A Key Enzyme for Biofuel Production: "Missing Link" between Oxidative Cellulose Degradation and Ethanol Fermentation by Microbes

Cellulose is the most abundant renewable biopolymer on Earth, and the establishment of cellulosic biomass degradation systems has attracted significant attention. Cellobionic acid phosphorylase is a recently-discovered enzyme that can catalyze the decomposition of a major product of oxidative cellulose degradation and increase the efficiency of microbial bioethanol production. We have determined the crystal structure of cellobionic acid phosphorylase. The enzyme has a unique binding site for the gluconic acid moiety of the substrate. This study provides a molecular insight into the energetically efficient metabolic enzyme for oxidized sugars that may overcome the bottleneck of current biofuel production systems.

The development of cost-efficient systems for degrading and converting cellulosic biomass is a challenging but essential task for establishing a sustainable society. Therefore, microbial cellulases have attracted significant research attention for a long time. The recent discovery of lytic polysaccharide mono-oxygenase, which oxidatively cleaves glycosidic bonds of cellulose, has changed the paradigm of this research area [1]. Oxidative enzymes such as lytic polysaccharide mono-oxygenase and cellobiose dehydrogenase synergistically act with orthodox hydrolytic enzymes such as cellobiohydrolase and endoglucanase to significantly enhance the degradation speed of the recalcitrant crystalline cellulose (**Fig. 1**), providing the most effective biomass degradation system to date. One of the main products of the joint cellulose degradation system is cellobionic acid (glucose-β1,4-gluconic acid). However, *Saccharomyces cerevisiae* and other yeasts generally cannot utilize cellobionic acid for ethanol fermentation. Nihira et al. recently discovered cellobionic acid phosphorylase (CBAP, EC 2.4.1.321) [2], which provides an effective enzymatic means of overcoming the bottleneck of current biomass degradation systems. CBAP catalyzes phosphorolysis (cleavage of the glycosidic bond by addition of inorganic phosphate) of cellobionic acid to produce α-D-glucose 1-phosphate and D-gluconic acid. Involvement of the phosphorolytic enzyme has an energy advantage in microbial catabolism because CBAP produces the phosphorylated sugar (α-D-glucose 1-phosphate) without consuming ATP.

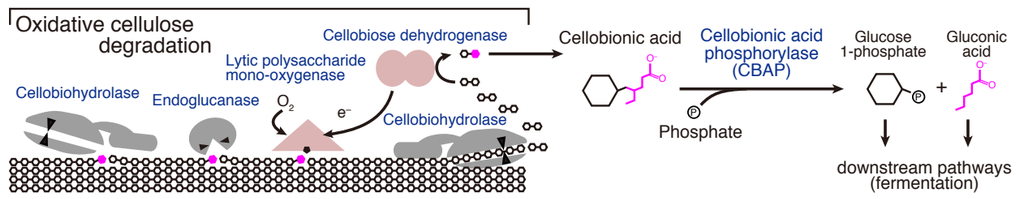

Figure 1: Schematic drawing of oxidative cellulose degradation system and the reaction of cellobionic acid phosphorylase.

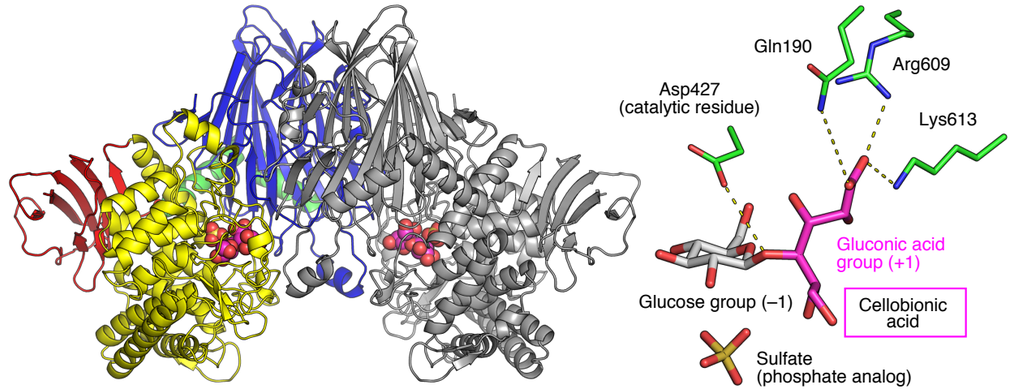

Figure 2: Overall structure **(left)** and the active site **(right)** of cellobionic acid phosphorylase.

We solved the crystal structures of CBAP from the cellulolytic marine bacterium *Saccharophagus degradans*. Structures of ligand-free and complex forms with cellobionic acid and gluconic acid were determined at resolutions up to 1.6 Å using BL-17A and AR-NW12A [3]. The active site is located near the dimer interface (**Fig. 2, left**). The glucose and gluconic acid moieties of cellobionic acid are recognized by subsite –1 and +1, respectively. Arg609 and Lys613 are key residues for the recognition of the carboxylate group of gluconic acid at subsite +1 (**Fig. 2, right**). Additionally, Gln190 from the neighboring subunit is involved in the recognition of the carboxylate group. A mutational analysis revealed that these residues are crucial for the substrate binding and catalysis. Structural analysis and sequence comparison with other phosphorolytic enzymes in the same enzyme family indicated that CBAP has a unique subsite +1 with a distinct amino acid residue conservation pattern at this site.

Our study provides the first structural basis of the key enzyme that connects the "missing link" of the oxidative cellulose degradation and downstream pathways. The conservation pattern of the three key residues at subsite +1 is a good indicator for finding more effective CBAP enzymes from microbial sources. In addition, the three-dimensional structure of CBAP will contribute to the future design and engineering of glycoside phosphorylases, which have the potential for application in large-scale production of functional oligosaccharides [4].

REFERENCES

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BEAMLINES

BL-17A and AR-NW12A

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