Chemico-Biological Interactions 203 (2013) 319–322

Contents lists available at SciVerse ScienceDirect

Chemico-Biological Interactions

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

Acetylcholinesterases of blood-feeding flies and ticks

Kevin B. Temeyer *, Alexander P. Tuckow, Danett K. Brake [1], Andrew Y. Li, Adalberto A. Pérez de León

Knipling-Bushland US Livestock Insect Research Laboratory, Agricultural Research Service, US Department of Agriculture, 2700 Fredericksburg Road, Kerrville, TX 78028-9184, United States

ARTICLE INFO

Article history:
Available online 1 October 2012

Keywords:
Rhipicephalus microplus
Ectoparasite
Acetylcholinesterase
Biting flies
Cattle tick

ABSTRACT

Acetylcholinesterase (AChE) is the biochemical target of organophosphate (OP) and carbamate pesticides for invertebrates, vertebrate nerve agents, and AChE inhibitors used to reduce effects of Alzheimer's disease. Organophosphate pesticides (OPs) are widely used to control blood-feeding arthropods, including biting flies and ticks. However, resistance to OPs in pests affecting animal and human health has compromised control efficacy. OP resistance often results from mutations producing an OP-insensitive AChE. Our studies have demonstrated production of OP-insensitive AChEs in biting flies and ticks. Complementary DNA (cDNA) sequences encoding AChEs were obtained for the horn fly, stable fly, sand fly, and the southern cattle tick. The availability of cDNA sequences enables the identification of mutations, expression and characterization of recombinant proteins, gene silencing for functional studies, as well as in vitro screening of novel inhibitors. The southern cattle tick expresses at least three different genes encoding AChE in their synganglion, i.e. brain. Gene amplification for each of the three known cattle tick AChE genes and expression of multiple alleles for each gene may reduce fitness cost associated with OP-resistance. AChE hydrolyzes the neurotransmitter, acetylcholine, but may have additional roles in physiology and development. The three cattle tick AChEs possess significantly different biochemical properties, and are expressed in neural and non-neural tissues, which suggest separation of structure and function. The remarkable complexity of AChEs in ticks suggested by combining genomic data from *Ixodes scapularis* with our genetic and biochemical data from *Rhipicephalus microplus* is suggestive of previously unknown gene duplication and diversification. Comparative studies between invertebrate and vertebrate AChEs could enhance our understanding of structure–activity relationships. Research with ticks as a model system offers the opportunity to elucidate structure–activity relationships for AChE that are important for advances in targeted pest control, as well as potential applications for medicine and biosecurity.

Published by Elsevier Ireland Ltd.

1. Introduction

Organophosphate pesticides (OPs) substantially replaced the use of organochlorines such as DDT in the 1960's and remain the most widely used class of insecticides in the world, approximating 32% of the total world pesticide market and 36% of the U.S. market in 2007 [1]. The mechanism by which OPs act is quasi-irreversible inhibition of acetylcholinesterase (AChE) in the central nervous system leading to functional collapse of the system and death of the arthropod pest [2]. A major mechanism of arthropod resistance to organophosphate pesticides is production of an altered acetylcholinesterase that is insensitive to the inhibitor as a result of one or more point mutations [3,4]. Studies in our laboratory are intended to elucidate mechanisms of pesticide resistance in arthropod ectoparasites of livestock, specifically ticks and biting flies. This research has implications for comparative studies aiming to manipulate AChE as a target for biomedical and biosecurity interventions.

2. Material and methods

Amino acid sequences of putative AChEs of *Ixodes scapularis* were downloaded from the NCBI protein database (available through http://www.ncbi.nlm.nih.gov/) and checked for correct annotation by BLASTp (protein–protein BLAST) analysis. Redundant sequences and those showing highest homology to identified non-cholinesterase proteins were omitted from further analysis. Amino acid sequences of *Rhipicephalus microplus* BmAChE1 (Gen-

Abbreviations: OP, organophosphate pesticide; AChE, acetylcholinesterase; CFTEP, cattle fever tick eradication program; cDNA, complementary DNA; dsRNA, double stranded RNA; BLASTp, Protein–protein BLAST.

