Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

The effect of the carbohydrate binding module on substrate degradation by the human chitotriosidase

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ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form 29 May 2015
Accepted 23 June 2015
Available online xxxx

Keywords:
Glycoside hydrolases
Recalcitrant polysaccharides
Human chitotriosidase
Carbohydrate binding modules
Transglycosylation

ABSTRACT

Human chitotriosidase (HCHT) is one of two active glycoside hydrolase family 18 chitinases produced by humans. The enzyme is associated with several diseases and is thought to play a role in the anti-parasite responses of the innate immune system. HCHT occurs in two isoforms, one 50 kDa (HCHT50) and one 39 kDa variant (HCHT39). Common for both isoforms is a catalytic domain with the $(\beta/\alpha)_8$ TIM barrel fold. HCHT50 has an additional linker-region, followed by a C-terminal carbohydrate-binding module (CBM) classified as CBM family 14 in the CAZy database. To gain further insight into enzyme functionality and especially the effect of the CBM, we expressed both isoforms and compared their catalytic properties on chitin and high molecular weight chitosans. HCHT50 degrades chitin faster than HCHT39 and much more efficiently. Interestingly, both HCHT50 and HCHT39 show biphasic kinetics on chitosan degradation where HCHT50 is faster initially and HCHT39 is faster in the second phase. Moreover, HCHT50 produces distinctly different oligomer distributions than HCHT39. This is likely due to increased transglycosylation activity for HCHT50 due the CBM extending the positive subsites binding surface and therefore promoting transglycosylation. Finally, studies with both chitin and chitosan showed that both isoforms have a similarly low degree of processivity. Combining functional and structural features of the two isoforms, it seems that HCHT combines features of exo-processive and endo-nonprocessive chitinases with the somewhat unusual CBM14 to reach a high degree of efficiency, in line with its alleged physiological task of being a "complete" chitinolytic machinery by itself.

1. Introduction

Chitin is an essential structural component in the exoskeleton of crustaceans, arthropods, and insects, and is also found in the cell walls of certain fungi, algae, and in parasitic nematodes [1]. This insoluble polymer is composed of β(1–4)-linked units of 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc; **A**-unit). Chitosans are a family of water-soluble, linear and binary heteropolysaccharides composed of β(1–4)-linked **A**-units and 2-amino-2-deoxy-β-D-glucopyranose (GlcN, **D**-unit), which can be prepared from chitin by chemical or enzymatic means, yielding varying extents of deacetylation. The metabolism of chitin in nature is controlled by enzymatic systems that produce and break down chitin, primarily chitin synthases and chitinases, respectively.

Humans do not possess chitin. Still they express two active human chitinases (EC 3.2.1.14) that are classified as family 18 glycosyl hydrolases (GH) in the CAZY database (www.cazy.org, [2]), human chitotriosidase (HCHT) and acidic mammalian chitinase (AMCase). Both chitinases are thought to play a role in anti-parasite responses of the innate human immune system [3,4] and they are associated with several diseases. AMCase is expressed in exaggerated quantities in human asthma [5], while HCHT is a biochemical marker of macrophage activation in some lysosomal diseases like Gaucher disease [6]. Furthermore, there are indications that both human chitinases play a role in the response to fungal infections. For example, elevated levels of mammalian chitinases have been reported in guinea pig blood following systemic infection with *Aspergillus fumigatus* [7]. A recombinant form of AMCase has been shown to inhibit fungal growth in vitro [8]. Engraftment of microcapsules containing cells transducted with chitotriosidase gene has

Abbreviations: HCHT, human chitotriosidase; HCHT50, the 50 kDa variant of HCHT; HCHT39, the 39 kDa variant of HCHT; AMCase, acidic mammalian chitinase; GH, glycoside hydrolase; GlcNAc (A), 2-acetamido-2-deoxy-β-D-glucopyranose; GlcN (D), 2-amino-2-deoxy-β-D-glucopyranose; CBM, carbohydrate-binding module; 4-MU, 4-methylumbelliferyl; DP, degree of polymerization; MBTH, 3-methyl-2- benzothiazolinone hydrazine; α, the degree of scission; F_a, fraction of acetylated sugar moieties; N_{cuts}, number of catalytic events before substrate dissociation

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http://dx.doi.org/10.1016/j.bbapap.2015.06.008
1570-9639/

the potential to combat infections caused by chitinous pathogens through the prolonged delivery of recombinant chitotriosidase [9].

HCHT is known to exist in two isoforms, one 50 kDa and one 39 kDa variant, hereafter referred to as HCHT50 and HCHT39. HCHT50 is synthesized and secreted in human macrophages. A portion of the produced enzyme is further routed to lysosomes where it is converted to HCHT39 isoform by carboxyl-terminal proteolytic processing [10]. Common for both isoforms is a catalytic domain with the $(\beta/\alpha)_8$ TIM barrel fold that is characteristics for chitinases belonging to the GH18 family (Fig. 1). HCHT50 has an additional proline-rich linker region, comprising approximately 29 residues, followed by a C-terminal carbohydrate-binding module (CBM) [10]. This CBM, consisting of 49 amino acids, belongs to the CBM14 family according to the CAZy database (www.cazy.org, [2,11]). At the time being only two NMR structures are solved (pdb codes 1dqc, Fig. 1, and 2mfk) and little is known about this particular CBM. The CBM has been shown to both interact with chitin, i.e. fungal cell walls, as well as chito-oligosaccharides [12,13]. A typical feature for this CBM is the presence of 6 conserved cysteine residues that are able to form three disulfide bonds [14]. It also appears that the residues of Cys, Pro, and Gly, all which have significant influence in the structural constructions, are well conserved in both tachycitin and HCHT50 [15].

