VU THI NHUAN. 2008. **Biochemical and crystallographic characterization of starch branching enzymes from the *Oryza sativa* L. seeds.** PhD Thesis. Laboratory of Biochemistry, Dept. of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Japan

ABSTRACT

I. With a view to understand the enzymatic properties of branching enzyme (BE), the recombinant of BEs in *E.coli* was characterized. In course of starch iodine assay, the greater effects were observed on amylose substrate than amylopectin in BE I and BE Ia, however, the higher effect examined on amylopectin than amylase in BE IIb. In amylopectin substrate, BE I could decreased up to 80% of that of the control, 22% observed in BE IIa in contrary, and 25% in BE IIb. In amylopectin substrate, BE I decreased to 70% of the control but little change in BE Ia and 35% of the control in BE IIb, respectively. Therefore, the rice BE isozymes differed in the BE I was more active than BE IIa and BE IIb on amylose as well as amylopectin. This results have suggested that, BE I was more active on amylose than amylopectin, whereas BE IIb was more active on amylopectin than amylase.

Phosphate has been reported to increase the branching activity of BEs close to maximal activation of BE I, was obtained at 60 mM phosphate with amylase as well as amylopectin; whereas BE IIa and BE IIb were 120mM phosphate in the same manner. On an amylase substrate, the activity of BE I performed in 60 mM phosphate was increased about 56% while in BE Ia was twofold and BE Ib was 86% of the control. On an amylopectin substrate, the activity of BE I performed was increased about 84% while in BE Ia was trace and BE II was 51% of the control.

Chain length profile of alpha polysaccharide for BEs also examined using the P/ACE MDQ Carbohydrate System. The study has shown BE I could produce the proportions of chains with $8 \le DP \le 13$ and $28 \le DP \le 38$ of the products while BE IIb could elevate the shorter chains than BE I with chains of $DP \le 11$. However, BE IIa has demonstrated no significant change on amylopectin chain profile.

II. To demonstrate the involvement of these amino acids in the catalytic activity of BE I, they were individually replaced by alanine by site-directed mutagenesis and amino acid at position 468 was deduced to be Asp in the previous study whereas the corresponding residue at position 468 in the cDNA clone is Gly. The CD spectra of the mutants were approximately the same as that of the wild type: the spectra of mutants are shown the backbone conformation of these mutants is practically tha same as that of the wild type BE, suggesting that replacements of amino acids with Ala do not appear to affect the integrity of the protein structure. The catalytic activity of the mutants were evaluated with amylose and amylopectine substrates. The study has shown, the mutants H275A, D344A, E399A, and H467A in BE I were changed to Ala, respectively, were virtually inactive against amylose and amylopectin. The site-directed mutagenesis of Y235A, D270A, and R342A drastically reduced the catalytic activity (10% - 15%), as compared to that of BE I. The present results indicated that seven amino acids: Tyr235, Asp270, His275, Arg342, Glu399 and His467, conserved in the alpha amylase family proteins play an important role in a catalytic activity in BE I from rice. In contrast, mutation of Gly458 with Asp had little influence on the enzymatic activity (94%), suggesting that Asp at position 458 in the alpha amylase family enzymes is not necessarily involved in catalytic activity of BE I.