FEBS Letters 589 (2015) 1807–1812

FEBS Letters

journal homepage: www.FEBSLetters.org

The directionality of processive enzymes acting on recalcitrant polysaccharides is reflected in the kinetic signatures of oligomer degradation

Anne Grethe Hamre, Daniel Schaupp, Vincent G.H. Eijsink, Morten Sørlie *

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

ARTICLE INFO

Article history:
Received 2 January 2015
Revised 17 April 2015
Accepted 20 May 2015
Available online 28 May 2015

Edited by Judit Ovadi

Keywords:
Processivity
Kinetic
Glycoside hydrolase
Recalcitrant polysaccharide

ABSTRACT

The enzymatic degradation of the closely related insoluble polysaccharides; cellulose (β(1–4)-linked glucose) by cellulases and chitin (β(1–4)-linked N-acetylglucosamine) by chitinases, is of large biological and economical importance. Processive enzymes with different inherent directionalities, i.e. attacking the polysaccharide chains from opposite ends, are crucial for the efficiency of this degradation process. While processive cellulases with complementary functions differ in structure and catalytic mechanism, processive chitinases belong to one single protein family with similar active site architectures. Using the unique model system of *Serratia marcescens* with two processive chitinases attacking opposite ends of the substrate, we here show that different directionalities of processivity are correlated to distinct differences in the kinetic signatures for hydrolysis of oligomeric tetra-N-acetyl chitotetraose.

1. Introduction

Chitin, a β-1,4-linked linear polymer of N-acetyl glucosamine (GlcNAc), and cellulose, comprised of β-1,4-linked glucose, are the two most abundant biopolymers in Nature with an annual production amounting to 100 billion and one trillion tons respectively [1,2]. Thus, these polymers are an almost unlimited source of raw material for environmentally friendly and biocompatible products. The enzymatic degradation of these recalcitrant polysaccharides is therefore of great biological and economical importance.

Enzymes catalyzing the hydrolysis of *O*-glycosidic bonds between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety are called glycoside hydrolases (GHs) (www.cazy.org; [3]). The enzymatic hydrolysis of glycosidic bonds requires a proton donor and a nucleophile/base and leads to either retention or inversion of the stereochemistry on the anomeric oxygen at C1 [4–6]. Moreover, enzymes acting on polysaccharides can have different modes of action. Endo-acting enzymes randomly cleave the polymer chains, whereas exo-acting enzymes have a preference for acting from either the reducing or the non-reducing chain end [4]. Both endo and exo mechanisms can be combined with processive action meaning that the enzyme hydrolyzes a series of glycosidic linkages along the same polymer chain producing dimeric products before dissociation. In order to bind to and guide the insoluble substrate through the active site cleft, many GHs have a path of solvent exposed aromatic residues leading from a carbohydrate binding domain to the active site cleft [7–12]. It has been suggested that these residues function as a flexible and hydrophobic sheath along which the polymer chain can slide during the processive mode of action [13,14].

There are 21 different GH families that contain one or more cellulose degrading enzymes. Most of these cellulases are classified into GH family 5, 6, 7, 8, 9, 12, 44, 45 and 48 [3,15]. Processive exo-acting cellulases are found in families 6, 7, and 48 [16]. Families 7 and 48 contain exo-cellulases moving from the reducing end using the retaining mechanism. Exo-cellulases moving in the opposite direction are found in family 6 and use the inverting mechanism [3,17]. Some processive endo-cellulases belonging to families 5 and 9 have recently been discovered [18,19].

Chitinases occur in GH families 18 and 19, and family 18 chitinases are thought to be Nature's primary instrument for degradation of recalcitrant chitinous biomass. Interestingly, while all GH18 enzymes use the same retaining substrate-assisted catalytic mechanism [20–22], members of the GH18 family differ in terms of endo versus exo-activity, processive versus non-processive action, and the directionality of processivity [7,23–26]. A specific example is

Author contributions: Sørlie and Eijsink planned project and experiments. Hamre and Schaupp executed experiments, Sørlie, Eijsink, Hamre, and Schaupp wrote the manuscripts.

* Corresponding author.
E-mail address: morten.sorlie@nmbu.no (M. Sørlie).

http://dx.doi.org/10.1016/j.febslet.2015.05.034
0014-5793/

the chitinolytic machinery of *Serratia marcescens* that includes three well-characterized GH18 chitinases [24]. Chitinase A (ChiA) is processive and moves toward the non-reducing end, while chitinase B (ChiB) also is processive but moves toward the reducing end (Fig. 1) [26]. Chitinase C (ChiC) is a less processive endo-acting enzyme [27,28]. The two processive chitinases have aromatic residues in their +1 and +2 subsites. In ChiB, these subsites interact with the substrate during processive hydrolysis while in ChiA the product of a processive hydrolysis, chitobiose, is displaced from these subsites (Fig. 1). In this study, we show, by kinetic analyses of site-directed mutants in subsite +1 and +2 in ChiA and ChiB, that different directionalities of processivity are correlated to distinct differences in the kinetic signatures for hydrolysis of oligomeric tetra-*N*-acetyl chitotetraose.

2. Materials and methods

2.1. Chemicals

Chito-oligosaccharides were obtained from Megazyme (Wicklow, Ireland). All other chemicals were of analytical grade.

