NREL Highlights SCIENCE

Probing Product Binding in Cellulase Enzymes

Simulations uncover potential new strategies for increasing the desired effects of enzymes for biofuels.

Large-scale computer simulations offer a new interpretation for the discrepancy in experimentally measured product inhibition constants in cellulase enzymes. The simulations also suggest new strategies for relieving product inhibition, which is key for the application of enzymes in the biofuels industry. Bacteria and fungi secrete enzyme cocktails consisting of processive and nonprocessive enzymes, which act synergistically to digest plant cell walls. As high-solids loadings are important for economically viable biomass conversion processes, the concentrations of glucose and cellobiose reached during enzymatic hydrolysis often cause a significant slow-down in cellulase activity through enzyme inhibition. Many studies have quantified cellulase product inhibition, but experimental measurements of product binding constants vary dramatically, and there is little consensus on the importance of this phenomenon. To provide molecular insights into cellulase product binding, scientists from the National Renewable Energy Laboratory (NREL) used computer simulation to demonstrate that the presence of cellodextrins bound in the substrate sites of cellulases, which are present during enzymatic catalysis, can increase cellobiose binding to processive cellulases by several orders of magnitude, whereas this presence has no effect on product binding in nonprocessive cellulases. These findings provide new insights into the large discrepancies reported for binding constants for cellulases. This work also suggests that catalytic engagement has an important effect on apparent inhibition constants for processive cellulases and should be taken into account to characterize product inhibition. Additionally, several computationally designed mutants of the processive cellulases are demonstrated to be able to reduce the cellobiose binding affinity, which suggests a means to engineer cellulases to improve the efficiency of biomass conversion.

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References: Bu, L.; Nimlos, M.R.; Shirts, M.R.; Stahlberg, J.; Himmel, M.E.; Crowley, M.F.; Beckham, G.T. (2012). "Product Binding Varies Dramatically between Processive and Nonprocessive Cellulase Enzymes." *J. Biol. Chem.* 287 (29), 24807-24813.

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Key Research Results

Achievement

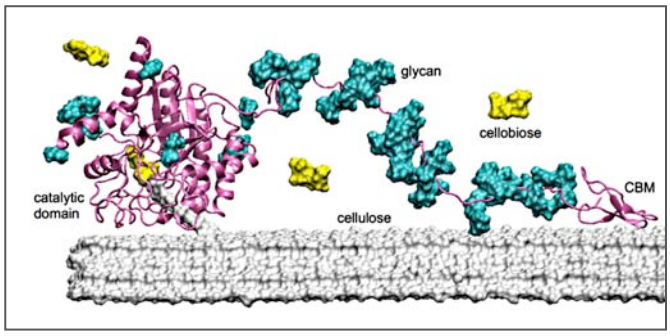
NREL researchers gained new insights into the differences in product inhibition constants for cellulase enzymes. These insights suggest new strategies for relieving product inhibition, a major factor in the use of enzymes for biofuels.

Key Result

Cellodextrin bound as substrate to cellulases increases product binding to processive cellulases but does not affect nonprocessive cellulases.

Potential Impact

Catalytic engagement should be taken into account to characterize product inhibition, as it may have an important effect on apparent inhibition constants for processive cellulases. Additionally, because designed mutants are shown to be able to reduce cellobiose binding affinity, cellulases potentially may be engineered to improve the efficiency of biomass conversion.



The T. reesei *Cel6A enzyme catalytically engaged on a cellulose crystal with the carbohydrate-binding module (CBM) bound on the cellulose surface.*

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NREL/FS-5100-56028 • August 2012