PE 6 - Crystal Structure and Enzymatic Properties of a Bacterial Family 19 Chitinase Reveal Differences with Plant Enzymes

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We describe the cloning, over-expression, purification, characterization and crystal structure of ChiG, a single domain family 19 chitinase from the Gram-positive bacterium *Streptomyces coelicolor* A3(2). Family 19 chitinases are commonly found in many plants, but were only recently discovered in bacteria. Studies of the enzymatic properties of ChiG revealed that the enzyme is active towards a variety of soluble chitinous substrates at rates similar to those observed for other chitinases, but has a very limited ability to degrade crystalline chitin. The crystal structures of ChiG and a related Streptomyces chitinase, ChiC, showed that bacterial family 19 chitinases lack several loops that extend the substrate-binding grooves in family 19 chitinases from plants. In accordance with these structural features, detailed analysis of the degradation of chito-oligosaccharides by ChiG showed that the enzyme has only four subsites (as opposed to six for plant enzymes). This finding, in combination with the fact that ChiG has a very open active site, can be an explanation for the low activity towards crystalline substrates. Previous studies have shown that chitinases with high activity against crystalline chitin, such as Bacillus circulans chitinase A1 and Serratia marcescens chitinase A, have deep substrate-binding grooves. In addition, these grooves contain linearly aligned aromatic residues that may play an important role in guiding a chitin chain from the crystalline chitin surface to the catalytic center. Such a stretch of aromatic residues is lacking in ChiG. The most prominent structural difference causing a reduced size of the substrate-binding groove is the deletion of a 13-residue loop in between the two putatively catalytic glutamates. The importance of these latter two residues for catalysis was confirmed by a site-directed mutagenesis study.