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Hemocyanin-derived phenoloxidase reaction products display anti-infective properties

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Abstract

Hemocyanin is a multi-functional protein located in the hemolymph (blood) of certain arthropods and molluscs. In addition to its well-defined role in oxygen transport, hemocyanin can be converted into a phenoloxidase-like enzyme. Herein, we tested the antimicrobial properties of horseshoe crab (*Limulus polyphemus*) hemocyanin-derived phenoloxidase reaction products using broad ranges of phenolic substrates (e.g. L-DOPA) and microbial targets (Gram-positive/negative bacteria, yeast). The enzyme-catalysed turnover of several substrates generated (by)products that reduced significantly the number of colony forming units. Microbicidal effects of hemocyanin-derived phenoloxidase were thwarted by the inhibitor phenylthiourea. Data presented here further support a role for hemocyanin in invertebrate innate immunity.

Keywords:

Horseshoe crabs; Innate immunity; Melanogenesis; Enzyme-substrate complexes; Multifunctional protein

1. Introduction

Broadly, invertebrate innate immunity is an assemblage of physical barriers, cellular defences coordinated by hemocytes, and diverse humoral (soluble) factors within the hemolymph. One of the most effective invertebrate responses to disease-causing agents – microbes or parasites – is the production of melanin via the pro-phenoloxidase (proPO) cascade (Cerenius *et al.*, 2008). Activated phenoloxidases (POs) convert mono/di-phenolic substrates into reactive quinones, which are further processed enzymatically/non-enzymatically into 5,6-dihydroxyindoles (DHI) and eventually eumelanin. Melanic polymers and toxic by-products of PO-associated

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