Archives of Biochemistry and Biophysics 652 (2018) 38–49

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Characterization of butyrylcholinesterase from porcine milk

Ashima Saxena[a,1], Tatyana Belinskaya[a,b], Lawrence M. Schopfer[c], Oksana Lockridge[c,*]

[a] Division of Biochemistry, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500, USA
[b] Infectious Diseases Research Directorate, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500, USA
[c] Eppley Institute, University of Nebraska Medical Center, Omaha, NE 68198, USA

ARTICLE INFO

Keywords:
Butyrylcholinesterase
Acetylcholinesterase
Porcine
Polyproline
Tetramer
Hupresin

ABSTRACT

Human butyrylcholinesterase (HuBChE) is under development for use as a pretreatment antidote against nerve agent toxicity. Animals are used to evaluate the efficacy of HuBChE for protection against organophosphorus nerve agents. Pharmacokinetic studies of HuBChE in minipigs showed a mean residence time of 267 h, similar to the half-life of HuBChE in humans, suggesting a high degree of similarity between BChE from 2 sources. Our aim was to compare the biochemical properties of PoBChE purified from porcine milk to HuBChE purified from human plasma. PoBChE hydrolyzed acetylthiocholine slightly faster than butyrylthiocholine, but was sensitive to BChE-specific inhibitors. PoBChE was 50-fold less sensitive to inhibition by DFP than HuBChE and 5-fold slower to reactivate in the presence of 2-PAM. The amino acid sequence of PoBChE determined by liquid chromatography tandem mass spectrometry was 91% identical to HuBChE. Monoclonal antibodies 11D8, mAb2, and 3E8 (HAH 002) recognized both PoBChE and HuBChE. Assembly of 4 identical subunits into tetramers occurred by noncovalent interaction with polyproline-rich peptides in PoBChE as well as in HuBChE, though the set of polyproline-rich peptides in milk-derived PoBChE was different from the set in plasma-derived HuBChE tetramers. It was concluded that the esterase isolated from porcine milk is PoBChE.

1. Introduction

Vertebrates have two types of cholinesterase (ChE) - acetylcholinesterase (AChE, E.C.3.1.1.7) and butyrylcholinesterase (BChE, E.C.3.1.1.8) [1]. AChE is present in cholinergic synapses in the brain, in autonomic ganglia in the neuromuscular junction, and in the target tissues of the parasympathetic system; its major function is to terminate neurotransmission [2,3]. Human BChE (P06276) on chromosome 3q26 [4] and human AChE (P22303) on chromosome 7q22 [5] share 70% sequence similarity and 52% sequence identity. Though BChE is widely distributed in organs and tissues, people with a hereditary absence of BChE have no symptoms, making it difficult to assign a physiological function to BChE [6]. BChE is primarily synthesized in the liver and is secreted into the plasma. It has been suggested that BChE in plasma functions as a bioscavenger, thereby protecting AChE from inactivation by naturally occurring toxins. This role of BChE is supported by many studies, which demonstrate that exogenously administered BChE can provide protection from the toxicity of organophosphorus (OP) nerve agents [7,8]. The involvement of brain BChE in neurotransmission has been demonstrated [9,10]. Each enzyme can be distinguished from the other on the basis of substrate specificity and sensitivity to various inhibitors [11]. Although AChE is most efficient at hydrolyzing acetylcholine, BChE exhibits less substrate specificity and efficiently hydrolyzes butyryl-, propionyl-, acetyl-, and benzoyl-choline. The two enzymes also can be distinguished by their sensitivity to inhibition by AChE-specific inhibitors BW284c51 and huperzine A, and BChE-specific inhibitors ethopropazine and iso-OMPA.

