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Research review paper

From bacteria to human: A journey into the world of chitinases

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ABSTRACT

Chitinases, the enzymes responsible for the biological degradation of chitin, are found in a wide range of organisms from bacteria to higher plants and animals. They participate in numerous physiological processes such as nutrition, parasitism, morphogenesis and immunity. Many organisms, in addition to chitinases, produce inactive chitinase-like lectins that despite lacking enzymatic activity are involved in several regulatory functions. Most known chitinases belong to families 18 and 19 of glycosyl hydrolases, however a few chitinases that belong to families 23 and 48 have also been identified in recent years. In this review, different aspects of chitinases and chi-lectins from bacteria, fungi, insects, plants and mammals are discussed.

Contents

1. Introduction

Chitin is the second most abundant natural carbohydrate polymer after cellulose and consists of β-(1 → 4)-linked units of *N*-acetylglucosamine (GlcNAc). It is a major component of fungal cell walls and invertebrate exoskeletons. In nature, chitin occurs in two different crystalline forms, α and β (Aam et al., 2010). α-Chitin is the dominant form and it is composed of linear chains of GlcNAc arranged in an antiparallel manner. On the other hand, β-chitin consists of parallel chains. Chitinolytic enzymes are produced by a wide range of organisms including bacteria, fungi, insects, plants and animals for different purposes such as nutrition, morphogenesis, and defense against chitin-containing pathogens (Adrangi et al., 2010). Many of theses organisms possess several genes that encode chitinolytic enzymes. For example, most filamentous fungi have 10 to 20 different chitinolytic genes, while in mycoparasitic species the number of such genes may reach 30 or even higher (Hartl et al., 2012). These enzymes act in a synergetic or successive manner to degrade chitin (Patil et al., 2000). Higher organisms such as *Arabidopsis* have also been reported to contain a large number of chitinolytic genes (Hossain et al., 2010). However, not all of these gene codes are for active enzymes. Many organisms including plants, invertebrates and higher animals express genes encoding so called chitinase-like lectins (chi-lectins) that are devoid of chitinolytic activity due to substitutions in their key catalytic residues (Arakane and Muthukrishnan, 2010; Hossain et al., 2010; Vega and

Abbreviations: AMCase, acidic mammalian chitinase; CBM, carbohydrate-binding module; GH, glycosyl hydrolase; GlcNAc, *N*-acetylglucosamine; IAD, innovation, amplification, and divergence; ISP, ice structuring protein; MDN, mutation during non-functionality; PR, pathogenesis-related.

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Kalkum, 2012). Despite lacking catalytic activity, these proteins retain the capacity to bind chitin. Owing to their widespread presence and diverse biological functions, chitinolytic enzymes have found several applications. For example they can be used in the production of single-cell proteins, isolation of fungal protoplasts, estimation of fungal biomass, development of 3D cell culture scaffolds, biocontrol of plant-pathogenic fungi and insect vectors, and the production of chitooligosaccharides, glucosamine, GlcNAc, neoglycoproteins and artificial polysaccharides (Adrangi et al., 2010; Jamialahmadi et al., 2011; Li et al., 2008; Lu et al., 2012; Ortiz-Rodriguez et al., 2010; Tajdini et al., 2010; Zakariassen et al., 2011). Recent studies in the field of chitinolytic enzymes have demonstrated that both the diversity and the physiological roles of these enzymes are far beyond those previously recognized.

2. Classification, structure and catalytic mechanism

Based on their mode of action, chitinolytic enzymes are classified into two categories: chitinases (EC 3.2.1.14) that cleave the chitin chain at internal sites in a random manner, and β-*N*-acetylhexosaminidases (EC 3.2.1.52) that catalyze the successive removal of GlcNAc residues from the non-reducing end of the chain (Adrangi et al., 2010). Chitinases occur in families 18, 19, 23, and 48 of glycosyl hydrolases (GH), while β-*N*-acetylhexosaminidases are included in GH3, GH18, GH20, and GH84 (Table 1). The classification of GH families is based on sequence homology and a continuously updated list of these families is available through the CAZy database (Cantarel et al., 2009). The catalytic domains of members of each GH family fold into a common three-dimensional structure (Fig. 1). Families GH18, GH20, and GH84 all have similar $(\beta/\alpha)_8$ barrel domains (Sumida et al., 2011). On the other hand, GH19 and GH23 enzymes adopt an $\alpha+\beta$ structure, while the catalytic domain of GH3 enzymes has a bipartite structure comprising a $(\beta/\alpha)_8$ barrel followed by an $(\alpha/\beta)_6$ sandwich (Wohlkonig et al., 2010; Yoshida et al., 2010). Some GH3 enzymes, however, lack the $(\alpha/\beta)_6$ sandwich (Yoshida et al., 2010). Finally, GH48 enzymes have an $(\alpha/\alpha)_6$ barrel structure characterized by six central α-helices surrounded by six external α-helices (Yennamalli et al., 2011). Most chitinases are modular proteins that, in addition to a catalytic domain, contain auxiliary domains such as the carbohydrate-binding module (CBM) (Guillen et al., 2010). The CBM enhances the activity of the enzyme towards insoluble substrates by anchoring the enzyme to the substrate and disrupting the crystalline structure of the substrate resulting in the formation of free chain ends (Guillen et al., 2010; Vaaje-Kolstad et al., 2005). Like catalytic modules, CBMs are classified into families of homologous proteins which may be accessed at the CAZy database. A second feature that helps to overcome the low accessibility of insoluble substrates is the presence of a deep and narrow substrate-binding cleft lined with aromatic residues in some chitinolytic enzymes (Fig. 2) (Horn et al., 2006a,b; Zakariassen et al., 2010). Such enzymes act in a processive manner, meaning once attached to a substrate chain, they thread the chain through their catalytic cleft performing several hydrolytic cuts instead of releasing the substrate after each cleavage. The aromatic residues provide the necessary environment for the flexible binding and movement of the substrate through the active site. Although processivity comprises an efficient strategy for the hydrolysis of insoluble substrates, it is usually associated with reduced activity towards soluble or more accessible polymeric substrates (Aam et al., 2010).

