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Organophosphate-resistant forms of acetylcholinesterases in two scallops—the Antarctic *Adamussium colbecki* and the Mediterranean *Pecten jacobaeus*

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Abstract

We describe the acetylcholinesterase polymorphisms of two bivalve molluscs, *Adamussium colbecki* and *Pecten jacobaeus*. The research was aimed to point out differences in the expression of pesticide-resistant acetylcholinesterase forms in organisms living in different ecosystems such as the Ross Sea (Antarctica) and the Mediterranean Sea. In *A. colbecki*, distinct acetylcholinesterase molecular forms were purified and characterized from spontaneously soluble, low-salt-soluble and low-salt-Triton extracts from adductor muscle and gills. They consist of two non-amphiphilic acetylcholinesterases (G_2, G_4) and an amphiphilic-phosphatidylinositol-membrane-anchored form (G_2); a further amphiphilic-low-salt-soluble G_2 acetylcholinesterase was found only in adductor muscle. In the corresponding tissues of *P. jacobaeus*, we found a non-amphiphilic G_4 and an amphiphilic G_2 acetylcholinesterase; amphiphilic-low-salt-soluble acetylcholinesterases (G_2) are completely lacking. Such results are related with differences in cell membrane lipid compositions. In both scallops, all non-amphiphilic AChEs are resistant to used pesticides. Differently, the adductor muscle amphiphilic forms are resistant to carbamate eserine and organophosphate diisopropylfluorophosphate, but sensitive to organophoshate azamethiphos. In the gills of *P. jacobaeus*, amphiphilic G_2 forms are sensitive to all three pesticides, while the corresponding forms of *A. colbecki* are sensitive to eserine and diisopropylfluorophosphate, but resistant to azamethiphos. Results indicate that organophosphate and/or carbamate resistant AChE forms are present in species living in far different and far away environments. The possibility that these AChE forms could have ensued from a common origin and have been spread globally by migration is discussed.

Keywords: Acetylcholinesterase; *Adamussium colbecki*; Azamethiphos; Diisopropylfluorophosphate; Membrane lipids; Molecular forms; Organophosphate resistance; *Pecten jacobaeus*; Polymorphisms

1. Introduction

Acetylcholinesterases (AChE, EC 3.1.1.7) are serine hydrolases, whose primary function in all living organisms is the specific hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. In vertebrates, AChEs are distributed in several tissues, where they are expressed as multiple molecular forms including asymmetric (A) and globular (G) enzymes as monomers or oligomers of either hydrophilic or amphiphilic catalytic subunits. These different AChE catalytic subunits likely arose from a single gene through alternative splicing in the 3′ region of the transcripts; such subunits show the same common catalytic domain, followed by distinct C-terminal peptides (Massoulié et al., 1993).

Only globular forms of AChE are present in invertebrates (Massoulié et al., 1993; Talesa et al., 1993, 1996; Pezzementi et al., 1989; Sanders et al., 1996). Such enzymes consist of

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monomers (G_1), dimers (G_2) and tetramers (G_4) of catalytic subunits, without the asymmetric collagenic-tailed forms typical of vertebrates. Generally, most AChEs described in invertebrates display a less defined substrate specificity and a marked variability in the kinetic behavior compared to vertebrates (Talesa et al., 1994). In particular, a dimeric AChE linked to the cell membrane by a phosphatidylinositol (PtdIns) anchor is always expressed in invertebrates. Such an enzyme is thought to fulfil a key role in cholinergic transmission, while the function of similar AChE forms is so far unknown in vertebrates.

In invertebrates, the genetics of AChEs differ in the various phyla and sometimes within the same phylum: the encoding of AChE subunits are encoded by one, two or several genes (Talesa et al., 1999, 2002), thus giving a complex polymorphism of the enzyme. In invertebrates, alternative splicing has been only evidenced in cotton aphid (*Aphis gossypii*) (Li and Han, 2002).

Previously, we found that some molluscs, cephalopods and bivalves (Talesa et al., 1995, 1998, 1999, 2001, 2002), display AChE forms with striking substrate specificity for acetylthiocholine (ATC) and high catalytic efficiency. These enzymes, similar to those of the vertebrate nervous system (Rosenberry, 1975), seem to be true AChEs that do not hydrolyze butyrylthiocholine (BTC). Again, these forms do not undergo inhibition by diisopropylfluorophosphate (DFP), an organophosphate (OP) neurotoxicant. In the optic lobe of the squid *Loligo opalescens* (Talesa et al., 1999), the deduced AChE amino acid sequence gave evidence for a polar amino acid residue, instead of a non polar one, in the acyl pocket of the enzyme active site. Such a replacement could explain the high substrate specificity, the absence of hydrolysis with butyrylthiocholine and the poor DFP inhibition of the enzyme, because it may prevent the access and/or binding of DFP to the active site (Talesa et al., 1999).

