

MOLECULAR CHARACTERISATION OF AN IMMUNE RELATED GENE, *CgBPI/LBP* ISOLATED FROM THE OYSTER *CRASSOSTREA GIGAS*

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Introduction

In the vertebrates and invertebrates, the defense mechanisms recognition of the lipopolysaccharides (LPS) is very important because it initiates the cellular and humoral responses. Different LPS binding proteins have been characterised in the mammalian system, two of these proteins are bactericidal increasing protein (BPI) and lipopolysaccharides binding protein (LBP). Both BPI and LBP showing the similar sequence homology, but different functions in the response immunity (Levy, 2000). BPI plays the key role in the recognizing bacterial LPS, exhibits a bactericidal activity and endotoxin-neutralizing properties (Weiss, 2003). Whereas LBP binds LPS and enhances cellular responses to LPS as in the induction of proinflammatory cytokines.

Materials and methods

By an EST approach (Gueguen et al., 2003), we have isolated from ARN haemocytes of the oyster *Crassostrea gigas* one cDNA (616 pb) that showed homologies with the C-terminal domain of the BPI/LBP super family protein. This clone was used as probe for rapid screening of the *C. gigas* cDNA library (18.432 clones). The same probe was used for northern blot analysis and *in situ* hybridization. After screening, full-length cDNA was obtained and cloned into pPIC9K vector. The recombinant plasmid was transformed into *Pichia pastoris* strain GS115. The recombinant protein, produced in the supernatant, was ultrafiltrated and finally purified by RP-HPLC. The ability of rCgBPI/LBP to bind the LPS was determined by surface plasmon resonance (SPR) with BIAcore 2000 biosensor instrument and the modification of outer membrane permeability in *E. coli* was evaluated by increased permeability to Actinomycin D assay.

Results

We have cloned one cDNA denoted as *CgBPI/LBP*, which was derived from the mRNA of haemocytes from the oyster *Crassostrea gigas*. This sequence reveal significant homology with LBP and BPI in mammals. The full-length cDNA of *CgBPI/LBP* is 1784 bp and encoded a protein 477 amino acids. This sequence was aligned with different LBP/BPI/CETP/PLTP family protein by using the Clustal W program. Sequence comparison showed that the *CgBPI/LBP* is most homologous to rainbow trout LBP/BPI and *C. intestinalis* LBP/BPI (49 and 46 % similarity respectively). Northern blot and *in*

situ hybridization analysis showed that CgBPI/LBP is expressed in the haemocytes and in several tissues. We detected high expression in the smooth and ridged epithelium of labial palps, digestive gland tubules and reproductive follicles. The levels of expression in haemocytes of CgBPI/LBP were modified by different conditions of bacteria stimulation. The purified recombinant protein CgBPI/LBP expressed in the *Pichia pastoris* system showed LPS binding capacity by SPR analysis with BIAcore 2000 using chips which were immobilized with recombinant protein. Finally the recombinant protein enhances access of Actinomycin D into bacteria.

Discussion

In this study we have identified a novel cDNA the type BPI /LBP gene present in the invertebrates. The protein deduced showed a high degree of structural similarity with mammal BPI and LBP protein. The mRNA expression in the haemocytes was modified by different bacterial challenge. In contrast, in the tissue characterised by a large surface area that is continuously exposed to invading organism present in the aquatic environment, the expression of CgBPI/LBP is constitutive. The biological function was deduced by the production and the characterisation of the recombinant protein. CgBPI/LBP may be involved in the innate immune response by recognition of the different LPS motif and its action on the permeability of the outer membrane of bacteria.

References

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