Amornrat Onpium 2007: Site-directed Mutagenesis in the Aglycone Binding Pocket of Thai Roeswood Beta-Glucosidase. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Mrs. Prachumporn Kongsaeree, Ph.D. 124 pages.

β-Glucosidases not only hydrolyze of β-glucosidic linkage, but some also catalyze reverse hydrolysis and transglucosylation. Dalcochinase (Thai rosewood β-glucosidase) was better for reverse hydrolysis, but poorer for transglucosylation, when compared with linamarase (cassava β-glucosidase). Both enzymes also exhibit specificities for their natural substrates (dalcochinin glucoside and linamarin, respectively). So they show differences in catalytic properties and substrate preferences, despite sharing 70% amino acid sequence identity. This research aims to identify key amino acid residues for function of β-glucosidases, using dalcochinase and linamarase as models. Amino acid residues in the aglycone binding pocket of dalcochinase were replaced with the corresponding residues of linamarase (M195V, H253F, N323Q and K402Y). Mutant enzymes were expressed in *Pichia pastoris* and purified. All mutants appeared similar to wild-type recombinant dalcochinase as judged by SDS-PAGE, western blot and activity staining on non-denaturing PAGE. Kinetic studies of all mutants, compared with recombinant wild-type dalcochinase, showed a decrease in K_m of M195V, N323Q and K402Y for hydrolysis of dalcochinin glucoside but the same K_m of H253F. K_m of all mutants decreased for hydroysis of *para*-nitrophenyl-β-D-glucopyranoside. No mutant could hydrolyze linamarin. From transglucosylation reaction studies, all mutants could use some primary and secondary alcohols as glucosyl acceptors better than wild-type natural and recombinant dalcochinase. M195V could transfer glucose to *iso*-propanol. H253F and N323Q could transfer glucose to methanol, *n*-butanol, *iso*-butanol and *iso*-propanol. K402Y could transfer glucose to *n*-butanol, *iso*-butanol and *iso*-propanol. Still, none of them could use tertiary alcohol as glucosyl acceptor. So, all mutations selected in this project are important for catalyzing substrate hydrolysis and transglucosylation since they could alter enzyme properties. It is possible that mutation at only 1 position is not enough to generate dalcochinase mutant that can hydrolyze linamarin and use tertiary alcochols as glucosyl acceptor. So, if mutations are made at more than 1 position, the resulting dalcochinase mutant may be able to hydrolyze linamarin and transfer glucose to primary, secondary and tertiary alcohols.

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