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Expression of membrane proteins from *Mycobacterium tuberculosis* in *Escherichia coli* as fusions with maltose binding protein

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Abstract

Sixteen of 22 low molecular weight integral membrane proteins from *Mycobacterium tuberculosis* with previously poor or undetectable levels of expression were expressed in *Escherichia coli* as fusions with both the maltose binding protein (MBP) and a His_8-tag. Sixty-eight percent of targeted proteins were expressed in high yield (>30 mg/L) in soluble and/or inclusion body form. Thrombin cleavage of the MBP fusion protein was successful for 10 of 13 proteins expressed as soluble proteins and for three proteins expressed only as inclusion bodies. The use of autoinduction growth media increased yields over Luria-Bertani (LB) growth media in 75% of the expressed proteins. Expressing integral membrane proteins with yields suitable for structural studies from a set of previously low and non-expressing proteins proved highly successful upon attachment of the maltose binding protein as a fusion tag.

Keywords: Membrane protein expression; Maltose binding protein; Fusion proteins

Production of membrane proteins for structural and functional studies remains a difficult task due to their highly hydrophobic nature [1,2]. During the last decade increasing efforts to express prokaryotic and eukaryotic membrane proteins have resulted in significant advancements for the expression of several bacterial transporter proteins [3–9], outer membrane proteins of bacteria [10], membrane protein complexes [11–13] and a few eukaryotic G-protein coupled receptors [14–21]. With others we have successfully expressed more than 70 membrane proteins from *Mycobacterium tuberculosis* in *Escherichia coli* using a His-tag [22]. However, a significant number of tested proteins (28%) were not expressed at detectable (Coomassie stain or Western) levels in *E. coli*. Expression of nonmembrane proteins by the *Methanobacterium thermoautotrophicum* structural genomics pilot project showed that almost 50% of cloned genes did not express when an N-terminal His-tag was used [23]. More importantly, 41% of our cloned membrane proteins with a His-tag that have a molecular weight less than 13.7 kDa did not express, and only 22% expressed well, such that a Coomassie stain was observed representing enough protein to proceed with structural studies. Here, we have set out to enhance the expression of small molecular weight proteins and to identify a robust protocol for doing this.

While many different vectors and tags have been used, the fusion construct with maltose binding protein (MBP)[1] has been found to be an effective fusion partner for increased solubility and expression yields for a variety of recombinant water-soluble proteins [3,24–28]. MBP has also been tried with a few eukaryotic and prokaryotic membrane proteins [3,15,29–31]. Grisshammer and colleagues

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[1] *Abbreviations used:* MBP, maltose-binding protein; LB, Luria-Bertani growth media; IPTG, isopropyl-β-D-thiogalactoside; MW, molecular weight.

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have achieved membrane localization and functional expression in *E. coli* for neurotensin and neurokinin-2, G-protein coupled receptors, upon fusion to the periplasmic MBP [1,14]. Expression as non-periplasmic MBP fusions in *E. coli* followed by purification and structural characterization by NMR was reported for two small membrane proteins: phospholamban and sarcolipin [30].

Here, we report a successful application of the MBP fusion expression system for expression of small molecular weight integral membrane proteins from *M. tuberculosis* in *E. coli*. Some of these proteins are expressed as soluble fusion proteins obviating the need for harsh solubilizing conditions in extracting membrane proteins from inclusion bodies, but many others were expressed in high yields in inclusion bodies from which we have demonstrated isolation and cleavage of the fusion.

