0.2 mM 5',5'-dithio-bis-(2-nitrobenzoic acid). The maximal rates of enzyme reaction and Michaelis constants ( $K_m$ ) were obtained by the Lineweaver - Burk method (13) using a computerized production control system.

The irreversible inactivation of the enzyme by OP and carbamates was measured with the help of bimolecular rate constant ( $K_2$ ) of interaction of the enzymes with inhibitors at pH 7.5 and 25°C in the 20 mM of phosphate buffer, containing 100 mM of potassium chloride and 0.2 M 5',5'- dithio-bis - (2-nitrobenzoic acid). In experiments were used the following organophosphates and carbamates. 1. Organophosphates: dipterex, DFP (diisopropylfluorophosphate), dichlorvos (DDVP), paraoxon, phosphamidon, dimethoate, mevinphos, sarin, soman. 2. Carbamates: physostigmine, aminostigmine (2-dimethylaminomethylene-3-dimethylcarbamoyloxy pyridine), bizerine (N,N'-hexamethylene-bis-[ N-methylcarbamic acid 3 - ( 2 - dimethylaminomethyl) pyridile ester] tetrahydrochloride), sevine, bis-quaternary carbamate (14) - X-129 (1-[ N- (3-[dimethylcarbamoyloxy] -  $\alpha$  - picolile) -N,N- dimethylammonio] - 10 - [N- oxyethyl - N,N - dimethylammonio]decabromine).

## RESULTS

The curve of the dependence between the hydrolysis rates of substrates by fish ChE (FChE) and the value of pH looks like a bell and has a maximum at pH 8.5. The curves for pH-dependence in the experiments with FChE differed only slightly from these for purified cholinesterases - AChE and BuChE. We investigated the activity of fish ChE (FChE) relatively to a choline and thiocholine esters hydrolysis as a function of substrate concentration. The optimal substrates for FChE are BuCh and BuThCh; the activity of enzyme rises with an increase of substrates concentration. The results of this and the following experiments indicate that the purified ChE of blue bream blood serum can be classified as a BuChE, so the velocity of butyrylcholine and butyrylthiocholine hydrolysis is more than other substrates. At the same time, this type of FChE differs from typical BuChE, so the hydrolysis rate of butyrylcholine by FChE is in 10 - 13 times more rapid as compared to hydrolysis of acetylcholine (Table 1).

On the other hand the hydrolysis rate of butyrylcholine by BuChE is only in 1.2 - 2.6 more rapid as compared to hydrolysis of acetylcholine. The comparison of Michaelis constants for different substrates confirms the differences between the FChE, horse blood plasma BuChE and human erythrocytes AChE. The value of  $K_m$  for FChE is 5 times less in the case of butyrylcholine and 30 times less for butyrylthiocholine as compared with ordinary BuChE.

The differences between FChE and other types of ChE are especially appreciable during the study of different inhibitors of cholinesterase. The FChE has a very high sensitivity to some organophosphorus compounds (Table 2). The sensitivity of FChE is 70 times higher for dipterex, that of DDVP - 1500 times, phosphamidon - 160 times, dimethoat - 90 times, mevinphos - 100 times, sarin - 50 times than the sensitivity of BuChE. It is very unexpectedly and unordinary that FChE has a very small sensitivity to active carbamates. The sensitivity of FChE for physostigmine is 200, aminostigmine - is 700, bizerine- is 250, bis-quaternary carbamate X-129 - is 15 000 times lower than the sensitivity of BuChE.

The results obtained with study of kinetic behaviour of FChE and its sensitivity to OP and carbamates suggested that there might be a essential difference between the active sites of FChE and another types of cholinesterases. It is very important that with the help of a new purified FChE the separate bioidentification of OP (especially the organophosphorus pesticides) and carbamates may be carried out. The extremely low sensitivity of new purified enzyme to carbamates is particularly valuable for these purposes.

