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Review

Chitinases of filamentous fungi: a large group of diverse proteins with multiple physiological functions

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ABSTRACT

Chitin is the second most abundant natural biopolymer and the main structural component of invertebrate exoskeletons and cell walls of filamentous fungi. Fungal chitinases have multiple physiological functions including the degradation of exogenous chitin and cell wall remodelling during hyphal growth, but the regulation of the chitinolytic systems of filamentous fungi is not well understood. Fungi have on average between 10 and 25 different chitinases, but only the increasing number of fungal genome sequencing projects in the last few years has enabled us to assess the whole range and diversity of fungal chitinases. In this review the variety, domain architecture and subgroups of chitinases of filamentous fungi are shown, and how these data integrate with that from molecular biological studies on chitinases are discussed.

1. Introduction

Chitin is a linear, insoluble homopolymer composed of β-1,4-linked subunits of the acetylated amino sugar *N*-acetylglucosamine. In nature two major types of chitin occur, which are characterized by an antiparallel (α-chitin) or a parallel (β-chitin) arrangement of the *N*-acetylglucosamine chains (Fig 1). After cellulose, chitin is the second most abundant polymer found in the biosphere (Tharanathan & Kittur 2003). It is the main compound of invertebrate exoskeletons and an essential structural component of the cell walls of filamentous fungi.

Previous reviews of fungal chitinases already emphasized the diversity of these enzymes as well as their multiple functions in fungi (Gooday 1990; Jollès & Muzzarelli 1999). However, only the recent advent of fungal genome sequencing projects has enabled us to assess the whole range and variety of fungal chitinases (http://genome.jgi-psf.org/mic_home.html, http://www.broad.mit.edu/seq/msc/). While in bacterial genome databases on average only between two and four chitinases can be found, the genomes of filamentous fungi typically contain between 10 and 25 different chitinases. The reasons why fungi have so many chitinases are not well understood. Potential physiological roles of fungal chitinases, as already discussed in previous reviews (Adams 2004; Cohen-Kupiec & Chet 1998; Duo-Chuan 2006; Gooday 1990; Jollès & Muzzarelli 1999; Yang *et al.* 2007), include: (i) degradation of exogenous chitin present in fungal cell walls of dead hyphal fragments or in the exoskeletons of dead arthropods, and the use of the degradation products as a nutrient source; (ii) cell wall remodelling during the fungal life cycle, which includes putative roles of chitinases during hyphal growth, branching, hyphal fusion and autolysis; and (iii) competition and defence against other fungi or arthropods in the fungal habitat. Some fungi have even developed lifestyles which

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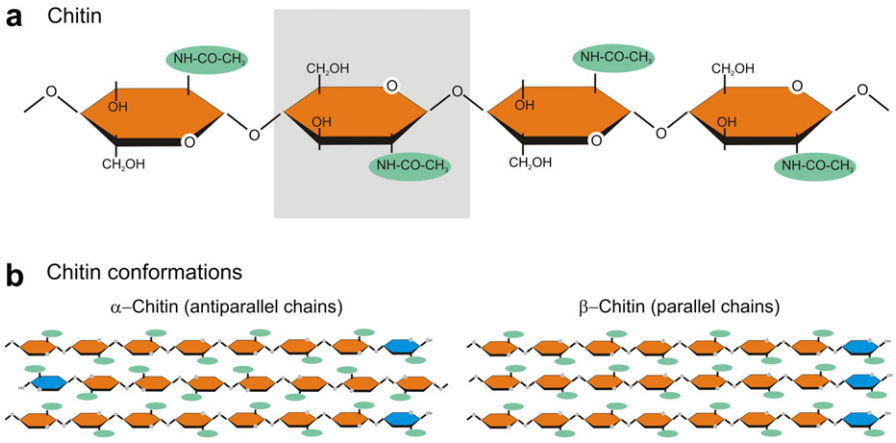

Fig. 1 – (a) Chemical structure of chitin. The grey box indicates one *N*-acetylglucosamine subunit of the chitin chain. (b) The two major types of chitin are characterized by an antiparallel (α-chitin) or parallel (β-chitin) arrangement of the chains.

involve the use of chitinases to actively attack of other fungi (mycoparasitism), insects (entomopathogenic fungi) or nematodes (nematode-trapping fungi).