BChE purified from human plasma (HuBChE) has been studied extensively due to its therapeutic applications [8]. Exogenously administered HuBChE can counteract the toxicity of OP nerve agents and pesticides, detoxify cocaine, and alleviate succinylcholine-induced apnea. Our laboratory has focused on developing HuBChE as a bioscavenger for the prophylaxis of OP nerve agent toxicity in humans. For ethical reasons, the efficacy of HuBChE cannot be investigated in humans. Therefore, the toxicity of OP nerve agents and the efficacy of HuBChE against multiple LD_{50} doses of OP nerve agents are evaluated in animal models. Results from animal studies are extrapolated to humans. Due to many similarities in anatomy and physiology to humans, pigs including minipigs are used for evaluating toxicity from percutaneous, intramuscular, intravenous, and inhalation exposure to OP nerve

* Corresponding author. Eppley Institute University of Nebraska Medical Center, Omaha, NE 68198 USA
E-mail addresses: ashima.saxena.civ@mail.mil (A. Saxena), tatyana.belinskaya.ctr@med.navy.mil (T. Belinskaya), lmschopf@unmc.edu (L.M. Schopfer), olockrid@unmc.edu (O. Lockridge).
[1] Present address U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, 20910.

https://doi.org/10.1016/j.abb.2018.06.006
Received 29 March 2018; Received in revised form 8 June 2018; Accepted 11 June 2018
Available online 15 June 2018

Abbreviations

AChE acetylcholinesterase
ATC acetylthiocholine iodide CAS 1866-15-5
BChE butyrylcholinesterase
BSA bovine serum albumin
BTC butyrylthiocholine iodide CAS 1866-16-6
BW284c51 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide CAS 402-40-4
ChE cholinesterase
decamethonium bromide decane-1,10-bis(trimethylammonium bromide) CAS 541-22-0
DEPQ 7-(*O,O*-diethyl-phosphinyloxy)-1-methylquinolinium methylsulfate
DFP diisopropyl fluorophosphates CAS 55-91-4
DTNB 5, 5′-dithiobis (2-nitrobenzoic acid) CAS 69-78-3
edrophonium chloride ethyl(m-hydroxyphenyl)dimethylammonium chloride CAS 116-38-1
ELISA enzyme-linked immunosorbent assay
ethopropazine hydrochloride 10-[2-diethylaminopropyl]phenothiazine hydrochloride CAS 1094-08-2
rHuAChE recombinant human acetylcholinesterase P22303
HuBChE Human butyrylcholinesterase P06276
iso-OMPA tetra(monoisopropyl)pyrophosphortetramide CAS 513-00-8
LC-MS/MS liquid chromatography-tandem mass spectrometry
OP organophosphorus toxicant
2-PAM pyridine-2-aldoxime methiodide CAS 94-63-3
paraoxon ethyl (*O,O*-diethyl *O*-(4-nitrophenyl) phosphate) CAS 311-45-5
PBS phosphate buffered saline
PNGaseF peptide-N-glycosidase F
PoBChE porcine milk butyrylcholinesterase
propidium iodide 3,8-diamino-5′-3'-(trimethylammonium)propyl-6-phenylphenanthridinium iodide CAS 25535-16-4
PTC propionylthiocholine iodide CAS 1866-73-5
tacrine 9-amino-1,2,3,4-tetrahydroacridine hydrochloride CAS 1684-40-8
TAPS N-[tris(hydroxymethyl)methyl]-3-amino propanesulfonic acid CAS 29915-38-6
TBS tris-buffered saline, 20 mM Tris.HCl pH 7.4 with 0.15 M NaCl
TBST tris-buffer saline plus 0.05% Tween-20

agents [12–15]. Pigs have also been used to evaluate protection against OP nerve agent toxicity afforded by pretreatment with bioscavengers such as HuBChE [16–18]. Pharmacokinetic studies showed that the mean residence time of plasma-derived, tetrameric HuBChE in minipigs was 267 h (data not shown), indicating that HuBChE was not rapidly cleared from the circulation of pigs, but remained in the circulation with a half-life similar to that in humans [19]. This suggests a high degree of similarity between human (Hu) and porcine (Po) BChE.

