Evolution of acetylcholinesterase and butyrylcholinesterase in the vertebrates: an atypical butyrylcholinesterase from the medaka oryzias latipes

Leo Pezzementi, Florian Nachon, Arnaud Chatonnet

To cite this version:
Leo Pezzementi, Florian Nachon, Arnaud Chatonnet. Evolution of acetylcholinesterase and butyrylcholinesterase in the vertebrates: an atypical butyrylcholinesterase from the medaka oryzias latipes. PLoS ONE, Public Library of Science, 2011, 6 (2), pp.e17396. 10.1371/journal.pone.0017396. hal-02646803

HAL Id: hal-02646803
https://hal.inrae.fr/hal-02646803
Submitted on 29 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Evolution of Acetylcholinesterase and Butyrylcholinesterase in the Vertebrates: An Atypical Butyrylcholinesterase from the Medaka _Oryzias latipes_

Leo Pezzementi¹*, Florian Nachon², Arnaud Chatonnet³,⁴,⁵

1 Department of Biology, Birmingham-Southern College, Birmingham, Alabama, United States of America, 2 Département de Toxicologie, Institut de Recherche Biomédicale des Armées, Antenne de la Tronche, La Tronche, France, 3 Institut National de la Recherche Agronomique, Unité Mixte de Recherche 866, Montpellier, France, 4 Université Montpellier 1, Montpellier, France, 5 Université Montpellier 2, Montpellier, France

Abstract

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are thought to be the result of a gene duplication event early in vertebrate evolution. To learn more about the evolution of these enzymes, we expressed _in vitro_, characterized, and modeled a recombinant cholinesterase (ChE) from a teleost, the medaka _Oryzias latipes_. In addition to AChE, _O. latipes_ has a ChE that is different from either vertebrate _AChE_ or _BChE_, which we are classifying as an atypical _BChE_, and which may resemble a transitional form between the two. Of the fourteen aromatic amino acids in the catalytic gorge of vertebrate _AChE_, ten are conserved in the atypical _BChE_ of _O. latipes_; by contrast, only eight are conserved in vertebrate _BChE_. Notably, the atypical _BChE_ has one phenylalanine in its acyl pocket, while _AChE_ has two and _BChE_ none. These substitutions could account for the intermediate nature of this atypical _BChE_. Molecular modeling supports this proposal. The atypical _BChE_ hydrolyzes acetylthiocholine (ATCh) and propionylthiocholine (PTCh) preferentially but butyrylthiocholine (BTCh) to a considerable extent, which is different from the substrate specificity of _AChE_ or _BChE_. The enzyme shows substrate inhibition with the two smaller substrates but not with the larger substrate BTCh. In comparison, _AChE_ exhibits substrate inhibition, while _BChE_ does not, but may instead show substrate activation. The atypical _BChE_ from _O. latipes_ also shows a mixed pattern of inhibition. It is effectively inhibited by physostigmine, typical of all ChEs. However, although the atypical _BChE_ is efficiently inhibited by the _BChE_-specific inhibitor ethopropazine, it is not by another _BChE_ inhibitor, iso-OMPA, nor by the _AChE_-specific inhibitor BW284c51. The atypical _BChE_ is found as a glycophosphatidylinositol-anchored (GPI-anchored) amphiphilic dimer (G2°), which is unusual for any _BChE_. We classify the enzyme as an atypical _BChE_ and discuss its implications for the evolution of _AChE_ and _BChE_ and for ecotoxicology.

Introduction

Acetylcholinesterase (AChE; EC 3.1.1.7) hydrolyzes acetylcholine at the neuromuscular junction of vertebrates. Higher vertebrates also contain an evolutionarily related cholinesterase (ChE), butyrylcholinesterase (BChE; EC 3.1.1.8). The function of _BChE_ is unknown but is suggested to play a role in growth and development and to act as a scavenger of cholinergic toxins as well as having an auxiliary role in synaptic transmission [1,2]. The two ChEs may be distinguished functionally both kinetically and pharmacologically: _AChE_ hydrolyzes acetylcholine (ACh) and is virtually inactive on the larger substrate butyrylcholine (BCh). _BChE_ is less selective, hydrolyzing both substrates comparably. _AChE_ exhibits inhibition at high substrate concentrations, while _BChE_ shows substrate activation instead [3]. The two enzymes may also be distinguished by their susceptibility to diagnostic inhibitors [4].

Within species, _AChE_ and _BChE_ have ~50% amino acid identity, and the overall tertiary structures of the two enzymes are similar [5,6]. Individual amino acid residues involved in determining the molecular basis of the differences in substrate and inhibitor specificity of _AChE_ and _BChE_ have been identified in the acyl pocket, located at the bottom of a deep catalytic gorge; the peripheral site, located at the lip of the gorge; the oxyanion hole; and the choline-binding site of the hydrophobic patch, also located within the gorge [7–14]. Although the dichotomy between _AChE_ and _BChE_ is generally clear in birds and mammals [1,15,16], the two enzymes often more closely resemble one another functionally in fish. In the cartilaginous fish, the electric ray _Torpedo marmorata_ [17], and the bony fishes, the placid _Pleuronectes platessa_ [18], the flounder _Platichthys flesus_ [19], and perhaps the surgeonfish _Acanthurus dussumieri_ [20,21], ChEs with properties intermediate to and atypical of _AChE_ and _BChE_ are found along with _AChE_. These enzymes have alternatively been considered atypical ChEs [18,19] or atypical pseudo-cholinesterases (pseudo-ChEs) [17,20]; we are designating them as atypical _BChEs_, as suggested by Whittaker [22]. Although a number of cDNAs have been cloned for _AChEs_ from these organisms, molecular information about the atypical _BChEs_ present is...
unavailable. Moreover, only a single ChE, AChE, has been identified functionally and molecularly in the jawless fish, the lamprey Petromyzon marinus [23] and the hagfish Myxine glutinosa [24]. These observations suggest that AChE is the ancestral ChE in the vertebrates and that an early gene duplication event and subsequent divergent structural and functional evolution produced the AChE and BChE of higher vertebrates [23,25].

AChE and BChE also exist in a variety of homomeric and heteromeric molecular forms. The catalytic subunit of AChE is found in different variants as a result of alternative splicing of the C-terminus, producing R, H, and T (or AChE R, AChE H, or AChE T) subunits [26,27]. The R, or read-through, transcript is rare and produces soluble non-ampiphilic monomers, G4 [29]. AChE/H has a hydrophobic C-terminus, which is replaced by a glycosphingolipid-inositol phospholipid (GPI) anchor and produces amphiphilic dimers, G4 [29]. AChE/T is capable of forming G4, G4, and G4 [29], as well as “tailed” forms (thus the T subunit) by associating with a transmembrane protein, the Proline-Rich Membrane Attachment (PRiMA) [30] and the triple helical collagen Q (ColQ, Q for queue, tail in French) [31,32]. In brain and at the neuromuscular junction, PRiMA localizes AChE to the cell membrane of synapses, forming G4 or G4. ColQ anchors AChE to the junctional basal lamina of the neuromuscular junction, producing A4, A8, and A12, which represent one, two or three tetramers attached to the ColQ triple helix. While AChE/T is found in all classes of vertebrates, AChE/H exists in cartilaginous fish (Torpedo spp.) [33], amphibians (Xenopus laevis) [34], and mammals [35], but has not been reported in jawless or bony fish, reptiles, or birds, raising questions about the evolution of this splice variant [26].

BChE does not exhibit alternative splicing and is considered to be found solely as a T variant (BChE/T) [36,37] that also associates with PRiMA and ColQ [30,36]. R and H variants of BChE have not been reported. However, according to the Xenopus tropicalis genome project [38] and other evidence [34,39–42], an H variant of BChE appears to be present in amphibian Xenopus species. The atypical BChEs of T. marmorata and A. dussumieri are T variants (BChE/T), assembling a collection of globular and asymmetric forms [17,20]. In remarkable contrast, the atypical BChE of P. flesus is BChE/T, assembling only into GPI-anchored G4 forms [19].

The medaka Oryzias latipes is a telost fish that is of interest as a vertebrate model system for developmental, genomic, and evolutionary biology [43–45]. It was previously reported that O. latipes possesses an AChE [46]. Here we report the cloning and characterization of an atypical BChE, which has properties intermediate to AChE and BChE, from O. latipes, and briefly discuss the implications of the structure and function of this enzyme for the evolution of the ChEs. Additionally, the presence of ChEs with anomalous inhibitor specificities has ecotoxicological implications for O. latipes [47,48] and other fish [49,50].

