Crystallographic Studies of β-glucanase and Exoglucanase

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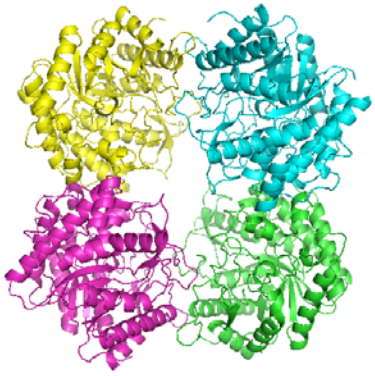
Global energy demand is estimated to grow by more than 50% by 2025. Due to the high energy prices and increasing energy imports, there is a heightened interest in the application of lignocellulose for cellulosic-biomass-based biofuel production. Cellulose, the main component of cell wall, is a chemically homogeneous linear polymer composed by D-glucose with β-1,4 linkage. The chemical uniformity which provokes spontaneous crystallization of the cellulose molecules and the tight conjunction with hemicellulose and lignin make cellulose-containing materials, like wood, hard to be hydrolyzed by a single enzyme. For producing biofuel from lignocellulose, degrading the "protector" of lignin and breaking up the crystal surface of cellulose to release soluble and fermentable cellulose molecules are the critical and expensive processing steps.

Anaerobic, thermophilic and cellulolytic bacteria, *Clostridium thermocellum*, produces large extra-cellular multienzymatic complexes termed cellulosomes that efficiently degrade crystalline cellulose and related plant cell wall polymers. This unique cellulose-decomposing system is comprised of many catalytic subunits, such as endo-β-glucanases, exoglucanases, xylanases, and lichenases. Advantages of using cellulosomes from *C. thermocellum* for biofuel production include (i) the cellulolytic and ethanogenic nature, allowing saccharification and fermentation in a single step, (ii) thermophilic fermentation being less prone to contamination, and (iii) thermophilic biomass-degrading enzymes enhancing protein stability. However, this strain is sensitive to ethanol and has lower tolerance than yeast.

The objective of our proposal is solving crystal structures of β-glucosidases and exoglucanases cloned from our other team member. The β-glucosidase from *Clostridium cellulovorans* has 50% identity and 70% homology with β-glucosidase from *Thermotoga Maritima*. After screen with commercial crystallization kits, three crystal forms were obtained. They are tetragonal form ($P4_12_12$) with cell dimensions a=b=155.22 c=347.84, a=b=128.498 c=264.06 and monoclinic form ($P2_1$) with cell dimensions a=96.15 b=161.76 c=136.39 beta=110.21. The first and third of them diffract to 2.9 A and have eight monomers in an asymmetric unit. Currently R=18% and Rfree =22% for Native $P2_1$ crystal form and R=25% and 37% for gluconolactone complex. And The R=16% and Rfree= 24% for $P4_12_12$ gluconolactone complex. The second tetragonal form has tetramer in asymetric unit. Currently model refine to 1.9A and R=18.7% and Rfree =22.4%.



The β-glucosidase from *Trichoderma reesei* (trbg1) has 55% identity and 68% homology with beta-glucosidase from *Basidiomycete Phanerochaete Chrysosporium.* After screen with commercial crystallization kits, one crystal form (C222 or $C222_1$) with cell dimensions a=94.36 b=357.72 c=104.27 and the other one is monoclinic form ($P2_1$) with cell dimensions a=93.53 b=103.86 c=183.62 beta=104.27. Currently model refine to 2.9A and R=33% and Rfree =36%. The high R factor is due to broken of detector at 13B.

The exo-glucanase from *Clostridium cellulovorans* is a gene for a major subunit of *Clostridium cellulovorans* cellulosome (ccExgS). ccExgS is one of most abundant component of its cellulosome. It has exoglucanase activities and show a greater activity in degrading Avicel than CMC. The majoe product produced by ExgS is cellobiose. It was cloned into pET 32 and expressed and purified by Ni column and S protein column. The final result was confirmed by Mass spectra. After screen with commercial crystallization kits, three crystal forms were obtained. They are tetragonal form ($P4_12_12$) with cell dimensions a=b=108.43 c=182.983. It has tetramer in asymetric unit. Currently model refine to 1.95A and R=16.7% and Rfree =19.3%.