

## CRYSTAL STRUCTURE ANALYSIS OF THE CATALYTIC DOMAIN OF CHITINASE A1 FROM *BACILLUS CIRCULANS* AT ATOMIC RESOLUTION

Chitinases are enzymes that hydrolyzes chitin, a homopolymer of N-acetylglucosamine. These enzymes exist in a wide range of organisms, including bacteria, fungi, higher plants, insects, crustaceans and some vertebrates, and play various roles both inside and outside the cell. *Bacillus circulans* WL-12 secretes at least six kinds of chitinases into the culture mediums [1].

Chitinase A1, the main chitinase of this bacterium, has the strongest chitin degradation ability and is produced most voluminously compared to the other five chitinases. Chitinase A1 is a typical multi-domain protein which consists of three different kinds of domains (a catalytic domain, two type III homology units of fibronectin and a C-terminal short segment). Classified by homology among the amino acid sequences of the catalytic domain, chitinase A1 belongs to family 18 of the glycosyl hydrolases.

The mutant, named CA6, which consists only of the catalytic domain of chitinase A1, is composed of 419 amino acid residues (*Mr* 45,489) and maintains

49% of the chitin degradation activity of intact chitinase A1. CA6 was crystallized at 20° C by the vapor diffusion method in 10(w/v)% aqueous solution of polyethylene glycol (average molecular weight is *ca.* 4,000) as a precipitant and 25 mM potassium dihydrogenphosphate as an additive. Crystals of about 0.20 X 0.20 X 1.75 mm<sup>3</sup> were obtained in two weeks' time. The space group was P1, and the lattice constants were *a*= 43.96 Å, *b*= 48.62 Å, *c*= 54.59 Å,  $\alpha$ = 108.90°,  $\beta$ = 95.06° and  $\gamma$  = 115.77°. One unit cell contained one molecule of CA6.

Initially, a structural analysis was attempted by the molecular replacement method using the X-ray diffraction data (completeness: 87.8%) up to 2.5 Å resolution collected in house by a Rigaku R-AXIS IIc oscillation diffractometer. A molecular model of CA6 based on the three-dimensional structure of chitinase A from *Serratia marcescens* with 33% amino acid sequence homology to CA6 was constructed by the homology modeling technique using the SWISS-MODEL. It was possible to determine the orientation of the CA6 molecule from a series of rotation searches with the program AMORE. However, there were many areas where an interpretation of the electron density map for the main chain was difficult, and accordingly the

refinement of the crystal structure failed. Afterwards, it became possible to interpret the density map since the phases were expanded and improved with the program ARP using the X-ray diffraction data (completeness: 74.7%) up to 1.5 Å resolution collected at the beamline 18B of the Photon Factory. We are currently refining the three-dimensional structure, adding anisotropic displacement parameters to the calculation (Figure 1), with the

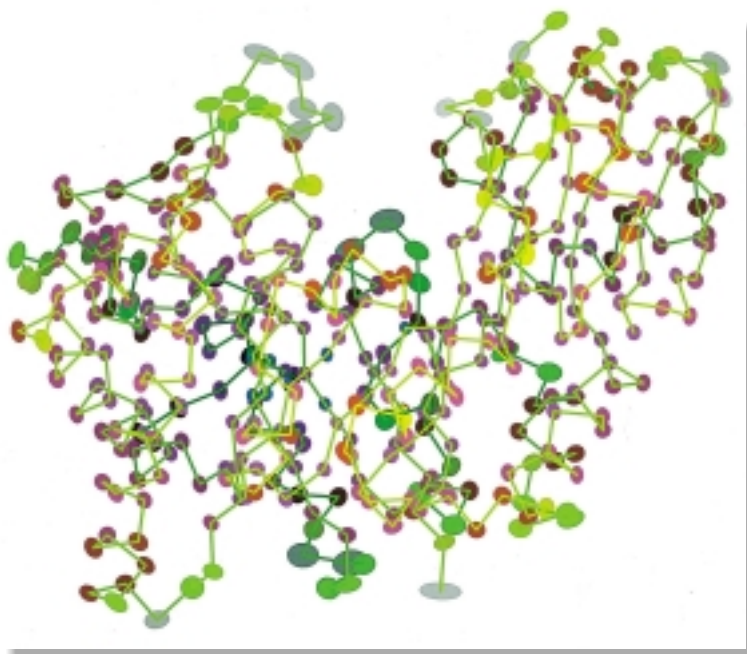


Fig. 1: Anisotropic displacement ellipsoids drawn at a 50% probability level for the 409  $\alpha$ -carbon atoms composing the catalytic domain of chitinase A1 (from *Bacillus circulans*).

program SHELX-97 using the diffraction data of up to 1.13 Å resolution [2]. The data were collected at room temperature by a Rigaku R-AXIS IV oscillation diffractometer installed at the beamline **BL44B2** (Figure 2). The current model contains 3,153 non-hydrogen protein atoms and 523 water molecules, and the current  $R$ -factor and the free  $R$ -factor are 0.196 and 0.212, respectively, against the 114,787 independent reflections (completeness: 0.826;  $R$ -merge: 0.032).