In the present study, we characterized AChE forms from an Antarctic scallop *Adamussium colbecki* and a Mediterranean counterpart *Pecten jacobaeus*, to analyze differences in expression of pesticide-resistant AChE forms in two phylogenetically close species, living in two different ecosystems, the former quite pristine and the latter highly anthropized environment. *A. colbecki*, a monospecific genus (Canapa et al., 2000), and *P. jacobaeus* belong to the family Pectinidae, as suggested by a previous analysis of 18S rRNA gene (Canapa et al., 1999). Both are filter-feeding species and are characterized by circular shells and flattened valves.

The habitat of *A. colbecki* provides a well-constrained experimental framework for assessing environmental coupling and climate-driven dynamics of marine and terrestrial ecosystem in a high-latitude region, where water is at transition between liquid and solid phases. In particular, even though Antarctica is commonly considered one of the least polluted areas of the world, recent studies have underlined the presence of man-made pollutants such as organic xenobiotics, including persistent pollutants and pesticides (Kennicutt, 1990; Focardi et al., 1995; Kennicutt et al., 1995). The susceptibility of the Antarctic marine environment to contamination is suggested by the slow recovery rates and short food chains which characterize such ecosystems. In addition, several studies have indicated a difference in sensitivity of aquatic polar organisms to pollutants in comparison with species from temperate areas (Ling et al., 1998; King and Riddle, 2001).

2. Materials and methods

2.1. Sampling

Specimens of *A. colbecki* were randomly collected from three coastal sites (Road Bay, Thethys Bay and Adelie Cove) near the Italian Antarctic Base "Baia Terra Nova" (BTN) (lat. 74410S, long. 164040E) (Terra Nova Bay, Ross Sea), during the Antarctic summer of 2001–2002. Specimens of *P. jacobaeus* were obtained in May from fishing operations on the northwest Adriatic Sea (Italy). Gills and adductor muscles from 15 individuals of each species were dissected, immediately frozen in liquid nitrogen to prevent enzyme deterioration and stored at −80 °C.

2.2. Materials

All reagents were analytical grade products from Sigma-Aldrich S.r.l. (Milano, Italy) and all solutions were made using twice-distilled water.

2.3. Buffers

Low-salt (LS) buffer contained 20 mM Tris–HCl, pH 7.4, 1 mM ethylene diamine tetraacetic acid (EDTA), 5 mM $MgCl_2$, 0.1 mg/ml bacitracin and 8×10^{-3} trypsin inhibitor unit (TIU)/ml aprotinin to minimize proteolysis. Low-salt-Triton (LST) and high-salt (HS) buffers contained LS buffer supplemented as above plus 1% Triton X-100 or 1.0 M NaCl, respectively. High-salt-Triton (HST) buffer contained HS buffer plus 1% Triton X-100.

2.4. Sequential extraction and purification of AChE

The extraction of AChE from the adductor muscle or gill tissues of *A. colbecki* and *P. jacobaeus* specimens followed a procedure similar to those previously described (Romani et al., 2005). It was aimed to recover sequentially non-amphiphilic spontaneously soluble (SS) AChEs, commonly present in the hemolymph, than low-salt-soluble (LS) and amphiphilic detergent-soluble (LST) AChE forms, both corresponding to tissue-specific enzymes. In detail, through distinct procedures for either species and operating at 5 °C, the adductor muscle and gills were separately recovered and pooled from 15 specimens. Tissues were minced by scissors into small pieces (1–2 mm) in 4 vol. of LS buffer, pH 7.4, at 5 °C and finally centrifuged at 100,000×*g* for 20 min. The supernatant, containing mostly blood and showing substantial AChE activity, was considered as SS extract and saved for subsequent enzyme purification procedures. The recovered pellet underwent two consecutive double cycles of homogenisation (Turrax homogenizer and Potter-Elvehjem glass–glass in succession; 1:5, w/v of LS and LST buffer) and centrifugation (100,000×*g* for 1 h, Beckman L60 ultracentrifuge with SW 41 Ti rotor), thus giving LS and LST