

Enzymes and accessory proteins for processing of recalcitrant biomass

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Understanding and Engineering Enzymes

In recent years, enzyme engineering efforts in the PEP group have been focused on chitinases, including studies of catalysis, inhibition, stability [1,2] and applicability for biomass turnover. Highlights include the discovery that aromatic residues in the substrate-binding cleft of Chitinase B from *Serratia marcescens* are essential for processive action (Fig. 1). While processive action contributes to efficient turnover of the insoluble substrate, we found that non-processive enzyme variants were up to 29-fold faster when degrading the water soluble chitin variant chitosan [3]. This leads to the important conclusion that it might be better to focus strategies for enzymatic depolymerization of polysaccharide biomass on improving substrate accessibility for non-processive enzymes rather than on improving the properties of processive enzymes. Other recent highlights include the development of enzyme variants and processes for the conversion of chitosan to defined mixtures of oligosaccharides (Fig. 1) [4, 5].

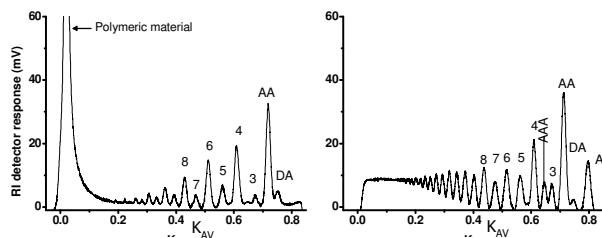


Figure 1. Size exclusion chromatography of products formed during degradation of chitosan (65 % acetylated water-soluble chitin with random distribution of acetylated units) with Chitinase B (A) and its W97A mutant (B), after cleavage of 14 % of the glycosidic bonds. The products are labelled by chain length or by sequence (A = acetylated unit; D = deacetylated unit).

Accessory Proteins

Like cellulases, chitinases often contain non-catalytic domains that help in binding and degrading the insoluble substrate. *S. marcescens* secretes a 18.8 kDa protein, CBP21 which belongs to the Carbohydrate-Binding Module (CBM) family 33 (Fig. 2A) [6].

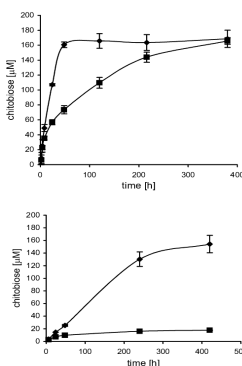
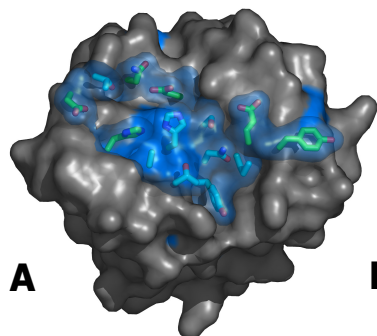


Figure 2. A) Surface representation of CBP21 illustrating a patch of conserved residues that is essential for functionality. B) Degradation of β -chitin with ChiC from *S. marcescens* (top) and ChiG from *Streptomyces coelicolor* (bottom) in the presence (diamonds) or absence (squares) of CBP21.

We have recently shown that binding of CBP21 to chitin leads to disruption of the crystalline substrate and to a dramatic increase in chitinase efficiency due to improved substrate accessibility (Fig. 2B) [7]. Proteins with such functions are of great interest for industrial conversion of recalcitrant polysaccharides such as cellulose. We are working on other CBM33 proteins, including cellulose-binders, following both genome mining and directed evolution strategies.

Applied Enzymology and Biofuels

Our applied enzyme work includes enzymatic processing of fish biproducts [8,9], barley and brewer's spent grain (www.repro-food.net). These substrates are being processed with different (industrial) proteases and glycosyl hydrolases, with the aim of producing protein hydrolysates and/or various poly- or oligosaccharide products.

Chitin and cellulose are similar both being linear recalcitrant insoluble polysaccharides. Based on the knowledge derived from our work on chitin turnover, we have recently entered the biofuel field. We focus on enzyme technology used in the production of second generation (wood-based) bioethanol. We advise Norwegian industrial consortia on process development. Own research is focused on the development of new enzymes and accessory proteins, using both discovery strategies (genome mining, proteomics) and enzyme engineering technology.

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Recent key references

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