Nudsara Thongthapthim 2009: Production and Characterization of Dalcochinase Mutants Containing Multiple Mutations in the Substrate Binding Site. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Prachumporn Kongsaeree, Ph.D.
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β-Glucosidase from Thai rosewood (dalcochinase) can synthesize alkyl glucoside using only primary and secondary alcohols, whereas *β*-glucosidase from cassava (linamarase) can use primary, secondary and tertiary alcohols as acceptors. Both enzymes show specificities for their natural substrates, which are dalcochinin glucoside and linamarin, respectively. Single mutations in the substrate binding pocket of dalcochinase to corresponding residues of linamrase decreased their specificities toward dalcochinin glucoside, but did not improve their activity toward linamarin. In addition, three dalcochinase mutants, I185A, N189F and V255F, showed improved transglucosylation activity. In this project, three double- and one triple mutations in the substrate binding pocket of dalcochinase to the corresponding residues of linamrase (I185A-N189F, I185A-V255F, N189F-V255F and I185A-N189F-V255F) were made to investigate the interactions of amino acid residues in substrate specificity. Residues I185, N189 and V255 in dalcochinase played important roles in the hydrolysis of dalcochinin glucoside, with I185A being more dominant than the other two residues. In hydrolysis of linamarin, residues A185-F189 and A185-F255 interacted to assist in hydrolysis of linamrin. In transglucosylation studies, mutations I185A-N189F, I185A-V255F and I185A-N189F-V2555F could improve transglucosylation efficiency using primary and secondary alcohols as acceptors. In particular, I185A-N189F-V2555F mutant gave significantly high yield of alkyl glucoside from *iso*-propanol. On the other hand, N189F-V255F mutant showed reduction in transglucosylation efficiency compared with single mutants. However, none of our four dalcochinase mutants could catalyze transglucosylation using tertiary alcohols as acceptors.

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