NEW ASPECTS OF THE ENZYMATIC BREAKDOWN OF CHITIN: A REVIEW

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Abstract

This review focuses on the enzymatic breakdown of chitin, taking into account the latest scientific reports on the activity of lytic polysaccharide monooxygenase (LPMO). Chitin is a natural, abundant polysaccharide of great practical importance in the environment. However, the insolubility in water and the tightly packed crystalline structure of chitin pose a serious obstacle to enzymatic degradation. This substrate can be converted into soluble sugars by the action of glycosidic hydrolases (GH), also known as chitinases. LPMO could prove to be helpful in enzymatic processes that increase the rate of chitin depolymerisation by improving the availability of substrates for chitinases. The unique action of LPMO is based on the ability to catalyse the oxidative cleavage of glycosidic chains present in complex, resistant crystal networks of chitin, and this cleavage facilitates the subsequent action of glycolytic hydrolases.

Keywords: *enzymatic degradation, chitinases, lytic polysaccharide monooxygenases*

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1. Introduction

One of the most abundant biopolymers in nature is chitin. This unbranched polysaccharide composed of β-*N*-acetyl-D-glucosamine molecules linked by β-1-4-glycosidic bonds occurs mainly in shells of crustaceans and the external skeleton of insects, as well as in the cell walls of fungi, moulds and yeasts. Every year, 120,000-200,000 tonnes of chitin are obtained from the waste of processing marine invertebrates [1-3]. Adjacent linear polymer molecules are linked by hydrogen bonds, which determines the great strength of chitin. In nature, it is found in three crystalline forms. α-Chitin is the most stable and common form and is insoluble in water; its polymer chains are arranged antiparallel, which favours the formation of strong hydrogen bonds between them. β-Chitin has polymer chains arranged in parallel, which causes the formation of weaker hydrogen bonds; it shows low affinity for water. γ-Chitin shows a mixed structure [4, 5].

Depending on the origin and method of obtaining chitosan, it is characterised by a different deacetylation degree. Due to the presence of the acetamido group present in the ring of the sugar residue, chitin can be used by microorganisms both as a source of carbon and nitrogen [5]. Chitin is difficult to depolymerise. In addition to chitinases, enzymatic degradation of chitin also involves the recently described lytic polysaccharide monooxygenases (LPMO), which are copper-dependent extracellular enzymes that oxidise β-1,4-glycosidic bonds in degradation-resistant polysaccharides [6, 7].

This review describes the latest scientific data on the chitin-degrading enzymes, considering the characteristics of the microbiological hydrolases and oxidoreductases involved in the degradation of chitin.

2. Occurrence, Substrate Specificity and Classification of Microbiological Chitinase and LPMO

Until now, several hundred different bacterial proteins involved in the chitin hydrolysis process have been characterised. Chitinolytic bacteria inhabit various environments such as the plant rhizosphere, soil, bottom sediments and water reservoirs. The ability to produce chitinases has been reported in bacteria of the genera *Arthrobacter*, *Aeromonas*, *Bacillus*, *Burkholderia*, *Serratia*, *Streptomyces*, *Stenotrophomonas*, *Vibrio* and *Xanthomonas*, among others [1].

Chitinases are widespread enzymes that belong to the subclass of *O*-glycosidic hydrolases (GH). They are concentrated in the 18, 19 and 21 families of GH [8, 9]. Due to the hydrolytic activity of enzymes, long chitin chains made of β-*N*-acetylglucosamine molecules linked by β-1,4-glycosidic bonds are degraded to shorter, water-soluble oligomers, such as chitohexosis, chitotetrosis or chitotriosis. In the next steps, the chitooligosaccharides are hydrolysed to single molecules of β-*N*-acetyl-D-glucosamine [9]. According to the guidelines of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), chitinase (EC.3.2.1.14) is classified based on the position of the attacked glycosidic linkage into two groups: endochitinases, which hydrolyse random glycosidic bonds inside the chitin chain, releasing soluble, low-molecular-weight *N*-acetylglucosamine oligomers, and exochitinases, acting from the non-reducing or reducing end of the chitin molecule and liberating *N*,*N*′-diacetylchitobiose disaccharides (Figure 1) [9].

Reports about unidentified oxidising agents supporting the degradation of polysaccharides appeared in the scientific literature quite early. However, a breakthrough in research on LPMO was the work published by Vaaje-Kolstad *et al*. [10], describing the oxidative catalytic mechanism and the structure of the chitin-specific metalloenzyme from *Serratia marcescens*. It has been shown that this enzyme uses atmospheric oxygen

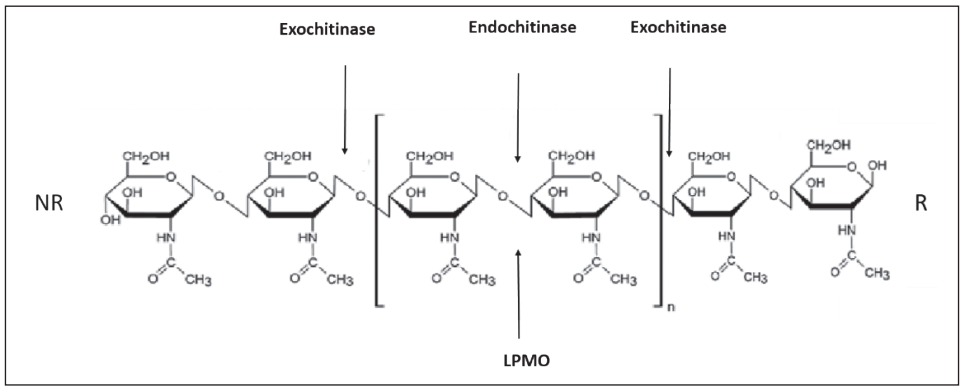

Figure 1. Site of action of chitin-degrading enzymes. Exochitinases degrade glycosidic bonds from the reducing or non-reducing end of the chitin chain. Lytic polysaccharide monooxygenases (LPMO) break chitin chains in the crystalline region by oxidative cleavages, creating new chain ends for exochitinases. Endochitinases randomly hydrolyse glycosidic bonds in the amorphous region of chitin, creating new chain ends for exochitinases. Abbreviations: NR, non-reducing end; R, reducing end of chitin chain

