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Structure of full-length class I chitinase from rice revealed by X-ray crystallography and small-angle X-ray scattering

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1 Introduction

Chitinases (EC 3.2.1.14) catalyze the degradation of chitin, a linear β-1,4-linked homopolymer of *N*-acetylglucosamine (GlcNAc). Chitin is a major component of fungal cell walls including those of fungal plant pathogens. Chitinases are believed to hydrolyze the chitin chains in cell walls and inhibit fungal growth.

OsChia1b is a plant class I chitinase from rice, and is composed of an N-terminal chitin-binding domain (ChBD) and a C-terminal catalytic domain (CatD) connected by a linker peptide. The antifungal properties of class I chitinases have been well characterized *in vitro*. These investigations showed that full-length class I chitinases exhibit 3–5 times higher antifungal activity than their CatDs alone corresponding to class II chitinases. Transgenic plants constitutively express OsChia1b showed increased resistance to fungal disease. However, the detailed mechanism of antifungal activity is not well understood.

Structural information for full-length class I chitinases is currently unavailable. Therefore, the spatial relationships between the two functional domains have not been reported. In this study, we report on the structure of full-length OsChia1b analyzed by X-ray crystallography and small-angle X-ray scattering (SAXS).

2 Experiment

Recombinant OsChia1b was overproduced in *Escherichia coli* and purified as described earlier [1]. SAXS experiment was carried out using a CCD based X-ray detector (Hamamatsu Photonics K. K., Shizuoka, Japan) installed at beamline BL-15A [2]. The detector was set at a distance of 1.0 m from the sample position, and the wavelength of the X-ray was 1.5 Å. The data were measured at a series of protein concentrations from 3.0 to 10.0 mg/ml at 25°C. Detailed experimental and data processing procedures were described previously [2]. Preliminary SAXS experiment was carried out at BL-10C on the proposal No. 2006G204.

3 Results and Discussion

Crystal structure of full-length OsChia1b was solved at 2.0-Å resolution [2]. In this structure, there were two regions constructed, Glu33-Cys74 and Asp87-Asn330, which approximately corresponded to ChBD and CatD, respectively. The linker peptide was not built due to the absence of electron density, suggesting its conformational flexibility.

To characterize the structure of full-length OsChia1b in solution (in the absence of crystal packing effects), SAXS experiment was performed. All scattering profiles of OsChia1b from different protein concentrations showed linear behavior at a small q^2 region (≤ 0.0025 Å^2) in the Guinier plot (Fig. 1a). The Radius of gyration (R_g) and molecular mass values were estimated to be 22.9 Å and 28.8 kDa after an extrapolation to zero concentration to eliminate the interference effect. The experimental distance distribution function, $p(r)$, displayed asymmetric bell-shaped profiles with a maximum dimension of 86 Å (Fig. 1b), which was longer than that of 75 and 65 Å calculated from the two full-length OsChia1b candidates in the crystal. The difference in maximum dimensions in the crystal and solution strongly suggests that the conformational flexibility of the linker peptide can be considered to cause the conformational extension and the variability of the domain arrangement of OsChia1b. These results imply the importance of flexibility of the linker for the antifungal activity of class I chitinases.

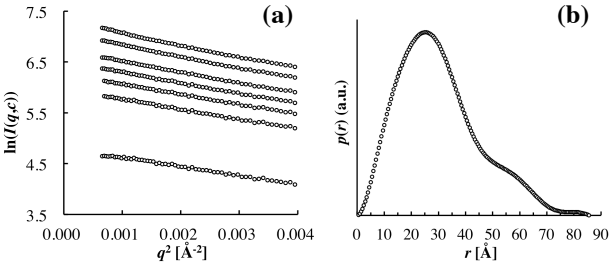

Figure 1 SAXS analysis. Guinier plot (a) and distance distribution function (b).

References

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