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Crystallization and preliminary crystallographic analysis of a native chitinase from the fungal pathogen *Aspergillus fumigatus* YJ-407

Hongyan Hu,[a] Ganggang Wang,[b] Haitao Yang,[b] Ju Zhou,[a] Lijuan Mo,[b] Kailin Yang,[b] Chunsheng Jin,[a] Cheng Jin[a]* and Zihe Rao[b,c]*

[a]State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China, [b]Laboratory of Structural Biology, Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, People's Republic of China, and [c]National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

Correspondence e-mail: jinc@sun.im.ac.cn, raozh@xtal.tsinghua.edu.cn

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Chitinase hydrolyzes chitin, a linear polymer of β-1,4-linked *N*-acetylglucosamine (NAG), and plays a variety of roles in the biological world. In addition to endo- and exo-hydrolytic activities, transglycosyl activity has also been observed in the extracellular chitinase (afCHI) from the airborne saprophytic fungi *Aspergillus fumigatus* YJ-407. Crystals of this native chitinase have been grown at 291 K using PEG 3350 as a precipitant. The diffraction data from the crystal extend to 1.7 Å resolution at BSRF, China. The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters a = 95.7, b = 100.5, c = 134.3 Å. The presence of two molecules per asymmetric unit gives a crystal volume per protein mass (V_M) of 3.6 $Å^3\ Da^{-1}$ and a solvent content of 65% by volume. A full set of X-ray diffraction data was collected to 2.1 Å resolution.

1. Introduction

Chitinases hydrolyze chitin, a linear polymer of β-1,4-linked *N*-acetylglucosamine (NAG), which is a structural component of the cell walls and coating of many organisms. Interestingly, chitinase plays a variety of roles in the biological world: bacteria produce chitinases for nutritive purposes (Brurberg *et al.*, 1996) and plants produce chitinases as part of pathogenesis-related proteins in response to fungal invasion (Leah *et al.*, 1994). Functional endogenous chitinase has also been identified in humans (Boot *et al.*, 1995); however, its physiological role is still unclear. Fungi produce chitinase throughout the growth process (Kuranda & Robbins, 1991), which is thought to contribute to a number of morphogenetic processes in filamentous fungi, including spore germination, side-branch formation, differentiation into spores and autolysis (Gooday *et al.*, 1992). Therefore, fungal chitinase is thought to be a putative virulence factor and a promising antifungal target molecule.

The catalytic domains of chitinases can be grouped into two families, called families 18 and 19, based on amino-acid sequence similarity. It was originally proposed that family 18 chitinases act with a retaining mechanism, which requires both a catalytic acid and a nucleophile (Armand *et al.*, 1994). However, these proteins lack a suitable nucleophile. Subsequently, a substrate-assisted catalytic mechanism was proposed for family 18 chitinases based on the crystal structure of hevamine (Terwisscha van Scheltinga *et al.*, 1995). With the accumulation of structural information, the catalytic mechanism for these glycoside hydrolases has been described in detail (Houston *et al.*, 2002; Vaaje-Kolstad *et al.*, 2004). However, the plant chitinase has a different subsite structure for catalysis from that of its microbial counterparts (Sasaki *et al.*, 2002). Furthermore, the microbial members of family 18 chitinases have been shown to exhibit transglycosyl activity, which is absent in the plant chitinases. To date, little effort has been made to probe the mechanism of the transglycosyl activity displayed by the microbial members of the family 18 chitinases.

As one of the most ubiquitous of the airborne saprophytic fungi, *Aspergillus fumigatus* has been shown to be a pathogen causing pneumonia and other fatal invasive infections (Latge, 1999). The extracellular chitinase from *A. fumigatus* YJ-407 possesses endo- and exo-hydrolytic activities and transglycosyl activity (Xia *et al.*, 2001). Structural studies of afCHI will provide insight into the mechanism of the transglycosyl activity of microbial chitinase. In addition, the physiological role of the sugar moiety of this native chitinase can be explored in detail using the structural information. Here, we report the crystallization and preliminary X-ray diffraction studies of native chitinase from *A. fumigatus* YJ-407.

