Chompoonuth Porncharoennop 2007: Production and Characterization of Mutant Forms of Thai Rosewood β-glucosidase. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Ms. Prachumporn Kongsaeree, Ph.D. 140 pages.

β-Glucosidases from different organisms exhibit diverse substrate specificities, especially for the aglycone moiety. Dalcochinase (a β-glucosidase from Thai rosewood) shows specificity for its natural substrate (dalcochinin glucoside), while linamarase (a β-glucosidase from cassava) has specificity for its natural substrate (linamarin). Apart from hydrolysis reaction, both enzymes can also catalyze reverse hydrolysis and transglucosylation reactions. Dalcochinase are capable of catalyzing reverse hydrolysis and transglucosylation by using primary and secondary alcohol as acceptor, but not tertiary alcohol. In contrast, linamarase is good at catalyzing transglucosylation by using primary, secondary and tertiary alcohol as acceptor, but poor in catalyzing reverse hydrolysis. In this project, we were interested in studying structure and function relationship of β-glucosidase, in order to identify the amino acid residues in the aglycone binding pocket that are important for substrate specificity and transglucosylation reaction. Four dalcochinase mutants, namely I185A, V255F, G367S and E455I, were generated by replacing amino acid residues that are likely located in the aglycone binding site of dalcochinase with the corresponding residues of linamarase. Mutant enzymes were cloned and expressed in yeast *Pichia pastoris*, and purified from culture media. Kinetic studies of all mutant enzymes showed that I185A, V255F, G367S and E455I did not improve the hydrolytic activity towards linamarin, but decreased K_m for hydrolysis of dalcochinin glucoside. I185A and E455I increased K_m for hydrolysis of *para*-nitrophenyl-β-D-glucopyranoside (*p*NP-Glc). In addition, all 4 mutations decreased the hydrolytic efficiency towards dalcochinin glucoside and *p*NP-Glc. In transglucosylation studies, I185A and V255F increase the enzymes' ability to transfer glucose to primary and secondary alcohol acceptors. However, all 4 dalcochinase mutants could not catalyze tranglucosylation by using tertiary alcohol as acceptor. Thus, it is expected that mutation at more than one position may generate new dalcochinase mutants which may function similarly to linamarase.

ชมพูนุท พรเจริญนพ — ประชุมพร คงแสงรี — 18 / 05 / 2007

Student's signature — Thesis Advisor's signature