This review aims at highlighting the lessons that we have learned about chitinases in the past few years from the increasing number of available fungal genome sequences and how these data integrate with the already available knowledge derived from molecular biological studies on chitinases.

2. Mechanisms of chitin degrading enzymes

Chitinolytic enzymes can be divided into *N*-acetylglucosaminidases and chitinases, which substantially differ in their cleavage patterns (Fig 2). *N*-acetylglucosaminidases (EC 3.2.1.52) catalyze the release of terminal, non-reducing *N*-acetylglucosamine (GlcNAc) residues from chitin, but in general they have the highest affinity for the dimer *N*,*N*′-diacetylchitobiose $(GlcNAc)_2$ and convert it into two monomers (Horsch *et al.* 1997). According to the CAZy classification (Carbohydrate Active Enzymes database, Coutinho & Henrissat 1999; http://www.cazy.org) *N*-acetylglucosaminidases belong to glycoside hydrolase (GH) family 20. It is important that those enzymes must not be referred to as exochitinases, which is unfortunately often confused in the literature. In contrast to that, chitinases (EC 3.2.1.14) are members of GH families 18 and 19 and catalyze the hydrolysis of the β-1, 4 linkages in chitin and chitooligomers, resulting in the release of short-chain chitooligomers. GH families 18 and 19 do not share sequence similarity, have different three-dimensional structures (Kezuka *et al.* 2006; Perrakis *et al.* 1994; Terwisscha van Scheltinga *et al.* 1996; van Aalten *et al.* 2000) and different catalytic mechanisms with ß-anomeric products being formed by GH 18 chitinases (retaining mechanism, Brameld *et al.* 1998), whereas α-anomers are produced by GH 19 chitinases (inverting mechanism, Brameld & Goddard 1998). Furthermore, depending on their cleavage patterns, chitinases can be divided into endo- and exochitinases (Fig 2). Endochitinases degrade chitin from any point along the polymer chain forming random-size length products while exochitinases cleave from the non-reducing chain end and the released product is $(GlcNAc)_2$. However, the enzymatic properties of chitinases are more complex and versatile than reflected in the exo-/endo classification. Detailed studies of the chitinolytic system of the bacterium *Serratia marcescens* demonstrated another way to classify the enzymatic properties of chitinases by grouping them into processive and non-processive enzymes (e.g. Horn *et al.* 2006; Sorbotten *et al.* 2005; Uchiyama *et al.* 2001). Processive chitinases do not release the substrate after hydrolytic cleavage but slide it through the active site-tunnel for the next cleavage step to occur. The presence of a carbohydrate binding domain can enhance processivity, but is not essential for it. Non-processive chitinases dissociate completely from the substrate after hydrolysis. This leads for

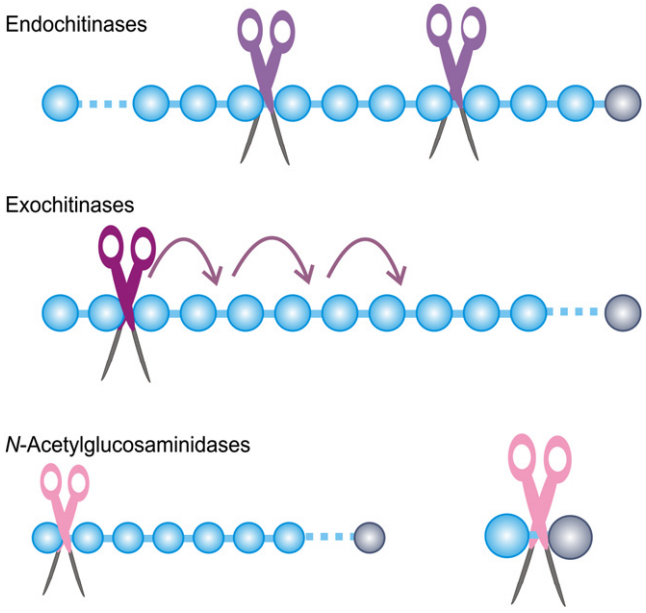

Fig. 2 – Schematic drawing of the predominant cleavage patterns of chitinolytic enzymes. The subunits of the chitin chain are shown in light blue and the reducing end sugar in dark blue. Dotted lines indicate that the polymer substrates are longer than shown in the figure.