2. Materials and methods

2.1. Crystallization

The extracellular chitinase from *A. fumigatus* YJ-407 (afCHI) was isolated as described previously (Xia *et al.*, 2001). The lyophilized protein was dissolved in double-distilled water to a concentration of 10 mg ml^{-1}. Crystallization trials were conducted at 291 K in

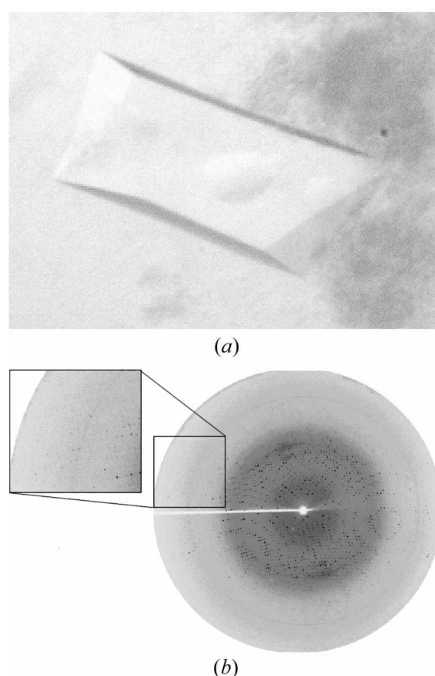

Figure 1
(*a*) Photograph of afCHI crystals. (*b*) A typical X-ray diffraction pattern of afCHI. The edge of the detector is at 1.7 Å resolution.

16-well plates using the hanging-drop vapour-diffusion method. Crystal Screens I and II (Hampton Research, Riverside, CA, USA) were used for initial screening. Drops consisting of 1 µl protein solution and 1 µl reservoir solution were equilibrated against 0.5 ml reservoir solution. Thin rod-like crystals of afCHI appeared in solution No. 43 of Crystal Screen II (50% MPD, 0.1 *M* Tris–HCl pH 8.5, 1.5 *M* ammonium phosphate), but the crystals failed to grow large enough to give any useful diffraction.

Further trials were set up using PEG/Ion kits (Hampton Research, Riverside, CA, USA) for crystal screening, as described above. Small crystals were grown from 0.2 *M* magnesium chloride, 20% polyethylene glycol (PEG) 3350 (solution No. 5 of the PEG/Ion kit). This condition was further optimized by variation of precipitant and protein concentration. The best crystals were obtained with a reservoir solution containing 36% PEG 3350 at a protein concentration of 5 mg ml^{-1}. The crystals reached dimensions of 0.4 × 0.15 × 0.05 mm (Fig. 1*a*).

2.2. X-ray crystallographic studies

Preliminary diffraction data sets were collected at room temperature in-house on a Rigaku Cu $K\alpha$ rotating-anode X-ray generator at 48 kV and 98 mA (λ = 1.5418 Å) with a MAR 345 image-plate detector. A complete set of data was collected on beamline 3W1A of the Beijing Synchrotron Radiation Facility (BSRF, Beijing, China) to 1.7 Å resolution. Crystals were immersed in a freezing solution for 5–10 s, picked up in a loop and then flash-cooled in a stream of nitrogen gas cooled to 100 K. The freezing solution contained 25% glycerol as cryoprotectant, but was otherwise identical to the reservoir solution. A Cryostream (Oxford Cryosystems, Oxford, UK) was used to maintain the crystal at 100 K during data collection. All intensity data were indexed, integrated and scaled with the *HKL*2000 programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses are for the outer shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 95.7, b = 100.5, c = 134.3
Solvent content (%)	65
Resolution (Å)	35.0–2.10 (2.18–2.10)
Total observations	309074
Unique reflections	74799
Redundancy	4.1
Average $I/\sigma(I)$	6.1 (2.4)
R_{merge}† (%)	14.9 (50.4)
Completeness (%)	98.8 (96.6)

† $R_{merge} = \sum |I - \langle I \rangle| / \sum I$.

3. Results

Diffraction data were collected to a Bragg spacing of at least 1.7 Å (Fig. 1*b*). Unit-cell parameters were determined to be a = 95.7, b = 100.5, c = 134.3 Å in space group $P2_12_12_1$. The asymmetric unit contains two molecules, each with a molecular weight of 45 000 Da, giving a crystal volume per protein weight (V_M) of 3.6 Å^3 Da^{-1} and a solvent content of 65% by volume (Matthews, 1968). As listed in Table 1, a data set was collected to 2.1 Å resolution. It consists of 309 074 measurements of 74 799 unique reflections with an overall R_{merge} of 14.9% ($R_{merge} = \sum |I - \langle I \rangle| / \sum I$) and an overall $I/\sigma(I)$ of 6.1. This represents 98.8% of the theoretically observable reflections at 2.1 Å resolution. The highest resolution shell consisting of data between 2.18 and 2.10 Å resolution is 96.6% complete.

The structure of afCHI was determined by the molecular-replacement method using the structural data of *Coccidioides immitis* chitinase 1 (PDB code 1d2k; Hollis *et al.*, 2000) and the structure has now been solved. Briefly, the afCHI monomer is glycosylated with one *N*-acetylglucosamine (NAG). Detailed analysis of the structure is now under way.

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