CHITINASE

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ABSTRACT

Chitin is one of the most abundant polymers in nature and an important component for many organisms. For catalyzing and hydrolyzing chitin into N-acetylglucosamine (GlcNAC) and $(GlcNAC)_n$, chitinases have being received much attention in many fields. This article mainly introduced the recent research on chitinase, including structure, function and application of the enzyme.

Keywords: chitinase, structure, function

INTRUDUCTION

Chitin is one of the most commonly occurring organic compounds on earth , a β-(1,4) polymer of N-acetylglucosamine (GluNAC),is a structural component of the arthropod exoskeleton and a common constituent of fungal cell walls [1, 2]. But plant, vertebrates and prokaryotes do not contain chitin. Chitinase which are hydrolytic enzymes that are responsible for the degradation of chitin into its monomer N-acetyl-D-glucosamine; however,are synthesized by a vast array

of organisms, including those who are not composed of chitin.

Chitinase can be classified in two major categories. Endochitinase (EC3.2.1.14) cleave chitin randomly at internal sites, generating soluble low molecular mass multimers of GlcNAc, such as chitotetraose, chitotriose and the dimmer di-acetylchitobiose [3]. Exo-chitinases can be divided into two subcategories: chitobiosidases (EC3.2.1.29) [4] which catalyze the progressive release of di-acetylchitibiose starting at the nonreducing end of the chitin microfibril; and 1-4-β-Nacetylglucosaminidases (EC3.2.1.30), which cleave the oligomeric products of endochitinases and chitibiosidases generating monomers of GlcNAc. Based on amino acid sequence similarity (which is indicative of folding similaritity,if sequence similarity is high and dispersed over the entire sequence) of chitinases from various organisms, these chitinases can be grouped into two families of glycosyl hydrolases, family 18and family 19 [5]. Chitinases from classes I, II, and IV are of plant original and make up the family 19 glycosyl hydrolases [6]. These chitinase share a homologous catalytic domain in addition to the signal peptide found in all of them. Class I chitinases consist of a cysteine-rich amino-terminal domain linked by a short glycine/ praline-rich region (signal peptide) to the catalytic domain [7]. Most of the class I chitinase contain a carboxy-terminal signal peptide that is essential for targeting into the plant cell vacuole. Class II chitinases found mainly in dicotyledons lack the cystine-rich domain and the carboxy-terminal vacuole targeting signal, indicating that these chitinases do not bind chitin and are secreted to the apoplast. Class IV chitinases, also identified mainly in dicotyledons, comprise a group of extracellular chitinases that share 42 - 47 % sequence identity with class I chitinases in the catalytic domain and also contain cysteine-rich regions resembling chitin-binding domains; however, class IV chitinases are smaller because of deletions in both domains [8]. Class III chitinases are mainly plant and fungal in origin. Together with class V chitinases they make up the family 18 glycosyl hydrolases [5]. Class III includes the bi-functional lysozyme/ chitinase enzyme of *Havea*

brasiliensis [9]. Class V is mainly comprised of bacterial chitinase.

Since 1921, Folpmers found bacteria can grow on the media which contain chitin and proved there were chitinase in the culture. Chitinase has been studied for more than 80 years. Lots of reports showed that many different organisms including bacteria, viruses, higher plants, and animals can excrete chitinases. However, the functions of these enzymes are believed to be different in the biological world.. Chitinases in higher plants are part of a defense mechanism against fungal pathogens [10]. Many fungi contain chitin as a major component of their cell walls and produce chitinase to modify chitin as a structural component [11]. Now many studies reported chitin is a very good and polyuseful material and chitinase play a good role in the plant pest control [12]. In order to promote study, about chinase, in this paper advance study on these is introduced.

Arthropod chitinase

In arthropods, chitinase are involved in cuticle turnover and mobilization and in nutrient digestion. The enzymes have been found in molting fluids, venom, gland and midgut of several insects, but only chitinase from the former two sources have been characterized extensively [13]. Insects periodically shed their old exoskeletons and either continuously or periodically shed their peritrophic membranes and resynthesize new ones [14]. This process is mediated by the elaboration of chitinase in the molting fluid that accumulates in the space between the old cuticle and the epidermis and in the gut tissue. The GlcNAc-containing products of htdrolysis are ultimately recycled for the synthesis of a new cuticle For example, the *Manduca sexta* chitinase gene is not active during the larval feeding period. The enzyme is expressed only during narrow time frames just before larval-larval, larval-pupal and pupal-adult moulting .The activity of this gene is regulated positively by ecdysteroid and affected negatively by juvenile hormone [15]. Often, the larvae will ingest and digest the old

cuticle or exuviun, the components of which are also recycled [13, 16]. Apparently, chitinase found in the gut have a digestive function in addition to their role in breaking down chitin present in the gut lining or peritrophic membrane. In the venom of some Hymenoptera, chitinase may expedite the spread of venomous compounds from the site of divery.

