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Determination of esterase activity and characterization of cholinesterases in the reef fish *Haemulon plumieri*☆

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Abstract

White grunt (*Haemulon plumieri*) has been proposed by the Mesoamerican Barrier Reef System (MBRS) Synoptic Monitoring Program as a bioindicator species. It is in this sense that the present study has a main goal to evaluate this organism's suitability as an indicator species. Individuals were captured during three seasons at the port of Sisal, Yucatan, Mexico which is located in an area that is considered to be weakly impacted by human activities such as agriculture or industry. Both cholinesterase (ChE) and carboxylesterase (CbE) activities were measured in brain, muscle, liver and eye of sampled individuals. Results indicated that ChE and CbE activities were greatest in the brain (256.3 ± 43) and in the liver (191 ± 21), respectively. Furthermore, ChEs detected in brain, liver and muscle were characterized, and results suggested that the acetylcholinesterase (AChE) type was more abundant relative to pseudocholinesterase (BChE) which was rare. In addition, K_m and V_{max} and IC_{50} values were calculated from the Michaelis–Menten equation. Finally, an additional experiment *in vitro* showed a significant decrease in both ChE and CbE activities when different tissues were exposed to model xenobiotics, such as benzo[*a*]pyrene and Chlorpyrifos. In conclusion, findings from this study confirm the potential suitability of *H. plumieri* as an organic pollution bioindicator species, and thus of practical use for environmental biomonitoring purposes.

Keywords: Monitoring; Biomarkers; Fish; Grunt; Cholinesterases; Carboxylesterases

1. Introduction

The increasing input of environmental contaminants to aquatic ecosystems has generated the need to understand and evaluate the biological effects of xenobiotics on aquatic biota. In this sense, a large number of studies have used biomarkers as functional tools to evaluate the toxicity of such compounds for natural populations and at subindividual levels. Such studies have also aimed at extrapolating implications from their results to higher levels of biological organization (Walker et al., 2001).

B-esterases are an enzyme group that is inhibited by organophosphorus pesticides (OPs) based on time and temperature-dependent reaction (Aldridge, 1953). Of this group, cholinesterases (ChEs) have been the most frequently used biomarkers to evaluate exposure and effects of several organophosphate and carbamate pesticides. Vertebrates have two types of ChEs: acetylcholinesterase (AChE) and butyrylcholinesterase or pseudocholinesterase (BChE), which differs in their substrate specificity. On the one hand, AChE hydrolyzes acetylcholine faster compared to other choline esters and is much less active with butirylcholine. In contrast, BChE is highly efficient at hydrolyzing both butirylcholine and acetylcholine. In fishes, AChE is predominant in brain and muscle tissues, whereas BChE is present mostly in the liver and plasma (Habig and Di Giulio, 1991). In addition, several studies have reported atypical ChEs in teleost marine fishes (Sturm et al., 1999; Varó et al., 2003; Rodríguez-Fuentes and Gold-Bouchot, 2004; Monteiro et al., 2005). The main role of AChE is to catalyze the hydrolysis of acetylcholine into choline and acetic acid at cholinergic synaptic sites (Walker and Thompson, 1991). On the other hand, the precise physiological function of BChE is not known principally because the inhibition of BChE is of no pharmacological

☆All experiments with fish in this study were conducted according to the institutional guidelines for the protection of animal welfare.

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importance. Despite the previous, BChE has a broad distribution across several types of tissue, including serum, liver, heart, brain, vascular endothelia and in the nervous system. Such widespread distribution might suggest that BChE has various roles across tissues within an organism (Mack and Robitzki, 2000). For instance, it has been proposed as a marking enzyme for support cells or other non-neuronal elements (Peakall, 1992). In addition, because BChE can hydrolyze hydrophobic and hydrophilic compounds containing carboxylic or phosphoric acid esters, it may work as an endogenous scavenger of anticholinergic compounds. In other words, BChE detoxifies the latter before they inhibit AChE at physiologically important target sites (Pezzementi et al., 1991; Cokugras, 2003). In practical terms, it is important to determine which type of ChE is most abundant in a candidate bioindicator species, as this will define which substrate and concentration are most appropriate for monitoring purposes.

