Figure 1. Homology model of Sfβgly. Active site residues are represented as sticks.

The rate of the enzymatic hydrolysis of cellulose decreases along the reaction time, which represents a drawback for the productivity of this process. One of the factors causing that problem is the cellobiose inhibitory action upon the “cellulases” (endoglucanases and cellobiohydrolases) that catalyze the hydrolysis. This project aims to stabilize the rate of the enzymatic hydrolysis of cellulose by developing β-glycosidases designed to reduce the cellobiose inhibitory effect on the “cellulases.” Such development will be based on the β-glycosidase (cellobiase) from the fall armyworm Spodoptera frugiperda (Sfβgly) and the “carbohydrate binding domain” of the endoglucanase EngXCA from Xanthomonas axonopodis pv citri (CBMXAC).

Firstly, a chimeric protein resulting from the fusion of Sfβgly and CBMXAC will be assembled. The targeting of Sfβgly to the surface of the cellulose fibers (due to the presence of a CBM) could decrease the cellobiose concentration directly in the microenvironment of action of the endoglucanases and cellobiohydrolases. Thus the action of Sfβgly-CBM could reduce the cellobiose inhibitory effect and sustain a high activity of endoglucanases and cellobiohydrolases for a longer time.

This project also intends to improve the participation of the β-glycosidases in the cellulose hydrolysis by selecting mutant Sfβgly that presents high hydrolytic activity upon cellobiose. Libraries of random mutant Sfβgly will be generated and screened based on the ratio of activity upon cellobiose versus synthetic substrates. Finally, amino acid residues networks involved in the determination of Sfβgly substrate specificity and catalytic activity will be identified by using structural analysis, site-directed mutagenesis studies and enzyme kinetic experiments.
SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Production of the chimeric protein Sf\(\beta\)gly-CBM

The DNA segment coding for the CBM (0.4 kb) of the endoglucanase EngXCA from *X. axonopodis* (AE011689) was amplified taking genomic DNA of that bacteria as template, whereas the segment coding for Sf\(\beta\)gly (AF052729; 1.4 kb) was amplified from a cDNA library of the *S. frugiperda* midgut. Following that, segments coding for CBMXAC and Sf\(\beta\)gly were fused by "overlapping pcr", which generated a product (1.8 kb) coding for the chimeric protein Sf\(\beta\)gly-CBM. This product was cloned into the vector pAE and this construction was introduced in BL21DE3 bacteria. In the next steps Sf\(\beta\)gly-CBM will be produced as recombinant protein and purified by Ni-binding chromatography. The catalytic activity of Sf\(\beta\)gly-CBM will be tested using p-nitrophenyl \(\beta\)-glycosides, whereas its cellulose-binding activity will be verified using avicel.

Enhancement of the cellobiase activity of Sf\(\beta\)gly

The search for Sf\(\beta\)gly mutants exhibiting high activity upon cellobiose was initiated by constructing libraries by random mutagenesis. A vector pCAL-Sf\(\beta\)gly was used as template according instructions of the kit GeneMorph II EZ Clone. In the next steps the library will be screened using a high-throughput procedure based on the recombinant expression and enzymatic assays in 96-well plates.

Identification of interaction networks in Sf\(\beta\)gly

In order to identify amino acid residues networks involved in the determination of substrate specificity and catalytic activity the tertiary structure of Sf\(\beta\)gly was represented as a graph and its central hubs were identified. In the next steps 10 of these central hubs will be separately removed by site-directed mutagenesis. The effect of these deletions on the Sf\(\beta\)gly substrate specificity and catalytic activity by enzyme kinetic experiments.

MAIN PUBLICATIONS


Sandro Roberto Marana

Instituto de Química
Universidade de São Paulo (USP)
Av. Prof. Lineu Prestes, 748 – Bl. 10 sup. – sala 1054
Cidade Universitária
CEP 05508-900 – São Paulo, SP – Brasil
+55-11-3091-3810 r. 224
srmarana@iq.usp.br

Figure 2. Amplification of the segments coding for CBMXAC (0.4 kb), Sf\(\beta\)gly (1.4 kb) and Sf\(\beta\)gly-CBM (1.8 kb), respectively