In vitro production of peroxynitrite by haemocytes from marine bivalves: C-ELISA determination of 3-nitrotyrosine level in plasma proteins from Mytilus galloprovincialis and Crassostrea gigas

ABSTRACT

Using the C-ELISA, we have shown that the phagocytosis of zymosan particles increased the 3-nitrotyrosine levels of plasma proteins from mussel M. galloprovincialis and oyster C. gigas 5.8 and 7.5 times respectively.

INTRODUCTION

Background Bivalves, unlike vertebrates, do not have humoral antigen specific active compounds such as antibodies and their self-defence systems are based on non-specific defensive compounds and phagocytosis by haemocytes. During phagocytic burst or after in vitro stimulation with PMA or LPS, haemocytes produce superoxide anions, i.e. the initial species of reactive oxygen intermediates (ROI) and nitric oxide (NO). ROI generation has been reported in Patinopecten vessoensis Crassostrea virginica, Crassostrea gigas, Ostrea edulis, Pecten maximus, Mytilus edulis and Mytilus galloprovincialis. NO-synthase activity was detected in haemocytes of M. edulis and C. gigas and peroxynitrite production by M. galloprovincialis haemocytes has been recently reported. In the presence of superoxide anions, nitric oxide generates peroxynitrite, a strong oxidant which kills bacteria and parasitic protozoa. Moreover, peroxynitrite is a nitrating agent, that converts tyrosine in 3-nitrotyrosine. Such nitration has been observed in proteins from human polymorphonuclear cells and 3-nitrotyrosine has been used as a marker to assess peroxynitrite involvement in pathological processes such as adult respiratory distress syndrome, rheumatoid arthritis and celiac disease. To determine levels of protein-associated 3-nitrotyrosine in human plasma or serum, Khan et al. developed a competitive enzyme-linked immuno-assay (C-ELISA) for 3-nitrotyrosine using a polyclonal anti-3-nitrotyrosine rabbit IgG raised against nitrated KLH. In the present study, we slightly modified this C-ELISA assay to investigate 3-nitrotyrosine levels in plasma proteins from mussel M. galloprovincialis and oyster C. gigas before and after zymosan phagocytosis.

CONCLUSION

The C-ELISA method we developed is sensitive enough to determine the amounts of 3-nitrotyrosine in plasma proteins of a single animal and to measure variations in 3-nitrotyrosine contents promoted by haemocyte stimulation or zymosan particle phagocytosis. However, this method remains semi-quantitative since the 3-nitrotyrosine antibody may not bind all 3-nitrotyrosine residues in a sample containing a mixture of proteins due to inaccessibility to some 3-nitrotyrosine residues because of the influence of adjacent aminoacids on antibody binding. We used the C-ELISA method to detect and quantify the stress of mussels and oysters exposed to environmental variations.