Improving the solubility of a recombinant human CBD (carbohydrate binding domain)

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Different expression systems have been developed, and are commercially available, aiming at producing recombinant proteins from different organisms, ranging from bacteria to man. However, in same cases, the solubility and stability of the produced protein can be a problem, especially for eukaryotic proteins, which need post translational modifications to be biologically active.

In the present work, different strategies were tested to increase the solubility of a human carbohydrate binding domain (CBD), present in laforin phosphatase. Namely, different expression systems, different hosts and fermentation conditions, the presence of additives and detergents during lyses, were used.

The DNA coding sequence was cloned by PCR into three prokaryotic expression systems: pET 29a, pET 25b; and two eukaryotic systems: pGAPZαC and pPICZαC.

The CBD was expressed at high level in pET system. In pET29a the CBD protein was obtained in inclusion bodies. In pET25b, a small amount of soluble protein was obtained in the presence of 0,6M of arginine, in the lyses buffer. Although a functional recombinant protein was obtained, it was not stable in solution, aggregating easily.

On the other hand, the utilisation of two expression systems of *Pichia pastoris* led to the production of soluble and stable CBD in extra cellular medium; however, this CBD was obtained at low expression level and its activity was not confirmed. Glycosilation of the expressed protein may explain the increased stability, at the expense of reduced functionality. Studies are underway to confirm this hypothesis.