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Fish & Shellfish Immunology 20 (2006) 12–19

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Phenoloxidase activity in three commercial bivalve species. Changes due to natural infestation with *Perkinsus atlanticus*

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Received 2 August 2004; revised 15 September 2004; accepted 15 February 2005
Available online 24 June 2005

Abstract

The phenoloxidase (PO) activity of the haemolymph and haemocytes from three clam species of commercial interest (*Ruditapes philippinarum*, *Chamelea gallina* and *Tapes decussatus*) has been compared. The activity was assayed spectrophotometrically by recording the formation of dopachrome from L-DOPA using sodium dodecyl sulphate, laminarin, trypsin or lipopolysaccharide as elicitors. Fewer PO units were observed in the haemolymph from *T. decussatus* than in the haemolymph from *R. philippinarum*, while the highest values were found in *C. gallina*. In all cases the activity was only significantly increased when sodium dodecyl sulphate was used as elicitor. PO activity in the haemocytes of all three clam species showed a very similar pattern to that found in the haemolymph from the same species. Furthermore, *T. decussatus* naturally parasitized by *Perkinsus atlanticus* (Protozoa, Apicomplexa) was used to study the influence of such infestation on PO activity, which was found to increase significantly in both haemolymph and haemocytes compared with non-infected (control) samples. PO activity in the haemocytes and in the haemolymph was higher when the level of parasitization was low or medium, respectively, and SDS was used as elicitor. No statistically significant differences were observed when the parasitization level was high. The present work constitutes the first report on the influence of this parasite on PO activity in haemolymph and haemocytes from *T. decussatus*.

Keywords: Phenoloxidase; Haemolymph; Haemocytes; Japanese littleneck (*R. philippinarum*); Striped venus clam (*C. gallina*); Carpet shell clam (*Tapes decussatus*); *Perkinsus atlanticus*; Parasites

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doi:10.1016/j.fsi.2005.02.002

1. Introduction

Phenoloxidase (PO) is a copper-containing enzyme with oxygenase activity, able to convert mono- and/or diphenols into quinones, which, in turn, can polymerise to form melanin [1]. This enzyme is widely distributed in microorganisms, plants and animals and is involved in several physiological processes, such as the sclerotisation of integument and egg capsules, the production of brown silks, eye pigmentation, neurotransmitter synthesis, and the production of bioadhesives and inks [2].

In invertebrates, including molluscs, PO is the last component of a reaction cascade called the "pro-PO-activating system", which consists of a complex pool of molecules released from immune competent cells into the haemolymph in response to external infestation or body injury [3–6].

While the biological role of PO has been studied intensely in arthropods, there are only a few reports which focus on its study in molluscs [7]. Furthermore, PO has been found [8–11] or not [12,13] in the different species of bivalve molluscs analyzed, where the fact that it is stimulated by different bacterial and fungal cell wall components suggests that PO plays a crucial role in defence mechanisms [11].

A major innate defence system in invertebrates is the melanization of pathogens and damaged tissues, a process which is also controlled by the enzyme PO, which, in turn, is regulated in a highly elaborate manner to avoid the unnecessary production of toxic and reactive compounds [14]. Several species of *Perkinsus* (Protozoa, Apicomplexa) have been described as parasitising and as causing a high degree of mortality in bivalves and some gastropods throughout the world, including the north-west coast of Spain in Galicia [15,16]. In Europe, for at least two decades, *Perkinsus* sp. trophozoites have been associated with mass mortalities of commercially important clams of the genus *Tapes* [16,17], a fact that underlines the interest of studying the immune status of naturally or experimentally infected mollusc specimens. In this sense, the inflammatory reaction caused by trophozoites of this parasite has been studied [18], as have several cellular (haemocyte density and phagocytic activity) and humoral (lysozyme and anti-bacterial activities, protein concentration and agglutination titre) immune parameters, in clams collected from enzootic areas for *Perkinsus atlanticus* [19,20]. However, the effect of such parasitization on PO activity has not yet been determined.

The aim of the present study was twofold. Firstly, to determine the presence of the phenoloxidase (PO) activity in the haemolymph and in the haemocytes of three bivalve species of commercial interest, Japanese littleneck (*Ruditapes philippinarum*), striped venus clam (*Chamelea gallina*) and carpet shell clam (*Tapes decussatus*), and to compare its in vitro activation by different elicitors. Secondly, to determine the influence of *P. atlanticus* natural infestation on PO activity in carpet shell clams, in an attempt to understand more fully the relationship between this parasite and the clam defence mechanisms.

2. Materials and methods

2.1. Animals

Market sized Japanese littleneck (*R. philippinarum*), striped venus (*C. gallina*) and carpet shell (*T. decussatus*) clams, obtained from local markets, were used to determine the presence of phenoloxidase (PO) and its activation (experiment 1). Carpet shell clams collected from Campelo, a *P. atlanticus* endemic area in Galicia (NW Spain), were used to determine the influence of this parasite on the pro-phenoloxidase (proPO) system (experiment 2). In both cases, animals were maintained in aerated recirculating aquaria, at 33‰ salinity and 15 °C, and were fed daily with algae: a mixture of *Tetraselmis* and *Isochrysis* (2.5×10^8 cells per day per clam, Instant Algae™, Reed Mariculture Inc., San Jose, California). Clams were allowed to acclimate for two weeks before being used in the experiments.