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Active site engineering of β-glucosidase

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Product inhibition of β-glucosidase is a rate limiting step in saccharification of cellulosic biomass. Till now, the exact mechanism of either inhibition or in some cases activation of β-glucosidase by glucose is very elusive. Herein we investigated the structural basis for glucose tolerance by comparing glucose tolerant β-glucosidases with a β-glucosidase from *Paenibacillus polymyxa*, Glu1C that has lower glucose tolerance. Comparative sequence alignment based on 3-D structure was carried out between glucose tolerant β-glucosidase (PDB: 3AHZ) from *Neotermes koshunensis* and β-glucosidase from *Paenibacillus polymyxa* (PDB: 2O9T). In silico studies suggested the importance of active site residues: L174, G373 and W412. Among these residues mutability for W412 was the highest as based on evolutionary relationships. In order to understand the role of W412, two charged mutations of opposite charges were created in previously cloned and characterized Glu1C gene of β-glucosidase and expressed in *E. coli* DH5α. Biochemical characterization of these two mutants revealed that the first mutant W412E was only 33% active in comparison of Glu1C and other mutant W412R was marginally active. A two fold increase in thermal stability was found in case of W412E ($t_{1/2}$=30 mins) at 50° C. However, the mutation resulted in marginal decrease in glucose tolerance as oppose to wild type. Hence, W412 residue, despite not being a part of catalytic residues, it was found that it was crucial to maintain optimal activity, thermal stability and glucose tolerance. Overall, presumbaly, β-glucosidase has a very delicate arrangements of structural elements that govern catalysis, stability and glucose tolerance.

Biography

Sneha Chandrakant Sawant is currently pursuing PhD Biotechnology from DBT-ICT Centre for Energy Biosciences; Institute of Chemical Technology, India. She has completed her Master of Science in Biotechnology from Mumbai University, India. Her area of research revolves around studying mechanistic behavior, structure- function relationships and protein engineering of enzymes involved in lignocellulose biomass depolymerization.

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