

## Immunological Properties Evidence by the *A. rubens* Sea Star Immunoglobulin Kappa Gene

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Rec date: Jul 02, 2014; Acc date: Aug 27, 2014; Pub date: Aug 29, 2014

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### Abstract

In 2013-2014, we had discovered a sea star Igkappa gene, showing 2 Ig sites, after star sea immunizations to HRP. By using this gene and introducing it into an *Escherichia coli* plasmid, we were able to verify that this gene had the property to induce a specific immune response to the enzyme HRP (Horse-radish peroxidase).

**Keywords:** Sea star IgKappa gene; Plasmid; Enzymatic assay; Immune reaction

### Introduction

The general idea that emerged from the experiments, made in our laboratories, was that Echinodermata, as exemplified by sea stars: *Asterina gibbosa* and *Asterias rubens*, possessed an immune system, with B sea star lymphocytes, able to mount cellular and humoral-specific responses [1]. *Asterias rubens* produced « An antibody » anti HRP, after injections to HRP: it was shown to correlate to kappa genes, in 2011 [2].

In 2013-2014 a sea star Ig Kappa gene to HRP was cloned [3,4]. But a question deserved to be put: did the cloned gene keep the property to induce a specific immune response managed specifically against the used antigen namely HRP (Horse-radish Peroxydase)? It is the object of this work which required, in the first place making an *Escherichia coli* plasmid and secondarily to perform an enzyme assay by the contribution of its substratum, to see if the « antibody » is bound in the HRP antigen.

### Materials and Methods

#### Animals

As precedently [3], we used sea stars immunized to HRP and their transcriptome.

Plasmid construction: GZK-2SMART cDNA was amplified in standard PCR conditions using primers: 5'TAAGGATCCTATGCGTGGCAACATGGCGT and 5'TATAAGCTTACGCAAACATCTGACAGCGG

PCR-amplified DNA was treated with restriction enzymes BamH I and Hind III and inserted into the corresponding sites of pTR-HIS (Lifetechnologies). Final construction was checked by sequencing.

#### Sonication

The sonication of control *E. coli* cultures and treated *E. coli* ones were performed with a sonicator. (VIBRACELL 75115). Control lysates and treated lysates were obtained and placed in PBS.

#### Enzymologic assay

Dosage of Horse-radish peroxydase was performed in microtitration plaque. Control and treated lysates were incubated in presence of 50 µl HRP/ well. Endogenous peroxydase was studied and directly revealed, in controls, by ABTS (3-ethylbenzthiazoline-6-sulfonic acid + H<sub>2</sub>O<sub>2</sub>)

Experiments were done in duplicate.

#### Results

Results were summarized in Figure 1. The comparison between control lysates (Bacterial positive lysates with peroxydase) and treated ones indicated a significant difference. Optical density at 405 nm in treated lysates showed the presence of bounding peroxydase (HRP), especially at a dilution of 1, in a degree upper to that of the controls (controls incubated in presence or not of HRP).

#### Conclusion

Our data showed that the *E. coli* plasmid, obtained from the sea star IgKappa gene, itself from the immunized sea stars to HRP genome, secretes a primitive antibody which is bound in the HRP antigen. In a general way, *E. coli* plasmid system may induce antibody [5]. A question deserves to be put, about the complexity of the antigen-binding domain, in invertebrate sea star antibody: this question, for the moment, remains enigmatic. Nevertheless, it can be said that the extracellular junction between two sea star Igkappa chains may play the rôle of Fab. We recall that the primitive sea star antibody would be composed of 4 kappa chains [6]. This deeply moving discovery deserved to be underlined from a point of view of the appearance of antibodies through the evolution of animal kingdom.

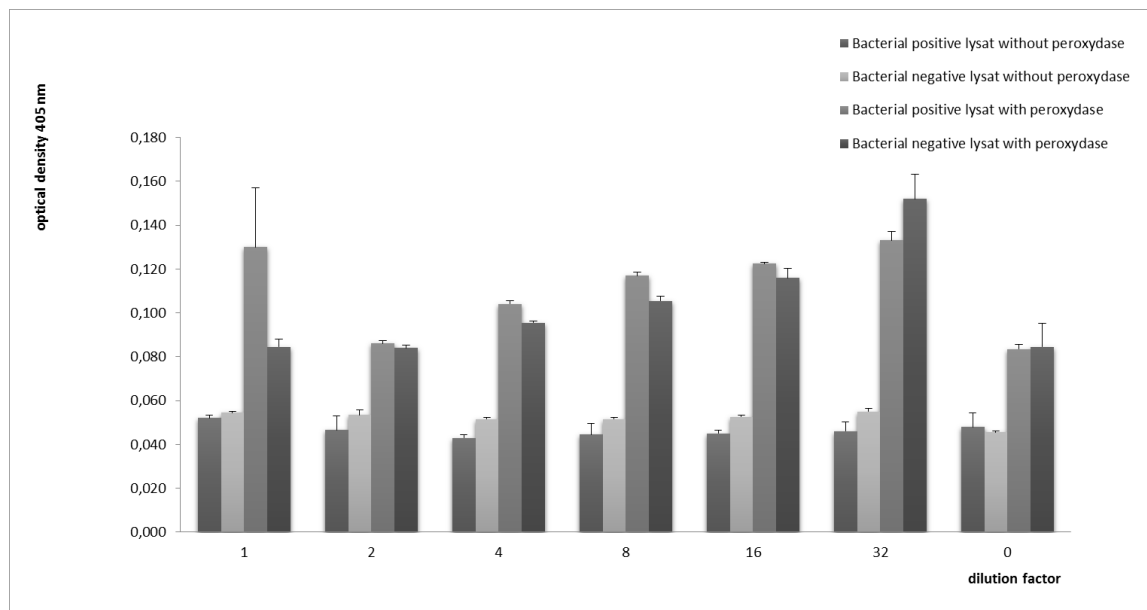


Figure 1: HRP « fixation » in control lysates and treated lysates

This work reveals a true progress in the comprehension of the sea star immune system which includes B and T sea star lymphocytes, primitive antibody and Igkappa gene. In the present case, when *A. rubens* was immunized to an antigen, when the sea star Igkappa gene was introduced in an *E. coli* plasmid a biological reaction occurred : immune properties were conserved. In itself it is a « revolution » in Invertebrate field. This gene could be of great interest in immune therapy.

## Acknowledgments

Thanks to Pr. Alain Legrand and Stéphanie Rose, Dr. Valérie Quesniau (CNRS Orléans) for their helpful assistance.

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