PE18 - Structure and Antifungal Activity of a Novel Chitinase from the Leaves of a Fern (*Pteris ryukyuensis*): The Role of LysM Domains in Antifungal Activity of the Chitinase

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There are several types of chitinase in flowering plants. On the basis of their amino acid sequences, plant chitinases have been classified into five classes: class I chitinases consisting of an N-terminal chitin-binding domain and a catalytic domain; class II chitinases with only a catalytic domain homologous to that of class I chitinases; class IV chitinases sharing homology with class I chitinases but smaller due to four deletions; and class III and V chitinases sharing no homology with class I, II, or IV chitinases but have distant sequence similarity to bacterial and fungal chitinases.
Researchers have primarily focused on chitinases from flowering plant. To study a basic physiological role of plant chitinases, we have examined structure and function of chitinases derived from evolutionarily older plants, such as cycas and fern. In this study, we have obtained a novel type of chitinase, consisting of two LysM domains (peptidoglycan binding motif) and a class III chitinase-like catalytic domain, from leaves of a fern. The novel chitinase exhibited antifungal activity. We examined the relationship between the structure and antifungal activity of the chitinase.
Two chitinases, designated *Pteris ryukyuensis* chitinase-A and -B (Pr Chi-A and -B) were purified from the leaves of a fern (*Pteris ryukyuensis*) using several column chromatographies. The molecular masses of Pr Chi-A and -B were 28 kDa and 42 kDa, respectively. The N-terminal amino acid sequences of Pr Chi-A and -B were similar to those of class IIIb chitinase and LysM domain, respectively. Pr Chi-B exhibited antifungal activity against *Trichoderma viride*, while Pr Chi-A did not. A cDNA encoding Pr Chi-B was cloned by rapid amplification of cDNA ends and PCR procedure. It consists of 1,459 nucleotides and encodes an open reading frame of 423 amino acid residues. The deduced amino acid sequences of Pr Chi-B indicate that Pr Chi-B consists of N-terminal two LysM domains and a C-terminal catalytic domain (similar to class IIIb chitinase) linked by proline, serine, and threonine-rich region.
To clarify the correlation between the structure and antifungal activity of Pr Chi-B, mutational analyses were done. The deletion mutant without LysM domains and the mutant E247Q (Glu247→Gln) without chitinolytic activity exhibited almost no antifungal activity. These results indicate that the LysM domains contribute significantly to the antifungal activity of Pr Chi-B and that the antifungal activity of Pr Chi-B is based on the chitinolytic activity.