Cholinesterase inhibition is widely used as a biomarker due to its specificity to organophosphorus and carbamates compounds (Galgani and Bocquene, 2000; Thompson, 1991). Additionally, other studies have also shown that ChEs are altered by PAHs, some heavy metals, and surfactants (Gill et al., 1990; Payne et al., 1996; Guilhermino et al., 2000; Moreira et al., 2004; Moreira and Guilhermino, 2005). The latter effect is not considered an inhibition process because it involves a different mechanism; although this has not been demonstrated for many species, positive results found in others are enough to suggest its use as a wide-ranging biomarker (Guilhermino et al, 1998).

On the other hand, carboxylesterases (CbE) also belong to the B-esterases group and are widespread across species, including fishes. Within an organism, CbEs are found ubiquitously, although they are most abundant in the liver. The natural substrate for most CbEs is not known; therefore, their physiological function has not been well understood. In spite of this, previous studies have reported that these enzymes are important for the metabolism of endogenous and exogenous compounds. Some isolated CbE isoenzymes have been shown to have a highly similar amino acid sequence and similar physicochemical and immunological properties (Jokanovic, 2001). Overall, CbEs are considered a group of esterases with wide substrate specificity (Walker and Thompson, 1991), and this makes them play a major role in the detoxification of many different types of xenobiotics such as OPs, pyrethroids, phthalate ester plasticizers, oil dispersants and other environmental pollutants that affect fishes and other aquatic organisms (Al-Ghais et al., 2000; Galloway et al., 2002; Wheelock et al., 2005b). CbEs have been shown to inactivate or detoxify OPs in different ways by: (i) hydrolyzing OP ester bonds; (ii) binding to OPs or binding OPs to other proteins, resulting in a decrease in the concentration of available OPs, and (iii) CbE phosphorylation by binding to serine hydroxyl group at its active site (Jokanovic, 2001).

Most studies looking at ChEs and other esterases have been conducted for temperate or cold water freshwater fishes. In contrast, almost no information is available for marine fishes, and even less so for tropical marine species. By studying these biomarkers in white grunt (*Haemulon plumieri*), the present work intends to fill this gap and generate information that allows the understanding of how these enzymes act. White grunt is a tropical marine fish that was selected as a bioindicator model organism by the Mesoamerican Barrier Reef System (MBRS) Synoptic Monitoring Program (Almada-Villela et al., 2003) because of its association to coral reefs, seagrass beds and mangroves. In addition, it fulfills a series of criteria required by this program such as: (i) it is easy to identify in the field, (ii) it is easy to capture and is abundant enough to be used as a bioindicator, (iii) it is not under any type of legal protection, (iv) it is found throughout the MBRS region, and (v) it is not migratory. The latter is inferred based on previous studies at the central-west coast of Florida which indicated that, at least such region, white grunt resides in one general area and does not undertake substantial movements or seasonal migrations (de Silva and Murphy, 2001). White grunts off the southern coast of Florida (Starck and Davis, 1966) and off the coast of St. Croix (Ogden, 1977, Ogden and Ehrlich, 1977) have been observed to undertake diurnal movements associated to foraging. In St. Croix, white grunts, together with other grunt species, move away from reefs shortly after sunset to forage over grass and sand flats, and then return to the reef for shelter before sunrise. Such movements make it more likely that these organisms eventually become exposed to land-originated pollutants.

Some studies have used white grunt to look at contaminant bioaccumulation and have shown that this species may be an appropriate bioindicator. For instance, it was used to measure mercury bioaccumulation in marine fishes due to gold mining in Suriname (Mol et al., 2001). Another survey employed *H. plumieri* to determine organochlorine pesticide residues (Glynn et al., 1995). Nevertheless, from an ecotoxicological viewpoint, a lack of basic biological knowledge on this species still remains, specifically that related to its response to pollutants (i.e., detoxification mechanisms).

The main goals of this study were (a) to improve the understanding on ChE and CbE activity in a marine fish (*H. plumieri*) and (b) evaluate the use of such compounds as biomarkers for environmental monitoring purposes in coral reef ecosystems. To achieve these goals, we assessed ChE and CbE activity in different organs in order to determine which tissues have the highest level of enzyme activity. Characterization of ChEs is important because their activity level varies greatly across organs within and individual, as well as across species. Specifically, in this study we characterized ChEs found in brain, liver and muscle by using specific substrates and inhibitors in different concentrations. Because ChEs also vary in terms of substrate specificity, we also estimated K_m, V_{max}