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Analysis of productive binding modes in the human chitotriosidase

Kristine Bistrup Eide, Anne Rita Lindbom, Vincent G.H. Eijsink, Anne Line Norberg, Morten Sørlie *

Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, PO 5003, N-1432 Ås, Norway

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ABSTRACT

Human chitotriosidase (HCHT) is a family 18 chitinase that is an innate part of the immune system. We have mapped preferred productive binding modes of chito-oligosaccharide substrates to HCHT and the data show that HCHT has strong binding affinity in the +3 subsite. Moreover, HCHT shows anomer-specific binding affinities in subsites +2 and +3. These features could endorse HCHT with higher endo-activity and a higher transglycosylation potential.

1. Introduction

Chitin, a crystalline polymer consisting of β-1–4 linked *N*-acetyl glucosamine units, is an abundant structural polysaccharide appearing in exoskeleton of arthropods and the cell wall of fungi [1]. Humans do not possess chitin, but do have active chitinases referred to as Acidic Mammalian Chitinase (AMCase; [2]) and Human Chitotriosidase (HCHT; [3]) (E.C numbers: 3.2.1.14), that are believed play roles in anti-parasite responses [4–6]. HCHT is synthesized and secreted as a 50-kDa two-domain protein in human macrophages. A considerable amount of produced enzyme is routed to lysosomes and processed into a 39-kDa isoform, lacking the C-terminal chitin binding domain.

The two human chitinases share considerable sequence and structural similarity, but current literature data indicate several (possible) functional differences. AMCase has a high acid tolerance which has been ascribed to the presence of His^{208}, His^{269}, and Arg^{145} near the catalytic residues, where HCHT has Asn^{208}, Arg^{269}, and Gln^{145} [7]. It has been suggested that HCHT is acting as an endo-enzyme, whereas AMCase probably primarily acts as an exo-enzyme [2,8–11]. Finally, there are indications that HCHT has a particularly high transglycosylation activity [12]. Endo-activity would benefit from an extended substrate binding cleft with more than two subsites on each site of the catalytic center where cleavage takes place. High oligosaccharide affinity in multiple positive subsites have been shown to be beneficial for transglycosylation activity [13].

While the structures of AMCase [7] and HCHT [14] are known, there are no structures of complexes with longer ligands that could provide comprehensive insight into the subsite structure of the enzymes. The presence of stretches of aromatic residues, including a conserved Trp-Tyr-Trp-Trp motif spanning an area corresponding to what could be subsites −6 to −1 and a Trp-Trp motif in the +1 and +2 subsites (using the nomenclature proposed by Davies et al. where subsites are labeled from −n to +n, −n represents the non-reducing end and +n the reducing end, and with cleavage taking place between the −1 and +1 subsites. [15]) suggests extended substrate-binding clefts in both enzymes (Fig. 1). A similar aromatic motif is found in the well studied ChiA of *Serratia marcescens* for which structural data have shown that a chito-octamer binds from −6 to +2 [16], and substrate degradation studies have shown that the polymer is degraded from the non-reducing end towards the reducing end [17,18]. Moreover, comparisons of thermodynamic signatures of ligand binding between HCHT and ChiA suggest that HCHT degrades chitin with the same directionality as ChiA [10,19]. This suggests that +1 and +2 are product release subsites in ChiA and HCHT in processive hydrolysis of polymeric chitin. Processive mechanism has been observed for both ChiA and HCHT [11,20]. The situation beyond subsite +2 for HCHT, AMCase, and ChiA, is less clear. Still, chito-oligosaccharide degradation by ChiA clearly show substrate binding affinity in a subsite +3 that has been linked to its endo-activity [21].

* Corresponding author. Fax: +47 64965901.
E-mail address: morten.sorlie@umb.no (M. Sørlie).

