Production of βglB-CBM, a chimeric protein that combines "beta-glucosidase" and "cellulose binding" domains

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Objectives

Cellobiose, formed in the enzymatic hydrolysis of cellulose, is actually a cellulase inhibitor. Hence, the cellobiose accumulation results in reduction of the cellulase activity along the reaction. Hence, the targeting of a beta-glucosidase (cellobiase) to the cellulose surface, site of the cellulase action, could in theory reduce the local concentration of cellobiose and prevent the cellulase inhibition. A possible strategy to target a beta-glucosidase to the cellulose surface is the utilization of a CBM, cellulose binding module. This project aims at to compare the efficiency of two beta-glucosidases (βglA e βglB) and their respective chimeras (βglA-CBM e βglB-CBM), which are formed by a catalytic domain and a cellulose binding module.

Materials and Methods

The wild-type and chimeric proteins were produced in BL21DE3 bacteria using the expression vector pLate51. Recombinant proteins were purified using Ni-NTA resin. An additional step of ion exchange chromatography was used for the chimeric enzymes. SDSPAGE was employed to attest the recombinant proteins purity. The substrate *p*-nitrophenyl beta-glucoside was used in the beta-glucosidase activity assays. Tests of cellobiohydrolase complementation were performed with crystalline cellulose (Avicel) and commercial cellobiohydrolase I (CBHI; Megazyme).

Results

The wild-type and chimeric proteins were produced and purified. However, this procedure was more effective for βglA and βglB than for the chimera proteins. Indeed, it was obtained 15 times more activity of βglA and 11 times more activity of βglB than their respective chimeras. Tests of enzymatic hydrolysis of cellulose using these enzymes are presented below.

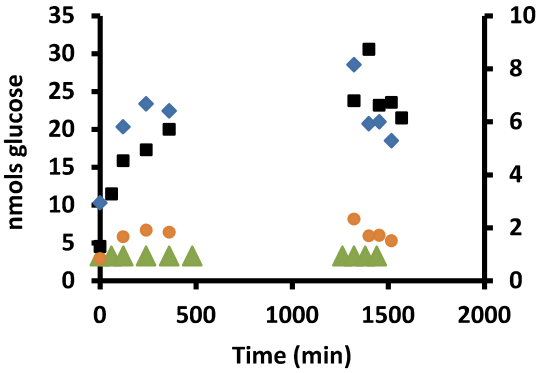

Figure 1: Glucose production from cellulose. βglA+CBHI (blue), βglACBM+CBHI (orange), βglB+CBHI (black) and βglBCBM+CBHI (green). Data for βglA on left; for βglB, on right.

Conclusions

Cellulose hydrolysis in the presence of wild-type beta-glucosidases was effective. However, the lower activity of the chimera enzymes precluded the glucose production. Thus, assays with more activity units of the chimeras should be performed for a real comparison with the wild-type enzymes.