

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

Through the use of C-ELISA, we have demonstrated that 3-nitrotyrosine levels of plasma proteins from mussel *M. galloprovincialis* and oyster *C. gigas* were raised by 5.8 and 7.5 times respectively, with the exception of the phagocytosis of zymosan particles.

## INTRODUCTION

The term 'peroxynitrite' is often used to describe a group of proteins found in various tissues of the body. Peroxynitrite is a reactive nitrogen species, and is produced by the conversion of nitric oxide to peroxynitrite. Peroxynitrite is commonly referred to as an 'oxidative stress' protein, due to its high affinity for the antioxidant glutathione.

Proteins containing peroxynitrite are classified into two groups:

1) those that are not directly related to the peroxynitrite, such as peroxynitrite- Bivalves lack the ability to produce humoral antigen specific active compounds like antibodies, unlike vertebrates, and their self-defence mechanisms depend on non-specific defensive compounds and phagocytosis by haemocytes. Superoxide anions, which are the first species of reactive oxygen intermediates (ROI) and nitric oxide (NO), are produced by haemocytes during phagocytic burst or after in vitro stimulation with PMA or LPS. Reports indicate that certain plants, such as *Patinopecten vesperum*, *Crassanthus virginica*, *C. rogiacteriorum*, *Ostrea edulis*, *Pectentinus maximus*, *Mytilus erythematosum*, and *Mytilostosis galloprovincialis* (*Pactus acidophilus*), have been found to generate an ROI, while recent reports indicate the presence of NO-synthase activity in haemocytes of *P. gallopsis* also produce peroxynitrite production by *M.* The presence of superoxide anions in nitric oxide results in the production of peroxynitrite, a potent oxidant that kills bacteria and parasitic protozoa. Additionally, peroxin is primarily enriched in 3-nitrobutyrosine (NTR), which has been found to convert tyridine into phosphorus in proteins from human polymorphonuclear cells and has also been used as 'mean-changeurs' for pathological processes such as respiratory distress syndrome, RTC and celiac disease Khan et al. developed a C-ELISA method to measure 3-nitrotyrosine levels in human plasma or serum, specifically for the presence of protein-3-nitrosine and other related factors.

## CONCLUSION

By using our newly developed C-ELISA method, we can determine the levels of 3-nitrotyrosine in plasma proteins of a single animal while also measuring changes to their contents due to haemocyte stimulation or phagocytosis via zymosan particle p535. However, this method remains semi-quantitative because some 3-nitrotyrosine residues in a sample of mixture proteins may not be bound by the 3-cytosine antibody at all (affected by adjacent aminoacids): for example, we used C-ELISA to assess and quantify the stress of mussels and oysters that were exposed to environmental variations.