ENGINEERING CHITIN DEACYTLASES FOR THE BIOTECHNOLOGICAL PRODUCTION OF PATTERNED CHITOSANS

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Chitin processing, mainly in the form of depolymerization and de-N-acetylation reactions by chitin-modifying enzymes (chitinases and deacytvlases), generates a series of derivatives including chitosans and chitosan-oligosaccharides (COS), which play remarkable roles in Nature. COS are particularly involved in molecular recognition events, including the modulation of cell signaling and morphogenesis, the immune response, and host-pathogen interactions. Chitosans and COS are also attractive scaffolds for the development of bionanomaterials for drug/gene delivery and tissue engineering applications. Most of the biological activities associated with COS seem to be largely dependent not only on the degree of polymerization but also on the acetylation pattern, which defines the charge density and the distribution of GlcNAc and GlcNH₂ moieties in chitosans and COS.

Chitin de-N-acetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and COS. The deacetylation pattern exhibited by CDAs and related carbohydrate esterase (CE4) enzymes active on COS is diverse, some being specific for a single position, others showing multiple attack. A major challenge is to understand how CDAs specifically define the distribution of GlcNAc and GlcNH₂ moieties in the oligomeric chain. By means of structural and biochemical studies, we have proposed a subsite capping model [1], which proposes that substrate specificity is governed by a series of variable and flexible loops that shape the binding cleft of CE4 enzymes.

Based on structural and bioinformatics analyses, here we will report the characterization of CE4 enzymes active of chitooligosaccharides and the engineering of loops by enzyme engineering approaches (rational and directed evolution) towards the production of paCOS with defined and novel deacetylation patterns.


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