Satrawut Charoenla 2014: Protein Engineering of a β-Glucosidase for Hydrolysis of Soybean Isoflavonoids. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Assistant Professor Prachumporn T. Kongsaeree, Ph.D. 134 pages.

Three leguminous isoflavone β-glucosidases, namely dalcochinase (from *Dalbergia cochinchinensis* Pierre), Dnbglu2 (from *Dalbergia nigrescen* Kruz) and GmICHG (from *Glycine max*), share about 60-80% sequence identity, but their catalytic efficiencies toward soybean isoflavone glucosides are different. Previous study revealed that changing single amino acid residues in the binding pocket of dalcochinase to the corresponding residues of GmICHG, namely A454F, E455A and S459V, could increase the catalytic efficiency for hydrolysis of soybean isoflavone glucosides, when compared with the wild-type dalcochinase. So, this study aims to construct the corresponding double and triple mutations in order to determine the interaction of amino acid residues, which may influence the hydrolytic efficiency toward soybean isoflavone glucosides. All dalcochinase mutants were approximately 66 kDa, which were similar to the wild-type enzyme. All double mutants showed improved hydrolytic efficiencies toward *p*-nitrophenyl-β-D-glucoside and dalcochinin-8′-*O*-β-D-glucoside when compared with the wild-type enzyme. For the hydrolysis of soybean isoflavone glucosides, the A454F/E455A mutant showed high hydrolytic efficiencies toward daidzin, genistin, malonyldaidzin and malonylgenistin. The A454F/S459V and E455A/S459V mutants showed increases in hydrolytic efficiencies toward genistin, malonyldaidzin and malonylgenistin. However, none of the triple mutants showed improved hydrolytic efficiencies toward those substrates. Moreover, the E455A/S459V mutant showed high ability for hydrolysis of soybean flour suspension. Therefore, the E455A/S459V mutant may be applied in food industry for releasing free isoflavone aglycones to improve the nutritional values of soybean food products.

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