Results

Sequence Analysis Reveals Two ChEs in O. latipes

Two expressed sequences for ChEs are present in the O. latipes genome: AChE (GenBank EST DK110600) and an enzyme that we are classifying as an atypical BChE [45] (GenBank cDNAs AV68390 and GU79251). The sequence for the AChE is truncated near the carboxyl terminus and contains 561 amino acids. The sequence of the mature polypeptide for the atypical BChE from O. latipes contains 564 amino acids (Fig. 1). Pair-wise BLAST alignments of sequences from the catalytic region of ChEs show that the AChE from O. latipes clearly resembles T. californica AChE rather than Homo sapiens BChE (68/80% identity/similarity to AChE compared to 54/70% for BChE), while the atypical BChE resembles both AChE and BChE more or less equally (46/68% for AChE and 49/67% for BChE). A phylogenetic tree of vertebrate and deuterostome invertebrate ChEs is shown in Fig. 2; the AChE of O. latipes is found in the AChE clade, while the atypical BChE of O. latipes is found in the BChE clade.

The members of the catalytic triad of ChEs are found as Ser203, Gln390, and His461 in the AChE, and Ser203, Gln390, and His443 in the atypical BChE. Of the fourteen aromatic amino acids that line the catalytic gorge of vertebrate AChE, all are conserved in the O. latipes AChE, and ten are conserved in the atypical BChE; in contrast, eight are conserved in vertebrate BChE (Fig. 1; Table 1). The O. latipes atypical BChE is missing two of the three aromatic residues of the peripheral site of AChE, while BChE lacks all three. Additionally, while AChE has two Phe residues in the acyl pocket and BChE none, the O. latipes atypical BChE has one Phe (Fig. 1; Tables 1, 2). As the O. latipes AChE conserves all ten aromatic residues, it has two Phe residues in its acyl pocket.

The three pairs of conserved cysteine residues involved in intra-chain disulfide bonding are also found as Cys69-Cys96, Cys257-Cys268, and Cys405-Cys543 in the AChE, and Cys66-Cys93, Cys257-Cys268, and Cys405-Cys529 in the atypical BChE of O. latipes. Another cysteine (Cys540), near the carboxyl terminal that normally mediates inter-chain disulfide bonding, is also conserved in the atypical BChE (Fig. 1). The carboxyl terminus of the enzyme is of the H-type (Fig. 3), characterized by a loosely defined GPI anchor signal, including an ω cleavage/attachment site followed by a stretch of hydrophobic amino acids [51]. The H-peptides show very little sequence homology to one another. We did not find evidence for the existence of T-type carboxyl terminus for the atypical BChE either as a T-exon in the genome or a T-type carboxyl terminus in the ESTS of O. latipes. The truncated AChE is missing its C-terminal sequence.

Diagnostic Inhibitors Show the Presence of Two ChE Activities in Adult O. latipes

To demonstrate the presence of two ChE activities in O. latipes, extracts from adult medaka were incubated with the inhibitors physostigmine, which inhibits all ChEs; BW204c51, which inhibits AChE selectively; and ethopropazine, which inhibits BChE preferentially, and assayed for activity with ATCh and PTCh [16,52,53]. Different dose-response curves were observed with the two substrates for each inhibitor, suggesting the presence of at least two ChE activities (Fig. 4). We did not find evidence for inhibition of P-type carboxyl terminus for the atypical BChE either as a T-exon in the genome or a T-type carboxyl terminus in the ESTS of O. latipes. The truncated AChE is missing its C-terminal sequence.

Kinetic Analysis of the Atypical BChE Indicates Its Anomalous Properties

As we were interested in the functional characteristics of the atypical BChE in O. latipes, we cloned and expressed in vitro a cDNA for the enzyme in COS-7 cells. To determine the substrate specificity of the enzyme, we assayed the hydrolysis of the substrates acetylthiocholine (ATCh), propionylthiocholine (PTCh), and butyrylthiocholine (BTCh) by the recombinant enzyme (Fig. 5). The smaller substrates ATCh and PTCh are hydrolyzed more or less equally, as indicated by the similar values of kcat/Substrate / kcat/ATCh (Table 3); the larger BTCh is hydrolyzed at about a quarter of the rate of the other two substrates. The Kms are inversely proportional to the length of the acyl group, with BTCh having the lowest Km. The highest catalytic efficiency (kcat/Km) is seen with PTCh. Additionally, ATCh and PTCh produce
An Atypical Cholinesterase from Medaka

Torpedo AchE

DDH3ELVWTXSQVNGVM--TRPVVLSSHISAFLGIPFAPFVGVMRFRFPEPKFWGSGV 58

Medaka AchE

QSEAEFLVQTQGSGFTSCGVRFQTVRDRPGVGSLGIPFAPFVGVMRFRFPEPKFWGSGV 60

Human BchE

--EDDIDIAATKVNGVRG--MNINVPGPTVTAFLGIFAYQPFLRPLRPFKFWGSLWDIW 56

Medaka BchE

--TIDRDLVINCTTHGQVG--KALISVLQGVEGARFPGYPKFGLKLRFRPAEPKEWAVK 57

Torpedo AchE

NASTYPNRCQPGYVDPFQGFGSPGEMWNPIIMSEDLCYLNIWPS--PFKSTT---VMWV 114

Medaka AchE

KADSYPNCACFQVPGFSGEMWNPIIMSEDLCYLNIWPSVSKHNL---VMWV 117

Human BchE

NATKYANSCQIQIDPSQFGFGSPGEMWNPIMLTEDLCYLNIWIPA--PFKSNAT---VLIW 112

Medaka BchE

DATKFPNCCQYQMPTAPFGFGAEMWNPIMLTEDLCYLNIWPTVFKTPQILPSAFLV 117

*:*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:*::*:**
None of these aromatic residues is conserved in the aromatic peripheral binding site and restrict access to the gorge.

The difference in volume is related to the lack of aromatic residues in inhibition by PTCh is weak (Fig. 5; Table 3). BTCh does not produce substrate inhibition. Overall, this pattern of substrate hydrolysis is not typical of either AChE or BChE.

Pharmacological Analysis of the Atypical BChE Confirms Its Anomalous Properties

Since the recombinant enzyme from *O. latipes* exhibited anomalous kinetic properties, to characterize further this atypical BChE activity, we determined the half maximal inhibitory concentration (IC50) values of the enzyme for the inhibitors physostigmine, which inhibits all ChEs; BW284c51, which inhibits AChE preferentially; and iso-OMPA and ethopropazine, which inhibit BChE preferentially. Physostigmine and ethopropazine inhibit the enzyme at sub-μM concentrations; by contrast, much higher concentrations of BW284c51 and Iso-OMPA are required for inhibition under the conditions tested (Fig. 6; Table 4). This pattern of inhibition is also not characteristic of either AChE or BChE.

Analysis of Molecular Forms of the Atypical BChE further Demonstrates Its Unusual Nature

ChEs exist in various homomeric and heteromeric molecular forms depending, in part, on the nature of their carboxyl termini. Since the amino acid sequence of the atypical BChE indicates an H-type C-terminus, we performed velocity sedimentation on sucrose gradients in the presence and absence of the non-ionic detergent Triton X-100 to determine the molecular forms of the recombinant enzyme produced *in vitro* by COS-7 cells. The extract contains G24a forms on the basis of the sedimentation coefficient (3.23±0.10 S; Mean±SE, N = 6) and its shift to higher values in the absence of detergent due to aggregation of the enzyme (0.96±0.05; Mean±SE, N = 6) (Fig. 7). Digestion of intact COS-7 cells with phospholipase C (PIPLC) releases ~90% of the surface enzyme activity. Spontaneous release of activity into the supernatant during incubation in the absence of PIPLC was ~10% (Fig. 8). These data indicate that the G24a produced is a glycosphingolipidionositol-anchored (GPI-anchored) form. It is unusual for any BChE to be found as a GPI-anchored form.

