Studies on Structure and Function of Bacterial α-1,3-Glucanases

Abstract

α-1,3-Glucanase from Bacillus circulans KA-304 (Agl-KA) is a multiple domain enzyme composted of N-terminal DS1, CBM6, DS2, UCD and catalytic domain. DS1, CBM6, and DS2 were determined as α-1,3-glucan-binding domain. Individual DS1, CBM6, and DS2 has an ability to bind to α-1,3-glucan- and fungal cell wall. However, the combined actions of DS1, CBM6, and DS2 contributed to increase cell-wall binding of Agl-KA and are indispensable for efficient cell-wall degradation by Agl-KA. Moreover, to understand the binding mechanism of these domains, CBM6 which showed the highest α-1,3-glucan- and fungal cell-wall binding ability among N-terminal domains of Agl-KA was further investigated. The critical residues were found to be three amino acids including aromatic amino acids that are conserved among CBM6s. However, two aromatic amino acid residues found only in CBM6 of α-1,3-glucanases were identified as critical residues. Catalytic mechanism of Agl-KA also was investigated. Agl-KA was determined as the endo-type enzyme that hydrolyzed α-1,3-glucan in retaining manner. Chemical modification and site-directed mutagenesis suggested that three acidic amino acid residues were essential amino acid residues in catalysis, and one of them might be the assist residue involved in substrate binding in active site of Agl-KA. In order to provide the much more information for further understanding the function of family 87 α-1,3-glucanase, the new type α-1,3-glucanases which were superior in properties such as thermal stability, pH dependence, and so on were screened. It has been found that the new type α-1,3-glucanase (Agl-FH1) produced by Paenibacillus glycanslyticus FH11 showed some biochemical properties different from those of Agl-KA. In addition, it had a unique catalytic domain which showed low similarity to that of known α-1,3-glucanases. Agl-FH1 was the first member of a new subgroup of family 87.

Objective

α-1,3-Glucanase from Bacillus circulans KA-304 (Agl-KA) is one of potent enzymes for preparing fungal protoplast and biological control of pathogenic fungi (1-2). Agl-KA conserved several non-catalytic domains (DS1, CBM6, DS2 and UCD) at N-terminal and catalytic domain at C-terminal. In the present study, the author aimed at analyzing the function of each modular structure of Agl-KA to provide much more beneficial information of cell-wall lytic type 87 α-1,3-glucanases. At first, the involvement of N-terminal domains of Agl-KA in α-1,3-glucan-binding and cell wall degradation was elucidated. Then, to gain the insight in to binding mechanism, α-1,3-glucan-binding site of N-terminal domains was identified with focusing on CBM6. Secondly, critical catalytic residues of Agl-KA were identified to elucidate the catalytic
mechanism of the enzyme.

Moreover, the present study also tried to find new α-1,3-glucanase producing sources, and to investigate characterization and structural analysis of a new type enzyme. The author expects that structural comparison of a new type enzyme with the known type enzyme (Agl-KA) might help to greatly understand the function of α-1,3-glucanase.

Summary of each Chapter

Chapter 1 deals with N-terminal region domains.

Section 1 evaluates the effect of deletion of each domain in N-terminal region of Agl-KA on α-1,3-glucan-binding ability and their contribution to enhancing hydrolyzing activity toward α-1,3-glucan and fungal cell wall.

Deleting DS1 and CBM6 from Agl-KA resulted in reduced hydrolyzing and binding activities, as was the case for deleting N-terminal domain of mutanase RM1 (3). In addition, deleting DS1 alone caused reduced hydrolyzing and binding activities, although the reduced activity by deletion of DS1 was lower than that by deleting both DS1 and CBM6. Moreover, deleting DS1, CBM6, and DS2 potentiated the reduced hydrolyzing activity. These results suggested that the DS1, CBM6, and DS2 independently formed domain structure and each of them had a particular role in substrate binding. For cell wall lysis, only a DS1 deletion also has dramatic effect on protoplast formation, indicating that the combination of these domains was indispensable for efficient substrate-binding and cell-wall degradation.

Section 2 confirms the binding ability of individual DS1, CBM6, and DS2 to α-1,3-glucan- and fungal cell wall.

An α-1,3-glucan binding assay with fluorescent fusion proteins revealed that DS1, CBM6, and DS2 could bind to α-1,3-glucan and fungal cell walls as binding efficiency was increased by their combined actions. This section confirmed the role of these N-terminal domain in α-1,3-glucan binding and their relevance to cell-wall lysis of the enzyme as described in Section 1.

On the other hand, UCD hardly influenced the catalytic reaction of Agl-KA, and UCD did not bind to insoluble substrates or S. commune mycelia. UCD might be a linker separating the binding domains from the catalytic domain in Agl-KA that provides flexibility to both.

Chapter 2 determines the critical amino acid residues that are involved in substrate binding of CBM6 from Agl-KA (AglCBM6) by mutagenesis analysis. In addition, homology structure model for AglCBM6 was constructed to clarify the role of amino acids might be involved in α-1,3-glucan binding.

