

Tracing the determinants of dual-substrate specificity in a diverse subfamily of family 5 glycoside hydrolases

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Project Goals:

To understand the determinants of specificity in a diverse family of glycoside hydrolases and engineer improved activity

Enzymes are traditionally viewed as having exquisite substrate specificity; however, recent evidence supports the notion that many enzymes have evolved activities against a range of substrates. The diversity of activities across glycoside hydrolase family 5 (GH5) suggests that this family of enzymes may contain numerous members with activities on multiple substrates. In this study, we combined structure- and sequence-based phylogenetic analysis with biochemical characterization to survey the prevalence of dual-specificity for glucan- and mannan-based substrates in the GH5 family. Examination of amino acid profile differences between the subfamilies led to the identification and subsequent experimental confirmation of an active site motif indicative of dual-specificity. The motif enabled us to successfully discover several new dually-specific members of GH5 and this pattern is present in over seventy other enzymes, strongly suggesting that dual endoglucanase-mannanase activity is widespread in this family. In addition, reinstatement of the conserved motif in a wild type member of GH5 enhanced its catalytic efficiency on glucan and mannan substrates by 175% and 1,600%, respectively. Phylogenetic examination of other GH families further indicates that the prevalence of enzyme multi-specificity in GHs may be greater than has been experimentally characterized.

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