Molecular Modeling of the Atypical BChE Illustrates Its Differences with AChE and BChE

We built a homology model of the atypical BChE from *O. latipes* based on the X-ray structures of *H. sapiens* BChE, and *T. californica* and *Drosophila melanogaster* AChEs, in order to get a structural understanding of the special pharmacological and enzymatic properties of the enzyme (Fig. S1). A comparison of the active site gorges is presented in Figure 9. The volume of the *O. latipes* atypical BChE catalytic gorge (630 Å3) is much closer to that of *H. sapiens* BChE (690 Å3) than that of *T. californica* AChE (410 Å3). The difference in volume is related to the lack of aromatic residues in three gorge subunits: the peripheral site, the acyl binding pocket, and the choline binding pocket. *T. californica* AChE possesses three conserved residues (Tyr70, Tyr121, and Trp279) that form an aromatic peripheral binding site and restrict access to the gorge. None of these aromatic residues is conserved in *H. sapiens* BChE and only one aromatic residue is present in the *O. latipes* atypical BChE (Tyr282), thus enlarging the gorge entrance (Table 1).

The acyl binding pocket of *T. californica* AChE is also smaller than that of the atypical BChE from *O. latipes*, which is in turn smaller than that of *H. sapiens* BChE, due to the respective decreasing number of the phenylalanines shaping the pocket. These differences are expected to change the selectivity of the enzymes for substrates and inhibitors bearing large chains oriented toward this pocket.

Another major difference between the *O. latipes* atypical BChE and *H. sapiens* BChE and *T. californica* AChE is located in the choline binding pocket. Phe330 (a tyrosine in mammalian AChE) is substituted by a non-aromatic residue in both BChEs (Ala328 in the *H. sapiens* BChE and Cys333 in the *O. latipes* atypical BChE). Phe330 can adopt different conformations in the presence of different ligands thus providing an efficient way to modulate the shape and size of the choline binding pocket, even acting as a molecular lid. The absence of a gating aromatic residue at this position in BChE leaves the gorge wide open at all times. Notably, just next to this position, the substitution of Met442 in the *H. sapiens* BChE by Ala440 in *O. latipes* BChE results in a significantly widened choline binding pocket.

All things considered, the active site gorge of the atypical BChE from *O. latipes* shares more structural features with that of *H. sapiens* BChE than that of *T. californica* AChE, and it appears legitimate to consider this enzyme as an atypical BChE from a structural point of view.

Discussion

Overview

In addition to possessing an AChE, the medaka *O. latipes* has an atypical BChE that is in many respects different from either vertebrate AChE or BChE (Table 5). Of the fourteen aromatic amino acids in the catalytic gorge of vertebrate AChE, ten are conserved in the atypical BChE from *O. latipes*; by contrast, eight are conserved in vertebrate BChE. These substitutions may account for the intermediate nature of the atypical BChE. Molecular modeling supports this interpretation. The enzyme hydrolyzes ATCh and PTCh preferentially, but BTCh to a considerable extent. In contrast, AChE is highly specific for ATCh compared to BTCh and even PTCh, while BChE hydrolyzes the larger substrates preferentially. The Ks for substrate hydrolysis by the atypical BChE are inversely proportional to the length of the acyl group, which is more a characteristic of BChE than AChE. The enzyme shows substrate inhibition with the two smaller substrates but not the larger substrate BTCh. By comparison, AChE exhibits substrate inhibition, while BChE does not, but may instead show substrate activation. The *O. latipes* enzyme also shows an atypical pattern of inhibition by diagnostic inhibitors. It is effectively inhibited by the ChE inhibitor physostigmine, typical of all ChEs. However, although the atypical BChE is efficiently inhibited by the BChE-specific inhibitor ethephonapirazone, it is not inhibited by another BChE inhibitor, iso-OMPA, nor by the AChE-specific bis-quaternary inhibitor BW284c51. The atypical BChE is found as a GPI-anchored G2 membrane-bound dimeric form, also unusual for a BChE. We consider the enzyme an...
atypical BChE has implications for the evolution of AChE and BChE in the vertebrates.

Comparison of Kinetic and Pharmacological Parameters of the Atypical BChE from *O. latipes* with AChE, BChE, and other Atypical BChEs in Vertebrates

Fluck [46] characterized the acetylcholine hydrolyzing activity of early embryos of *O. latipes* and found that ATCh, PTCh, and BTCh were hydrolyzed at the relative rates of 1.0, 0.4, and 0.14, respectively; that ATCh, at least, produced substrate inhibition; and that 10 μM BW284c51 inhibited 90% of the activity, while 10 μM iso-OMPA inhibited only 10% of the activity. He concluded that the enzyme present was AChE. Given the results from the *O. latipes* genome project, which indicate two ChEs in the genome; our results for BW284c51 and ethopropazine inhibition from the *O. latipes* genome project, which indicate two ChEs in the genome, also suggest two ChEs; and our data for the recombinant atypical BChE from *O. latipes*, it seems likely that both AChE and BChE were present in the embryos studied by Fluck [46].

The $K_s$ for substrate hydrolysis by the atypical BChE from *O. latipes* is inversely proportional to the length of the acyl chain of the substrate (Table 5). This relationship is seen in *H. sapiens* BChE, which is representative of the typical vertebrate BChE; but not *T. marmorata*, which is representative of vertebrate AChE. This pattern is the most conserved characteristic of the atypical BChEs from the flounder *P. flesus*, the placid *P. platessa*, the surgeonfish *A. dussumieri*, and the ray *T. marmorata*, suggesting that the non-covalent stabilization of binding of substrate is determined similarly in these enzymes. The acyl pocket, Phe288 and Phe290 in *Topecko* spp. AChE, is implicated in this binding [8,12-14]. A detailed discussion of the molecular basis of $K_s$ and other parameters of substrate and inhibitor specificity is given in the next section; since sequence information is only available for the atypical BChE from *O. latipes*, it alone, among the atypical BChEs, can be compared to AChE and BChE at the molecular level.

In contrast to the clear series seen for $K_m$ the substrate hydrolysis specificities of the enzymes, as defined by relative $V_{max}$ or $k_{cat}$ values, do not show as consistent a pattern. Most of the atypical BChEs hydrolyze ATCh preferentially and BTCh the least. An exception is the *O. latipes* atypical BChE, which hydrolyzes PTCh maximally, albeit only slightly faster than ATCh. However, there are two additional exceptions to this generalization: the *P. platessa* atypical BChE hydrolyzes BTCh faster than PTCh, and the *A. dussumieri* atypical BChE hydrolyzes BTCh only marginally better than does AChE. The variation in relative $V_{max}$ or $k_{cat}$ values suggests that there are differences in the stabilization in the covalent Michaelis complexes of substrates in the various atypical BChEs. Such differences could be due to differences in the nature of the conformation of the histidine of the catalytic triad, which appears to be determined by different sets of structural interactions in AChE and BChE [9,54].

There are also four different patterns of substrate inhibition among the five atypical BChEs, indicating another heterogeneity among the enzymes. The physiological relevance and molecular mechanism of substrate inhibition are unclear [55–57]. Nevertheless, these differences in the enzymes are probably due to alterations of amino acids, probably non-aromatic substitutions, comprising the peripheral site and/or other regions of the catalytic gorge in the various enzymes that are important for substrate inhibition [9,13,38,59]. It is notable that the substrate hydrolysis curve for PTCh hydrolysis by the *O. latipes* atypical BChE, particularly in the region of substrate inhibition resembles the curves for ATCh and PTCh hydrolysis by the atypical BChE from *A. dussumieri*, as the substrate inhibition levels off in both of the enzymes [21].

The atypical BChEs also show different patterns of sensitivity to inhibitors; however, given the range of inhibitors used, and focusing on the atypical BChE from *O. latipes*, it is possible to compare their inhibition only for the AChE and BChE diagnostic inhibitors BW284c51 and ethopropazine, and even for these inhibitors the data are incomplete and a consistent pattern elusive.

---

**Table 1. Aromatic Amino Acids in the Catalytic Gorge of Vertebrate ChEs.**

| Subsite          | Torpedo AChE | Oryzias AChE | Oryzias BChE | Homo BChE |
|------------------|--------------|--------------|--------------|-----------|
| Peripheral Site  | Tyr70        | Phe72        | Met69        | Asn68     |
| Hydrophobic Patch | Trp121     | Tyr124       | Val124       | Gly119    |
|                  | Trp279        | Trp282       | Tyr282       | Ala277    |
|                  | Trp84         | Trp86        | Trp83        | Trp82     |
|                  | Trp130        | Trp133       | Trp133       | Tyr128    |
|                  | Phe330        | Phe333       | Cys333       | Ala128    |
| Acyl Pocket      | Phe288        | Phe291       | Leu291       | Leu286    |
| Wall of Gorge    | Phe290        | Phe293       | Phe293       | Val288    |

Conserved aromatic residues are shown in bold. Torpedo AChE is representative of all vertebrate AChEs and Homo BChE is representative of all vertebrate BChEs.

