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Crystal structure of a novel mannose-binding lectin from *Gastrodia elata* with antifungal activity

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Introduction

Gastrodia elata B1. belongs to orchid family and has been widely used as a anodyne and tranquilizer with no side effect in Chinese medicinal science for about two thousand years. This plant does not contain chlorophyll and leads a parasitic life on the fungus *Armillaria mellea*. The fungal hyphae infect ancestor or nutritive corms of the plant, but are arrested and digested in the cortical cells. The released nutrients are mainly transported into the terminal corms for its growth. Morphology study of *Gastrodia elata* found that the terminal corm of this plant has never been infected by the fungi. Hu *et al.* (1988) isolated and purified an anti-fungal protein named Gastrodia Antifungal Protein (GAFP1) or gastrodianin from the terminal corm of *Gastrodia elata*, which was showed to be of strong anti-fungal activity to seprophytic fungi, such as *Armillaria mellea* and *Trichoderma viride*. GAFP1 does not process any chitinase or β-1,3-glucanase activity. The amino acid sequence of GAFP1 showed that it share high homology with those of other mannose-binding lectins from orchids such as *Listera ovata* and *Epipactis helleborine* (Van Damme *et al*, 1994). Those data suggest that GAFP1 could be a lectin-like protein with strong inhibitory activity against certain fungal pathogens.

The structure of GAFP1 will be important to our understanding of the mechanism of this kind of protein. In this work, we report the crystallization, data collection at PF and structural solution of GAFP1.

Experimental

GAFP1 (Gastrodia antifungal protein 1) was purified from new-born terminal corms of the orchid *Gastrodia elata* B1 f. elata as described by Hu *et al*. (1988).

Hang-drop vapor-diffusion method was employed for the crystallization of GAFP1. Best crystals suitable for X-ray diffraction analysis were grown in the following condition: drops formed by mixing equal volume (3 ul) of 15 mg/ml protein in water and reservoir solution containing 1.7 M ammonium sulfate, 1%-5%(v/v) dioxane, 0.1M k/sodium tartrate, 0.1 M MES pH6.5. Crystals grown under this condition were long rod-like with maximum crystal dimensions of around 0.5 ×0.2 × 0.1mm^3 (Liu *et al*., 2002).

X-ray data of GAFP1 to a resolution of 2.0Å were collected from one crystal by using CCD detector (ADSC Quantum 4R) at beamline BL18B of the Photo Factory at KEK (Trukuba, Japan). The collection was performed at room temperature with a crystal-to-detector distance 573 mm, $\Delta\varphi$=1°, λ=1.0 Å and 60 s exposure time. Data were processed and scaled with DPS/Mosflm (Rossmann & van Beek, 1999). The crystal belongs to space group $P2_12_12$ with unit cell parameters:a = 61.087Å, b = 91.488 Å, c = 81.132 Å, $\alpha=\beta=\gamma=90°$. The dataset consists of 31377 unique reflections with a completeness of 99.8% and the R_{merge} of 9.7%. Assuming that four molecules are presented in the asymmetric unit, the Matthews coefficient, V_m (Matthews, 1968), is estimated as 2.3 Å^3 Da^{-1} with a corresponding solvent content of 46.2%.

Result and discussion

The structure of GAFP1 was determined by molecular replacement and refined using CNS1.1 to a final R and free R factors of 16.68% and 20.57%, respectively. Four monomers, which formed two dimers, were found in the asymmetric unit of GAFP1 crystal. The two dimers were related by a non-crystallography 2-fold axis.

GAFP1 is a typical β protein. The topological diagram of the secondary structures shows that the monomer has twelve β strands but no helix. Every four β strands form an anti-parallel β sheet. The three anti-parallel β sheets are arranged around a pusdo-3-fold axis and so form a β barrel. Structural comparisons show that the mannose-binding sites of GAFP1 were located at the centre of each anti-parallel β sheet. The possible antifungal mechanism and its relationship with the obvious different oriented C terminal segment of GAFP1 are proposed.

References

[1] W. Liu et al., *Acta Cryst.* **D58,** 1833-1835 (2002).
[2] Z. Hu et al., *Acta Bot. Yunnan* **21**, 131-138 (1988).
[3] M. G. Rossmann et al., *Acta Cryst.* **D55**, 1631-1640 (1999).
[4] E. J. Van Damme et al., *Glycoconj. J.* **11**, 321-332 (1994).
[5] B. W. Metthews, *J. Mol. Biol*. **33**, 491- 497 (1968).

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