

Molecular characterisation of an immune related gene, *Cg* LBP/BPI isolated from the oyster *Crassostrea gigas*



M. Gonzalez, Y. Gueguen, J. Fièvet, J. M. Escoubas, F. Vandembulcke* et E. Bachère

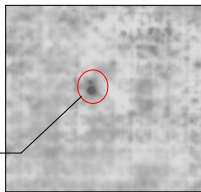
IFREMER/CNRS/ Université de Montpellier 2, UMR 5171, Place E. Bataillon, CC 80 - 34095 Montpellier, France. *Laboratoire Ecologie Numérique et Ecotoxicologie, Batiment SN3, Biologie Animale, Université des Sciences et Technologies de Lille, France.

ABSTRACT: In the vertebrates and invertebrates, the mechanism of recognition the lipopolysaccharide (LPS) are very important because it initiates the cellular and the humoral responses. Different LPS binding proteins have been characterised in the mammalian system. Two of these proteins are the bactericidal increasing protein (BPI) and the lipopolysaccharide binding protein (LBP). By an EST approach, we have isolated from haemocytes of the oyster *Crassostrea gigas* one cDNA, named *Cg* LBP/BPI, which showed significant homology with LBP and BPI proteins from mammals. Northern blot and *in situ* hybridization analyses showed that *Cg* LBP/BPI is expressed in the haemocytes and tissues and that, the level expression of *Cg* LBP/BPI was modified by different conditions of bacterial stimulation. In addition, to characterize the biological activities of the encoded protein, we cloned the gene and expressed the *Cg* LBP/BPI protein in *Pichia pastoris*. Preliminary results showed that the partially purified recombinant *Cg* LBP/BPI protein exhibits antibacterial and LPS binding activities.

RESUMEN: En los vertebrados e invertebrados los mecanismos de reconocimiento de lipopolisacáridos (LPS) son muy importantes ya que comienzan las respuestas de tipo humoral y celular. Diferentes proteínas de unión a los LPS han sido caracterizadas en mamíferos como por ejemplo, la proteína aumentadora de la permeabilización (BPI) y la proteína de unión a los LPS (LBP). A partir de un programa de EST, logramos aislar desde hemocitos de la ostra *Crassostrea gigas* el ADNc, denominado *Cg* LBP/BPI, el cual muestra significativas homologías con las proteínas de mamíferos tipo LBP y BPI. El análisis por Hibridación *in situ* y Northern blot revelaron que *Cg* LBP/BPI se expresó en hemocitos y diferentes epitelios, los niveles de expresión del gen pueden ser modificados con respecto a diferentes tipos de estimulación con bacterias. Para poder caracterizar las actividades biológicas de la proteína, esta se clonó y expresó en el sistema *Pichia pastoris*. Resultados preliminares mostraron que la proteína recombinante parcialmente purificada presenta actividades antibacterianas y de unión a los LPS.

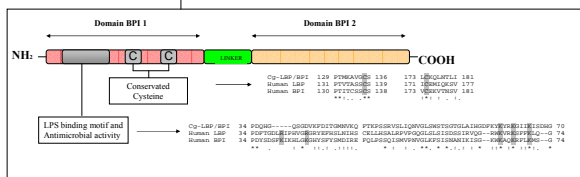
1. EST approach

By an EST approach, we have isolated from hemocytes of the oyster *Crassostrea gigas* one partial cDNA (*Cg* LBP/BPI) fragment cDNA, named *Cg* LBP/BPI, which showed significant homology with LBP and BPI proteins from mammals



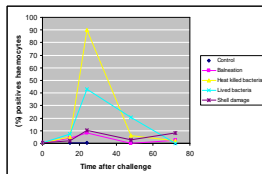
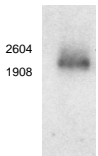
Using rapid screening on high density membrane

Full cDNA (*Cg*-LBP/BPI) was obtained (1784 nt)

