Expression of a Chimeric Protein containing both a Phosphatase and a Carbohydrate Binding Domains

Gonçalves, L.M., Hannun, P.G.C., Cuccovia, I. M., Chaimovich, H., Marana, S.R.
Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (IQUSP).

Introduction and Aims. Protein-polysaccharide interaction is a key step in the microbial hydrolysis of cellulose. This interaction in many cellulose or hemicellulose degrading enzymes depends on carbohydrates binding domains (CBD) that are noncatalytic, structurally and functionally independent. . Our aim is to obtain chimeric proteins containing phosphatase domains linked to a CBD as proof of concept, leading to application of the CBDs by facilitating protein immobilization on cellulose supports. Such chimeric proteins could be useful for the development of low cost, phosphatase-based cellulose materials for the simultaneous adsorption and degradation of stored or spilled organophosphate wastes. ***Results***. The DNA segments coding for CBD of a cellulase from *Xhantomonas axonopodis pv citri* (AE011689) and acid phosphatase (APPA; M58708) from *Escherichia coli* were fused using an "overlapping primer" strategy resulting in a segment coding for a chimeric protein APPA-CBDII. The correctness of this procedure was attested by DNA sequencing. The chimeric construction was then cloned in the expression vector pT7-7 to produce APPA-CBDII as a recombinant protein in BL21DE3 bacteria. The expression of APPA-CBDII was attested by enzymatic assays using *p*-nitrophenyl phosphate as the substrate. Enzymatic assays were also used to demonstrate that APPA-CBDII was immobilized onto a variety of cellulose matrixes like microcrystalline cellulose (Avicel), cotton fabric and paper. The binding specificity was confirmed by comparison with the native APPA. In conclusion we have obtained a cellulose-binding chimeric protein containing fully functional phosphatase activity.

Supported by CNPq and FAPESP