FEBS Letters 588 (2014) 4620–4624

journal homepage: www.FEBSLetters.org

Enzyme processivity changes with the extent of recalcitrant polysaccharide degradation

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ARTICLE INFO

Article history:
Received 25 August 2014
Revised 16 October 2014
Accepted 16 October 2014
Available online 5 November 2014

Edited by Peter Brzezinski

Keywords:
Processivity
Recalcitrant polysaccharides

ABSTRACT

Glycoside hydrolases depolymerize polysaccharides. They can subtract single carbohydrate chains from polymer crystals and cleave glycosidic bonds without dissociating from the substrate after each catalytic event. This processivity is thought to conserve energy during polysaccharide degradation. Herein, we compare the processivity of components of the chitinolytic machinery of *Serratia marcescens*. The two processive chitinases ChiA and ChiB, the ChiB-W97A mutant, and the endochitinase ChiC were analyzed for the extent of degradation of three different chitin substrates. Moreover, enzyme processivity was assessed on the basis of the [(GlcNAc)2]/[GlcNAc] product ratio. The results show that the apparent processivity (Papp) greatly diminishes with the extent of degradation and confirm the hypothesis that Papp is limited by the length of obstacle free path on the substrate.

1. Introduction

To understand the mechanisms behind enzymatic hydrolysis of recalcitrant polysaccharides such as cellulose (a 1,4-β-linked polymer of D-glucose) and chitin (a 1,4-β-linked polymer of *N*-acetyl-D-glucosamine (GlcNAc) (Fig. 1) is of great biological and economic importance. Enzymes acting on cellulose or chitin face the challenges of associating with the insoluble substrate, disrupting the crystal packing, and guiding a single polymer chain into the catalytic center. Many polymer active enzymes act in a processive manner meaning that they bind individual polymer chains in long tunnels or deep clefts and hydrolyze a series of glycosidic linkages along the same chain before dissociation [1–5]. The general idea is that catalytic efficiency is improved by keeping the enzyme closely associated to the substrate in between subsequent hydrolytic reactions. In the case of crystalline substrates, calculations show that the enzymes face a free energy penalty of 5.6 kcal/mol pr. chitobiose unit and 5.4 kcal/mol pr. cellobiose unit in decrystallization energies signifying the importance for processive enzymes being capable of keeping once-detached single chains from re-associating with the insoluble material [3,5–8].

Intrinsic processivity of polymer active enzymes is governed by the rate constants k_{off} and k_{cat} meaning that it is the enzyme dissociation from the polymer chain and the catalytic constant that is important. This is summarized in the formula $P^{Intr} = (k_{cat} + k_{off})/k_{off}$ [9,10]. P^{Intr} values estimated for cellobiohydrolases are in the range of 1000 whereas the measured values of processivity, also referred to as apparent processivity (P_{app}), are more than an order of magnitude lower [9,11]. These findings have led to the hypothesis that P_{app} (number of cleavages per one productive binding event) is limited by the length of obstacle free path on the substrate [9,11–13]. Because processive enzymes have intrinsically low k_{off} values the encounter of an obstacle will cause an enzyme to halt. This limits the rate of enzyme recruitment [12] and causes so called traffic jams [14,15] on the polymer surface thus slowing the overall rate of polymer degradation. To test the possible correlation between the rate of polysaccharide degradation and P_{app}, we studied the changes of P_{app} upon a large range of the degree of chitin conversion. The well-characterized chitinolytic machinery of *Serratia marcescens* that contains two processive chitinases (ChiA and ChiB) and an endochitinase (ChiC) [16] was used. Chitin is an insoluble and heterogenous substrate, and therefore it is challenging to determine the processivity quantitatively [17]. The simplest way to measure P_{app} is to follow the profile of soluble products from hydrolysis. It has been proposed that the first cleavage from a polymer chain end will result in the release of an odd numbered oligosaccharide (e.g. mono- or trisaccharide) whereas all subsequent processive cleavages result in the release of disaccharides (Fig. 2). Assuming that the first product is a trisaccharide that is subsequently hydrolyzed to a mono- and a disaccharide the P_{app} is given by P_{app} = [disaccharide]/[monosaccharide]. This approach has several pitfalls, like the assumption of the exclusive formation of

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http://dx.doi.org/10.1016/j.febslet.2014.10.034

odd numbered oligosaccharides from the first cleavage which may not hold as different enzymes may have different preferences for the orientation of the chain end relative to the polymer surface or different probability of endo-mode initiation. Despite these possible pitfalls, a recent high speed atomic force microscopy (HS AFM) study has revealed a good consistency between P_{app} values of cellobiohydrolases measured using product profiles and HS AFM results [18]. However, one may speculate that the fraction of enzymes performing very short runs on the polymer is underestimated in single molecule tracking studies leading to an overestimate of the degree of processivity. Here we quantified the soluble hydrolytic products $(GlcNAc)_2$ and GlcNAc in hydrolysis of chitin by *S. marcescens* chitinases and used their ratio as a measure of P_{app}. Although the absolute values of P_{app} should be treated with caution our results demonstrated the drastic reduction of P_{app} with the degree of chitin conversion.

