Production of the human carbohydrate binding module from laforin protein: A comparative study between bacterial and yeast expression systems

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The goal of this work is the production of a recombinant Carbohydrate Binding Module (CBM) from human origin aiming to improve the functional properties of biomaterials based on polysaccharides. When fused with the bioactive peptides, CBMs may dramatically improve the therapeutic efficiency of biomaterials such as biocompatibility, biomimetism and/or biodegradability. Laforin is a human protein associated with glycogen metabolism, composed of two distinct structural and functionally independent domains: a phosphatase and a substrate binding module. The Laforin-CBM sequence was originally cloned by PCR from a human muscle cDNA library. Commercially heterologous expression systems of *Escherichia coli* (pET 29a, pET 25b and pGEXT4-1) and of *Pichia pastoris* (pGAPZ alpha C and pPICZ alpha C) were used in order to obtain high levels of soluble protein that can be purified by affinity chromatography using 6xHis-tag or GST-tag.

With pGEXT4-1 expression system, CBM was fused with the GST protein, which in theory increases solubility, but the amount of recombinant protein obtained was very low. In pET29a the CBM was obtained in inclusion bodies, which after solubilization and refolding processes was soluble but easily aggregated. Using pET25b, in the presence of arginine and CHAPS in the lyses buffer, the amount of soluble protein was higher but again formed aggregates. Two *P. pastoris* expression systems, both with secretion signal alpha- factor, were utilized: pGAPZ alpha C (containing a constitutive promoter) and pPICZ alpha C (containing an inductive promoter). The integration of the CBM coding sequence, in yeast genome, was confirmed by slot-blot. Expression was analysed by northern-blot and confirmed by western-blot. Fermentations carried out at 18ºC were critical to achieve production in both systems. The utilisation of *P. pastoris* expression systems led to the production of soluble and stable CBM in extra cellular medium; however, this CBM was obtained at low expression level. Post-translation modifications, such as glycosylation and phosphorylation of the protein may explain the increased stability, at the expense of reduced functionality. Studies are underway to confirm this hypothesis.

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