484b Examining Beta-Glucosidase Reaction Kinetics by Isothermal Titration Microcalorimetry

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Beta-glucosidases, key components of a cellulase system, play a critical role during biomass degradation by simultaneously minimizing product inhibition of cellobiohydrolases and by providing a glucose stream for fermentative microorganisms. Increasing reaction temperatures and using enzymes with enhanced thermal tolerance is one strategy for improving overall biomass degradation efficiencies. Using directed evolution, we have recently identified nine mutants of *Thermobifida fusca* BglC with increased thermal tolerance. The effect of the mutations on the cellobiase activity of *T. fusca* BglC was examined by isothermal titration microcalorimetry (ITC). ITC is a highly sensitive and non-destructive assay for homogeneous enzyme-substrate reactions. Real-time data from the ITC kinetics assays corroborated the application of the pseudo-steady state assumption for obtaining Michaelis-Menten approximations to the data. The BglC enzymes were found to be susceptible to substrate inhibition but the 'inhibited enzymes' appeared to retain catalytic activity. Within the set of *T. fusca* BglC mutants, improved thermal tolerance did not correlate with cellobiase activity.