1Includes the choline-binding site.

doi:10.1371/journal.pone.0017396.t001
Table 2. Amino Acid Sequences in the Region of the Acyl Pocket of Vertebrate AChE and BChE.

| Enzyme | Class          | Species       | Sequence<sup>b</sup> |
|--------|----------------|---------------|-----------------------|
| AChE   | Mammalia       | Felis catus   | FPFVPVV...DINVICP     |
|        |                | Bos taurus    | FPFVPVV...DINVICP     |
|        |                | Torpedo spp.  | FPFVPVV...DINVICP     |
|        |                | Homo sapiens  | FPFVPVV...DINVICP     |
| Aves   |                | Gallus gallus| FPFVPVV...DINVICP     |
| Reptilia |                | Bungarus fasciatus | FPFVPVV...DINVICP |
| Amphibia |                | Xenopus tropicalis | FPFVPVV...DINVICP |
| Osteichthyes | Electroharus electricus | FPFVPVV...DINVICP |
|        |                | Danio rerio   | FPFVPVV...DINVICP     |
|        |                | Oryzias latipes| FPFVPVV...DINVICP     |
| Chondrichthyes | Torpedo spp. |               | FPFVPVV...DINVICP     |
| Agnatha |                | Myxine glutinosa | FPFVPVV...DINVICP     |
| BChE   | Mammalia       | Felis catus   | LLSVNFPGPV...DINIICP  |
|        |                | Bos taurus    | LLSVNFPGPV...DINIICP  |
|        |                | Torpedo spp.  | LLSVNFPGPV...DINIICP  |
|        |                | Homo sapiens  | LLSVNFPGPV...DINIICP  |
| Aves   |                | Gallus gallus| LLSVNFPGPV...DINIICP  |
| Amphibia |                | Xenopus tropicalis | LLSVNFPGPV...DINIICP |
| Osteichthyes | Gasterosteus aceulatus | LLSVNFPGPV...DINIICP |
|        |                | Fugu rubripes | LLSVNFPGPV...DINIICP  |
|        |                | Oryzias latipes| LLSVNFPGPV...DINIICP  |

---

Of the atypical BChEs, those from *O. latipes* and *P. flesus* resemble most closely BChE, being sensitive to ethopropazine, but not BW284c51; the BChE from *T. marmorata* is equally in/sensitive to both inhibitors, while the BChEs from *P. platessa* and *A. dussumieri* appear to be sensitive and resistant, respectively. These inhibitors are sensitive to the presence or absence of aromatic amino acids present in the choline-binding and peripheral sites, as well as to the volumes of the catalytic gorges [12,14,60,61], which could be and probably are different in the various atypical BChEs. Overall, the variety of kinetic and pharmacological properties of these atypical BChEs could be the result of natural selection exploring the adaptive landscape for the various enzymes.

Molecular Basis of Substrate and Inhibitor Specificity in the Atypical BChE from *O. latipes* in Comparison to AChE and BChE

The atypical BChE from *O. latipes* maximally hydrolyzes ATCh and PTCh almost equally and the larger substrate BTCh at about a quarter of the rate of the two other smaller substrates. This substrate specificity is not typical of either AChE, which hydrolyzes ATCh maximally, PTCh adequately, but not BTCh at all; nor BChE, which hydrolyzes the three substrates more or less equally [15]. An important molecular determinant of ChE specificity is the acyl pocket, which in vertebrate AChE is characterized by two Phe residues (Phe288 and Phe290 in *F. catus* [6], while in BChE these aromatic residues are replaced by smaller aliphatic amino acids (Leu286(288) and Val288 in *T. marmorata* [5]. Site-directed mutagenesis studies suggest that the smaller amino acids relax the steric hindrance of the aromatic rings and allow the accommodation and proper positioning of larger substrates in the catalytic gorge for nucleophlic attack by the active site serine [8,12,14]. The simple fact that the atypical BChE from *O. latipes* has one of the two Phe residues, Phe291 (Phe290 in *T. marmorata* BChE) but not the other, present as Val288 (Phe290), seems sufficient to explain the intermediate substrate specificity of the enzyme. Our molecular modeling is consistent with this explanation.

In the mouse *Mus musculus* AChE, the F295L (F288L) mutation has little effect on the *Km* for ATCh but lowers the *Km* for BTCh 10-fold [14]. In *H. sapiens* AChE, F295L has little effect on the *Km* for ATCh or PTCh but decreases *Km* for BTCh 30-fold [14]. In *H. sapiens* AChE, F295L has little effect on the *Km* for ATCh or PTCh but decreases *Km* for BTCh 30-fold [14]. In contrast, F295A also spares the *Km* for ATCh or PTCh but decreases *Km* for BTCh and BTCh 4-fold and 3-fold, respectively. With respect to
Figure 3. Alignment of peptide sequences of C-termini of representative vertebrate AChEs and BChEs. Conserved (*) and similar (:. ) residues are indicated. Putative Ω-cleavage sites are underlined. Putative hydrophobic transmembrane regions are boldly underlined. H. sapiens (human), F. catus (cat), C. familiaris (dog), M. musculus (mouse), R. norvegicus (rat), X. tropicalis (clawed toad), O. latipes (medaka), G. aculeatus (electric ray).

doi:10.1371/journal.pone.0017396.g003

$k_{\text{cat}}$, the F295L M. musculus AChE mutant decreases $k_{\text{cat}}$ 4-fold for ATCh and increases it 14-fold for BTCh. In H. sapiens AChE, the F295L A mutants do not affect appreciably the $k_{\text{cat}}$ for ATCh but increase $K_m$ 400-fold. For wild type H. sapiens and M. musculus AChE, the highest catalytic efficiency ($k_{\text{cat}}/K_m$) by far is seen for ATCh; whereas, for the F295L A mutants, the highest efficiency is found for BTCh, with the efficiency for all three substrates within a factor of three. These data are consistent with, if not identical to, the results that we have obtained for the O. latipes atypical BChE, which lacks the homologous Phe: all three substrates are hydrolyzed appreciably, the $K_m$ is lowest for BTCh but lower for PTCCh compared to ATCh, and the catalytic efficiency for all the substrates are within a factor of two. Thus, it appears that the residue corresponding to Phe288 affects both the binding and hydrolysis of substrate.

Furthermore, the two phenylalanines (Phe288 and Phe290) that shape the acyl binding pocket of AChE, along with the aromatic peripheral site residues, form an aromatic continuum with Phe330 and Phe331 of the hydrophobic patch. This aromatic network in AChE has been suggested to play an important role in the stabilization of the catalytic histidine [54]. But Phe288 and Phe290 are not conserved in H. sapiens AChE, and Phe288 is not conserved in the atypical BChE from O. latipes (Leu291). Thus, this stabilizing network is absent in BChE. However, the catalytic histidine of BChE is well stabilized by interactions with an adjacent phenylalanine, Phe398 in H. sapiens BChE or Phe403 in O. latipes atypical BChE, which is absent in AChE (Val400 in T. californica AChE). As a matter of fact, the catalytic histidine of H. sapiens BChE has never been observed in an alternate conformation, whereas such a conformation is not unusual in liganded AChE (See X-ray structure of VX-AChE [pdb entry 2VXR], tabun-AChE [pdb entry 3DL4] and the NMR study of Masiah et al. [62]). Interestingly, when the acyl loop of a cholinesterase bears an aliphatic residue at the position equivalent to Leu291 in O. latipes atypical BChE, there is an aromatic residue facing it, Phe401. Reciprocally, when there is an aromatic residue at this position, as in Phe288 in T. californica AChE, there is an aliphatic residue facing it, Val400 (Table 2). This symmetrical situation probably translates into a difference in the stability of the catalytic histidine and the acyl pocket loop, which in turn might affect the stability of the transition state during catalysis. It should be noted that in the vast majority of invertebrate AChEs the acyl pocket appears to be constructed in a different way with additional or alternative aromatic residues playing a role in substrate specificity [63]. Thus, modifications of the acyl pocket appear to occur throughout animal evolution.