The amino acid sequences of AglCBM6 and a number of the structural known CBM6s were compared to determine the functional amino acids involved in ligand binding. The amino acid residues, two tyrosine, two phenylalanine, three tryptophan residues and one asparagine residue selected for substitution were located at the region where could be predicted to be ligand binding site based on the report by Abbott et al
The results of mutagenic analysis showed that substitution of one phenylalanine, three tryptophan residues and one asparagine residue to alanine decreased the affinity to \( \alpha-1,3 \)-glucan, suggesting that these amino acid residues might be involved in \( \alpha-1,3 \)-glucan binding of Agl-KA. Moreover, homology structure model of AglCBM6 also shows that these were located in cleft where is considered to be ligand binding site of the known CBM6s. The model supported the involvement of these amino acids in \( \alpha-1,3 \)-glucan binding.

**Chapter 3** identifies essential amino acid residues in \( \alpha-1,3 \)-glucanase activity of Agl-KA. Essential amino acid residues for catalytic function of Agl-KA were identified by chemical modification and site direct mutagenesis. The inactivation of \( \alpha-1,3 \)-glucanase caused by ECD modification indicated the importance of carboxylic amino acids in catalytic function of Agl-KA. The amino acid sequences of \( \alpha-1,3 \)-glucanases were aligned. Seven aspartic acid and three glutamic acid residues of Agl-KA that are in position equivalent to the completely conserved carboxylic amino acids among bacterial \( \alpha-1,3 \)-glucanases were subjected to substituted to alanine. The substitution of three aspartic acid residues to alanine dramatically decreased \( \alpha-1,3 \)-glucanase activity, suggesting that these aspartic acid residues might be involved in catalytic function of the enzyme. The results of kinetic analysis of the mutants proposed that these aspartic acid residues might be involved in substrate-binding and direct catalytic event. However, the role of conserved glutamic acid residues as nucleophile catalysts and acid/base catalysts, respectively, could not be clearly determined in the present study.

**Chapter 4** deals with \( \alpha-1,3 \)-glucananase isozymes from *Paenibacillus glycanilyticus* FH11. To obtain the new type \( \alpha-1,3 \)-glucanase which has superior properties such as thermalstability, pH dependence and so on, new source organisms of \( \alpha-1,3 \)-glucanase were screened and \( \alpha-1,3 \)-glucan-assimilating bacterium *P. glycanilyticus* strain FH11 was selected.

**Section 1** describes the production and purification of \( \alpha-1,3 \)-glucananases from *P. glycanilyticus* FH11. The enzymes were purified from supernatants of *P. glycanilyticus* FH11 using several column chromatography. The results indicated that *P. glycanilyticus* FH11 produced two \( \alpha-1,3 \)-glucananases (Agl-FH1 and Agl-FH2). Two enzymes exhibited similar characteristics such as optimal pH, pH stability, optimal temperature, thermostability, and molecular masses on SDS-PAGE. However, a great difference between them was found on their hydrolysis product. These amino acid sequence analysis revealed differences between the amino acid sequences of Agl-FH1 and Agl-FH2, confirming that these enzymes are the isozyme.

**Section 2**, Genes of Agl-FH1 and Agl-FH2 were cloned and expressed in *E. coli*. The recombinant Agl-FH1 and Agl-FH2 exhibited the same enzymatic properties as those of each wild-type enzyme, and both of the recombinants showed the activity on
the protoplast formation of *S. commune* mycelia. Comparison of amino acid sequences among α-1,3-glucanases showed that the C-terminal region of Agl-FH1 has only slight similarity to them (approximately 20% identity). Phylogenetic analysis revealed that type 87 α-1,3-glucanase was divided into three subgroups and Agl-FH1 was grouped into a new subgroup.

**Section 3** deals with cloning and expression of the catalytic domain of Agl-FH1.

The gene of catalytic domain of Agl-FH1 was failed to expression in *E. coli*. To enhance the expression of catalytic domain of Agl-FH1, N-terminal fusion tags such as T7-tag, thioredoxin-tag, GST-tag were used. GST tagging protein was successfully expressed in *E. coli*, but its activity was very low than that of Agl-FH1. The predicted secondary structure shows that overall structure of catalytic domain of Agl-FH1 might be significant different from the know α-1,3-glucanases. Thus the catalytic domain of might require N-terminal binding domains not only to enhance its catalytic efficiency, but also to stabilize the structure of this enzyme by interacting between domains.

**Conclusion**

The present thesis revealed the involvement of individual N-terminal domain of bacterial α-1,3-glucanases in both substrate- hydrolyzing and binding activities. The results also suggested that N-terminal domain might be necessary for stabilization of folding structure for some α-1,3-glucanases. Further investigation such as X-ray crystallographic analysis remains for elucidation of catalytic mechanism of α-1,3-glucanase. However, the findings in present study provided the biochemical and structural information of type 87 cell-wall lytic α-1,3-glucanases that may be beneficial to clarify the function of the enzyme in the future.

**References**