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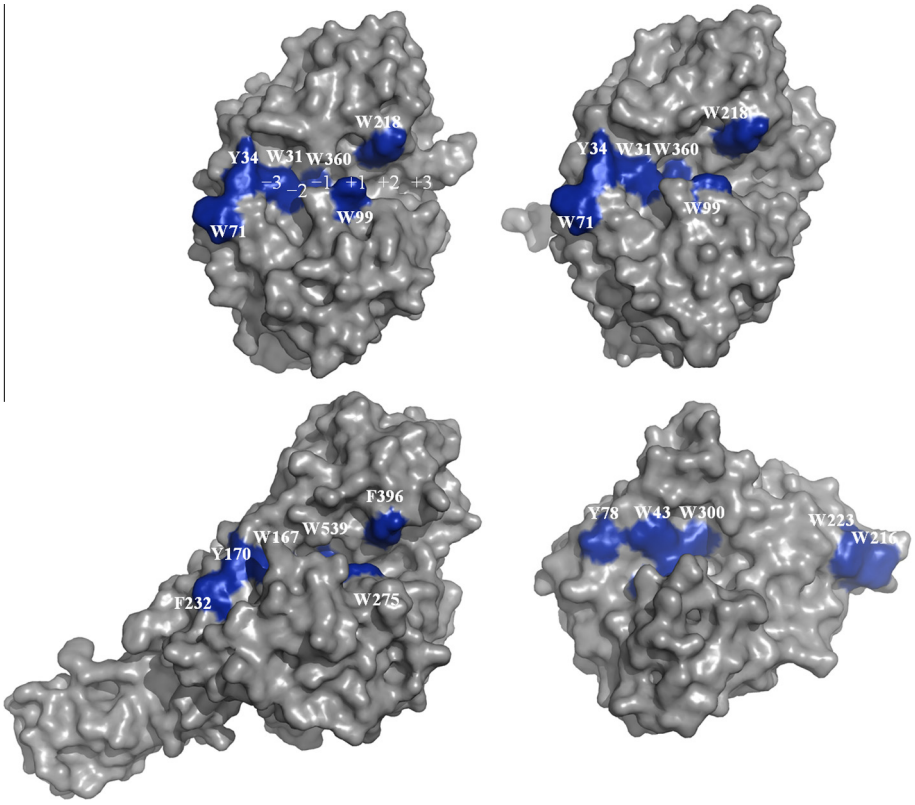

Fig. 1. Aligned crystal structures of HCHT, (top left [9], 1guv) AMCase, (top right [7], 3fxy), ChiA from *S. marcescens* (bottom left [36], 1ehn), and ChiC (bottom right [30], 4axn). Aromatic amino acids lining the substrate-binding cleft and known to be important determinants of enzyme properties [37,38] are colored blue. Individual subsites are marked for HCHT.

In this work, we have investigated productive binding of chito-oligossaccharides to assess potential substrate affinity in positive subsites outside of the substrate binding cleft in HCHT.

2. Materials and methods

2.1. Chemicals

N-Acetylated chito-oligosaccharides were purchased from Seikagaku (Tokyo, Japan). Purified bovine serum albumin (BSA) was purchased from New England Biolabs (Ipswich, MA, USA). Dihydroxy benzoic acid (DHB), and all other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. HCHT expression and purification

The HCHT gene was expressed in *Pichia pastoris* and purified as described previously [11].

2.3. Degradation of N-acetylated-glucosamine oligomers

$(GlcNAc)_4$ to $(GlcNAc)_6$ were dissolved to a concentration of 100 μM in 50 mM sodium acetate buffer pH 4.2 containing 0.1 mg ml^{-1} BSA. HCHT was added to a concentration between 1 nM and 25 nM depending on the substrate. Hydrolysis reactions were run in a shaking water bath at 37 °C. Aliquots where withdrawn at several time points and enzyme activity was quenched by adding an equal volume of 100% acetonitrile.

2.4. High performance liquid chromatography (HPLC)

Immediately after quenching, the reaction products were analyzed by HPLC using an Amide 80 column (Tosoh Bioscience, Montgomeryville, PA, USA) coupled to a Gilson Unipoint HPLC system (Gilson; Middleton, WI, USA). The reaction products were eluted isocratically at 0.7 ml/min with 70% acetonitrile at room temperature. UV absorbance of the chito-oligosaccharides was measured at 210 nm and quantified by measuring peak areas. Chromatograms were collected and analyzed using the Chromeleon software (Dionex, Germiering, Germany). Peak areas were compared to peak areas obtained by use of standard samples with known concentrations of chitooligosaccharides. Peak areas versus concentration of oligosaccharides produced a linear plot in the concentration range measured in this work. To minimize mutarotation of α/β anomers of the reaction products the samples were analyzed immediately after quenching.

2.5. Initial hydrolysis of $(GlcNAc)_5$ in $H_2^{18}O$

To determine substrate positioning in the active site of HCHT initial hydrolysis assays of $(GlcNAc)_5$ were performed in $H_2^{18}O$ (Larodan Fine Chemicals, Malmö, Sweden). The hydrolysis were executed at 37 °C and 600 rpm in $H_2^{18}O$ containing 0.05 M NaAc (pH 4.2), 300 μM $(GlcNAc)_5$ and 25 nM HCHT. Aliquots were withdrawn at several time points within 120 s. The reactions were quenched by mixing with DHB and then spotted directly on the MALDI target.

As a control experiment $(GlcNAc)_5$ were incubated without the enzyme at 37 °C and 600 rpm to assess the rate of nonenzymatic incorporation of ^{18}O at the anomeric center. This experiment showed no incorporation of ^{18}O within 120 s.

2.6. Matrix assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry

Immediately after quenching the reaction with acetonitrile, 1 μl of the resulting solution was mixed with 1 μl matrix solution