Penporn Sujiwattanarat 2006: Production and Site-directed Mutagenesis of Recombinant β-Glucosidase from Thai Rosewood. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Miss Prachumporn Kongsaeree, Ph.D. 119 pages.
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Dalcochinase, a β-glucosidase from Thai rosewood, can hydrolyze dalcochinin β-glucoside that is its natural substrate, whereas linamarase, a β-glucosidase from cassava, hydrolyzes linamarin. Dalcochinase can catalyze reverse hydrolysis well, but shows low efficiency in transglucosylation. On the other hand, linamarase catalyses transglucosylation better than dalcochinase, but was not efficient in catalyzing reverse hydrolysis. Despite these differences, both enzymes have 60% amino acid sequence homology. Thus, this project is interested in studying the relationship between structure and function of β-glucosidase, particularly the identification of the amino acid residue that is important for hydrolysis and transglucosylation. The coding sequence of dalcochinase was cloned, expressed in yeast *Pichia pastoris*, and purified. The recombinant enzyme exhibits similar enzymatic properties to natural dalcochinase. Mutant forms of dalcochinase (namely N189F and A454N) were generated by replacing amino acid residues located in the aglycone binding pocket of dalcochinase with the corresponding residues of linamarase. Kinetic analysis of both enzymes showed that both N189 and A454 were not involved in hydrolysis of linamarin, but N189 could be important for hydrolysis of *p*NP-Glc and dalcochinin glucoside. In transglucosylation studies, N189F mutant could improve transglucosylation efficiency using primary alcohols as acceptors. However, neither N189 nor A454 was likely to be involved in transglucosylation using secondary and tertiary alcohols as acceptors.

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