Like other glycosyl hydrolases, chitinolytic enzymes generally catalyze the depolymerization of their substrate through one of the two pathways known as single- and double-displacement mechanisms (Aam et al., 2010; Andersen et al., 2005; Cantarel et al., 2009; Li and Greene, 2010; Slamova et al., 2010; Tang et al., 2004; Udaya Prakash et al., 2010). In both pathways, two distinct catalytic groups are involved. One of these is a carboxyl group that acts as a proton donor and is usually provided by a conserved glutamate residue at the active site of the enzyme, although in some cases, for example in family GH84, an aspartate residue may fulfill this role (Table 1). The second catalytic group may act either as a base (as in the single-displacement mechanism) or a nucleophile (as in the double-displacement mechanism). This group may be a carboxyl moiety provided by a conserved glutamate or aspartate residue, or it may be the *N*-acetyl group of the sugar positioned in the −1 subsite of the enzyme (Aam et al., 2010; Udaya Prakash et al., 2010). Subsites are numbered from $-n$ to $+n$, where negative sign represents the non-reducing end of the chain with cleavage occurring between the −1 and +1 subsites (Davies et al., 1997). Since the single-displacement mechanism results in the inversion of the anomeric configuration of the hydrolyzed GlcNAc residue, it is also known as the inverting mechanism. On the other hand, in the double-displacement mechanism, also referred to as the retaining mechanism, the anomeric configuration is retained.

3. Bacterial chitinases

Bacterial chitinases occur in families GH18, GH19, and GH23 (Dahiya et al., 2006; Udaya Prakash et al., 2010; Ueda et al., 2009). Most bacterial chitinases belong to the GH18 family (Larsen et al., 2011) (Fig. 3). Based on sequence homology, bacterial GH18 chitinases are classified into three subfamilies A, B and C (Li and Greene, 2010). It should be noted that the nomenclature of bacterial chitinases does not follow this classification; for example, chitinase B from *Serratia marcescens* belongs to subfamily A while *Bacillus circulans* chitinase D is classified in subfamily B (Watanabe et al., 1999). Some bacterial GH18 chitinases besides catalytic and CBM domains contain a fibronectin type III-like domain which plays a role in substrate binding (Horn et al., 2006a). On the other hand, the distribution of GH19 chitinases among bacteria appears to be restricted to actinobacteria and purple bacteria (Udaya Prakash et al., 2010). It has been proposed that actinobacteria and purple bacteria may have acquired GH19 chitinase genes from plants and, in turn, transferred them to arthropods and nematodes (Udaya Prakash et al., 2010). To date, only one GH23 chitinase has been identified in bacteria (Ueda et al., 2009). This enzyme was isolated from *Ralstonia* sp. A-471 and comprises an N-terminal chitin-binding domain linked to a C-terminal catalytic domain that shows homology to goose-type

Table 1
Characteristics of chitinolytic enzymes belonging to different GH families.

Family	Catalytic mechanism	Proton donor	Base/nucleophile	Structure	Reference
Chitinases					
GH18	Retaining	Glu	Substrate acetamido group	$(\beta/\alpha)_8$	CAZY; Sumida et al. (2011)
GH19	Inverting	Glu	Glu/asp	$\alpha+\beta$	CAZY; Wohlkonig et al. (2010)
GH23	Inverting	Glu	Not known	$\alpha+\beta$	CAZY; Wohlkonig et al. (2010); Arimori et al. (2013)
GH48	Inverting	Glu	Not known	$(\alpha/\alpha)_6$	CAZY; Yennamalli et al. (2011)
β-N-acetylhexosaminidases					
GH3	Retaining	Glu	Asp	$(\beta/\alpha)_8+(\alpha/\beta)_6$	CAZY; Yoshida et al. (2010)
GH18	Retaining	Glu	Substrate acetamido group	$(\beta/\alpha)_8$	CAZY; Sumida et al. (2011)
GH20	Retaining	Glu	Substrate acetamido group	$(\beta/\alpha)_8$	CAZY
GH84	Retaining	Asp	Substrate acetamido group	$(\beta/\alpha)_8$	CAZY

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