* Corresponding author. Tel.: +1 (830) 792 0330; fax: +1 (830) 792 0314.
E-mail addresses: kevin.temeyer@ars.usda.gov (K.B. Temeyer), alex.tuckow@ars.usda.gov (A.P. Tuckow), danett.bishop@med.navy.mil (D.K. Brake), andrew.li@ars.usda.gov (A.Y. Li), beto.perezdeleon@ars.usda.gov (A.A. Pérez de León).
[1] Present address: Medical Service Corps, US Navy, Wound Infections Dept., Naval Medical Research Center, 503 Robert Grant Ave., Silver Spring, MD 20910-7500, United States.

0009-2797/$ - see front matter Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.cbi.2012.09.010

Bank accession CAA11702) BmAChE2 (GenBank accession AAC18857) and BmAChE3 (GenBank accession AAP92139) were included as reference sequences. The protein sequence alignment and phylogenetic tree were generated using CLC Main Workbench version 6.6.2 (CLC bio, Aarhus, Denmark). Alignment was performed utilizing the slow, but very accurate algorithm using the default parameters (gap open cost of 10 and gap extension cost of 1). The phylogenetic tree was generated using the neighbor joining algorithm with 1000 iterations in the bootstrap analysis.

3. Results and discussion

3.1. Ticks

The OP coumaphos remains a key component of the Cattle Fever Tick Eradication Program (CFTEP) to prevent reentry and eradicate outbreak infestations of *Rhipicephalus* (*Boophilus*) *microplus* and *R.* (*B.*) *annulatus* ticks within the United States [5]. These ticks transmit the causative agents of bovine babesiosis and anaplasmosis, each of which is capable of producing up to 90% mortality in naïve animals. *R. microplus* and *R. annulatus* were eradicated from the United States by a protracted effort beginning in 1906 under the CFTEP [6]. The ticks were declared eradicated from the United States in 1943; however, the ticks remain endemic in Mexico and subtropical areas throughout much of the world. Emergence and increasing incidence of tick resistance to OP exposure in Mexico and other countries threaten the US cattle industry and complicate efforts by the CFTEP to maintain an effective entry barrier and to eradicate outbreak infestations [7]. The major mechanism of resistance to OP acaricides in ticks was biochemically characterized as AChE insensitivity to OP inhibition in extracts of tick synganglia [8–10] and resistance may also have a metabolic detoxification component [11].

Baxter and Barker first reported identification of a cDNA presumptively encoding AChE in *R. microplus* and other ticks, but searches to identify AChE mutations conferring OP insensitivity were not successful [12]. A second cDNA presumptively encoding an AChE of *R. microplus* was subsequently reported [13], and attempts to identify AChE mutations associated with acaricide resistance remained unsuccessful [14]. A third cDNA presumptively encoding *R. microplus* AChE was reported [15], subsequently expressed in baculovirus for biochemical confirmation of identity as an AChE [16], and at least one mutation was shown to result in production of an altered AChE with decreased sensitivity to OP inhibition [17]. Further studies utilized baculovirus expression to produce enzymatically active recombinant protein for each of the three presumptively identified AChE cDNAs of *R. microplus* (*BmAChE1*, *BmAChE2*, and *BmAChE3*) and allowed biochemical confirmation that each of them encoded authentic AChEs [18]. The recombinant baculoviral constructs also confirmed that mutations in *BmAChE1* and *BmAChE3* produced OP-insensitive enzymes. Genotyping surveys identified a number of mutations in each of the three confirmed *BmAChEs* that appeared to be associated with OP-resistant strains [17–21]. Identification of OP-insensitive mutations is important to allow development of molecular assays for rapid diagnosis of OP-resistance, to guide selection of control options, and to determine effects on enzyme structure and activity resulting from specific amino acid substitutions.