Since 1986, Kramer purified from the moulting fluid and integumrnt of the tobacco homworm, *Manduca Sexta*, many reports about these aspects have been presented. So far the chemical, physical, and kinetic properties of these enzymes have been characterized [16]. Molecular mass of chitinase in most insect is 40 - 85 kD and higher than in plants or microorganism. The pH and pI of the chitinolytic activity is 4 - 8 and 5 – 7, respectively. Chitinase purified from insect are much higher glycosylated in the insect than in the plant [17]. The structure of invertebrates chitinase is composed of catalytic domain, Cys-rich chitin binding demain (CBD) and Ser/Thr-rich domain that is glycosylated. Generally chitinase from insect normally have a catalytic domain in N terminal and a CBD in C terminal. However, chitinase of *Penaeus japonica* has a CBD in N terminal and chitinase of *Caenorhaditis elegans* have two CBD in C terminal. CBD of chitinase from insect have 65 amino acids, 6 amino acids among them are most conservative and can be found in all the insect CBD and sequence of CBD of chitinase from different insect is obviously similar [18].

Plant chitinase

Plant chitinase distribute many parts such as seed, root and flowerl. Normally chitinase activity from plant is very low, but the activity can be increased rapidly after induction. Molecular weight of chitinase from plant is small about 12 – 55 kD, it is not sensitive to heat, optimized pH more than 7 and pI is 3 – 10 [19]. The proposed role of plant chitinase is a defense mechanism against chitin-containing organism. Since 1986 Schlumbaum reported chitinase purified from kidney bean has anti fungal activity, many studies in the field have

been reported [12]. Fbroe Kaetr indicated that chitinase from mandala can inhibit the growth of mycelia and germination of spores of *Trichoderma*. Chitinases of Class III is dual-function enzymes which can not only degrade cell wall of bacteria but also catalyse glycosylation, so they inhibit growth of bacteria. Heterologous chitinase gene expression is used in various plants to enhance their defense mechanisms against fungal pathogens [20]. Plant chitinases play an important role during plant development. For instance, chitinase IV are found only in the fruit in stead of the leaf, root and seed during mature of the grape. Chitinase activity increased markedly at the onset of ripening in grape *(Vitis vinifera* 1) and continued to increase through-out the sugar accumulation phase of berry development [21]. Kragh reported that a chitinase (32 kD) can rescue somatic embryos of the temperature-sensitive carrot variant ts II and development of asexual embryo mutant will be inhibited if quantity of this chitinase excrete to the culture are decreased [22]. In order to study the structure –function relationships of chitinase, the structure of chitinase from *Brassica juncea* have been determined. It is proved that chitinase from *Brassica juncea* has two CBDs which inhibit growth of plant pathogenic bacteria [23].

Now we know that precursor of plant includes a signal domain in N terminal composed of 20 amino acids, catalytic domain and extend domain in C terminal. Sometime binding domain riched with cystein locate behind signal domain. Binding domain and catalytic domain is linked by variable domain. Signal domain in N terminal of different chitinase has different function. Its function of plant chitinase is transportation chitinase into endoplasmic reticulum. Plant chitinase have half, one or two binding domains. They are commonly composed of about 40 amino acids .8 of them are fixed cysteines. Their location is not changeable and can make into disulfide bonds between them [24]. Binding domain is essential for binding chitin, but not for chitinase activity [25]. Variable domain is abundant in glycine and proline and its length of different chintinase is very different. For example poplar chitinase do not have this domain,

beet chitinase has this domain which composed of 133 amino acids , 90 amino acids of which are pralines. Now execpt that linkage whether or not having other functions is not clear [26]. Catalytic domain contains 300 amino acids and its active center proton acceptor is Glu. For instance proton acceptor of rubber plant chitinase and barley chitinase is Glu 27 and Glu 67 respectively and two proton acceptor, Glu 39 and GLu 67, of barley chitinase are necessary during catalyzing [27]. The test of mutation of either of two essential glutamates converts the catalytic domain of tabacco class I chitinase into a chitin-binding letin has been proved [25]. Endochitinase from chestnut seeds has two essential amino acids (Glu 24 and Glu 46) and it is found that catalytic domain is not essential for antifungas activity as the result that binding domain can inhibit directly the mycelia growth [28]. Chitinase sited in vacuole only has extended domain. Compared with extracellar chitinase PR-P amino acids of C-terminal of chitinase sited vacuole from tabacco are more than 6. If 7 amino acids of C-terminal of chiA were got rid of, the chiA would be excreted interstitial space [29].

