The Structure and Specificity Landscape of a Glycoside Hydrolase Family

Kirk A. Vander Meulen,[1,2] Evan M. Glasgow,[1,2] Craig A. Bingman,[1,2] Taichi E. Takasuka,[1,2,3] Christopher M. Bianchetti,[1,2,4] Lai F. Bergeman,[1,2] Samuel Deutsch,[5] and **Brian G. Fox**[1,2]

[1]Great Lakes Bioenergy Research Center, Madison, WI; [2]Department of Biochemistry, University of Wisconsin – Madison; [3]Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan; [4]Department of Chemistry, University of Wisconsin – Oshkosh, USA; [5]DOE Joint Genome Institute, Walnut Creek, CA, USA

https://www.glbrc.org

Project Goals: Great Lakes Bioenergy is a U.S. Department of Energy-funded research center dedicated to developing sustainable biofuels and bioproducts. Studying enzymes capable of hydrolyzing the numerous polysaccharide types contained in plant cell walls will facilitate development of biomass deconstruction strategies.

To deepen the understanding of the structural and evolutionary driving forces underlying specificity patterns in β-1,4 endo-acting GH family 5, we have characterized structure and function across subfamily 4 (GH5_4) and closely related enzymes. GH5_4 is an expansive subfamily consisting of three major clades, and here we mapped activity profiles and structures onto these phylogenetic groupings. In a quantitative enzymatic screen of the catalytic core domains of 243 enzymes on multiple polysaccharide substrates, members from one of these clades (Clade 3) possessed consistently and significantly elevated activities. A subfamily-wide correlation between lichenase and xylanase specific activity values was also observed, suggesting that the ancestral enzyme's structural framework may enforce a linkage between changes in either activity. Crystal structures were determined for 11 members of subfamily 4, and in concert with previous structures depict a diverging binding cleft morphology. Across this backdrop, two cleft residues correlate with the most active enzymes, a histidine residue that hydrogen bonds with the -1 glycosidic subunit and a +1-subsite stacking tryptophan.

References

Glasgow, EM, Vander Meulen, KA, Takasuka, TE, Bianchetti, CM, Bergeman, LF, Deutsch, S, and Fox, BG. Extent and Origins of Functional Diversity in a Subfamily of Glycoside Hydrolases. Journal of Molecular Biology. In Press.

This material is based upon work supported in part by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Numbers DE-SC0018409 and DE-FC02-07ER64494. EG is supported by the NIGMS Biotechnology Training Program (NIH 5 T32 GM008349) at the University of Wisconsin – Madison.