The atypical BChE of O. latipes is efficiently inhibited by physostigmine and ethopropazine but not BW284c51 or iso-OMPA, a pattern of inhibition different from AChE or BChE. However, we think that the pattern more closely resembles BChE rather than AChE with the inhibition by iso-OMPA being exceptional. All ChEs are inhibited by physostigmine, so its effective inhibition simply confirms that the enzyme is a ChE [16] and does not need discussion. The sensitivity of AChE to the slender, elongated bisquaternary inhibitor, BW284c51, is due to its bivalent binding via cation-π and π-π interactions to aromatic amino acids of the choline-binding site at the bottom of the gorge and the peripheral site at its rim. In contrast, BChE has a number of these residues replaced by aliphatics. In M. musculus AChE, the peripheral site double mutant Y72N/Y124Q (Y70N/Y121Q) increases $K_i$ for BW284c51 69-fold [13]. Comparably, in H. sapiens AChE, the same double mutation increases $K_i$ for the inhibitor 35-fold. Moreover, the choline-binding site mutation Y337A (Phe330A) increases the $K_i$ 5-fold [60]. Making the reasonable assumption of a synergistic effect for the mutations [60], a triple Y72N/Y124Q/Y337A mutation encompassing both the peripheral and choline-binding sites could increase $K_i$ by over two orders of magnitude. Thus in the O. latipes atypical BChE, which has the aliphatic substitutions Met69, Val124, and Cys333 at the homologous sites, preventing the necessary cation-π and π-π interactions via the aromatic residues, one might expect inefficient inhibition by BW284c51, which is exactly what is observed. Notably, the structure of the acyl pocket does not appear to influence the binding of BW284c51 [13,14,60], consistent with the situation in the atypical BChE of O. latipes.
The F330A mutation of *Torpedo* spp. AChE increases the lower gorge volume to 338 Å³ and allows ethopropazine to bind. In contrast, the A328Y and A328F mutations in the *H. sapiens* BChE model only decrease the volume of the lower gorge to 410 and 406 Å³, respectively, which allow the gorge to still be large enough to bind ethopropazine easily [61].

Our own volume calculations using the narrower part of the bottleneck to define the gorge entrance and taking into account the conserved structural water molecule, gives an overall volume of 690 Å³ for *H. sapiens* BChE, 630 Å³ for *O. latipes* atypical BChE and 410 Å³ for *Torpedo* spp. AChE, in good agreement with the observed trend for ethopropazine inhibition.

Iso-OMPA is an effective organophosphate inhibitor of BChE but not AChE [52], where it is over 10,000 times less reactive [14], and this selectivity appears dependent on the dimensions of the active center of the enzyme, particularly the acyl pocket, affecting the affinity of the enzymes for inhibitor. In *M. musculus* AChE, the acyl pocket mutation F295L (F288L) increases $k_i$ 90-fold, F297I (F290I) 200-fold, and the double mutant, over 500-fold for iso-OMPA [14]. In *H. sapiens* AChE, similar, although more complex, results were seen for the less bulky organophosphates, diisopropyl phosphorofluoridate (DFP), diethyl phosphorofluoridate (DEFP), and paraoxon [64]. Replacement of aromatic amino acids in the acyl pocket with aliphatic residues increases $k_i$ up to 130-fold, with substitutions at Phe295 (Phe288) having the greater effect. The differences in $k_i$ were primarily due to decreases in $K_d$ with $k_2$ relatively unaffected, suggesting that the substitutions relieved steric interference in the binding of the inhibitors and enhanced enzyme activity.

Figure 4. Concentration dependencies for inhibition of ATCh and PTCh hydrolysis by extract from adult *O. latipes*. Adult fish were extracted in HIS buffer and assayed with ATCh and PTCh in the presence of various concentrations of the inhibitors (A) physostigmine, (B) ethopropazine, and (C) BW284c51. Extracts were incubated with inhibitor for 20 minutes prior to being assayed for activity with ATCh (●) or PTCh (○). doi:10.1371/journal.pone.0017396.g004

Figure 5. Substrate concentration dependencies for hydrolysis of ATCh, PTCh, and BTCh by recombinant BChE from *O. latipes*. Transfected COS-7 cells producing BChE were extracted in HIS buffer and assayed with ATCh (●), PTCh (□), or BTCh (▲) and fit as described in Materials and Methods. doi:10.1371/journal.pone.0017396.g005
affinity, but did not alter the rate of phosphorylation. Unlike 
ethopropazine, converse site-directed mutagenesis of the acyl pocket 
has not been reported for BChE and iso-OMPA. The atypical BChE 
from *O. latipes* is relatively insensitive to, but is inhibited by iso-
OMPA. This result, as well as the substrate specificity of the enzyme, 
is in good agreement with the reduction in size of the acyl pocket, 
compared to BChE, associated with the presence of only one of the 

Two aromatic residues in the subsite in our molecular modeling. 

This explanation, as well as the others offered on the molecular basis of 
substrate and inhibitor specificity could be tested by site-directed 
mutagenesis. In any event, it should be kept in mind that vertebrate 
AChEs, including *Torpedo* spp. and mammalian AChE are found as 
AChET and AChEH as the result of alternative splicing, all 
vertebrate BChEs are considered to be of the T-type [26]; the 
atypical BChEs from *O. latipes* and *P. flesus* are the only certain 
exceptions to this rule. The nature of the ChE activity in *Xenopus* 
spp. is perplexing. A PIPLC-sensitive G₂ AChEH has been 
reported in *X. laevis* muscle [34,41], even though its substrate 
specificity is characteristic of BChE [41], but the enzyme has been 
classified as an AChE on the basis of diagnostic inhibition [39,40]. 

The ChE in *X. laevis* tadpoles was found to be resistant to 
various carbamate and organophosphate inhibitors and to not 
show inhibition by excess substrate [42]. Moreover, the genome 
project for *X. tropicalis* indicates that only AChET sequences and 
not AChEH sequences are present. In striking contrast though, a 
BChEH sequence is found. The H-peptide is widespread in the 
invertebrates nor with the vertebrates, 

AChEs of invertebrates, although these sequences are not 
BChEH sequence is found. The H-peptide is widespread in the 
invertebrates nor with the vertebrates,
ChE as BChE gradually evolved from an ancestral AChE in the vertebrates subsequent to a gene duplication event early in vertebrate evolution. Assuming Darwinian gradualism, one would expect that if AChE were the ancestral vertebrate ChE, with two Phe residues in its acyl pocket, that upon gene duplication the two residues would be replaced sequentially with an intermediate enzyme having one of the Phe residues and decreased substrate specificity, a property that is consistent with the putative role of BChE in detoxification mechanisms and our data [1,2,65–67]. Subsequently, under selection pressure, the enzyme would lose its second acyl pocket Phe residue to obtain the substrate specificity of BChE in the higher vertebrates. Likewise, the atypical BChEs show different intermediate patterns of substrate inhibition or lack thereof, which could be interpreted as a transition to the complete loss of substrate inhibition with all substrates, and the acquisition of substrate activation, also a property consistent with the detoxifying role of BChE. While substrate inhibition may be physiologically relevant for the role of AChE in synaptic transmission, it would not be adaptive toxicologically. The issue of inhibitor sensitivity is more complicated. One might expect selective pressures on a detoxifying enzyme to be directed towards increased resistance to inhibitors. While non-aromatic substitutions decrease the sensitivity of the enzyme to some inhibitors, they increase the volume of the catalytic gorge in BChE, literally opening it up to inhibition by bulkier inhibitors that cannot gain access to AChE. Thus, there may be a tradeoff between decreased substrate specificity and substrate inhibition, and decreased inhibitor inhibition with the maximization of all three impossible. By contrast, the larger gorge and increased sensitivity to some inhibitors allows BChE to act as a stoichiometric scavenger of natural and man-made carbamate and organophosphate inhibitors [2]. Clearly, other non-aromatic substitutions in the acyl pocket, in other subsites in the catalytic gorge, and in the peripheral site, also producing kinetic and pharmacological differences between the two enzymes have occurred in this transition as the two enzymes diverged structurally and functionally subsequent to gene duplication [9,54,68,69].

