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Ciona intestinalis peroxinectin is a novel component of the peroxidase–cyclooxygenase gene superfamily upregulated by LPS

Aiti Vizzini, Daniela Parrinello, Maria Antonietta Sanfratello, Valentina Mangano, Nicolò Parrinello, Matteo Cammarata *

Marine Immunobiology Laboratory, Department of Biological Chemical Pharmaceutical Science and Technology, University of Palermo, Via Archirafi 18, Palermo, Italy

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ABSTRACT

Peroxinectins function as hemoperoxidase and cell adhesion factor involved in invertebrate immune reaction. In this study, the ascidian (*Ciona intestinalis*) peroxinectin gene (*Ci*Pxt) and its expression during the inflammatory response have been examined. *Ci*Pxt is a new member of the peroxidase–cyclooxygenase gene superfamily that contains both the peroxidase domain and the integrin KGD (Lys-Gly-Asp) binding motif. A phylogenetic tree showed that *Ci*Pxt is very close to the chordate group and appears to be the outgroup of mammalian MPO, EPO and TPO clades. The *Ci*Pxt molecular structure model resulted superimposable to the human myeloperoxidase. The *Ci*Pxt mRNA expression is upregulated by LPS inoculation suggesting it is involved in *C. intestinalis* inflammatory response. The CiPxt was expressed in hemocytes (compartment/morula cells), vessel epithelium, and unilocular refractile granulocytes populating the inflamed tunic matrix and in the zones 7, 8 and 9 of the endostyle, a special pharynx organs homolog to the vertebrate thyroid gland.

1. Introduction

Enzymes that have peroxidase domains are spread among all the living kingdoms. Phylogenetic analysis clearly reveals that almost all heme peroxidase coding genes have an early origin, and two ubiquitous heme peroxidase superfamilies (peroxidase–cyclooxygenase superfamily and peroxidase–catalase superfamily) represent the main lineages of their development (Zamocky and Obinger, 2010). Although the numerous representatives of the peroxidase–cyclooxygenase superfamily exert a variety of function, the importance of this superfamily is underlined by the numerous enzymes (including human myeloperoxidase, eosinophil peroxidase, and lactoperoxidase) critical in the innate immune responses (Zamocky and Obinger, 2010). Seven main clades representing distinct subfamilies are well segregated in the unrooted phylogenetic tree reconstructed by Zamocky et al. (2008). Even though distinctive mosaic structures can be found, the members of this superfamily share a catalytic domain of about 500 amino acid residues that contains a heme molecule to exert the peroxidase activity.

Peroxinectin, component of the peroxidase–cyclooxygenase superfamily, is characterized by a peroxidase domain and an integrin-binding motif (KGD: Lys-Gly-Asp), and it is mainly spread among various arthropod and nematode species. First, peroxinectin was detected in crayfish hemolymph as a cell adhesion and migration molecule with peroxidase activity (Johansson and Söderhäll, 1988; Johansson et al., 1995; Lin et al., 2007). Cell adhesion and migration are essential mechanisms for development, homeostasis and immunity (Gumbiner, 1996; Ruoslahti and Brink, 1996; Dong et al., 2009). This protein appeared to be involved in hemocyte degranulation (Johansson and Söderhäll, 1989; Cerenius et al., 2008), invasive microorganisms immobilization, phagocytosis, encapsulation, nodule formation (Johansson, 1999; Kobayashi et al., 1990), opsonization (Thornqvist et al., 1994), and transduction pathway regulating the expression of antibacterial peptide genes (Dong et al., 2009). In addition, shrimp peroxinectin activity can be generated by proteolysis related to the activation of the prophenoloxidase system (Sritunyalucksana et al., 2001).

In the phylogenetic tree of peroxidase–cyclooxygenase superfamily, two main invertebrate clades were recognized in peroxinectin subfamily (Zamocky et al., 2008). The first clade includes nematode and squid peroxinectins, whereas the second is formed by insect and crustacean peroxinectins. Among Deuterostomia only a minor subclade of echinozoa (sea urchin *Lytechinus variegates*, *Strongylocentrotus purpuratus* and *Haplochromis pulcherrimus*) peroxinectin sequences have been found, and they resulted very distantly related with any known sequence of vertebrate peroxidases (Zamocky and Obinger, 2010). So far, no sequences from vertebrate peroxinectins are known.

Ascidians occupy a key phylogenetic position and, recently, have been retained the sister group of vertebrates (Swalla et al., 2000; Zeng and Swalla, 2005; Delsuc et al., 2006; Tsagkogeorga

* Corresponding author. Tel.: +39 09123891805.
E-mail address: matteo.cammarata@unipa.it (M. Cammarata).

