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IMMUNE PROPERTIES IN THE PRODUCTED RECOMBINANT SIP YOUNG 6 HIS PROTEIN FROM THE SEA STAR IGKAPPA GENE THROUGH HEK 293 EBNA CELLS REVEALING AGAINST SPIKE-RBD PROTEIN AND HRP ONE CHANGES IN SEA STAR GENE EXPRESSION?

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ABSTRACT

In 2014 we have isolated and cloned the sea star Igkappa gene which induced an anti-HRP primitive invertebrate antibody. We attempt, in the present work, to produce a SIP Young 6 His protein through HeK 293 EBNA cells after Igkappa gene cloning. This protein bound HRP by the use of Escherichia coli as vector of cloning but not HRP with HeK cells as vector. Is there changes in sea star IGKappa gene expression? We notice also that the SARS-CoV-2-SPIKE RBD Protein didn't bind to SIP Young 6 His protein when compared to HRP.

Keywords: Invertebrates, Seastar Igkappa Gene, Hek 293 Cells, Primitive Antibody.

INTRODUCTION

The sea star IGKappa gene was discovered in Osteraset et al. (2014). It is an IPA (Invertebrate Primitive Antibody) which produce an anti-HRP protein. We recall it was obtained from sea star Asterias rubens immunized to HRP (Horse-radish peroxydase). In Leclerc and Otten (2014) it was inserted in an Escherichia coli plasmid and showed a specific binding to the antigen HRP (Leclerc & Otten, 2014). Recently, (Leclerc, 2021) we have also inserted the sea star IGKappa gene in Hek 293 EBNA cells as it was done by Kujirai et al. (2014). In the present work we attempt to observe if these last cells produce the anti-HRP protein immobilzed in magnetic beads by contact to HRP. A control which is made of another protein: the recombinant SARS-CoV-2-Spike .RBD was used to verify the binding of this last one to anti-HRP protein issued from HeK 293 cells. A comparison is performed between the binding of anti-HRP protein to HRP and the binding of anti-HRP to spike RBD protein.

MATERIALS AND METHODS

Subcloning in Expression Vector

The genes coding for the target proteins were chemically synthesized with optimization for expression in HEK293 cells. The sequence is illustrated below (Osteraset et al., 2014).

Sequences Information

> SIP_Young_6His cDNA - 384 bp

ATGGGCGGAATGAGAGGCAACATGGCCAGCCTGTGGATGTTCTTTTTCGTGGTGGGC
ATCACACTGCAGAGGAGC
CTGGCCATCTACACATTCAGAGAGCAGCCTTCCGACACCTCCGCCCTGCAGGGCAGC
ACAGTGGTGCTGCACTGT
AGCGTGGAGCAGTACATCAACACCACCGCCATCGTGTGGTGGAGCAGGGATTCCGT
GATCTCCCACAACAACGAC
CTGAACCTGAGCAGCCTGAACACAGATCAGCTGCAGAGCTACAGCATCAGCGGCGA
CGCCTCCAGGGGCGAGTTC
AATCTGAATATCGTGAATTTCACAGCCACCGATGCCGCCAGCTACAGATGTCAGATG
CTGGAGCACCACCACCAC
CATCACTGA

Expected Protein Sequence
> SIP_Young_6His – 127 AAs – 14.30 kDa

MGGMRGNMASLWMFFFVVGITLQRSLAIYTFREQPSDTSALQGSTVVLHCSVEQYINTT
AIVWWSRDSVISHNND
LNLSSLNTDQLQSYSISGDASRGEFNLNIVNFTATDAASYRCQMLE
HHHHHH Features:
SIP: [1:27]
His tag [122:127]

Assay was performed as a co-immunoprecipitation by using the sea star anti-HRP protein instead a classical antibody. The binding of sea star protein (Bait protein) and target proteins or Prey proteins (HRP, Recombinant Sars-Cov-2 Spike RBD Flag Tag (R and D Products) were assessed by Western-blots. Flag Tag was the following amino-acid terminaison: DYKDDDDK.

Experiments were realized by the use of magnetic beads in column. These last ones were bound to bait protein. Secondly the bait protein linked or not to prey one. A same molar amount of proteins was used for bait and prey proteins sometimes a ratio: 1/2 was performed At this point only the sea star protein and the protein bound to it remain immobilized to the magnetic beads. A specific elution with buffer containing Imizadole was performed and determined the specificity of the binding when compared to negative controls.

RESULTS

Small-scale Expression and Purification Tests

Short Protein Purification Protocol Description

Culture medium was purified by affinity against His-tag (IMAC) by a standard method:

-Equilibration with PBS, pH 7.5

-Wash 1/2/3 with PBS, pH 7.5, 0 mM, 30mM and 50mM imidazole buffer

-Elution with PBS, pH 7.5, 200mM and 400mM imidazole buffer

-Analysis by SDS-PAGE of fractions of interest

-Final sample QC: qualitative by SDS-PAGE, quantitative by Bradford method

E2-E6 were pooled, buffer exchanged vs PBS, pH7.5 by dialysis method. The purification test results and QC are illustrated in Figure 1 and Figure 2.

