Analysis of the anisotropic displacement parameters of the catalytic domain of chitinase A1 from *Bacillus circulans*

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Chitinases are enzymes for the hydrolysis of the chitin, which is the homopolymer of N-acetylglucosamine. These enzymes exist in a wide range of living things, including bacteria, fungi, higher plants, insects, crustaceans, and some vertebrates, and play various roles inside and outside the cell. *Bacillus circulans* WL-12 secretes at least six kinds of chitinases into the culture medium (Watanabe *et al.*, 1990). 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 domain protein which consists of three 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 the 419 amino acid residues (*Mr* 45,489) and maintains 49% chitin degradation activity of intact chitinase A1.

CA6 was crystallized at 20° C by the vapor diffusion method using polyethylene glycol as a precipitant. The optimal crystallization conditions are 10(w/v)% aqueous solution of polyethylene glycol (the 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^3 were obtained under these conditions in two weeks. The space group of the crystals is *P*1, and the lattice constants are a=43.96Å, b=48.62Å, c=54.59Å, α=108.90°, β=95.06°, and γ=115.77°. One molecule of CA6 exists in the asymmetric unit, *i.e.* the unit cell.

First of all, a structural analysis was attempted by a molecular replacement method using the X-ray diffraction data (completeness: 87.8%) up to 2.5Å resolution collected by the Rigaku R-AXIS IIc oscillation diffractometer in house. 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 the series of rotation search with the program AMORE. However, there were many areas where it was difficult even to interpret the electron density distribution for the main chain, and accordingly the refinement of the crystal structure failed. Afterwards, interpretation of the electron density map became possible since the phases were expanded and improved with the program ARP using the X-ray diffraction data (completeness: 74.7%) up to 1.5Å resolution collected at the beamline 18B of Photon Factory.

We are refining the three-dimensional structure, adding the anisotropic displacement parameters to the calculation, with the program SHELX-97 using the diffraction data up to 1.13Å resolution (Matsumoto *et al.*). The data were collected at room temperature by the Rigaku R-AXIS IV oscillation diffractometer installed at the beamline 44B2 of SPring-8. The current model contains 3,153 non-hydrogen protein atoms and 523 water molecules, and the current $R_{\text{-factor}}$ and the free $R_{\text{-factor}}$ are 0.196 and 0.212, respectively, against the 114,787 independent reflections (completeness: 0.826; $R_{\text{-merge}}$: 0.032).

As a result of the 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. The three-dimensional structure of CA6 is composed of an α / β domain with TIM barrel structure and two beta domains. The TIM barrel structure is common to all enzymes belonging to the family 18 whose three-dimensional structures are known.

References

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