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学位論文内容の要旨

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学位論文題名

Structure-function relationship of fungal α-glucosidases belonging to glycoside hydrolase family 31

（Glycoside hydrolase family 31に属する真菌類由来α-glucosidaseの構造と機能に関する研究）

α-Glucosidase from *Podospora anserina* (PAG) and α-glucosidase from *Schwanniomyces occidentalis* (SOG) belong to Glycoside Hydrolase family 31 (GH31). PAG revealed high regioselectivity for α-1,3- and α-1,4-glucosidic linkages in hydrolysis as well as transglycosylation, while SOG preferred α-1,4-glucosidic linkage in hydrolysis and generated α-1,4- and α-1,6-glucosidic linkages in transglycosylation. The mutational study on both enzymes elucidates the structural elements important for the recognition of α-1,6-glucosidic linkage and α-1,3-glucosidic linkage.

1. Purification and characterization of PAG

A filamentous fungus *P. anserina* possesses an α-glucosidase (PAG) which belongs to GH31. Recombinant PAG harbouring six histidine residues (His-tag) at C-terminal was produced in *Pichia pastoris*. The recombinant PAG showed high regioselectivity for α-1,3- and α-1,4-glucosidic linkages in hydrolysis as well as transglycosylation. In addition, it has strong transglycosylation ability. However, limited proteolysis has been occurred during cultivation and recombinant PAG was composed of two subunits. Therefore, original PAG was purified and its enzymatic properties were investigated.

P. anserina was cultivated in liquid medium containing 10% soluble starch as a carbon source and PAG was purified from culture supernatant. Original PAG also revealed high regioselectivity for α-1,3- and α-1,4-glucosidic linkages in hydrolysis as well as transglycosylation. Original PAG was also composed of two subunits and the N-terminal amino acid sequences are identical to those of recombinant PAG. A full length PAG was produced by site-directed mutagenesis and the properties were investigated. The results indicate that the proteolysis has no effect on the enzyme characteristics.

2. Investigation of transglycosylation properties of SOG W324Y

α-Glucosidase from *Schwanniomyces occidentalis* (SOG) belongs to GH31. SOG displays broad substrate specificity in hydrolysis and high regioselectivity for α-1,4- and α-1,6-glucosidic linkages in transglycosylation. In previous study, it has been elucidate that Trp324 on β→α loop 1 of SOG is important for hydrolysis of α-1,6-glucosidic linkage.

In transglycosylation reaction with 100 mM maltose, W324Y generated α-1,4- and α-1,2-glucosidic linkages and lost the ability of the formation of α-1,6-glucosidic linkage. That is, Trp324 is also critical for the recognition of α-1,6-glucosidic linkage in transglycosylation as well as hydrolysis. In addition, a novel product was detected with considerable amount. Mass spectrometry and NMR determined its structure as 2,4-di-*O*-(α-D-glucopyranosyl)-D-glucopyranose. The maximum production yield of 2,4-di-*O*-(α-D-glucopyranosyl)-D-glucopyranose was calculated as 16, 19, and 16% under 50, 100, and 200 mM of substrate concentration, respectively.

3. Elucidation of structure-function relationship between SOG and PAG by mutational study

SOG and PAG have different regioselectivity though their high similarity in amino acid sequence (35%). SOG variants were produced by site-directed mutagenesis focused on active pocket based on the primary structures of SOG and PAG and three dimensional structure of human maltase which belongs to GH31. The active pocket of human maltase is composed of $(\beta/\alpha)_8$ catalytic domain, insert 1 and insert 2 (long protruding region between β_3 and α_3 and between β_4 and α_4, respectively), and N-loop protruding from N-terminal domain. The mutations were introduced into β_3, β→α loop 1, β→α loop 2, β→α loop 8, insert2, and N-loop.

The kinetic parameters and transglycosylation reactions of single or multiple mutants were investigated. The relative k_{cat}/K_m values of a triple mutant and a quadruple mutant for maltose, nigerose, kojibiose, and isomaltose were 100, 1.7, 4.6, and 0.1%, and 100, 31, 17, and 0.1%, respectively (wild type: 100, 3.4, 7.9, and 7.3%). From these results, important structural elements for the recognition of α-1,6-glucosidic linkage and α-1,3-glucosidic linkage are elucidated.