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Table 1. The kinetic parameters ( $K_m$   $\mu$ M, and relative rate of substrates hydrolysis  $-V$ ) of different cholinesterases (Rates of hydrolysis expressed as percentages of the acetylcholine rate).

| Enzymes           | ACh  | PrCh | Substrates<br>BuCh | AThCh | PrThCh | BuThCh |
|-------------------|------|------|--------------------|-------|--------|--------|
| <b>FChE</b><br>V  | 100  | 450  | 1140               | 500   | 800    | 1250   |
| <b>Km</b>         | 1300 | 560  | 170                | 430   | 57     | 16     |
| <b>AChE</b><br>V  | 100  | 70   | 5                  | 89    | 19     | 5      |
| <b>Km</b>         | 190  | 260  | -                  | 88    | 160    | -      |
| <b>BuChE</b><br>V | 100  | 173  | 260                | 141   | 127    | 210    |
| <b>Km</b>         | 910  | 550  | 770                | 540   | 510    | 430    |

Table 2. The bimolecular rate constant ( $k_2$   $M^{-1} \text{ min}^{-1}$ ) of interaction of the enzymes with organophosphates and carbamates.

| Compounds     | Types of cholinesterases |                  |                  |                  |                  |
|---------------|--------------------------|------------------|------------------|------------------|------------------|
|               | AChE                     | BuChE            | PrChE-1          | PrChE-2          | FChE             |
| Dipterex      | $2 \cdot 10^3$           | $2.6 \cdot 10^3$ | $1.2 \cdot 10^3$ | $2 \cdot 10^4$   | $1.8 \cdot 10^5$ |
| DDVP          | $1.1 \cdot 10^4$         | $2.1 \cdot 10^4$ | $2.3 \cdot 10^4$ | $2.5 \cdot 10^5$ | $3.1 \cdot 10^7$ |
| Paraoxon      | $3 \cdot 10^6$           | $1.3 \cdot 10^6$ | $2 \cdot 10^6$   | $7 \cdot 10^6$   | $3.4 \cdot 10^7$ |
| DFP           | $1.1 \cdot 10^4$         | $4.2 \cdot 10^6$ | $4.5 \cdot 10^5$ | $5 \cdot 10^6$   | $8 \cdot 10^7$   |
| Phosphamidon  | $5 \cdot 10^3$           | $5 \cdot 10^3$   | $5 \cdot 10^4$   | $8 \cdot 10^4$   | $8 \cdot 10^5$   |
| Dimethoate    | $6 \cdot 10^3$           | $1 \cdot 10^4$   | $2 \cdot 10^4$   | $3.5 \cdot 10^4$ | $9 \cdot 10^5$   |
| Mevinphos     | $5 \cdot 10^4$           | $5 \cdot 10^3$   | $2.5 \cdot 10^3$ | $4.5 \cdot 10^4$ | $5.5 \cdot 10^5$ |
| Sarin         | $1.2 \cdot 10^7$         | $4.2 \cdot 10^6$ | $4.8 \cdot 10^7$ | $1.9 \cdot 10^8$ | $2.5 \cdot 10^8$ |
| Soman         | $7.8 \cdot 10^7$         | $1.5 \cdot 10^7$ | $3 \cdot 10^7$   | $3 \cdot 10^8$   | $9 \cdot 10^7$   |
| Aminostigmine | $5 \cdot 10^6$           | $7.7 \cdot 10^5$ | $1 \cdot 10^5$   | $3.5 \cdot 10^5$ | $1 \cdot 10^3$   |
| Physostigmine | $8 \cdot 10^6$           | $2 \cdot 10^6$   | $1.2 \cdot 10^6$ | $1.6 \cdot 10^5$ | $1.1 \cdot 10^4$ |
| Bizerine      | $3.3 \cdot 10^6$         | $9 \cdot 10^5$   | $4 \cdot 10^5$   | $8 \cdot 10^5$   | $4 \cdot 10^3$   |
| X-129         | $2.6 \cdot 10^9$         | $4 \cdot 10^7$   | $6 \cdot 10^6$   | $5 \cdot 10^6$   | $2.4 \cdot 10^3$ |
| Sevine        | $2.5 \cdot 10^4$         | $2.5 \cdot 10^3$ | $1 \cdot 10^3$   | $1 \cdot 10^3$   | $4 \cdot 10^2$   |