Poster Presentation

MS82.P01

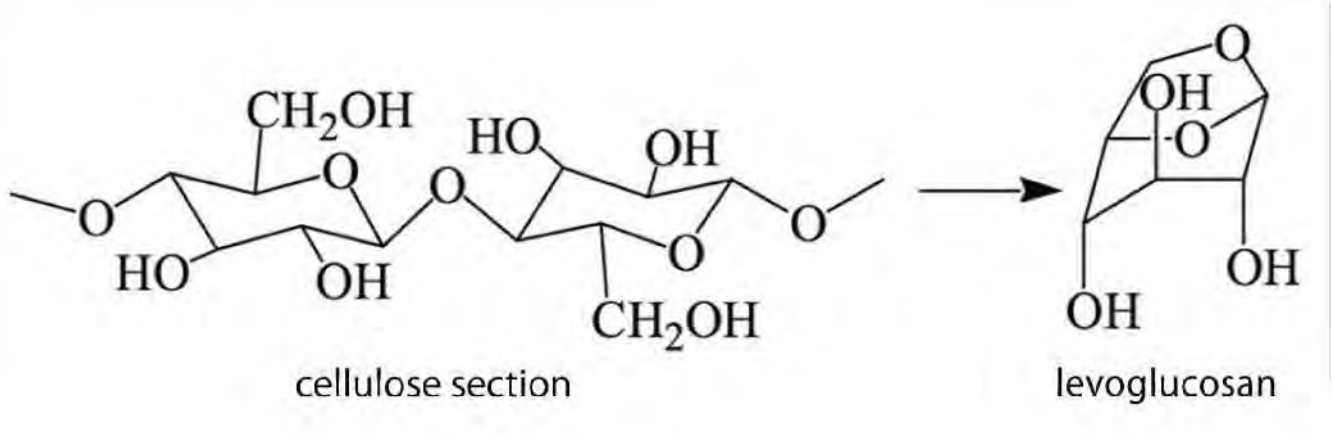
Structure and mechanism of LGK: insights into the bioconversion of levoglucosan

J. Bacik[1], B. Broom-Peltz[1], L. Jarboe[2], B. Mark[3], R. Michalczyk[1], Z. Fisher[1], C. Unkefer[1]

[1]Los Alamos National Laboratory, Biosciences Division, Los Alamos, NM, USA, [2]Iowa State University, Department of Chemical and Biological Engineering, Ames, IA, USA, [3]University of Manitoba, Microbiology Department, Winnipeg, MB, Canada

Lignocellulosic biomass is an abundant source of carbohydrates that can be used for the production of biofuels. Additional processing of biomass-derived sugars arises from the fact that the most abundant sugar product from biomass pyrolysis is the unusual anhydro-ring containing sugar, levoglucosan (LG). LG does not fall within the native substrate range of commonly used biocatalysts such as Escherichia coli and Saccharomyces cerevisiae. However, LG can be further converted to glucose-6-phosphate through the activity of the sugar kinase known as levoglucosan kinase (LGK), which has been isolated from a variety of fungal sources. Integration of recombinant LGK from Lipomyces starkeyi into an engineered ethanologenic Escherichia coli strain has been shown to allow for the utilization of LG as the sole carbon source for ethanol production [1]. However, challenges associated with effective utilization of LG include a high Km of LGK for LG, and the relatively low activity of LGK at physiological pH. In addition to the practical applications of LGK for biofuel production, the enzyme is an exceptional target for structural and mechanistic studies since it appears to possess dual hydrolase and kinase functionality. In order to gain a better understanding of the structure and mechanism of LGK, we have crystallized and determined several high-resolution X-ray structures of Lipomyces starkeyi LGK bound to reaction substrates and products. We have also recently collected low-resolution neutron diffraction for an LGK crystal, and further optimization of LGK crystals is currently underway to improve crystal size and diffraction. Neutron diffraction will reveal the protonation states of key residues in the active site of LGK and provide highly detailed information about hydrogen bonds, including water-bonding interactions. The rational design of new LGK constructs will be used to improve applications of this enzyme towards levoglucosan derived biofuel production.

[1] *D. Layton, A. Ajjarapu, D. Choi, et al., Bioresource Technology, 2011, 102, 8318-22*



Keywords: levoglucosan kinase, protein crystallography, structural enzymology