（学位第３号様式）

# 学 位 論 文 要 旨

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| 題　目 | The roles of domains of plant multi-domain chitinases in chitin recognition and antifungal activity<br>（植物由来マルチドメインキチナーゼのキチン認識および抗真菌活性における各ドメインの役割） |


Plant chitinases are defense-related enzymes that hydrolyze β-1,4-glycosidic linkages of chitinous components localized to the cell wall of fungal pathogens. Multi-domain chitinases consist of chitin-binding domain and catalytic domain. The catalytic domains are classified into either glycoside hydrolase family 18 (GH18) or 19 (GH19) based on their amino acid sequences. In plant multi-domain chitinases, there are two types of chitin-binding domains consisting of carbohydrate-binding module family 18 (CBM18) and 50 (CBM50). It has been reported that multi-domain chitinases have stronger hydrolytic activity against insoluble chitin and antifungal activity than the catalytic domains. However, the mechanism of substrate recognition and antifungal action by multi-domain chitinase has not been elucidated. Here, the roles of domains of plant multi-domain chitinases in chitin recognition and antifungal activity were studied.

CJP-4 is a two-domain chitinase from Japanese cedar (*Cryptomeria japonica*) pollen, consisting of an N-terminal CBM18 domain and a GH19 catalytic domain (Cat). CJP-4 exhibited higher activity toward chitin nanofiber, an insoluble substrate, than did CJP-4-Cat. Fungal growth was strongly inhibited by CJP-4 but not by CJP-4-Cat. These results indicate that the CBM18 domain assists the hydrolysis of insoluble substrate and the antifungal action of CJP-4-Cat by binding to chitin. The substrate binding to an inactive mutant protein of full-length CJP-4, in which the catalytic acid Glu108 was mutated to glutamine, CJP-4(E108Q), was analyzed by NMR spectroscopy. Based on the chemical shift perturbations of $^{1}H$-$^{15}N$ HSQC signals of Gly26 (CBM18 domain) and Trp185 (GH19 domain), the association constants for individual domains of CJP-4(E108Q) toward soluble chitin hexamer were determined to be 2300 and 3500 $M^{-1}$, respectively. When chitin nanofibers were added to the CJP-4(E108Q) solution, strong line-broadening was observed for the majority of the backbone resonances in CBM18 domain but not in GH19 domain, indicating a binding preference of CBM18 domain to the insoluble chitin. We here demonstrated importance of CBM18 domain in insoluble chitin recognition based on the NMR binding data obtained for full-length CJP-4.

PrChiA is an antifungal chitinase obtained from *Pteris ryukyuensis*, a fern plant. It consists of two N-terminal lysin motif (LysM = CBM50) domains and a C-terminal catalytic domain of glycoside hydrolase family 18. Previous studies have shown that the deletion of LysM domains or loss of hydrolytic activity causes the loss of the antifungal activity of chitinases. In this study, we produced LysM-domain multimers (LysMn, n=2−5) and the respective multimer fusion chitinases (LysMn-Cat, n=1−4), and characterized their enzymatic and antifungal properties. LysMn and LysMn-Cat showed a higher affinity to insoluble chitin than single LysM domain and single catalytic domain alone, respectively. LysMn-Cat hydrolyzed insoluble chitin more efficiently than the catalytic domain alone. Surprisingly, LysMn showed antifungal activity without chitinolytic activity. Further, LysMn-Cat exhibited a stronger antifungal activity than LysMn. Microscopic observation revealed that LysMn attacked only the tips of the fungal hyphae; LysMn-Cat attacked not only the tips, but also the lateral walls around the septa of the fungal hyphae. It is suggested that the LysMn act on the growing point of the hyphal tip through their chitin-binding ability and that the LysMn-Cat act on not only the hyphal tips, but also on the lateral walls through their chitin-hydrolyzing and -binding activities.