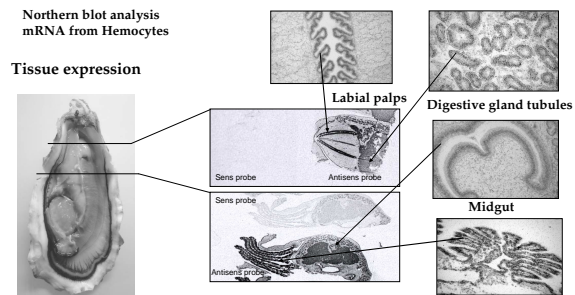


Cg LBP/BPI showed similarity with the BPI (46 % similarity), LBP (44 % similarity). Lysine residues are greatly conserved (42, 48, 92, 95) and contains a positive charges that may be a potential site of interaction with LPS. *Cg* LBP/BPI showed the single conserved cysteine residue

4. Expression of *Cg* LBP/BPI



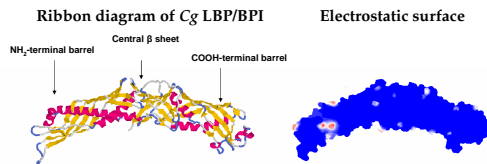
Cg LBP/BPI expression was induced by bacterial challenge



In situ hybridization demonstrated that *Cg* LBP/BPI mRNA is also expressed constitutively in tissues

2. Structure modelling

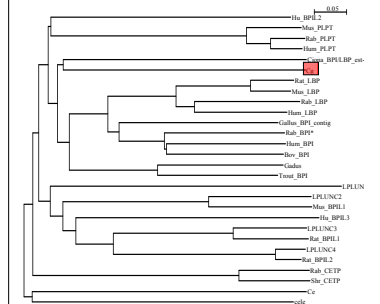
Three-dimensional structural model of the *Cg* LBP/BPI was generated with the automated server (<http://bioserv.cbs.cnrs.fr>) for threading optimisation modelling and evaluation.



The structure obtained after automated modelling showed that *Cg* LBP/BPI is similar to human BPI and LBP

High concentration of positive charge on *Cg* LBP/BPI is similar to human BPI

3. Phylogenetic analysis of LBP/BPI family

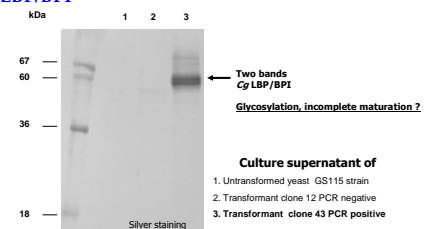


The phylogenetic tree (neighbor-joining method) suggests that the *C. gigas* and *Ciona intestinalis* LBP/BPI gene are orthologous to fish and mammalian gene LBP/BPI.

5. Cloning and expression of recombinant protein *Cg* LBP/BPI

Expression of *Cg* LBP/BPI in *P. pastoris*

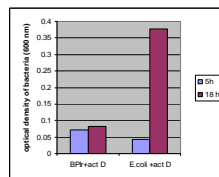
- Cloning into pPIC9K
- Transformation in *E. coli* DH5 alpha
- Linearization of pPIC9K-*Cg* LBP/BPI plasmid with SalI
- Transformation into *Pichia pastoris* GS115
- Selection of Histidine and Geneticin resistant clones
- Growth on MD medium and induction 48 h using methanol



Culture supernatant of
1. Untransformed yeast GS115 strain
2. Transformant clone 12 PCR negative
3. Transformant clone 43 PCR positive

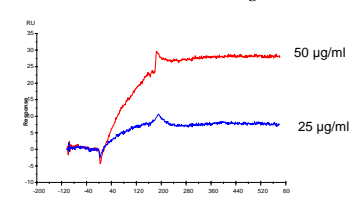
6. Biological Activity

Assay permeability activity



E. coli bacterial envelopes are impermeable to actinomycin D (Act D). After incubation with recombinant *Cg* BPI/LBP, the bacterial outer membrane is modified that increases bacterial sensitivity to Act D.

Surface Plasmon Resonance Analysis (BIAcore) of the interaction between *E. coli* LPS and *Cg* LBP/BPI



Two concentration of LPS from *E. coli* was applied to a *Cg* LBP/BPI coated chip. BIAcore analyses confirms the *Cg* LBP/BPI interaction with LPS to *E. coli*

Conclusions:

- *Cg* LBP/BPI presents similar structural characteristics with the mammalian protein family LBP/BPI/CETP/PLTP.
- The expression of *Cg* LBP/BPI is induced by bacterial challenge in hemocytes whereas is expressed constitutively in epithelia.
- The recombinant protein presents affinity with *E. coli* LPS and has an effect on bacterial permeability.

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