Structure-Function Studies on the Chitinolytic Enzymes of *Serratia marcescens* Chitinase and Chitobiase

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Chitin is the second most abundantly distributed biological polymer throughout nature. This homopolymer of *N*-acetyl-D-glucosamine is not only the major constituent of the fungal cell wall and the arthropod exoskeleton, but also it is an important nutrient source of carbon and nitrogen in the marine environment.

Our interest is the elucidation of the mechanism of chitin degradation by chitinolytic enzymes. These enzymes, chitinases (EC 3.2.1.14) and chitobiases (EC 3.2.1.30), are produced and secreted from chitinolytic bacteria. Chitinases have been classified into families 18 and 19 of glycosyl hydrolases.[1] They hydrolyze chitin to oligosaccharides, of which *N,N'*-diacetyl-glucosamine is the predominant product; this is the substrate for chitobiase (trivial name for *N*-acetyl-glucosaminidase), which is classified into family 20 of glycosyl hydrolases.[1]

As a first step on this project, we applied crystallographic techniques to investigate the molecular structure of these enzymes and gain information in order to elucidate the mechanism of chitin degradation on the molecular level.

THE STRUCTURE OF CHITINASE A

The X-ray structure of chitinase A from the chitinolytic bacterium *Serratia marcescens* has been solved by multiple isomorphous replacement (MIR) and refined at 2.3-Å resolution, resulting in a crystallographic *R*-factor of 16.2%.[2] The structure of chitinase A consists of three domains (see FIGURE 1). The amino-terminal domain (aa [amino acids] 24–137), which consists only of β-strands, connects through a hinge region (residues 138–158) to the main αβ barrel domain (residues 159–442 and 517–563). The third domain has an α + β fold and is formed by an insertion in the barrel motif (residues 443–516).

MODE OF ENZYMATIC DEGRADATION

To date, four structures of family 18 chitinase enzymes have been reported. These include hevamine, a plant defense protein with combined chitinase and lysozyme activity;[3] chitinase A from *Serratia marcescens*;[2] and endo-β-*N*-acetylglucosaminidases F1[4] and H.[5] In these four structures, the main and catalytic domain is

an αβ barrel, which establishes a common fold for all family 18 enzymes.[6] They possess a long groove for the binding of the substrate, on the C-terminal end of the αβ barrel. The hydrolysis of chitin by chitinases belongs to the general acid-base catalysis. From primary structure comparisons, mutagenesis, and structural data, it is clear that the proton donor is a glutamate residue (127 in hevamine, 315 in chitinase, and 132 in endo-β-*N*-acetylglucosaminidases F1 and H). The acidic character of this residue is further stabilized by a strong hydrogen bond of an aspartate in position n-2. The intermediate is likely stabilized by the *N*-acetyl group of the substrate itself.

THE STRUCTURE OF CHITOBIASE

The chitobiase gene encoding an 885-amino-acid protein was cloned and sequenced; the protein was produced in *Escherichia coli,* purified, characterized, and crystallized as described in reference 7. The 3D structure of chitobiase was solved by

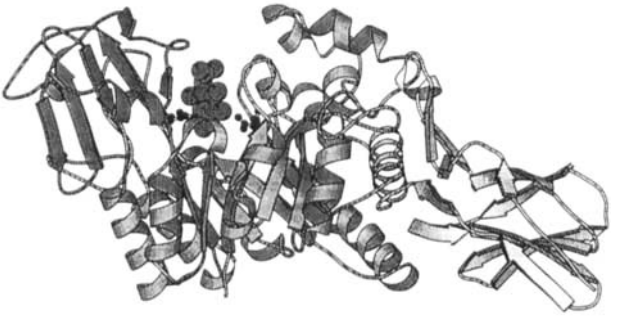

FIGURE 1. Ribbon diagram illustrating the structure of chitinase A, its three domains, and the groove of the active site where the sugar ring is bound.

MIR and refined to a resolution of 1.9 Å. Chitobiase has an eight-strand αβ-barrel structure (domain III, see FIGURE 2) with three additional domains. The N-terminal domain I, 154 aa, comprises two β-sheets. Domain II, 122 aa, has α + β topology. Domain III, 483 aa, an αβ-barrel motif, is the core of the structure, around which the other domains are organized. The active site is on the C-terminal end of the barrel, from where long loops interacting with domain I extend. The eight β-strands are surrounded by seven α-helices. Several deviations from the classical barrel motif have been identified. Domain IV, like domain I, comprises two β-sheets, but has only 67 aa. Overall dimensions of the protein are roughly 90 × 80 × 60 Å.

MODE OF ENZYMATIC ACTION OF CHITOBIASE

The enzymatic mechanism of *N,N'*-diacetyl-glucosamine degradation was determined from the crystal structure of several substrate/inhibitor complexes. The

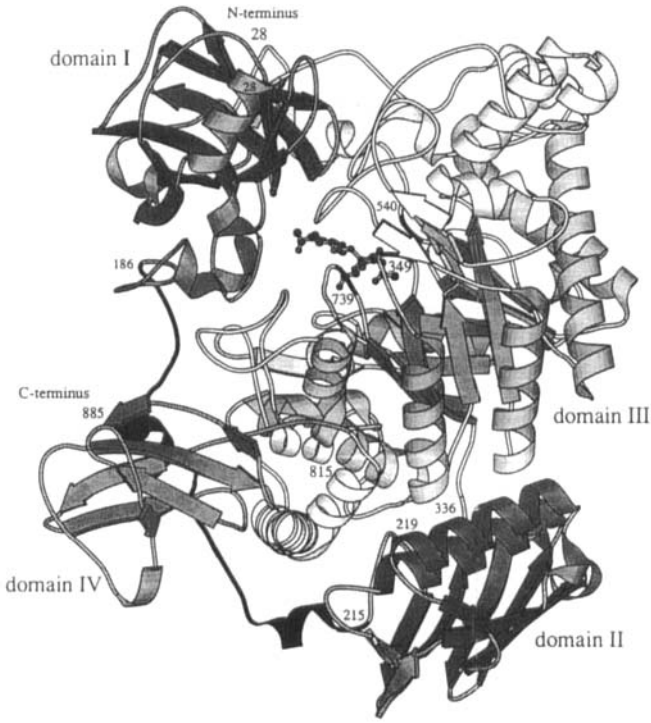

FIGURE 2. Schematic diagram illustrating the structure of chitobiase and its domains. The amino acid residue responsible for catalysis (glutamate 540) is shown in ball-stick symbols.

substrate lies in a pocket with the arginine 349 at its base, binding the nonreducing terminal sugar. There is only one protein carboxyl side chain as a catalytic group (glutamate 540), with the nucleophile being provided by the acetamido group of the substrate itself.

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