Although the biochemical properties of HuBChE are well-characterized, the properties of PoBChE are largely unknown. A choline ester hydrolyzing enzyme, partially purified from porcine milk [20] and porcine parotid gland [21] was identified as PoBChE based on substrate and inhibitor specificity. In agreement with Augustinsson and Olsson [20] we found that porcine milk was a richer source of BChE than porcine plasma. BChE activity was 1–3 U/mL in milk and 0.2 U/mL in plasma, where Units of activity are μmoles butyrylthiocholine hydrolyzed per min. Therefore milk was chosen as the source of PoBChE for the current studies. PoBChE was purified using high speed centrifugation followed by procainamide affinity and gel permeation chromatography. A side-fraction was purified by Hupresin affinity chromatography. The catalytic and inhibitory properties of PoBChE were compared with those of HuBChE and recombinant human acetylcholinesterase (rHuAChE). The bioscavenging properties were evaluated by comparing inhibition by diisopropyl fluorophosphate (DFP) and the aging and reactivation of DFP-inhibited enzyme. The amino acid sequence of PoBChE and the identity of polyproline-rich peptides embedded in PoBChE tetramers were determined by mass spectrometry analysis. Enzyme-linked immunosorbent assays identified 3 anti-HuBChE monoclonal antibodies that recognize PoBChE.

2. Materials and methods

2.1. Materials

HuBChE was purified from Cohn fraction IV-4 paste, as described [17]. Recombinant human acetylcholinesterase (rHuAChE) expressed in CHO cells and purified using procainamide Sepharose 4B affinity chromatography, was provided by Dr. Nageswararao Chilukuri (Division of Biochemistry, Walter Reed Army Institute of Research). Porcine milk was from Waltz farms (Hagerstown, MD). (7-(*O,O*-diethyl-phosphinyloxy)-1-methylquinolinium methylsulfate (DEPQ) was provided by Drs. Yacov Ashani and Haim Leader (Israel Institute for Biological Research, Ness-Ziona, Israel). Pyridine-2-aldoxime methiodide (2-PAM) was obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research. Bio-Spin® 6 chromatography columns and Biogel A 1.5 m column were from Bio-Rad Laboratories (Hercules, CA). YMC-Pack Diol-300 column for HPLC was from Waters Corp. (Milford, MA). Procainamide Sepharose 4B was from Sigma Chemical Co. (St. Louis, MO). Hupresin Sepharose 4B was synthesized by Emilie David at CHEMFORASE, 76130 Mont Saint-Aignan, France. Mouse anti-HuBChE monoclonal antibodies 11D8 (accession KT189147 and KT189148), mAb2 (accession KJ141199 and KJ141200), and B2 18-5 (accession KT189143 and KT189144) are described [22,23]. The commercially available mouse anti-HuBChE monoclonal antibody 3E8 (HAH 002-01) was from BioPorto Diagnostics, Denmark via Antibody Shop. All reagent grade chemicals including acetylthiocholine iodide (ATC), butyrylthiocholine iodide (BTC), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3 (BW284c51), decamethonium bromide, edrophonium chloride, ethopropazine hydrochloride, tetraisopropyl pyrophosphoramide (iso-OMPA), propidium iodide, tacrine, N-[tris(hydroxymethyl)methyl]-3-amino propanesulfonic acid (TAPS), sodium phosphate, and bovine serum albumin (BSA) were from Sigma Chemical Co. (St. Louis, MO).

2.2. Isolation and purification of cholinesterase from porcine milk

Porcine milk (1200 mL) was defatted by centrifugation at 5200 × g for 20 min at 4 °C. The purification of BChE from defatted milk was conducted essentially as described [24]. Defatted milk was combined with 25 mL of procainamide-Sepharose 4B affinity gel and stirred overnight at 4 °C. The gel was washed with 500 mL of 50 mM sodium phosphate, pH 8.0, and packed into a 1.5 cm × 20 cm column. Bound PoBChE was eluted with 0.1 M procainamide in 50 mM sodium phosphate, pH 8.0. Fractions containing BChE activity were pooled and dialyzed against 10 mM sodium phosphate, pH 8.0. The enzyme was loaded onto a 1 cm × 20 cm column packed with 10 mL of procainamide-Sepharose 4B gel, washed until the A_{280} of the effluent was < 0.01 and eluted with 0.1 M procainamide in 50 mM sodium phosphate, pH 8.0. Fractions containing BChE activity were pooled, concentrated in an Amicon stirred cell with a PLGC 30 membrane (Amicon, Beverly, MA) and loaded onto a Biogel A 1.5 m column (1.5 cm × 170 cm) equilibrated in 50 mM sodium phosphate, pH 8.0. Fractions containing