Because of the possibility of three extensive (even genome-wide) gene duplication events early in vertebrate evolution at (1) the origin of the vertebrates, (2) the emergence of the jawed fish, and (3) the appearance of the ray-finned fishes [70–72], the timing of the putative gene duplication event producing vertebrate BChE from AChE is uncertain. The jawless vertebrates, the lamprey P. marinus and the hagfish M. glutinosa, appear to have only one ChE, AChE [24,73], and there is evidence for two ChEs in the cartilaginous jawed fish T. marmorata, AChE, and an atypical BChE [17]. Therefore, it is possible that a duplication of an ancestral AChE gene accompanied the wide-spread gene duplication event that coincided with the emergence of the jawed fish, with the atypical and typical BChEs of bony fish and other derived vertebrates descendents of this gene. However, given the genome duplication event in the ray-finned fish lineage, it is possible that these atypical BChEs are unique to the ray-finned fish and not ancestral to the BChE of higher vertebrates, as the land vertebrates descended from the lobe-finned fishes, represented today by the lungfish and the coelacanth, which diverged from the ray-finned fish prior to the duplication event [74]. Currently there is not any information about ChEs from the coelacanth genome project, but such information could shed light on the timing of the gene duplication event and the evolutionary origins of BChE in tetrapods. In any case, for the time being, the atypical BChE of O. latipes can serve as a model for the evolutionarily intermediate ChE between AChE and BChE.

Given that the poorly conserved H-transcripts are widespread in the AChEs of invertebrates, while in vertebrates they are present only in the AChE of the clams and the teleosts P. flesus and O. latipes. Combes et al. [73] speculated that the conservation of splice sites at the C-terminus of AChE in insects, nematodes, and vertebrates suggests that exon shuffling has occurred at the 3‘end of ChE genes at various times throughout evolution. Such shuffling is one mechanism for appearance of an alternatively spliced exon [76]. Another explanation is exonization [77,78]; indeed, the presence of read-through or R-transcripts [79–
in AChE could be considered a nascent or abortive exonization event, and the low abundance of such R-transcripts is consistent with an exonization process [78]. Thus, it is possible that the evolution of the C-termini of ChEs is independent of the evolution of the catalytic subunit. Further discussion of the evolution of ChEs can be found in Pezzementi and Chatonnet [82].

Ecotoxicological Implications

Pesticide use is a major concern in aquatic environments, where runoff from agricultural and urban ecosystems impacts their ecology. The major pesticides in use today are organophosphate and carbamate acetylcholinesterase inhibitors, and pyrethroid ion-channel agents [83,84]. These toxins are transported from terrestrial to aquatic ecosystems, placing the latter at risk; thus, it is important to understand the effects of these compounds on the resident vertebrate and invertebrate fauna [85]. Inhibition of ChE activity in fish generally correlates with mortality. There may also be sub-lethal behavioral and physiological effects, including reduced swimming ability, and altered feeding and social behavior. However, there are marked species differences in these effects [86]. These differences could be due to different levels of AChE and BChE in the nervous system.

Figure 9. Active site gorges of *H. sapiens* BChE (hBChE; pdb code 1p0i), *T. californica* AChE (TcAChE; pdb code 1ea5) and a homology model of *O. latipes* atypical BChE (OlBChE). The side chains of key residues lining the gorges are represented as sticks (carbon in orange, oxygen in red, and nitrogen in blue). The top of the gorge and the entrance to and exit from the enzyme are shown at the top of the figures. The acyl pocket, which accommodates the acyl group of substrates, is comprised of residues F288 and F290 in *T. californica* AChE, L286 and V288 in *H. sapiens* BChE, and L291 and F293 in *O. latipes* AChE. The central residue of the choline binding site at the bottom of the gorge is W84 in *T. californica* AChE, W82 in *H. sapiens* BChE, and W83 in *O. latipes* atypical BChE. Substrates are bound between these two subsites. The solvent accessibility surface of the gorge was calculated by taking into account highly conserved structural water molecules, using the software "HOLLOW" and represented as grey dots [99].

do:10.1371/journal.pone.0017396.g009
system and the blood, different catalytic abilities of the enzymes, and differential sensitivity of the ChEs to inhibitors, including the more recently used enantioselective organophosphates [48,50,87]. The presence of atypical BChEs in some species of fish probably contributes to these species-specific effects, and a better understanding of the kinetics and pharmacology of these atypical enzymes could provide insights into the toxic effects on fish of ChE poisoning.

Materials and Methods

Ethics Statement

All animal procedures were conducted in strict adherence to the European Council Directive of November 24, 1986 (86-609). Approval for this study was provided by Comité Regional d’Ethique Languedoc Roussillon C34-172-10.

Materials

Dulbecco’s modified Eagle medium, fetal bovine serum, OptiMEM medium, and phosphatidylcholinositol-specific phospholipase C (PIPLC) were purchased from Invitrogen, Carlsbad, California. FuGene was obtained from Roche, Indianapolis, Indiana. Acetylthiocholine (ACh), butyrylthiocholine (BCh), propionylthiocholine (PTCh), recombinant human butyrylthiocholine (rHuBCh, FuGene) were purchased from Sigma, St. Louis, Missouri. The high-affinity ligand 7-[(diethoxyphosphoryl)oxy]-1-methylquinolinium iodide (DEPQ) was a gift from Yacov Ashani.

Table 5. Comparison of Available Kinetic and Pharmacological Parameters and Splice Variants of Vertebrate AChE, BChE, and Atypical BChEs.

| Parameter/Species | T.marmorata AChE | O.latipes BChE | P.flesus BChE | P.platessa BChE | A.dussumieri BChE | T.marmorata BChE | H.sapiens BChE |
|-------------------|------------------|----------------|---------------|----------------|------------------|------------------|---------------|
| $K_m$ ACh | 0.05 | 0.27 | 2.20 | 0.90 | 1.24 | 0.4 | 1.4* |
| $K_m$ PTCh | 0.2 | 0.16 | 1.03 | 0.15 | 0.24 | 0.15 | 0.97* |
| $K_m$ BCh | N.M. | 0.07 | 0.32 | 0.14 | 0.06 | 0.05 | 0.91* |
| $V_{max}$ PTCh | 0.25 | 1.11 | 0.29 | 0.41 | 0.61 | 0.59 | 1.66* |
| $V_{max}$ BCh | <0.01 | 0.25 | 0.22 | 0.58 | 0.03 | 0.28 | 2.41* |
| Substrate Inhibition | +A,−P | +AP,−B | +APB | −AP,B | +APB | −APB | −APB |
| IC50 Ethopropazine | 158 | 0.90 | 12.6 | N.D. | 100 | 15.3 |
| IC50 BW284C51 | 0.04 | 480 | 63 | 0.79 | Weak | 100 | 651 |
| Splice Variant | H, T-type | H-type | H-type | N.D. | T-type | T-type | T-type |

aData are from Toutant et al., [17]; the IC50 values are estimates.

bData are from Davies et al. [100].

cData are from Leibel and inhibition by BW284C51 is based on qualitative observations of the inhibition of ATCh staining of enzyme following non-denaturing gel electrophoresis [21]. Splice variant data are from Leibel [20].

data from Toutant et al. [17]; the IC50 values are estimates.

data are from Stieger et al. [19].

Materials cloning and sequence analyses

The cDNA clone Ola.23452 (Genbank AV668390) from an O. latipes library was obtained from UniGene. The clone was extended by PCR on the basis of the gene structure on scaffold2582 in UCSC genome project, and the full cDNA sequence (Genbank GU797251) was cloned into the expression vector pCMV SPORT 6.1 (Invitrogen).

Sequences were aligned with Clustal W or Clustal X for molecular modeling or phylogenetic analysis by the neighboring method [89]. Putative cleavage/attachment sites and downstream stretches of hydrophobic amino acids of H-type C-termini of ChEs were predicted with PredGPI [89] and ProtScale [90], respectively.

In vitro expression and extraction of enzyme

COS-7 monkey cells (American Type Culture Collection) were grown in Dulbecco’s modified Eagle medium containing 10% fetal calf serum. Cells were plated at a density of $2.5\times10^5$ cells/$75\ cm^2$ culture flask, incubated overnight, and transferred to OptiMEM medium. FuGene was then used to transfect the cells with 7.8 µg of DNA. The cells were then incubated for 48 h at 37°C before the medium was removed and the cells extracted in high ionic strength (HIS) buffer: 10 mM NaHPO4, pH 7, 1 M NaCl, 1% Triton X-100, 1 mM EDTA. Extracts were centrifuged at 20,000 g for 20 min, and the supernatants were assayed for ChE activity.

