

Cloning and production of ITIM-containing bacterial proteins

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Thematic topic: (Natural Sciences)

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INTRODUCTION/BACKGROUND

Understanding the immunological mechanisms preventing disease development in non-allergic individuals and evidence for healing of altered regulatory mechanisms in allergic diseases opens perspectives for innovative interventions in immunological signalling pathways. Multi-subunit cell-surface receptors involved in signal transduction consist of a surface exposed receptor domain responsible for binding of the ligand, a transmembrane domain anchoring the molecule in the cell membrane and an intracellular effector domain, responsible for signal transduction. The intracellular domains of a family of signalling proteins with inhibitory effects on cells of the immune system contain loosely conserved motifs termed “ITIMs” for **i**mmunoreceptor **t**yrosine-based **i**nhibitory **m**otifs (ITIM) [1]. Recently small protein domains, referred to as protein transduction domains (PTD) [2], have been developed for the delivery of proteins into eukaryotic cells. They represent attractive pharmacological and biochemical tools to target peptides/proteins inside of cells. The goal of this ongoing diploma thesis is to produce cell membrane translocation PTD fusion proteins derived from ITIM containing proteins of the gram-negative bacteria *Shigella flexneri* [3], and development of fluorescent control constructs based on an engineered cysteine-rich motif [4]. Cloning and expression of ITIM containing bacterial proteins is an integral part of a large ongoing research project devoted to study the effects of ITIM- domains containing proteins in immunomodulating the host’s innate immune response during bacterial infections.

MATERIAL & METHODS

Cloning and expression of OspF and OspG fusion proteins

The sequences of interest were PCR-amplified with specific primers, digested with restriction endonucleases and ligated in frame into high level expression vectors. The constructs were transformed into competent *Escherichia coli* cells and selected on agar plates containing antibiotics. Single colonies were picked and the insert sequence was checked by restriction fragment length analysis and sequencing. Purified plasmid-containing vectors were transformed into *E. coli* expression strains and the target proteins were small-scale expressed as his₆tag N-terminal fusion proteins, followed by immobilized metal affinity chromatography (IMAC). Large-scale expression samples were purified on a 5 ml HisTrap FF crude affinity column (GEHealthcare) on an ÄKTA HPLC/FPLC device (GEHealthcare). Expression products were tested for correct size and purity by SDS-PAGE, dialyzed and stored at -20°C for further experiments.

RESULTS

We successfully engineered the *S. flexneri* protein OspF as 31.3 kDa PTD -fusion protein and as 33.7 kDa PTD, TetCys (tetra-cysteine residue)-fusion protein. Additionally we engineered the bacterial kinase OspG as 26 kDa PTD- and as 28.4 kDa PTD, TetCys-fusion protein. We were able to express PTD- and the PTD, TetCys-fusion proteins in the *E. coli* strain BL21 DE3 (Fig. 1) and the PTD-only fusion proteins in the strain M15 pREP4 (Fig. 2) and in BL21

DE3 (data not shown). According to our experiments both strains are able to produce similar, however relatively low quantities of our target proteins. Non-induced control cultures showed a prominent amount of basal expression, therefore, modified expression systems will be tested for reducing basal expression and increasing production yields.

