Poster

Multiple high-resolution structures providing the insights of glucose tolerance in GH1 family β-glucosidases

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Cellulases are a group of enzymes that includes endoglucanase, exoglucanase, and β-glucosidase that play an important role in the hydrolysis of lignocellulosic biomass. The combined effect of these three major enzymes determines hydrolysis efficiency [1]. The hydrolysis of cellulose produces simple sugars that are further fermented to bioethanol [2]. β-glucosidase is a rate-limiting enzyme of the pathway and gets inhibited at a high concentration of glucose, which results in a slowdown of the cellulose hydrolysis process [3]. This feedback inhibition is one of the primary reasons for the low catalytic efficiency of β-glucosidase. Therefore, developing an efficient β-glucosidase with high glucose tolerance is essential. In our research, a β-glucosidase (sBGl) gene from the GH1 family has been cloned and overexpressed in *E. coli* BL21(DE3) cells. The overexpressed protein is purified to obtain a homogenous population. The biochemical properties of the pure sBGl suggested that the enzyme has maximum activity at pH 6.0 at 55 °C. The glucose tolerance level of the wild-type enzyme is 0.9 M, which is insufficient as the glucose tolerance for β-glucosidases to be used for industrial bioethanol production is 1.0 M. The high-resolution (1.2 Å) structure of sBGl complexed with glucose molecules provided detailed information of the binding sites of glucose in the catalytic crater of the sBGl (Fig. 1). From structural analysis, Cysteine at +1 subsite was found to be stabilizing the glucose near the catalytic site. Upon mutation of Cysteine to Valine (M1), we found no change in biochemical property but exhibited a higher glucose tolerance of 1.8 M (Fig. 2). The structure of mutant sBGl was determined at 1.3 Å resolution. We have also explored +2 subsite, where histidine residue was mutated to tryptophan (M2) to increase the substrate specificity of the enzyme. The M2 mutant exhibited activity at wide range of pH from pH 5.0 to pH 7.0 with better kinetic properties. Also, +2 subsite mutant showed increased glucose tolerance level of 1.5 M (Fig. 2) as compared to the native sBGl. Cellobiose and glucose complexed M2 mutant structures were determined at 1.7 Å and 1.8 Å resolution respectively. Finally, the M3mutant with the combined effect of +1 and +2 subsite alteration showed the highest glucose tolerance. The mutants of sBGl produced in this research have high glucose tolerance, and therefore can aid in improving the efficiency of bioethanol production. The rationale engineering strategy used in our study can be applicable for rationale design of GH1 family β-glucosidases to improve their glucose tolerance and use of those enzymes in industrial bioethanol production for green energy.

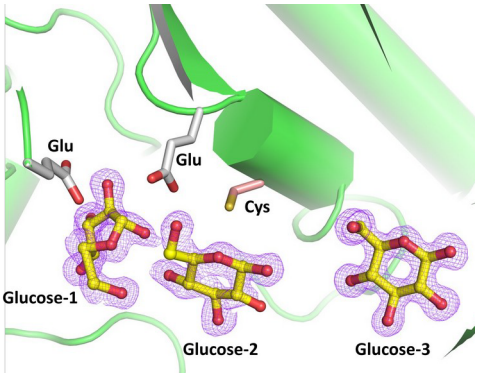

Figure 1. Product binding sites of native sBGL

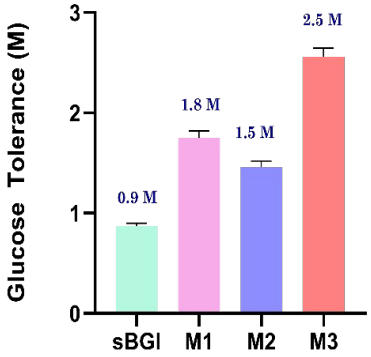

Figure 2. Glucose tolerance levels of native and mutant sBGLs

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