Mechanism of enzymatic degradation of recalcitrant structural polysaccharide with crystalline structure

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Research aims

Chitin is the second-most-abundant biopolymer in nature and is composed of β-1,4-linked *N*-acetylglucosamine (GlcNAc). It is a common constituent of insect exoskeletons, crustacean shells, and fungal cell walls. Chitin and its derivatives have many potential applications owing to a multitude of useful functionalities. However, enzymatic hydrolysis of chitin is particularly difficult because of a rigid crystalline structure. To utilize this valuable and plentiful biomass resource, it is necessary to understand the mechanism of crystalline chitin hydrolysis that occurs naturally in bacterial chitinases. Recently, we have succeeded in observing chitinase molecules hydrolyzing chitin chains on the surface of crystalline chitin using High-Speed Atomic Force Microscopy (HS-AFM). This has added significant weight to a model of crystalline chitin hydrolysis by bacterial chitinase[1)] that was proposed by our group several years ago[2)]. We are now investigating:

① Local structures in the chitinase molecule that are essential for crystalline chitin hydrolysis.

② The importance of CBP21 in chitin degradation and utilization, in addition to understanding its expression and physiological function in chitinolytic bacteria.

Methods

① Investigating the local structure of the chitinase molecule, which is essential for crystalline chitin hydrolysis

From our recent studies on chitinase, it is becoming apparent that aromatic amino acid residues, both on the surface and inside the substrate-binding cleft, play a vital role in crystalline chitin hydrolysis. To understand the details of their function, aromatic amino acid residues, either on the surface or within the substrate-binding cleft, were substituted with other amino acid residues via site-directed mutagenesis. The effects of these substitutions were analyzed by HS-AFM and conventional enzymological analysis.

② The importance of CBP21 in chitin degradation and utilization, in addition to understanding its expression and physiological function in chitinolytic bacteria

The chitin binding protein CBP21 from *S. marcescens* has been reported to enhance chitin degradation by chitinases. CBP21 is an oxidative enzyme (lytic polysaccharide monooxygenase, LPMO) that generates new ends on chitin chains at the surface of crystalline chitin. These new ends enable chitinases to more easily bind and process chitin chains. Various chitinolytic bacteria appear to possess proteins similar to CBP21. In order to elucidate the importance of CBP21 in chitin utilization and its physiological function in chitinolytic bacteria, a *CBP21* deletion mutant was constructed. Using this mutant, the effects that *CBP21* deletion had on the utilization of various type of chitin, bacterial growth, production of chitinases, and pathogenicity in the insect host, were investigated.

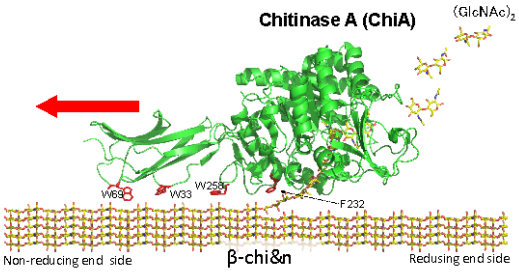

Fig 1. Processive hydrolysis model of crystalline β-chitin.

Results

① Investigating the local structure of the chitinase molecule, which is essential for crystalline chitin hydrolysis

To elucidate the importance and function of aromatic amino acid residues exposed on the surface of chitinase molecules, we focused on the two Tyr residues of ChiB. Tyr240, most proximal to the substrate-binding cleft, and Tyr481, most distal to the substrate-binding cleft, were substituted with Trp. The effects on the binding and hydrolytic activities of the highly crystalline β-chitin microfibrils were investigated.

Substitution of either Tyr240 or Tyr481 by Trp did not significantly affect chitin-binding activity. On the other hand, Tyr241→Trp mutation significantly decreased hydrolytic activity against β-chitin microfibrils, indicating that the two aromatic residues possess different functions. These results suggest that Tyr240 has additional roles in the degradation of highly crystalline β-chitin, possibly by guiding a chitin chain into the catalytic cleft.

To study the function of the aromatic amino acid residues within the substrate-binding cleft, we focused on the Trp167 residue in ChiA. Trp167 was substituted with Tyr, and the effect on binding and hydrolytic activity was examined. This mutation not only affected the binding of ChiA to crystalline β-chitin microfibrils, but also the binding of $(GlcNAc)_6$. No significant differences were observed in any thermodynamic binding parameter examined. Conversely, hydrolytic activity against highly crystalline β-chitin microfibrils decreased significantly (approximately 30% of wild-type ChiA). We predict that the Trp167→Tyr mutation reduces the rate at which the chitin chain enters the substrate-binding cleft towards catalytic site.

② Importance of CBP21 in chitin degradation and utilization and understanding its expression of physiological function in chitinolytic bacteria

Clearing zone formation by the CBP21 deletion mutant on agar plates containing β-chitin microfibrils was significantly smaller and more ambiguous compared to that of the wild-type strain. This indicates that this protein is particularly important for degradation and utilization of β-chitin microfibrils. In addition, deletion of CBP21 had a more severe effect on the degradation and utilization of chitin fragment flakes larger than fine powder. Unexpectedly, deletion of CBP21 did not affect pathogenicity in the insect host.

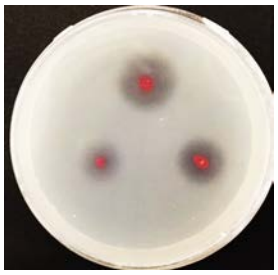

Fig 2. Clearing zone of β-chitin. Wild type (top), CBP-deficient mutant (left), and complemented strain (right).

Conclusions

In the processive hydrolysis of crystalline chitin, aromatic amino acid residues play an important role. The functions of individual residue are very different from each other, and depend on the position and type of aromatic side chain. CBP21 enhances degradation of chitin by chitinases via a sophisticated mechanism. Our observation that CBP21 acts more effectively on chitin in a more native state suggests that the enzyme plays a critical role in recycling chitin in nature.

References

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2) Uchiyama, T., Katouno, F., Nikaidou, N., Nonaka, T., Sugiyama, J. and Watanabe, T. (2001) Roles of the exposed aromatic residues in crystalline chitin hydrolysis by chitinase A from *Serratia marcescens* 2170. J. Biol. Chem. 276(44):41343-41349.

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