2.2. Protein expression and purification

The chitinases used were from *S. marcescens* strain BJL200 [29,30]. ChiA-F396A and ChiA-W275A genes were expressed in *Escherichia coli* as described previously [25]. For protein purification, periplasmic extracts were loaded on a column packed with chitin beads (New England Biolabs) equilibrated in 50 mM Tris–HCl pH 8.0. After washing the column with the same buffer, the enzymes were eluted with 20 mM acetic acid. The buffer was then changed to 100 mM Tris–HCl pH 8.0 using Amicon Ultra-Centrifugal filters (Millipore). Enzyme purity was verified by SDS–PAGE and estimated to be > 95%. Protein concentrations were determined by using the Bradford Protein Assay from Bio-Rad.

2.3. Kinetic analysis

The kinetic constants k_{cat} and K_m of the ChiA mutants were determined essentially as described previously [31,32]. In each experiment, 8–10 different $(GlcNAc)_4$ concentrations varying from 2 to 200 μM in 20 mM sodium acetate buffer, pH 6.1 and 0.1 mg/ml BSA were pre-incubated in 10 min at 37 °C in an Eppendorf thermo mixer at 800 rpm before the reactions were started by adding purified enzyme to the reactions. Final enzyme concentrations were 1 nM for ChiA-W275A and 0.5 nM for ChiA-F396A. Seven samples of 75 μl were withdrawn at regular time intervals up to 20 min, and the enzyme was inactivated by adding 75 μl 20 mM H_2SO_4. At such mildly acidic conditions and short time intervals before analysis, there are no significant acid catalyzed hydrolysis in line with the work of Einbu and Vårum where such rate constant has been found to be 1.5×10^{-4} s^{-1} in concentrated acid (12 M) [33]. Prior to HPLC analysis, all samples were filtrated through a 0.45 μm Duapore membrane (Millipore) to remove denaturated protein. All samples were stored at −20 °C until HPLC analysis.

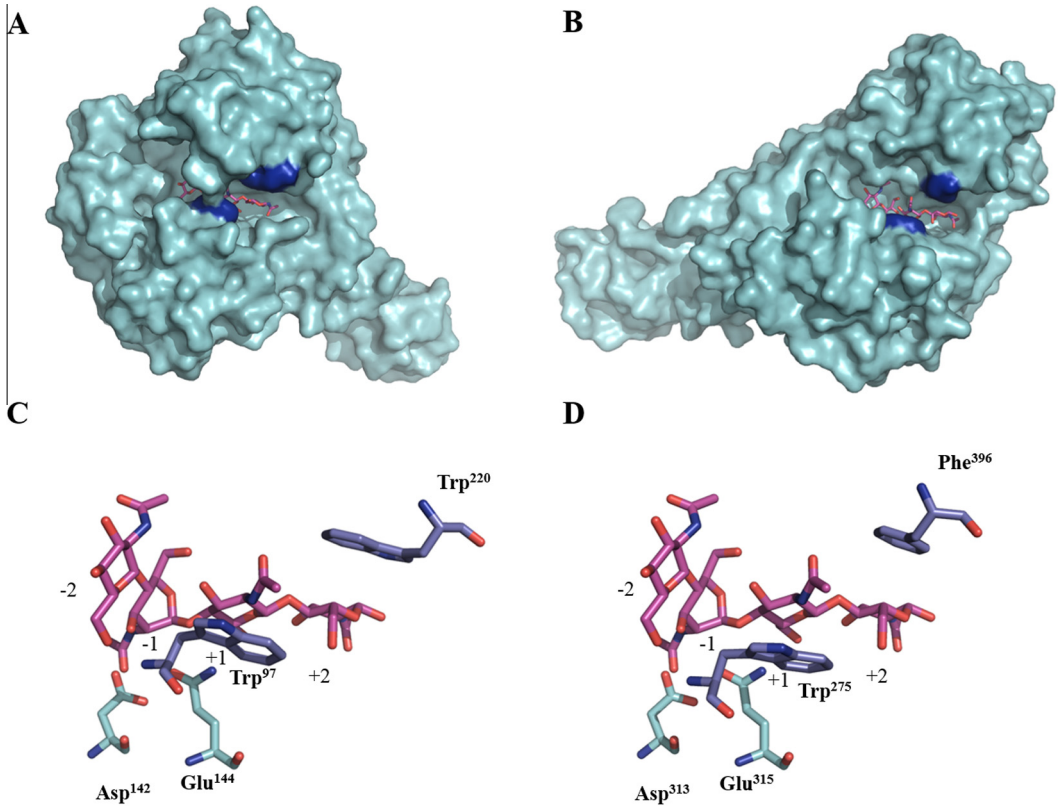

Fig. 1. Enzyme–substrate interactions for ChiA and ChiB. Panel A and C show the structure of exo-processive ChiB (PDB ID code 1e6n, [22]) that degrades chitin from the non-reducing end. Panels B and D show the structure of exo-processive ChiA (PDB ID code 1ehn, [45]) that degrades chitin from the reducing end. Panels A and B show surface representations of the complete protein; the surface-exposed aromatic amino acids in subsites +1 and +2 are highlighted in blue, whereas crystallographically observed substrate molecules are shown in magenta. Both chitinases contain a carbohydrate-binding module, a CBM5/12 pointing to the right in ChiB and a FnIII domain pointing to the left in ChiA (for more details, see Vaaje-Kolstad et al., 2013 [24]) (C) Close up of the active site of ChiB. Asp^{142} and Glu^{144} are part of the diagnostic DXDXE motif containing the catalytic acid/base (Glu144). (D) Close up of the active site of ChiA.