The catalytic domain of HCHT belongs to family 18 of the glycoside hydrolases. A common feature for this family is that it employs a substrate-assisted catalytic mechanism that involves the *N*-acetyl group of the sugar moiety bound in the −1 subsite. The *N*-acetyl group acts as the catalytic nucleophile and its attack on the anomeric carbon results in the formation of an oxazolinium ion reaction intermediate [16–20]. A nucleophilic attack of a water molecule on the oxazolinium ion reaction intermediate completes the hydrolytic reaction in what is referred to as the deglycosylation step. In principle, a chitin fragment could replace the water molecule in the deglycosylation step and the outcome of the reaction is a tranglycosylation, rather than hydrolysis. GH18 chtinases vary in terms of their tendency to catalyze transglycosylation, a variation that has been attributed to variation in both negative and positive subsites, as well as variation in the catalytic machinery [21–23]. Notably, due to the substrate-assisted catalytic mechanism, substrate binding to family 18 chitinases is only productive if subsite −1 is occupied by an acetylated sugar.

GHs can cleave polymeric substrates at the chain ends (exo-action) or at random positions (endo-action) [24]. Each of these modes of action can also be combined with processivity. Processive GHs tend to have long and deep substrate binding clefts or even tunnels lined with aromatic amino acids [25–29]. The general idea is that processivity improves catalytic efficiency by keeping the enzyme closely associated to the substrate in between subsequent hydrolytic reactions [29].

Although HCHT seems to play a role in several diseases [30], relatively little is known about its physiological role, functional properties, and the effect the CBM14 has on substrate degradation. To gain further insight into enzyme functionality, we have expressed both forms of the chitotriosidase and compared their catalytic properties, including the degradation rates and conversion efficiencies on both soluble and non-soluble substrates and the degree of processivity.

2. Experimental

2.1. Protein expression and purification of the two isoforms of human chitotriosidase

For production in HEK293-6E cells two vectors were constructed designated pHCHT50 and pHCHT39 expressing HCHT including its native signal peptide and with and without the C-terminal chitin binding

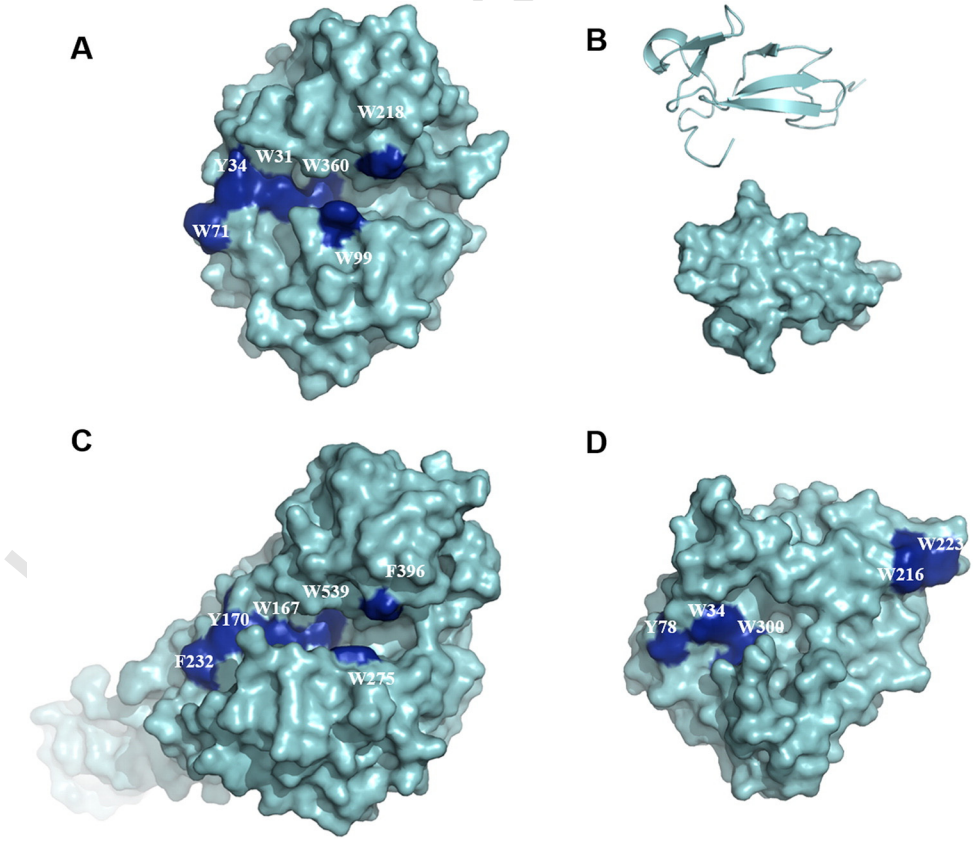

Fig. 1. Structures of HCHT39 (A, [57], pdb code 1guv), tachycitin, an example of CBM family 14 (one of only two available structures of CBM14) (B, [15], pdb code 1dqc), ChiA (C, [63], pdb code 1ctn), and ChiC (D, [66], pdb code 4axn). Both HCHT and ChiA have solvent-exposed aromatic amino acid motif in the active site cleft, which are highlighted in blue. The active site openness of HCHT is intermediate to that of ChiA and the nonprocessive endo active ChiC.

Please cite this article as: L.W. Stockinger, et al., The effect of the carbohydrate binding module on substrate degradation by the human chitotriosidase, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbapap.2015.06.008