Enzymatic Deconstruction of Cellulosic Biomass

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Project Goals: The goal of this project is to expand the understanding of enzymatic deconstruction of cellulosic plant biomass by carbohydrate active enzymes on both the atomic and macromolecular level. This project is using solid-state nuclear magnetic resonance (ssNMR), crystallography, and enzyme catalysis to contextualize enzyme hydrolysis and synergistic deconstruction experiments at the molecular level.

Abstract text:

Enzymatic deconstruction of plant biomass has been the focus of much research, especially with increasing interest in using plant biomass as a renewable resource to build various value-added products such as biofuels, bioplastics, and other platform chemicals. Research in this area has not yet produced a model that incorporates detailed, atomic level resolution and understanding across the scope of possible options. For example, the molecular basis for synergy between glycoside hydrolases that target different polysaccharides (e.g., an endo-xylanase and an exo-cellulase) is still not fully understood across the breadth of potential substrates and enzymes. This represents a significant knowledge gap, which this project is working to fill.

We established an analytical index of the interactions between biomass and a model cellulase (CelR) through the strong correlation between the ratio of the two peaks in the split C4 resonance observed by ssNMR (called X_{NMR}) and the final yield of enzymatic hydrolysis [1]. The correlation between X_{NMR} and enzymatic hydrolysis is diagnostic across biomass from different plant species and also with different co-solvents used in biomass pretreatment.

Our current work is focused on understanding the reactivity of CelR on pure cellulose and pretreated biomass treated with γ-valerolactone (GVL). With both crystalline and amorphous cellulose, CelR shows similar k_{cat} while K_M changes; however, with biomass, CelR exhibits bimodal kinetic behavior (rapid initial velocity followed by slower phase of hydrolysis over an extended time period). This potentially indicates that CelR is operating on two distinct populations of cellulose in the GVL-treated material. Amplitudes of the two kinetic phases and X_{NMR} are being analyzed with a panel of biomasses and pretreatments to identify how different populations of cellulose might respond to hydrolysis.

To better understand the interaction of CelR with cellulose, we solved its crystal structure. Similar to an earlier structure of a related enzyme from *Thermomonospora fusca* [2],

CelR has an open binding cleft and active site that would allow access of amorphous cellulose strands, but this single domain of the enzyme shows only weak reactivity with polysaccharides. When the natural carbohydrate binding module (CBM) is present, the crystal structure shows a tightly packed, extensive surface lined with aromatic residues that has a roughly linear orientation toward the active site. This form of the enzyme is ~10x more reactive than the catalytic domain alone and appears to behave as a processive endocellulase. When the enzyme is engineered to contain a second non-native CBM attached by flexible linker, the reactivity is doubled again. A combination of structural and catalytic contributions to this progressive improvement in reactivity will be reported.

References/Publications

1. Walker T.W., Kuch N., Vander Meulen K.A., Clewett C.F.M., Huber G.W., Fox B.G., Dumesic J.A. 2020. Solid-State NMR Studies of Solvent-Mediated, Acid-Catalyzed Woody Biomass Pretreatment for Enzymatic Conversion of Residual Cellulose. *ACS Sustainable Chemistry & Engineering* **2020** *8* (16), 6551-6563.
2. Sakon J., Irwin D., Wilson D.B., Andrew Karplus P. 1997. Structure and mechanism of endo/exocellulase E4 from Thermomonospora fusca. *Nature Structural Biology* **1997** *4*(10), 810-818.

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