and exogenous reducing agents (e.g. ascorbate) in the reaction [11]. As research progressed, this group of enzymes has been called polysaccharide monooxygenases (PMOs) or LPMO, which oxidise β-1,4-glycosidic bonds in hydrolysis-resistant polysaccharides. Therefore, they support the action of GH [12, 13]. The substrates for LPMO can be soluble and insoluble polysaccharides. These enzymes are active against chitin, cellulose, hemicellulose, cellodextrin and starch. The known LPMO in most cases use both α- and β-chitin as a substrate, but some enzymes from this group prefer only the β-form [14].

LPMO have only been described in a few fungal and bacterial species. They have been included in the CAZy database as a new group of carbohydrate-active enzymes with auxiliary activity (AA) and divided into different substrate specificity and origin of the AA family: 9, 10, 11, 13, 14 and 15. The AA9, AA11, AA13 AA14 and AA15 families are fungal enzymes, while the AA10 family is composed of bacterial enzymes [15, 16]. So far, LPMO active against chitin have been described in, among others, bacteria of the genera *Bacillus*, *Streptomyces* and *Serratia* [10, 17, 18], and in fungi of the genera *Thermobia* and *Fusarium* [19, 20].

3. Structure and Catalytic Mechanism of Chitin-Degrading Enzymes

Hydrolysis of the β-1,4-glycosidic bond in chitin follows the acid catalytic mechanism. Two possible reaction mechanisms have been observed. The first leads to the so-called inversion of the anomeric conformation of the product, so it is opposite compared with the initial one. In the latter case, retention takes place and the product maintains the original conformation of the substrate. Characteristic for chitinases from the GH 18 family is catalysis with conformation retention, which results in the formation of a product in the form of a β-anomer. As a result of the activity of chitinases from the GH 19 family, inversion occurs and the product formed is an α-anomer [21].

Chitinase is modular, with at least two and more often several functional domains. The shuffling of these domains in various microorganisms has led to the biochemical diversity of chitinases, resulting from the variable position in the molecule of several domains: catalytic (CatD), chitin-binding (CHBD), cadherin type (Cadh), fibronectin type

III (FN3) and PKD [22, 23]. CatD plays a major role in the degradation of chitin because it contains the active centre. CatD of the GH 18 family have a (β/α)8-barrel structure, with a longitudinal slit being the active centre of the enzyme. Family 19 enzymes contain α-helical structures resembling those found in lysosomes [24, 25]. ChBD can be located at the N-terminus or C-terminus of the bacterial chitinase and binds to the crystalline or insoluble substrate chitin [26]. The FN3 domain together with the cadherin-type domain plays a role in the degradation of bound chitin. The FN3 domain, like that of the cadherin type, influences the chitin-binding domain and the spatial organisation of the catalytic domain. This part of chitinase mediates protein-protein interactions and performs a structural function by acting as a linker. The FN3 domains are randomly distributed in multiple copies in bacterial glycohydrolases, not only in chitinases but also in cellulases and amylases [27-29]. The exact role of the bacterial PKD domain is unknown, but its similarity to FN3 domain suggests that both domains may perform a similar function [22].

The active site of LPMO contain a copper ion (Cu^{2+}), which is coordinated by the so-called histidine clamp comprising two histidine residues [30, 31]. The active centre of LPMO resembles a pyramid, the base of which, acting as a substrate-binding surface, is composed of aromatic amino acid residues. The catalytic mechanism involves the reduction of Cu(II) to Cu(I) by an external electron donor, resulting in the formation of copper peroxide. This compound participates in the hydroxylation of the carbon atom at the C1 or C4 position of the polysaccharide [32]. During the decomposition of cellulose, carbon is oxidised in the C1 and C4 position, while during the decomposition of chitin, carbon is only oxidised in the C1 position [33]. LPMO synthesised by *Serratia marcescens* (SmCBP21) oxidises the C-H bond on the C1 carbon to C-OH, followed by breakdown of the glycosidic bond and cleavage of the chitin chain. This reaction uses atmospheric oxygen and an external electron donating reducer [16].

4. Conclusions

Chitin, a polymer of *N*-acetylglucosamine molecules linked by β-1,4-glycosidic bonds, is one of the largest natural sources of organic carbon. Mechanically strong chitin is an essential component of the shell structure of crustaceans, insect shells and fungal cell walls. The decomposition products of this polysaccharide in the form of soluble sugars have potential application as an alternative substrate for the production of biofuels. For many years it was believed that only chitinases, hydrolytic enzymes belonging to GH, are involved in the breakdown of chitin. The results of research from the last dozen or so years have shown that the breakdown of β-glycosidic bonds can also take place through oxidation, with the participation of LPMO, which are used in enzyme preparations in biotechnology. These enzymes have proved to be helpful in enzymatic degradation by increasing the rate of chitin depolymerisation, thereby increasing the efficiency of chitinolytic enzymes.

5. References

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