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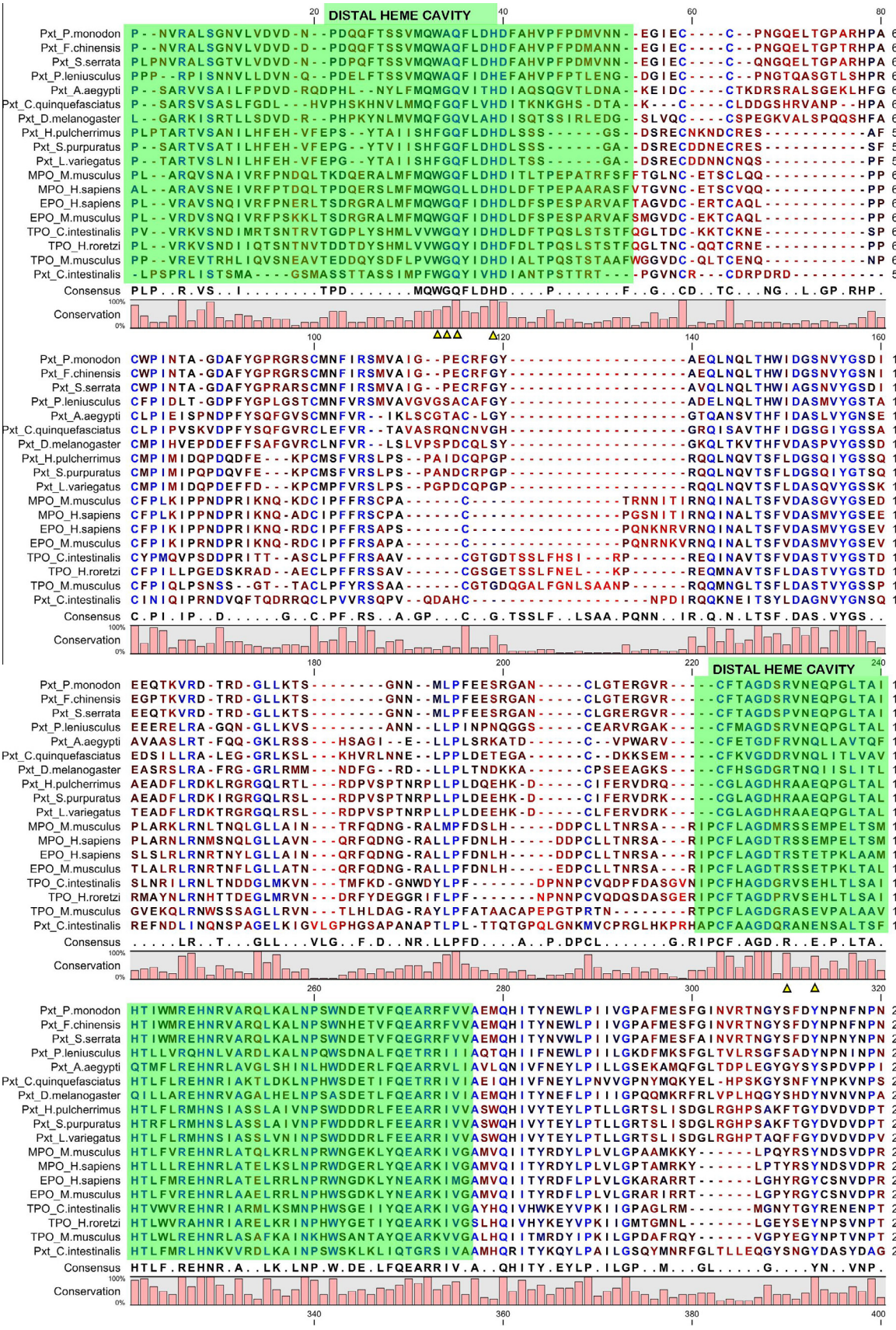

Fig. 1. Alignment of the *Ci*PXT peroxidase domain deduced aminoacid sequence with sequences of peroxidase–cyclooxygenase superfamily components: myeloperoxidase (MPO); eosinophil peroxidase (EPO); thyroid peroxidase (TPO); invertebrate peroxinectins (Pxt). Yellow triangles indicate the conserved amino acids that are known to interact with heme group or calcium. The conservation of amino acid is represented by letter background color gradients (from red to blue). The green boxes indicate the distal (panel A) and proximal (panel B) heme-binding site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)