学位論文内容の要旨

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学位論文題名

Function and structure of β1-3-glucan-degrading enzymes from the pink snow mold fungus, *Microdochium nivale*
（紅色雪腐病菌 *Microdochium nivale* 由来 β1-3-グルカン分解酵素の機能と構造に関する研究）

β1-3-Glucan is a polysaccharide with a β1-3-D-glucosidic main chain, distributed in various organisms with various structures. Fungi use β1-3-glucan-degrading enzymes to degrade their own and external β1-3-glucans for growth and to obtain carbon sources. The pink snow mold fungus, *Microdochium nivale*, is a phytopathogenic filamentous fungus that infects grasses and cereals. The β1-3-glucan-degrading enzymes of this fungus have not been characterized yet. In this study, the extracellular β1-3-glucan-degrading enzymes (endo-β-1,3-glucanase and β-glucosidase) were identified, and functionally and structurally characterized.

1. Function and structure of an extracellular endo-β-1,3-glucanase from *M. nivale*

The endo-β-1,3-glucanase, MnLam55A, was purified from the culture supernatant of *M. nivale* and identified as a glycoside hydrolase family 55 (GH55) protein. GH55 includes exo-β-1,3-glucosidases and endo-β-1,3-glucanases, acting on laminarin, which is a β1-3/1-6-glucan consisting of a β1-3/1-6-linked main chain and β1-6-linked branches.

MnLam55A hydrolyzed internal β-D-glucosidic linkages of laminarin but no other β1-3-glucans lacking β1-6-linkages in the main chain. NMR analysis of the reaction products from laminarin showed that MnLam55A specifically hydrolyzes the non-reducing terminal β1-3-linkage of the β1-3-glucan moiety on the reducing end side of the internal β1-6-linkage in the main chain. MnLam55A liberates D-glucose from laminaritriose and longer laminarioligosaccharides, but the k_{cat}/K_m value to them were much lower than to laminarin, indicating that β-glucan binding to the minus subsites is required for high hydrolytic activity.

A crystal structure of MnLam55A was determined. The overall structure and the catalytic site are similar to those of exo-β-1,3-glucosidases. The docking simulation of MnLam55A with 6^{III}-*O*-D-glucosyl laminaritriose supported the endo-type hydrolysis of MnLam55A based on the protein structure involving the gentiobiosyl moiety-binding subsites −1 and −2 and an extended cleft for the non-reducing end side binding. Sequence comparison suggested that this cleft is shared in GH55 endo-type enzymes. The laminarin degradation mechanism is presumably common to GH55 endo-β-1,3-glucanases.

2. Chemical synthesis of three monomeric units for the synthesis of β1-3/1-6-glucooligosaccharides

For the detailed analyses of β1-3-glucan-degrading enzymes including GH55 enzymes and the structure-function analysis of β1-3/1-6-glucans, β1-3/1-6-glucooligosaccharides with fine structure are desired. In this study, the method for the chemical synthesis of β1-3/1-6-glucooligosaccharides was developed.

2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside served as an acceptor in the formation of β1-3-linkages. Phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside and phenyl 2,3-di-*O*-benzoyl-4,6-di-*O*-levulinyl-1-thio-β-D-glucopyranoside acted as donors, synthesizing acceptors suitable for the formation of β1-3- and β1-6-linkages, respectively, in the next condensation. By sequential connections of these units from the reducing end side, the tetrasaccharide derivative (Glcβ1-6Glcβ1-3Glcβ1-3Glc) was synthesized. It was suggested that the linear β1-3/1-6-glucooligosaccharides with favorable sequence can be synthesized using this method.

3. Identification and characterization of an extracellular β-glucosidase from *M. nivale*

The β-glucosidase, MnBG3A, was purified from the culture supernatant. Sequence analysis identified MnBG3A as a GH3 protein and shows the high sequence identity to the *Aspergillus aculeatus* β-glucosidase, AaBGL1. Among *p*-nitrophenyl β-D-glycosides, MnBG3A showed activity to β-D-glucoside (pNP-Glc) and slight activity to β-D-xyloside. In the pNP-Glc hydrolysis, MnBG3A showed the more severe inhibitions by substrate and D-glucose than AaBGL1. D-Glucose competitively inhibited the reaction. MnBG3A acted on β-glucobioses with β1-3, -6, -4, and -2 linkages, in descending order of k_{cat}/K_m. In contrast, the regioselectivity for newly formed products was limited to β1-6 linkage.

The pNP-Glc hydrolysis was analyzed in the presence of monosaccharides. The reaction rates were increased with D-galactose and D-xylose, and decreased with D-mannose. Kinetic analyses suggested that the water molecule, the second pNP-Glc, and monosaccharides competitively bind to the glucosyl enzyme intermediate. D-Glucose is released from the monosaccharide complex, and each monosaccharide showed different competitiveness with the water molecule, leading to the different reaction rates.

In summary, two β1-3-glucan-degrading enzymes from *M. nivale* were characterized. MnLam55A exhibited specific endo-type hydrolysis of laminarin, the mechanism of which was demonstrated by its function and structure. MnBG3A showed the high specificity for the hydrolysis of β1-3- and β1-6-linkages and for the formation of β1-6-linkage, and the reaction rates were affected by monosaccharides. These enzymes were suggested to act in cooperation for the degradation of the fungal cell wall containing laminarin-like β1-3/1-6-glucan.