Khuanjarat Choengpanya 2014: Investigation of Substrate Specificity in Glycoside Hydrolases using *In Silico* and Experimental Approaches. Doctor of Philosophy (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Prachumpron T. Kongsaeree, Ph.D. 201 pages.

Glycoside hydrolases catalyze the breakdown of glycosidic bond between a carbohydrate (glycone) and a non-carbohydrate (aglycone) moieties. These are classified into family on the basic of sequence similarity. Members in each family show various specificities for both glycone and aglycone parts of their substrates.

These specificities of GH1 *β*-glucosidases were predicted and explained by molecular modeling and docking approaches. Residue F196 of dalcochinase might play an important role in glycone specificity by directing the mannoside substrate in a suitable position for catalysis. Residues A201, F205, F269-F271 of linamarase might provide hydrophobic pockets, which was important for aglycone specificity. Mutations of the dalcochinase to these corresponding residues of linamarase (I185A/N189F/V255F mutant) might create the A185, F189, H253-F255 hydrophobic pockets that could bind to linamarin and tert-butyl alcohol acceptors.

The substrate specificity of GH3 enzyme was conducted with AnBX. AnBX was successfully cloned and expressed in *Pichia pastoris*. The enzyme properties of the recombinant AnBX were similar to the natural enzyme. Residue D288 of AnBX was confirmed as catalytic nucleophile, and residue E88 of AnBX was likely to be the acid/base catalyst.

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