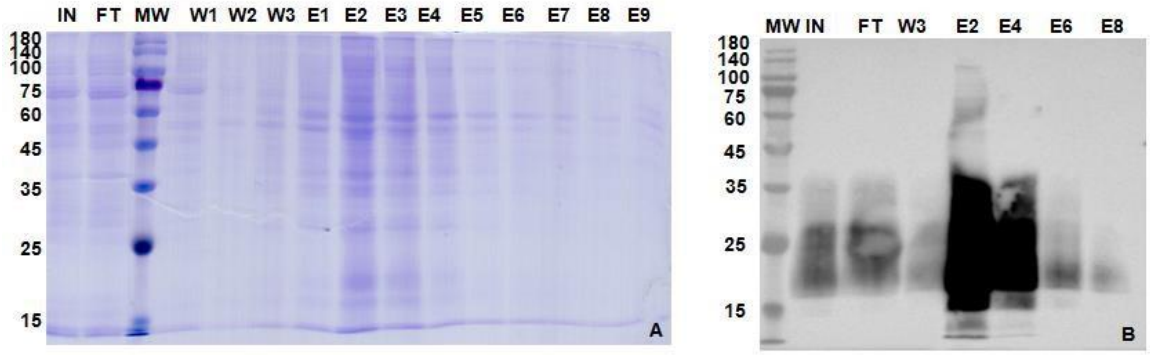


Figure 1. Target protein purification profile. *Coomassie blue staining.* **A.** Reduced PAGE analysis.
B. Western blot with anti-his antibody (ECL revelation).

MW. Molecular weight marker. **IN.** Input. **FT.** Flow through. **W1-W3.** Washes. **E.** Eluted fractions.

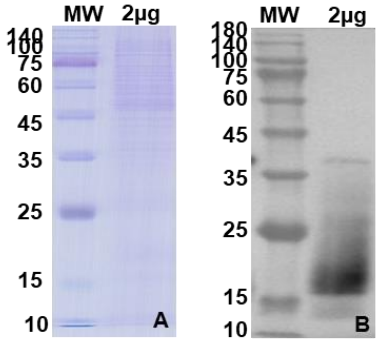


Figure 2. The final QC gel. *Coomassie blue staining.* **A.** Reduced PAGE analysis.
B. Western blot with anti-his antibody (ECL revelation). **MW.** Molecular weight marker.

These figures show clearly that the eluates E2,E3, E4 contain mainly the SIP Young 6 His protein which is revelated by the anti-His antibody (ECL revelation)

SIP Young -6 HIS Does Not Bind Spike RBD Protein

Immunoprecipitation assay doesn't reveal a binding between SIP Young 6 HIS protein and Spike RBD one. Eluted fractions don't contain a labelling at all in Western-blots.

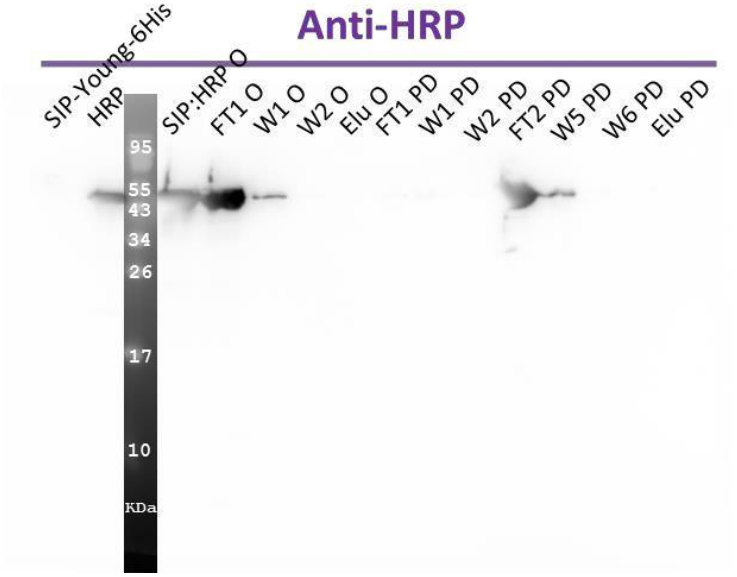
SIP Young-6 HIS and HRP Protein

Two assays were performed: A pull-down (PD) and an outside (O) ones.

Unfortunately they show no specific binding between the 2 proteins in a ratio 1/1 and in the ratio 1/2 (2 HRP for 1 SIP Young-6 HIS) in Eluates: EluO and Elu PD.

All the fractions have been loaded in a Western-Blot. The excess of proteins is named FT1 in Pull-Down assay, FT2 in the case of Outside assay. W1 corresponds to washing 1 with buffer and W5 to washing 5

Results are summarized as following in Figure 3.



Signals weigh around 40kDa (consistent with HRP weight) and are found on:

- Positive control lane of HRP only
- SIP: HRP, FT1 and W1 lanes for the binding outside the beads.
- FT2 and W5 fractions for the pull-down assay

There is no signal on the elution lanes for both assays.

Figure 3. Anti-HRP Western-blot fractions

CONCLUSION

It appears clearly that the immune property of Anti-HRP Protein (which was described in 2014(2) through E.coli cloning) disappears with HeK 293 ENBA cloning in 2021.

Mainly we may think that changes of the sea star gene expression occur in HeK cells and anti-HRP function was altered. Such changes exist in Biology: We do reference to the excellent paper of Magdalena Malm. The publication of which was in Malm et al. (2020) with the title: « Evolution from adherent to suspens-systems biology of HeK 293 cell line development »

We note, on the other hand that SIP-Young -6HIS protein doesn't bind SARS-CoV -2-Spike RBD protein: this result is more logic in our mind.

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