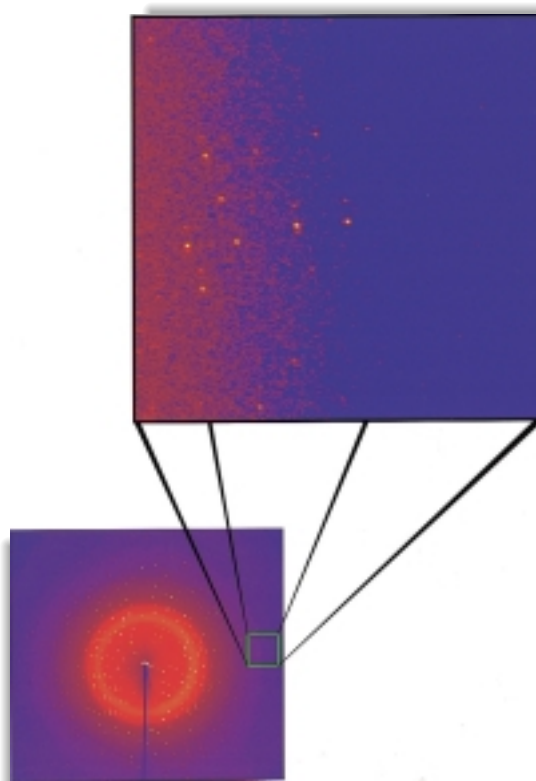


Fig. 2: X-ray diffraction image from a crystal of the catalytic domain of chitinase A1 (from *Bacillus circulans*) at the atomic resolution (1.13Å), measured by a Rigaku R-AXIS IV oscillation diffractometer.

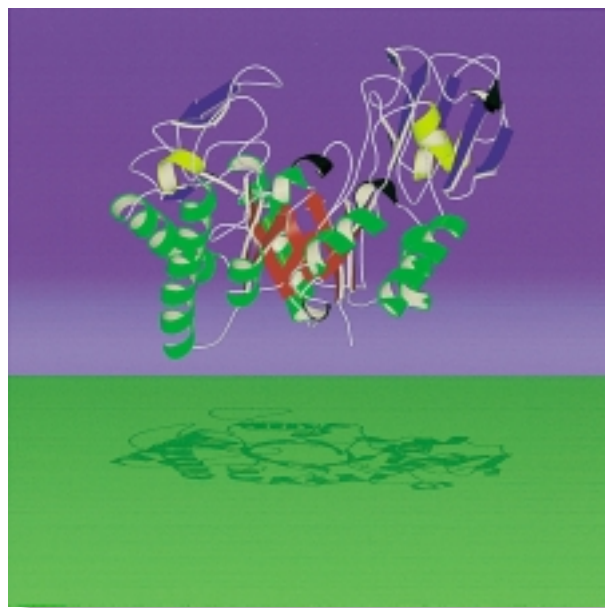
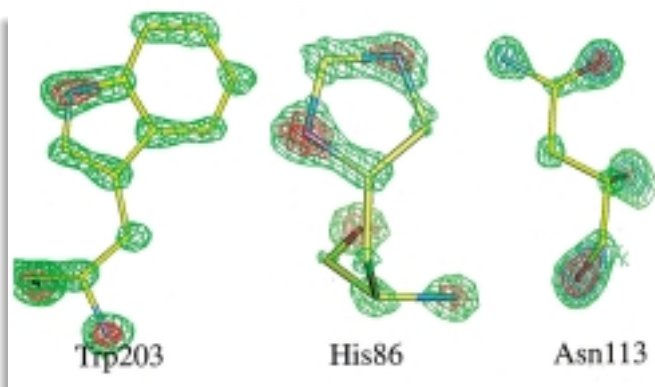


Fig. 4: Overall structure of the catalytic domain of chitinase A1 (from *Bacillus circulans*).

As a result of refinement at the atomic resolution, it became possible to identify the orientation of the imidazole ring of the histidine residues and to distinguish the nitrogen atom from the oxygen atom composing the side chain of the asparagine and glutamine residues (Figure 3). The three-dimensional structure of CA6 is composed of an  $\alpha / \beta$  domain with a TIM barrel structure and two beta domains (Figure 4). The TIM barrel structure is common to all of the enzymes belonging to the family 18, whose three-dimensional structures are known.

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**References**

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- [2] T. Matsumoto *et al.*, to be published.

Fig. 3: Electron density maps for some of the amino acid residues of the catalytic domain of chitinase A1 (from *Bacillus circulans*).