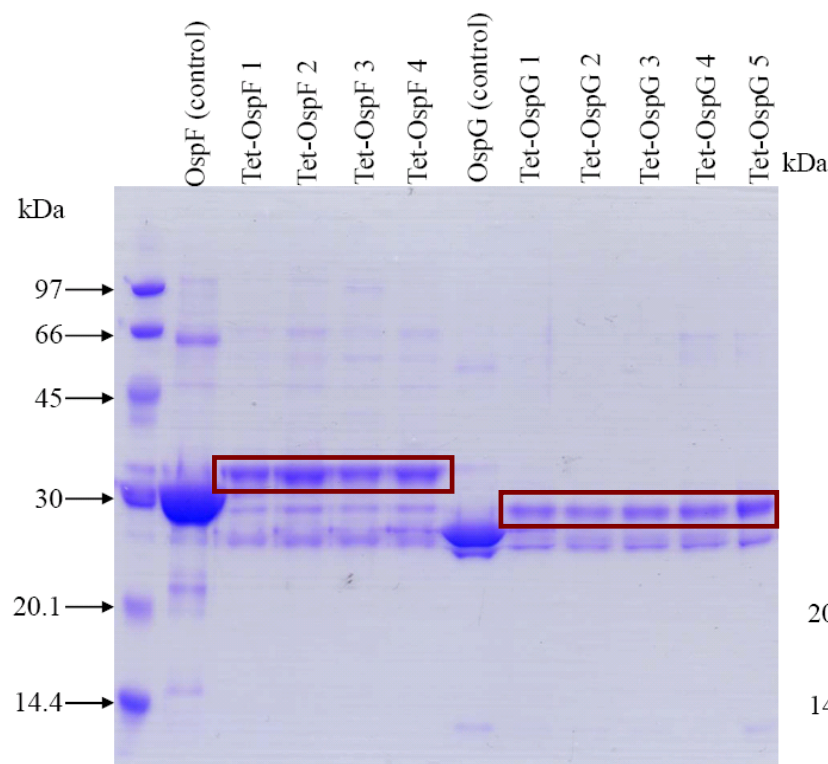


Fig. 1: SDS-PAGE gel stained with Coomassie blue® showing small-scale expression of OspF and OspG as PTD, TetCys fusion protein

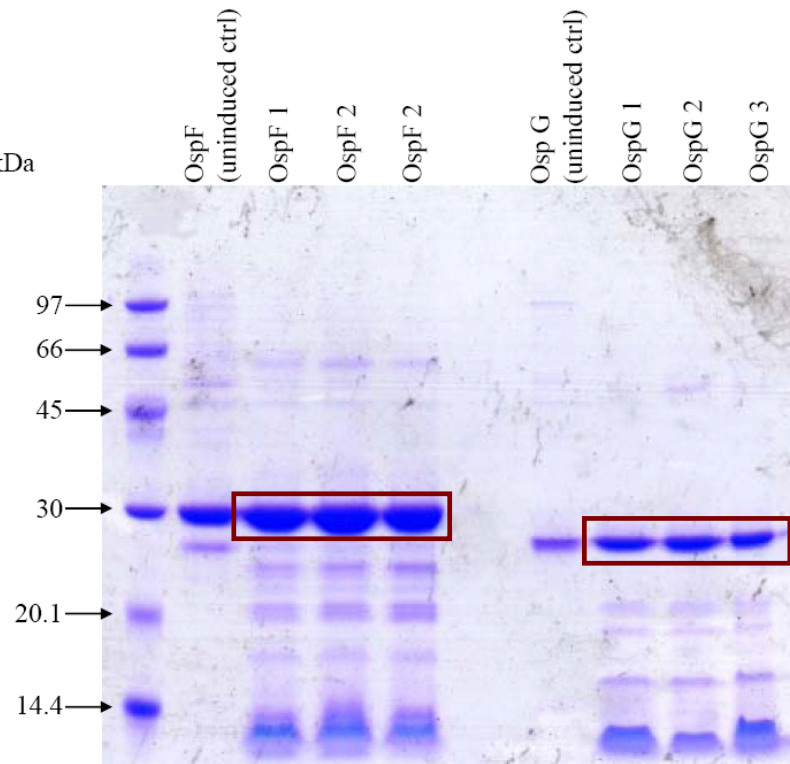


Fig. 2: SDS-PAGE gel stained with Coomassie blue® showing small-scale expression of OspF and OspG as PTD fusion protein

CONCLUSIONS & OUTLOOK

During this diploma work we were able to engineer and produce in small-scale the cell membrane translocation fusion proteins OspF and OspG from *Shigella flexneri*. In addition a construct of each protein containing a tag enabling labeling of the protein with a fluorescent dye was generated. For future studies and applications e.g. biological activity of the protein constructs, we will strive to scale-up the production and to fine tune the purification protocols with special focus on bacterial endotoxin removal. Endotoxin free proteins will be used to investigate the immunomodulatory effects of the ITIM-containing bacterial proteins OspF and OspG on Peripheral Blood Mononuclear Cells (PBMCs). The fluorescent control proteins will be used to investigate the efficiency of transmembrane translocation and cellular localization by confocal microscopy.

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