Reverse-transcription PCR demonstrated that transcripts encoding each of the three BmAChEs were expressed in tick synganglia [18] and quantitative PCR demonstrated that each of the three *BmAChE* genes were present in multiple copies in genomic DNA [21]. Sequencing of cDNAs from individual ticks further demonstrated that multiple alleles were expressed in tick synganglia for each of the three *BmAChEs*. Results of gene silencing experiments by microinjection of dsRNA (double stranded RNA) strongly suggested that the three BmAChEs functionally complement one another *in vivo* [20]. It is hypothesized that the presence of multiple copies and multiple alleles for each of the *BmAChEs* presents the opportunity for recombination to rapidly develop enhanced resistance combinations. Additionally, the ability to express multiple alleles may allow mitigation of potential fitness costs associated with OP-insensitivity by allowing survival in the presence of selection while retaining fully functional wild-type alleles in the absence of OP selection [20,21]. These studies revealed that OP-resistance in *R. microplus* is highly complex.

Genomic data from *R. microplus* and *I. scapularis* strongly suggest the presence of additional genes encoding AChE in ticks. Our investigations indicated that AChE transcripts are expressed in non-neural tick tissues, including salivary glands. Preliminary results suggest the presence of AChE enzymatic activity in tick saliva. AChE is known to be secreted by other parasites [22,23], including the saliva of blood feeding insects [24,25]. AChE is known to be involved in vertebrate immune function [26,27], and secretion of AChE in tick saliva may be involved in promoting feeding by hydrolyzing platelet activating factor [24], or inhibiting infiltration of lymphocytes [28] and wound healing [29] by reducing local tissue concentration of acetylcholine. In addition, salivary AChE may provide modulation of the host immune response [30–32], or protection of the tick from effects of acetylcholine contained in the large volume of ingested blood from the host. The phylogram depicting amino acid sequences purportedly encoded by AChE genes of *I. scapularis* and confirmed AChEs of *R. microplus* shown in Fig. 1 suggests the adaptive multiplicity and diversity of AChEs in ticks. Ticks are well-known vectors of diverse viruses and microorganisms. Duplication of tick genes may have arisen from viral reverse transcriptase activity consistent with our observation that most *BmAChE1* genomic sequences of *R. microplus* amplified by PCR lack a specific intron present in a small minority of such amplicons (unpublished). Integration of cDNA copies generated by reverse transcription could allow amplification by recombination and/or replicational stuttering followed by subsequent structural and functional diversification of some of the amplified copies to give rise to the multiplicity of presumptive AChE genes. In addition to potential functions facilitating feeding or manipulating host immune response previously mentioned, diversity of tick AChE functions could include regulation of developmental, metabolic, or adaptive behavioral (e.g., seeking a host or mate) processes. Amplification of AChE genes allows ticks to maintain multiple alleles for each of the BmAChEs expressed in the *R. microplus* synganglion (brain) which may allow the ticks to maintain AChE activity in the presence of inhibitors sufficient for survival, while also maintaining fully functional allelic copies thereby reducing or eliminating potential fitness cost associated with *BmAChE* mutations producing altered forms of the enzymes insensitive to inhibition. In addition, if each of the BmAChEs plays an essential role, mutations in multiple genes may be necessary to generate the acaricide-resistant phenotype. New studies are planned to confirm the biochemical identity of additional tick AChEs and to elucidate the physiological roles of tick AChEs.

In vertebrates, a single AChE gene is subject to alternative splicing to give rise to variant forms of AChE [33,34]. In addition to the canonical role in termination of neural impulses by catalytic hydrolysis of acetylcholine, AChE in vertebrates is postulated to play many additional roles in physiology and development [35,36]. The presence of multiple genes encoding AChE in ticks may provide separation of structure and function (see Fig. 1). Tick AChEs therefore present an attractive and opportune model system for elucidation of non-canonical roles of AChE, with substantial implications for biomedical research.