INTENDED INSOLUBLE EXPRESSION OF RECOMBINANT PROTEIN WITH A PULL-DOWN TAG IN ESCHERICHIA COLI FOR SIMPLIFYING PRODUCT PURIFICATION AND INCREASING YIELD

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The markets for recombinant proteins are growing and for most applications there is a demand for soluble recombinant protein. However, recombinant protein production in Escherichia coli often triggers insoluble protein production. Insoluble protein is either degraded or accumulates in solid inclusion bodies with high specificity. This means there is a high purity grade of the recombinant protein in inclusion bodies that can reach up to 90%. Moreover, protein in inclusion bodies is hardly degraded. However, the protein is mostly not accessible in an easy way and has to be denatured to resolubilize the inclusion body structure. The native protein structure has to be reestablished in an empirical manner via refolding procedures which account for high yield losses. There are two general possibilities to avoid the aforementioned struggles of insoluble protein production. First, the target protein can be designed by targeted mutations that can increase its solubility or by fusing the target protein to tags that convey solubility. There is a high chance to get a bioactive protein, but the advantage of high initial product purity and product stability is lost. Second, the protein can be designed with certain tags that convey accumulation in inclusion bodies under known molecular circumstances. These tags can also enable an easy resolubilization without denaturants. Thus, it is also possible to obtain bioactive protein and one profits by the high initial product purity and stability in inclusion bodies. One tag of special interest is the 158 amino acid Cry4AaCter-tag from Bacillus thuringiensis israelensis [1, 2]. It pulls down the protein of interest into inclusion bodies and resolubilization is easily achieved by transfer to basic pH milieu. This study aims for the recombinant production of an antimicrobial peptide (amp) in inclusion bodies in E. coli using the Cry4AaCter-tag. The applied model amp is the 70 amino acids Galleria mellonella insect metalloproteinase inhibitor (IMPI), which contains five disulfide bonds [3]. The production of this disulfide-rich peptide in E. coli was accomplished applying a genetically engineered strain with a non-reducing cytoplasmic milieu. High yields of fusion protein were already obtained at lab-scale in shaking flasks, which were almost completely resolubilized by simple change into basic pH milieu. The stability of IMPI enabled acid precipitation after tag cleavage, because IMPI stays soluble in presence of trichloroacetic acid while the cleaved tag and the protease precipitate. The activity of the product was confirmed in a fluorescence-based assay where IMPI inhibits a metalloprotease. An eukaryotic potential drug candidate protein was produced in E. coli in its bioactive form with high purity via this inclusion body-based production strategy with rendering denaturants, refolding procedures and chromatography steps obsolete. This system holds great promise for increasing demands of recombinant protein and will be further investigated with other proteins of interest.