Title

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Publication Date

2009-12-15

jbei
Joint BioEnergy Institute

Characterization of a Hyperthermophilic Cellobiohydrolase from *Caldicellulosiruptor saccharolyticus*: Enzymatic Hydrolysis of Cellulose Mediated by Substrate Binding

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We cloned, expressed, purified, and characterized a recombinant cellobiohydrolase (EC 3.2.1.91) domain from celB, a modular cellulolytic gene from *Caldicellulosiruptor saccharolyticus* that contains glycoside hydrolase family 10 (GH10), carbohydrate binding module family 3 (CBM3), and GH5 domains. The deletion analysis of *celB* confirmed that the constructs containing the GH5 domain were able to hydrolyze the soluble substrates carboxymethyl-cellulose (CMC) and *p*-nitrophenyl-β-D-cellobioside (*p*NPC). Therefore, we focused our study on the recombinant CBM3-GH5 and GH5. The recombinant proteins were expressed in *E. coli*, and purified to homogeneity by affinity and ion-exchange chromatography methods. The functional stability and melting temperature measurements demonstrated that both CBM3-GH5 and GH5 are highly stable up to 80°C at pH 5.5. CBM3-GH5 and GH5 were also able to hydrolyze microcrystalline cellulose (Avicel), ionic liquid (IL)-pretreated cellulose, and IL-preated corn stover to produce cellobiose; CBM3-GH5 produced more cellobiose than GH5 did from these insoluble substrates. We employed fluorescence confocal microscopy and total internal reflective fluorescence (TIRF) methods to investigate whether the binding interaction between the enzyme and substrate was attributed to the product yield from the insoluble substrates. We observed stronger binding interaction between CBM3-GH5 and cellulose (both microcrystalline and amorphous) than that between GH5 and cellulose. Thus, the higher product yields from the enzymatic hydrolysis of microcrystalline cellulose, IL-pretreated cellulose, and IL-pretreated corn stover by CBM3-GH5 were possibly mediated by the interaction between the CBM3 domain and the substrates. The recombinant CBM3-GH5 is a thermostable and active cellobiohyrolase that could be used with other types of cellulolytic enzymes for degradation of IL-pretreated biomass to produce fermentable sugars.

This work was part of the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.