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STRUCTURE AND FUNCTION OF GLYCOSIDE HYDROLASE FAMILY 19 CHITINASES

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Chitinases (EC 3.2.1.14) are the nature's tool to breakdown chitin, β-(1->4) linked N-acetylglucosamine units (GlcNAc). Chitinases are classified into two main glycoside hydrolase (GH) families, 18 and 19. Family 19 enzymes consist of enzymes from plants, bacteria and viruses playing roles in defense from pathogens, growth and development. The plant chitinases are divided into classes I-VI where classes I, II, IV and V belong to family 19.

There are 8 family 19 chitinase crystal structures, 6 from plants and 2 from bacteria, reported to date. Family 19 enzymes have a highly α-helical bi-lobed structure with a wide cleft lined by conserved residues. The catalytically important residues and 2 disulfide bonds are conserved. Considering *Brassica juncea* chitinase as the reference structure (PDB code 2Z37)[1], Glu212 and Glu234 have been identified as the proton donor and the general base respectively. The mutational studies have shown that Arg361 and Glu249 can work together as a catalytic triad altering properties of Glu212 especially changing the pKa to activate it [2]. The distance between Glu212 and Glu234 is ~9Å showing a feature of inverting hydrolases.

The main difference between the structures is in loop regions named I-V (Fig. 1). Class I/II structures possess these loops where class IV enzymes lack loops II, IV and V. Bacterial enzymes do not have loops I, II and V. The loop III is fairly conserved and includes Glu234, the putative general base. Flexibility of these loops was obvious among the molecules of the same structures as well as in different structures. Papaya chitinase bound to GlcNAc (PDB code 3CQL) is more closed compared to other structures without bound ligands. Catalytic residue in class IV enzyme structure from Norway spruce (PDB code 3HBE) shows conformational changes displaying that the side chain can move if necessary during the reaction keeping the interaction with Arg in the triad [3]. The catalytic clefts are varying in the length, probably accommodating 3 to 5 GlcNAc units depending on the enzyme.

Some of these enzymes contain chitin-binding modules (CtBM). A linker connects CtBM to the catalytic module (CM). CtBM's function is to locate the CM close to the substrate, which promote the activity on solid substrates. There are conserved disulfide bonds in CtBMs and possess a patch of conserved residues for substrate binding.

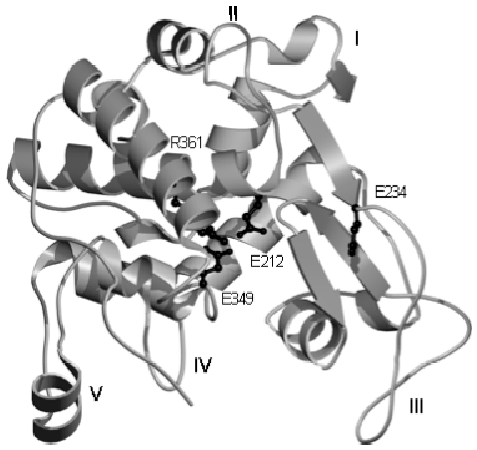

Fig. 1. *Brassica juncea* chitinase structure (PDB code 2Z37) showing loops and catalytically important residues

The catalysis of family 19 chitinases takes place after binding an undistorted chair conformation of the chitin chain in the open cleft at the vicinity of the proton donor (Glu212). Then, the movements both in the lobes and the loops tighten the grip on the substrate make it easier for the catalysis. First, Glu212 donates a proton to O1 of sugar activating it. Then, loop III moves bringing Glu234 closer to the substrate, where Glu234 abstract a proton from a water molecule and direct the attack on the anomeric carbon of the sugar inverting the anomeric configuration in the product. It is also suggest that the Glu234 has a role in stabilizing the oxocarbenium ion intermediate of the reaction. Mutational studies have shown the importance of the role of Glu212 rather than the Glu234 suggesting that it actives a water molecule in the bulk solvent, which can attack the sugar directly. The product must leave the enzyme freely since there is no product inhibition observed.

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