学位論文内容の要旨

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学位論文題名

Structural and functional studies of novel α-L-glucosidase from *Cecembia lonarensis*

（*Cecembia lonarensis*由来新規α-L-グルコシダーゼの構造と機能に関する研究）

Natural L-glucose has not been found, and nature is believed to have a homochirality of D-glucose. However, an L-glucose-utilizing bacterium in soil has been reported, and its unique catabolic pathway of L-glucose has been elucidated. Moreover, our group's discovery of two α-L-glucosidases, ClAgl29A and ClAgl29B, in the *Cecembia lonarensis* genome has opened the possibility of α-L-glucoside in nature. This dissertation presents an X-ray crystallographic analysis of ClAgl29A and ClAgl29B, shedding light on their unique substrate specificity and the structural differences from α-L-fucosidases, which are their homologs and abundant in nature. It also explores the specificities of ClAgl29A and ClAgl29B for substrates other than α-L-glucoside. The glycosidases catalyze the formation of glycosidic linkages, often referred to as transglycosylation, which is used for the industrial production of oligosaccharides. For efficient α-L-glucoside synthesis using α-L-glucosidases, several mutant enzymes are prepared and evaluated.

1. Structural Characterizations of α-L-Glucosidases

The crystal structures of ClAgl29A and ClAgl29B were determined. Their structure comprised catalytic TIM $(\beta/\alpha)_8$ barrel and β-sandwich domains. The ClAgl29B complex obtained with β-L-glucose suggested that α-L-glucosidase had gained the α-L-glucoside recognition mechanism during molecular evolution. A significant finding was a substantial difference in the recognition sites of the equatorial hydroxyl group at carbon 4 of L-glucose (O4) compared to the evolutionarily related α-L-fucosidases. While α-L-fucosidases recognized axial O4 of L-fucose with two His residues located at the C-terminus of β-strand 1 and β-strand 2 of the TIM barrel, respectively, α-L-glucosidase recognized O4 of L-glucose with different orientation from L-fucose with an Asp located on the long loop connecting β-strand 1 and α-helix 1 of the TIM barrel. Recombinant proteins of five amino acid sequences with α-L-glucosidase-like sequences, identified by the structural analysis, were prepared. However, several of these showed

only weak α-L-glucosidase activity.

2. Substrate Specificity of α-L-Glucosidases

This section evaluated the substrate specificity of the two α-L-glucosidases for *p*-nitrophenyl α-L-rhamnoside, α-L-6-deoxyglucoside, and α-L-xyloside. Both enzymes hydrolyzed α-L-6-deoxyglucoside a little more efficiently than α-L-glucoside. They had little activity toward α-L-rhamnoside, widely found in nature. Together with the previous results of weak substrate specificity of ClAgl29A and ClAgl29B for α-L-fucoside, these enzymes were found not to act on glycosides that are abundant in nature but on glycosides whose existence is undetermined.

3. α-L-Glucoside Synthesis Using α-L-Glucosidases

To investigate the cause of the low transglucosylation activity of ClAgl29B, mutant ClAgl29B enzymes mimicking ClAgl29A were produced. The mutant enzymes exhibited greater transglucosylation activity than the wild type, indicating that the mutated residues are involved in directing the reaction toward transglucosylation or hydrolysis. The mutant ClAgl29A and ClAgl29B having a mutation at the catalytic amino acid residue catalyzed α-L-glucosylation of D-xylosides and D-glucosides, using β-L-glucosyl fluoride as a substrate. The reactions formed α-L-Glc-(1→4)-D-Glc and α-L-Glc-(1→4)-D-Xyl.