Measurement and analysis of BChE activity and inhibition

AChE activity was measured according to the method of Ellman et al. [91] as modified by Doctor et al. [92] in 100 mM NaHPO4,
pH 7, 0.3 mM DTNB, 167 mM NaCl, and 258 μM Triton X-100; some BChEs are inhibited by Triton X-100 [63], but the BChE from *O. latipes* is not at the concentrations used in this study (data not shown). ATCh, BTCh, and PTCh were used as substrates at various concentrations; for pharmacological analyses and assays of sucrose gradients, the concentration of ATCh was 1 mM. The kinetic parameters $k_{inact}$, $K_s$, $b$, and $V_{max}$, were determined by using SigmaPlot to fit the data to the equation below as described by Radić et al. [13] and Kaplan et al. [9]. $k_{inact}$ is the dissociation constant for the binding of substrate to a second site on the enzyme, and the parameter $b$ indicates the relative catalytic efficiency of the SES complex compared to SE. If $b < 1$, the enzyme shows substrate inhibition; if $b > 1$, the enzyme shows substrate activation, and if $b = 1$, Michaelis-Menten kinetics is observed.

$$v = \frac{(1 + b[S]/K_{inact})V_{max}}{1 + S/K_{S}}$$

The turnover number $k_{cat} (V_{max} / [Enzyme])$ was determined by enzyme titration with DEPQ [93] as described previously [63]. Values of IC$_{50}$ for the inhibitors used were determined by incubating enzymes with various concentrations of drug for 20 min and then assaying for enzyme activity in the presence of ATCh. SigmaPlot was then used to fit the data to a three-parameter logistic function, yielding IC$_{50}$. Biphasic inhibition curves were fit with GOSAfIt. Since we were just looking for classical diagnostic differential inhibition, it was not necessary to determine $k_i$ or $K_i$ values for the inhibitors [4,24,52].

### Velocity Sedimentation on Sucrose Gradients; PIPLC Digestion

The molecular forms of ChE were analyzed by velocity sedimentation in 5–25% isokinetic sucrose gradients prepared in HIS buffer (with or without Triton X-100) containing 1 mg/ml bovine serum albumin. Sedimentation was in an SW 41 rotor at 30,000–37,000 rpm for times satisfying the equation [(rpm)$^2 \times t$ (h)] = 2.5 × 10$^{10}$ as described previously [24]. Apparent sedimentation coefficients were calculated relative to the sedimentation of catalase (11.3 S). Data were plotted as fractional activity of total ChE activity on the gradient as a function of sedimentation coefficient; fractional activity on gradient $= (activity$ in a given fraction/total activity on gradient); sedimentation coefficient $= (fraction$ number $)[11.3$/fraction number of catalase peak].

### Supporting Information

**Figure S1** The pdb dataset for the model of *O. latipes* BChE. (DOC)

### Acknowledgments

We thank Florence Sabatier for the generous gift of *O. latipes*. We also thank GIS-AMAGEN CNRS/INRA for the gift of the initial UniGene EST.

### Author Contributions

Conceived and designed the experiments: AC FN LP. Performed the experiments: AC FN LP. Analyzed the data: AC FN LP. Contributed reagents/materials/analysis tools: AC FN LP. Wrote the paper: AC FN LP.
15. Walker CH, Thompson HM. Phyletic distribution of cholinesterases and related esterases, in Cholinesterase-inhibiting insects; their impact on wildlife and the environment. Mineau P, ed. 1991, Elsevier: New York: pp 1–17.

16. Morrell SN, Rozergent EV (2007) Comparative enzymology of cholinesterases. La, Chironomidae. In: NA. Comp Biochem Physiol Part C 22: 2183–90.

17. Toutant JP, Massouile J, Bon S (1985) Polymorphism of pseudocholinesterase in Torpedo marmorata tissues: Comparative study of the catalytic and molecular properties of this enzyme with acetylcholinesterase. J Neurochem 44: 380–92.

18. Lundin SJ (1986) Properties of a cholinesterase from body muscle of plaice, Pleuronectes platessa. Acta Chem Scand B 40: 577–79.

19. Stieger S, Gentinetta R, Brodech U (1989) Cholinesterases from floundered muscle. Purification and characterization of glycosyl-phosphatidylinositol-anchored and collagen-tailed forms differing in substrate specificity. Comp Biochem Physiol 110: 631–42.

20. Lebel WS (1983) Antiherb probes to an atypical pseudocholinesterase from surgoenfish reveal immunocchemical variability and tissue-specific molecular polymorphism. J Exp Zool 249: 209–23.

21. Leibel WS (1988) Characterization of a pseudocholinesterase purified from surgoenfish tissues confirms the atypical nature of this enzyme. J Exp Zool 249: 198–208.

22. Whittaker VP (2010) How the cholinesterases got their modern names. Chem Biol Interact 181: 55–64.

23. Pezzementi L, Reinheimer EJ, Pezzementi ML (1987) Acetylcholinesterase from the skeletal muscle of the lamprey Petromyzon marinus exists in globular and asymmetric forms. J Neurochem 48: 1753–60.

24. Sanders M, Mathews B, Sutherland D, Soong W, Giles H, et al. (1996) Biochemical and molecular characterization of acetylcholinesterase from the naghfish Myxine glutinosa. Comp Biochem Physiol B Biochem Physiol Mol Biol 115: 97–109.

25. Chatonnet A, Lockridge O (1989) Comparison of butyrylcholinesterase and acetylcholinesterase in solution. Biochem J 261: 625–34.

26. Massouile J, Perrier N, Nourdelinde H, Liang D, Bon S (2000) Old and new questions about cholinesterases. Chem Biol Interact 157: 30–44.

27. Silman I, Sussman JL (2005) Acetylcholinesterase: ‘classical’ and ‘non-classical’ functions and pharmacology. Curro Opin Pharmacol 5: 293–302.

28. Muensterer E, Soreq H (2006) Virtues and woes of ache alternative splicing in stress-related neuropathologies. Trends Neurosci 29: 216–24.

29. Duval N, Massouile J, Bon S (1992) H and t subunits of acetylcholinesterase from Torpedo, expressed in COS cells, generate all types of globular forms. J Biol Chem 267: 53–57.

30. Perrier AL, Massouile J, Krecji E (2002) PRIJMA: The membrane anchor of acetylcholinesterase in the brain. Neuron 33: 275–85.

31. Krecji E, Cousens F, Duval N, Chatel JM, Legacy C, et al. (1991) Primary structure of a collagen tail peptide of Torpedo acetylcholinesterase: Co-expression with catalytic subunit induces the production of collagen-tailed forms in transfected cells. EMBO J 10: 1285–93.

32. Krecji E, Thomine S, Bocchetti N, Legacy C, Skeltj E, et al. (1997) The mammalian gene of acetylcholinesterase-associated collagen. J Biol Chem 272: 29140–7.

33. Futerman AH, Low MG, Michaelson DM, Silman I (1985) Solubilization of membrane-bound acetylcholinesterase by a phosphatidylinositol-specific phospholipase C. J Neurochem 45: 1487–94.

34. Isnoufa NC, Fuentes ME, Anglister J, Futerman AH, Silman I (1988) A membrane-associated dimer of acetylcholinesterase from Xenopus skeletal muscle is solubilized by phosphatidylinositol-specific phospholipase C. Neurosci Lett 96: 109–12.

35. Roberts WL, Kim BH, Rosenbury TL (1987) Differences in the glycolipid membrane anchors of bovine and human erythrocyte acetylcholinesterases. Proc Natl Acad Sci USA 84: 7817–21.

36. Altamirano CV, Lockridge O (1999) Association of tetramers of human butyrylcholinesterase with acetylcholinesterase. J Biol Chem 274: 641–7.

37. Fluck RA (1982) Localization of acetylcholinesterase activity in young embryos of the Japanese medaka Oryzias latipes. J Comp Physiol 148: 491–501.

38. El-Alfy A, Sreelak D (1998) Potential mechanisms of the enhancement of aldicarb toxicity to Japanese medaka, Oryzias latipes, at high salinity. Toxicol Appl Pharmacol 152: 175–83.