Chitinase from microorganism

Microorganism including bacteria and fungi in sea and soil play an essential role of degrading chitin. Many of them have been reported mostly from bacteria, such as *Serratia marcescens, Bacillus circulans, Alteromonas* sp, *Enterobacter agglomerans, Aeromonas hydrophila* et al and fungas, such as *Mucorales, Deuteromycetes* and *Ascommycetes*.

Bacteria produce chitinases to meet nutritional needs. They usually produce several chitinases, probably to hydrolyze the diversity of chitins found in nature [30]. Benecke reported that bacteria *Seratia marcescens* can excrete 5 different molecular weight chitinases [31]. Chitinase from bacteria is stable in pH3-10 and has enzyme activity in temperature 4-60 or after kept for two years; highest activity in 40-50 °C [32]. The structure of Chitinase from bacteria also includes catalytic domain, binding domain and type III domain. On the

basis of homology of catalytic domain, chitinase from bacteria is separated into 5 groups, such as group I 93%, group II 85%, group III 83%, group IV 71% and group V 50%, furthermore asp 197, asp 200, asp 202 and Glu 204 are important for keeping high chitinase activity. The chitin-bingding domain in bacterial chitinase canbe located either in the amino-terminal or carboxy-terminal domains of the enzymes [32]. Through compared homology of different chitinase bingding domain, homologous sequence has 17 amino acids which belong to aromatic amino acid. Wanatable reported that *B. circulans*' bingding domain can bind unsolvable chitin, but not deacetylchitin. Type III domain is found during studying chitinase from *B. circulans*. Its losing does not affect chitinase combination, but depress activity of degrading colloidal chitin [33]. Most of the bacterial chitinase isolated and sequenced so far are included in family 18 of glycosyl hydrolases; however, there is one report of a chitinase isolated from Streptomyces griseus HUT6037 that belongs to family of 19 of glycosyl hydrolased [34].

Fungal chitinase have multiple functions. Similar to the bancterial chitinase,they play a role in nutrition, but they are also active in gungal developmental processes and in morphogennesis, because chitin is a major cell wall component in fungi. Chitinase also play a key role in the mycoparasitic activity of *Trichoderma* species against several plant pathogenic fungi, making *Trichoderma* a biocontrol agent [35]. The chitinolytic system of *Trochoderma harzianum* is composed of seven chitinases:two N-acetylglucosaminidases (chit102 and chit 73) and five endochitinases (chit 52, chit42, chit 36, chit 33 and chit 31). The expression pattern of each of these enzymes depends on the host fungus, and on the time course of the mycoparasitic interaction [35]. Chit102 and chit73 are also induced by GlcNAc together with other chitinases, while chit102 is constitutively secreted by *T. harzianum* under non-inductive conditions at a low level [36]. Fungal chitinase share high amino acid homology with plant chitinases [6];however, chit36 shares a high similarity (87 %) to a putative chitinase from

Streptomyces coelicolor[37]. Most fungal enzymes contain a signal peptide, some contain a chitin binding domain [38], whereas others do not [39]. Based on amino acid sequence comparison with the yest chitinase binding domain, similar domains were claimed to be present in *Rhizopus niveus* and *Rhizopus oligosporus* chitinases. In order to enhance chitinase activity of Chit42, M.Ccarmen *et al.* have produced hybrid chitinases with stronger chitin-binding capacity by fusing to Chit42 a chitinase binding domain from Nicotiana tabacum, Chia chitinase and the cellulose-binding domain from cellobiohydrolase II of *Trichoderma reesei*. The result showed that chimeric chitinases had similar activities towards soluble substrate but higher hydrolytic activity than the native chitinase on high molecular mass insoluble sunsteates such as ground chitin or chitin-rich fungal cell walls [47].

In the marine environment there are many organisms can secrete chitinases and they share only little homology with other chitinases. The chitinolytic system of the maeing bacterium *Vibrio furnissii* has three chitinase, such as designated Exol, Endo I and ExoII. ExoII can hydrolyze p-nitrophenol (PNP)-β-GlcNAc and 4-methylumbelliferone, 7-hydroxy-4-methylcoumarin (MUF)-β-GlcNAc. *Aeromonas hydrophila* has capability of degrading flake-chitin (as opposed to colloidal chitin) and can secrete five chitinase and one β-N-acetylglucosaminidase [41].

From the knowledge above, we know that chitin is a very good resource which is not used up for ever. Hence, studies on chitinase which can catalyze and degrade chitin appear important. So far lots of chitinases from different organisms have been investigated and multiple different chitinase genes have been detected. This can explain why most of the molecular biology research carried out on chitin digestion concentrated on isolating chitinase-encoding genes and classifying them. Chitin digestion is a regulated process and many questions regarding the regulation of this complex process still need to be elucidated. With studying chitinase and chitinase genes deeply, especially how to use chitinase in plant

pest bio-control, we will benefit from them.

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