Poster

Structure-guided improvement of glucose tolerance and catalytic efficiency of β-glucosidase from *Clostridium thermocellum* for industrial bioethanol production

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Cellulases are utilized by bioethanol production industries for converting complex cellulose into fermentable sugars. β-glucosidase, constituting the last enzyme of the cellulose degradation pathway, is inhibited by its product – glucose [1]. This inhibition becomes a bottleneck of the industrial cellulose hydrolysis process. Hence, engineering β-glucosidases with high glucose tolerance along with high catalytic efficiency is a need of the hour in bioethanol production industries. Our current study encompasses the characterization of β-glucosidase from *Clostridium thermocellum* (WT-CtGH1). The purified WT-CtGH1 showed its higher specificity towards cellobiose over pNPG as a substrate. However, WT-CtGH1 showed a low glucose tolerance of 380 mM glucose compared to the desired industrial criterion of > 800 mM of glucose. Hence, we proceed with improving the glucose tolerance of WT-CtGH1. For this, the crystal structure of WT-CtGH1 elucidated by us at 3 Å resolution was used as a reference for the rational design approach (Figure 1a). A comprehensive understanding of the mechanism by which glucose binds to the active site pocket was obtained through sequence and structural analyses of WT-CtGH1 with other glucose-tolerant β-glucosidase enzymes [2 - 4]. These sequence and structure comparisons aided us in introducing two point mutations – SDM1 (Gly to Trp) and SDM2 (Ser to Trp) in the active site crater for improved glucose tolerance [5]. The purified SDM1 and SDM2 mutant enzymes showed enhanced glucose tolerance of ~ 800 mM and ~ 600 mM compared to WT-CtGH1 while retaining the kinetic properties (Figure 1b). Surprisingly, a point mutant – SDM3 (Ser to Leu) showed a more than two-fold increase in the catalytic efficiency (with cellobiose as compared with WT-CtGH1. A combinatorial double mutant of earlier mentioned mutations – SDM4 (Gly to Trp and Ser to Leu) showed a 1.5-fold increase in the catalytic turnover as well as the catalytic efficiency with cellobiose as a substrate along with the enhanced glucose tolerance of ~750 mM glucose (Figure 1b). Along with glucose tolerance, the temperature (45°C – 60°C) and pH (4.8 -5.5) of the saccharification bioreactor limit the bioethanol production in separate hydrolysis and fermentation process (SHF). WT-CtGH1, along with all the above-mentioned mutants, showed considerable thermal stability at industrial operational conditions (Figure 1c). The mutant β- glucosidases developed in this research work would stand as potential candidates to be employed for the saccharification process in biofuel production, hence, have immense commercial value.

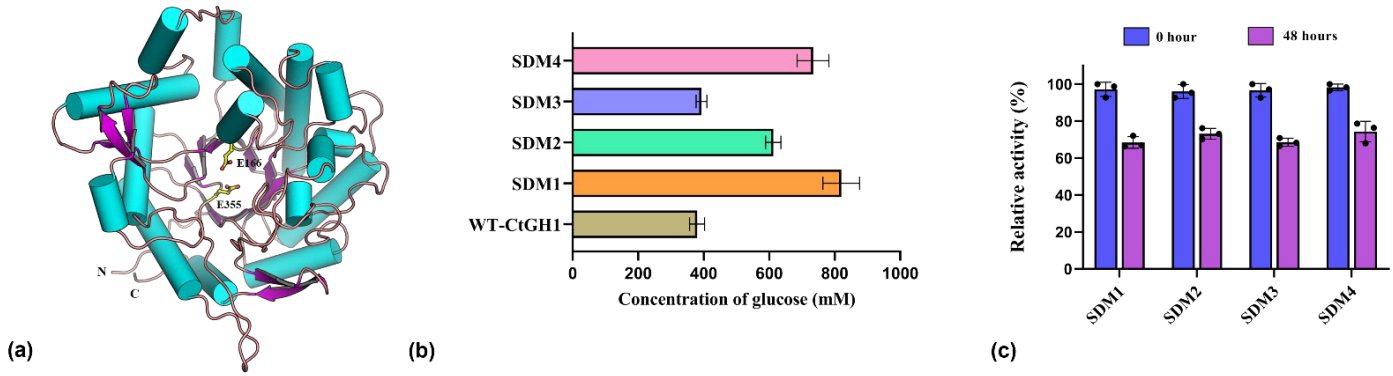

Figure 1. (a) Overall structure of WT-CtGH1 elucidated using X-ray crystallography, showing $(\alpha/\beta)_8$ barrel fold with catalytic glutamates – E166 and E355; **(b)** Comparison of glucose tolerance of WT-CtGH1 with point mutants – SDM1, SDM2, SDM3 and a double mutant – SDM4; **(c)** Comparison of thermal stabilities of mutants – SDM1, SDM2, SDM3 and SDM4.

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