Materials and methods

Cloning

The DNA fragments encoding membrane proteins from *M. tuberculosis* were cloned into a modified pMALc2 plasmid [32] by PCR. Cloning was done according to standard techniques. Plasmid DNA encoding selected proteins were used as a template for PCR. All primers were purchased from IDT, Inc., USA. Pairs of gene specific primers were used to amplify DNA using standard PCR conditions and the enzyme *Pfu Turbo* DNA polymerase (Stratagene, USA), shown to have lower probability for introducing unwanted mutations. Two pairs of the restriction endonucleases were used to digest generated PCR fragments or vector DNA: *Bam*HI and *Hin*dIII or *Bam*HI and *Pst*I. Sites for restriction endonucleases were introduced in the primer sequences. PCR products were gel purified after digestion with restriction enzymes and ligated into the prepared expression vector. Correct insertions into the vector were confirmed by PCR screening followed by DNA sequencing to check for in-frame insertion and lack of PCR-introduced point mutations.

Expression constructs

The plasmid results in the expression of the MBP fusion protein with eight histidine residues and the thrombin protease cleavage site at the N-terminus of the protein of interest to improve cleavage of MBP (Fig. 1). Importantly, two affinity tags, MBP and the His-tag, allow for a choice of the alternative purification approaches.

Protein expression

Escherichia coli BL-21(DE3) codonPlus-RP strain (Stratagene, USA) was used to express recombinant MBP fusion proteins. This strain is designed to compensate for codon usage differences between *M. tuberculosis* and *E. coli* and was successfully used in our previous expression of membrane proteins.

Fig. 1. *Mycobacterium tuberculosis* membrane proteins were expressed as fusions to the C-terminus of non-secreted MBP. The fusion construct has eight histidines and a thrombin recognition and cleavage site positioned between MBP and the fused polypeptide. The thrombin recognition site is composed of LVPRGS amino acids. Two amino acid residues (glycine and serine) remain with the N-terminus of the expressed protein after thrombin digest. Molecular weight of MBP with the C-terminal His-tag generated after thrombin digest is 42.5 kDa.

The *E. coli* cultures were initially grown in standard liquid media with appropriate antibiotic [33]. Briefly, 20 ml of LB growth media supplemented with 50 mg/ml ampicillin was inoculated with a 1:100 dilution of an overnight bacterial culture and incubated with agitation at 37 °C to an OD_{600} of ~0.5. Protein expression was induced with 0.4 mM IPTG. Cells were harvested 3 h after induction by centrifugation at 5000*g* in an Avanti J-20 XP centrifuge (Beckman Coulter, Inc., USA). The cells were resuspended in 1 ml of water and lysed by sonication using a Sonic Dismembrator, Model 100 (Fischer Scientific, Inc.). The lysate was clarified by centrifugation for 15 min at 10,000*g* using a Microfuge 18 Centrifuge (Beckman Coulter, Inc.). The pellet was resuspended in 1 ml of 1% SDS and 4M urea. Insoluble and soluble fractions were checked for expression by the appearance on the SDS–PAGE gel stained with Coomassie R-250. Samples were loaded on the gel on the basis of equal volume, and the expression outcomes were assessed by visual inspection. Furthermore, the amount of expressed fusion protein was estimated for each sample during the purification step when the fusion protein was eluted from the amylose or His-tag affinity columns. The concentration was determined via the absorbance at 280 nm.

Bacterial autoinduction growth system, which utilizes lactose for the induction of protein expression has also been used for all expressed fusion proteins in an effort to increase yields [34]. Briefly, TB growth media was supplemented with 50 mg/ml ampicillin, 100 mM dibasic sodium phosphate, 50 mM monobasic potassium phosphate, 25 mM ammonium sulfate, 0.5% glycerol, 0.05% glucose, and 0.2% lactose. Twenty microliters of prepared media were inoculated with a 1:100 dilution of an overnight bacterial culture and incubated with agitation at 37 °C overnight (16 h of growth). Cells were harvested by centrifugation followed by lysis and sample preparation as described above for the bacterial cells grown in LB media.

Protein purification

Membrane proteins from *M. tuberculosis* fused with the C-terminus of MBP were expressed in two forms: as soluble fusion proteins and as proteins directed into inclusion bodies. Dual affinity properties of the recombinant fusion protein, MBP and His-tag, allow the purification of both a