39. Niitao MG, Rodrigues-Fuentes G, Sannel J, Silman I (1997) Enantioselective acetylcholinesterase inhibition of the organophosphorous insecticides profenofos, fonofos, and crotoxynos. Environ Toxicol Chem 26: 1949–54.

40. Chiuco G, Podgornaya VA (2005) Acetylcholinesterase and butyrylcholinesterase of freshwater teleosts: Cross-species and cross-family differences. Comp Biochem Physiol B Biochem Systemat Biol 135: 53–61.

41. Delcea C, Munken AO (2007) Atypical cholinesterase from phylogenetic and evolutionary perspectives. PLoS ONE 2: 806–11.

42. Shapira M, Seidman S, Livni N, Soreq H (1998) In vivo and in vitro resistance of Anopheles gambiae to organophosphorous cholinesterase inhibitors. J Insect Physiol 44: 395–405.

43. Wittbrodt J, Shima A, Schartl M (2002) Medaka—a model organism from the far east. Nat Rev Genet 3: 53–64.

44. Lundin SJ (1968) Properties of a cholinesterase from body muscles of plaice, Pleuronectes platessa. Acta Chem Scand 22: 2183–90.
86. Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, et al. (2005) Individual
85. Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and
84. Scholz NL, Hopkins WA (2006) Ecotoxicology of anticholinesterase pesticides:
83. Singh BK, Walker A (2006) Microbial degradation of organophosphorus
82. Pezzementi L, Chatonnet A (2010) Evolution of cholinesterases in the animal
81. Sikorav JL, Duval N, Anselmet A, Bon S, Krejci E, et al. (1988) Complex
80. Legay C, Bon S, Massoulie J (1993) Expression of a cDNA encoding the
79. Li Y, Camp S, Taylor P (1993) Tissue-specific expression and alternative
78. Sorek R (2007) The birth of new exons: Mechanisms and evolutionary
77. Schmidt EE, Davies CJ (2007) The origins of polypeptide domains. Bioessays
76. Liu M, Grigoriev A (2004) Protein domains correlate strongly with exons in multiple eukaryotic genomes—evidence of exon shuffling” Trends Genet 20:
399–403.
75. Combes D, Fedon Y, Grauso M, Toutant JP, Arpagaus M (2000) Four genes
74. Meyer A, Schartl M (1999) Gene and genome duplications in vertebrates: The
one-to-four (to-eight in fish) rule and the evolution of novel gene functions. Curr
Opin Cell Biol 11: 699–704.
73. Combes D, Fedon Y, Grauso M, Toutant JP, Arpagaus M (2000) Four genes
encode acetylcholinesterases in the nematodes Caenorhabditis elegans and
Caenorhabditis briggsae. cDNA sequences, genomic structures, mutations and in vivo expression. J Mol Biol 308: 727–42.
72. Liu M, Grigoriev A (2004) Protein domains correlate strongly with exons in multiple eukaryotic genomes—evidence of exon shuffling” Trends Genet 20:
399–403.
71. Schmidt EE, Davies CJ (2007) The origins of polypeptide domains. Bioessays
29: 262–70.
70. Sorek R (2007) The birth of new exons: Mechanisms and evolutionary
consequences. RNA 13: 1603–8.
69. Li Y, Camp S, Taylor P (1993) Tissue-specific expression and alternative
mRNA processing of the mammalian acetylcholinesterase gene. J Biol Chem
268: 5780–7.
68. Legay C, Bon S, Massoulie J (1993) Expression of a cDNA encoding the
glycolipid-anchored form of rat acetylcholinesterase. FEBS Lett 315: 163–6.
67. Sikorav JL, Duval N, Anselmet A, Bon S, Krejci E, et al. (1988) Complex
alternative splicing of acetylcholinesterase transcripts in Torpedo electric organ;
primary structure of the precursor of the glycolipid-anchored dimeric form. EMBO J 7: 2983–93.
66. Pezzementi L, Chatonnet A (2010) Evolution of cholinesterases in the animal
kingdom. Chem Biol Interact 167: 27–33.
65. Singh BK, Walker A (2006) Microbial degradation of organophosphorus
compounds. FEBS Microbiol Rev 30: 429–71.
64. Scholz NI, Hopkins WA (2006) Ecotoxicology of anticholinesterase pesticides:
Data gaps and research challenges. Environ Toxicol Chem 25: 1185–6.
63. Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and
invertebrates as an indicator of organophosphorus insecticide exposure and
data gaps and research challenges. Environ Toxicol Chem 20: 37–45.
62. Liu M, Grigoriev A (2004) Protein domains correlate strongly with exons in multiple eukaryotic genomes—evidence of exon shuffling” Trends Genet 20:
399–403.
61. Schmidt EE, Davies CJ (2007) The origins of polypeptide domains. Bioessays
29: 262–70.
60. Sorek R (2007) The birth of new exons: Mechanisms and evolutionary
consequences. RNA 13: 1603–8.
59. Li Y, Camp S, Taylor P (1993) Tissue-specific expression and alternative
mRNA processing of the mammalian acetylcholinesterase gene. J Biol Chem
268: 5780–7.
58. Legay C, Bon S, Massoulie J (1993) Expression of a cDNA encoding the
glycolipid-anchored form of rat acetylcholinesterase. FEBS Lett 315: 163–6.
57. Sikorav JL, Duval N, Anselmet A, Bon S, Krejci E, et al. (1988) Complex
alternative splicing of acetylcholinesterase transcripts in Torpedo electric organ;
primary structure of the precursor of the glycolipid-anchored dimeric form. EMBO J 7: 2983–93.
56. Pezzementi L, Chatonnet A (2010) Evolution of cholinesterases in the animal
kingdom. Chem Biol Interact 167: 27–33.
55. Singh BK, Walker A (2006) Microbial degradation of organophosphorus
compounds. FEBS Microbiol Rev 30: 429–71.
54. Scholz NI, Hopkins WA (2006) Ecotoxicology of anticholinesterase pesticides:
Data gaps and research challenges. Environ Toxicol Chem 25: 1185–6.
53. Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and
invertebrates as an indicator of organophosphorus insecticide exposure and
data gaps and research challenges. Environ Toxicol Chem 20: 37–45.
52. Liu M, Grigoriev A (2004) Protein domains correlate strongly with exons in multiple eukaryotic genomes—evidence of exon shuffling” Trends Genet 20:
399–403.
51. Schmidt EE, Davies CJ (2007) The origins of polypeptide domains. Bioessays
29: 262–70.
50. Sorek R (2007) The birth of new exons: Mechanisms and evolutionary
consequences. RNA 13: 1603–8.
49. Li Y, Camp S, Taylor P (1993) Tissue-specific expression and alternative
mRNA processing of the mammalian acetylcholinesterase gene. J Biol Chem
268: 5780–7.
48. Legay C, Bon S, Massoulie J (1993) Expression of a cDNA encoding the
glycolipid-anchored form of rat acetylcholinesterase. FEBS Lett 315: 163–6.
47. Sikorav JL, Duval N, Anselmet A, Bon S, Krejci E, et al. (1988) Complex
alternative splicing of acetylcholinesterase transcripts in Torpedo electric organ;
primary structure of the precursor of the glycolipid-anchored dimeric form. EMBO J 7: 2983–93.
46. Pezzementi L, Chatonnet A (2010) Evolution of cholinesterases in the animal
kingdom. Chem Biol Interact 167: 27–33.
45. Singh BK, Walker A (2006) Microbial degradation of organophosphorus
compounds. FEBS Microbiol Rev 30: 429–71.
44. Scholz NI, Hopkins WA (2006) Ecotoxicology of anticholinesterase pesticides:
Data gaps and research challenges. Environ Toxicol Chem 25: 1185–6.
43. Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and
invertebrates as an indicator of organophosphorus insecticide exposure and
data gaps and research challenges. Environ Toxicol Chem 20: 37–45.
42. Liu M, Grigoriev A (2004) Protein domains correlate strongly with exons in multiple eukaryotic genomes—evidence of exon shuffling” Trends Genet 20:
399–403.
41. Schmidt EE, Davies CJ (2007) The origins of polypeptide domains. Bioessays
29: 262–70.
40. Sorek R (2007) The birth of new exons: Mechanisms and evolutionary
consequences. RNA 13: 1603–8.
39. Li Y, Camp S, Taylor P (1993) Tissue-specific expression and alternative
mRNA processing of the mammalian acetylcholinesterase gene. J Biol Chem
268: 5780–7.