2. Materials and methods

2.1. Chemicals

Chitooligosaccharides were obtained from Megazyme (Wicklow, Ireland). Squid pen β-chitin was purchased from France Chitin (180 μm microparticulate, Marseille, France) and α-chitin was purchased from Yaizu Suisankagaku Industry (Tokyo, Japan) and was sheared using a converge mill to a crystallinity of 74% as described by Nakagawa et al. [19]. All other chemicals were of analytical grade.

2.2. Protein expression and purification

The ChiA and ChiB [20], the ChiC [21], and ChiB-W97A [8] genes were expressed in *Escherichia coli* as described previously. The 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.

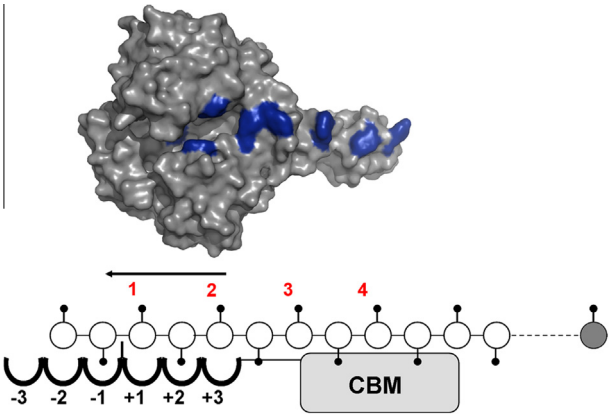

Fig. 2. Crystal structure of ChiB (top) and a schematic picture of ChiB in complex with a single chitin chain. Highlighted in blue are surface exposed aromatic amino acids that stacks with sugar moieties (being individual subsites). The glycosidic bond between the sugar residues in subsite −1 and +1 is enzymatically cleaved. A correctly positioned N-acetyl group (shown as sticks) in the −1 subsite is essential for the substrate-assisted catalysis. Due to that the smallest structural unit of chitin is a disaccharide, the product of repeated processive enzymatic actions will be dimers, $(GlcNAc)_2$. Monomers, GlcNAc, originate from initial productive binding when the sugar in the non-reducing end occupies a subsite with an odd number. For these reasons, a high ratio of $[(GlcNAc)_2]/[GlcNAc]$ indicates a high degree of apparent processivity.

2.3. Degradation of chitin

Hydrolysis of chitin (2.0 mg/ml) was carried out in 50 mM sodium acetate buffer at pH 6.1. The chitin samples were sonicated for 20 min in a sonication bath (Transsonic, Elma) to increase the surface of the substrate and thereby increase the availability of

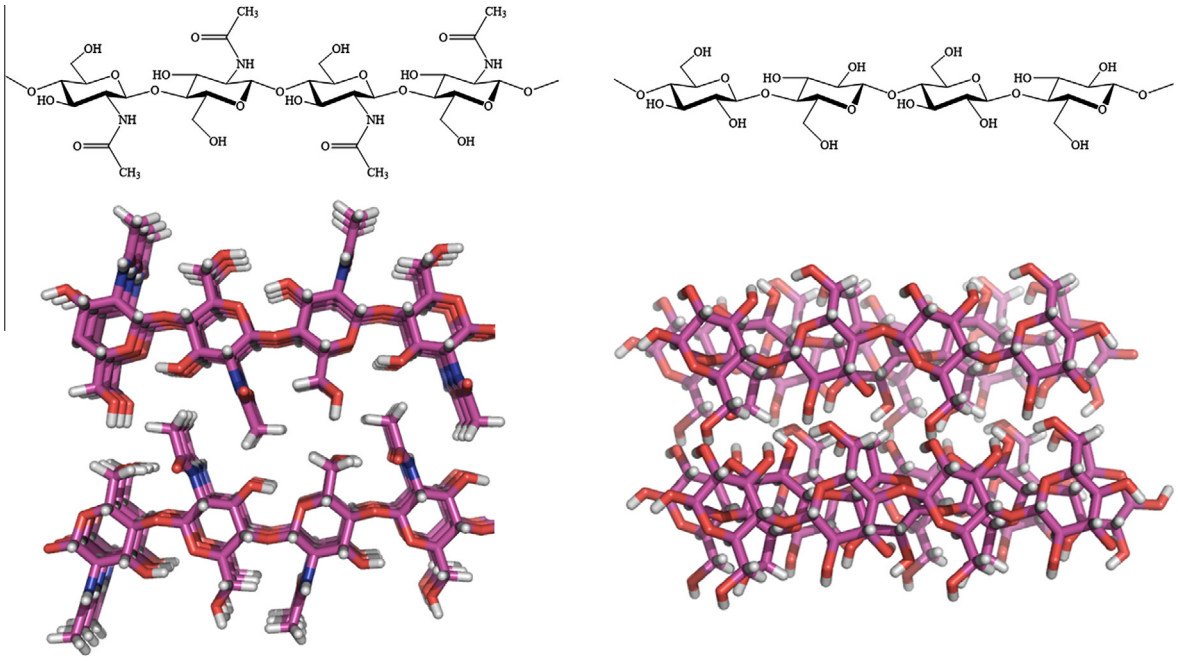

Fig. 1. Left: Chemical structure of chitin and how it stacks in an α-chitin polymer crystal structure. Right: Chemical structure of cellulose and how it stacks in a cellulose II polymer crystal structure [30]. Both chitin and cellulose have the sugar units rotated 180° relative to their neighboring residues, so that the smallest structural unit is a disaccharide.