FEBS Letters 584 (2010) 4063–4068

journal homepage: www.FEBSLetters.org

Inverting character of family GH115 α-glucuronidases

Katarína Kolenová, Olena Ryabova, Mária Vršanská, Peter Biely *

Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 1 June 2010
Revised 21 July 2010
Accepted 19 August 2010
Available online 7 September 2010

Edited by Judit Ovádi

Keywords:
α-Glucuronidase
Inverting glycoside hydrolase
^{1}H NMR spectroscopy
Pichia stipitis
Schizophyllum commune

ABSTRACT

α-Glucuronidases of glycoside hydrolase family 115 of the xylose-fermenting yeast *Pichia stipitis* and wood-destroying fungus *Schizophyllum commune* liberate 4-*O*-methyl-D-glucuronic acid residues from aldouronic acids and glucuronoxylan. The specific activities of both enzymes depended on polymerization degree of the acidic xylooligosaccharides and were inhibited by linear β-1,4-xylooligosaccharides. These results suggest interaction of the enzyme with several xylopyranosyl residues of the xylan main chain. Using ^{1}H NMR spectroscopy and reduced aldopentaouronic acid (MeGlcA^{3}Xyl$_{4}$-ol) as a substrate, it was found that both enzymes are inverting glycoside hydrolases releasing 4-*O*-methyl-D-glucuronic acid (MeGlcA) as its β-anomer.

1. Introduction

Wood and agricultural residues represent a large reservoir of renewable biomass. The main structural polysaccharides in this material are cellulose and xylan. Xylan constitute up to 35% of the dry weight therefore its degradation to fermentable xylose is essential for economic conversion of biomass to value-added products including biofuels [1,2]. Xylan consists of a main chain of β-1,4-linked xylopyranosyl residues that can be substituted by α-1,2-linked glucuronic or 4-*O*-methyl-D-glucuronic acid (MeGlcA), α-1,3-linked arabinofuranose and esterified by acetic acid at positions 2 and 3. Arabinofuranosyl residues of cereal arabinoxylans are partially esterified by cinnamic acids at position 5 [3]. Complete degradation of such heteropolysaccharide requires cooperation of several enzymes. Endo-β-1,4-xylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37) cleave the main chain of xylan, while α-arabinofuranosidase (EC 3.2.1.55), α-glucuronidase (EC 3.2.1.139), acetylxylan esterase (EC 3.1.1.72) and feruloyl esterase (EC 3.1.1.73), so called accessory xylanolytic enzymes, remove the main chain substituents [4]. The accessory xylanolytic enzymes can be divided into two groups. Enzymes of the first group liberate side chains only from short branched oligosaccharides generated by endoxylananses. Enzymes of the second group attack both polymeric and oligomeric substrates.

α-Glucuronidases assigned to glycoside hydrolase (GH) family GH67 [5,6] belong to the first group. They liberate only uronic acids linked to the terminal non-reducing xylosyl residues of xylooligosaccharides. The members of a recently established family of α-glucuronidases GH115 differ from the α-glucuronidases of family GH67 by the ability to release MeGlcA linked not only to the non-reducing terminal xylopyranosyl residues, but also those linked to the internal xylosyl residues [5,7]. To date, only two members of this family have been purified and partially characterized; α-glucuronidase of *Schizophyllum commune* (*Sc*Agu115) [8] and α-glucuronidase of *Pichia stipitis* (*Ps*Agu115) [7]. From polymeric glucuronoxylan these enzymes were reported to release 50% and 75% of the total MeGlcA present in polymer, respectively. In this work we present further characterization of these two enzymes of the new GH115 family, focusing on their action on a series of aldouronic acids differing in the length and on the stereochemistry of hydrolysis of the glycosidic linkage.

2. Materials and methods

2.1. Enzymes

α-Glucuronidase of *P. stipitis* CBS 6054 was purified as described earlier [7]. α-Glucuronidase of *S. commune* ATCC 38548 was purified from a cellulose-spent medium [9] according to Tenkanen and Siika-aho [8], with one modification: the last step of purification was done on Superose 12 instead of Sephacryl S-100 HR. Both purified enzymes were concentrated in 50 mM acetate buffer (pH

Abbreviations: MeGlcA, 4-*O*-Methyl-D-glucuronic acid; GH, Glycoside hydrolase

* Corresponding author. Fax: +421 2 59410222.
E-mail addresses: chempbsa@savba.sk, chemptk@savba.sk (P. Biely).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.08.031

5) using Microcon centrifugal filter device (Millipore, Bedford, MA, USA) with10 kDa cut-off membrane. Both enzymes were stored at −20 °C until use.

2.2. Substrates

Beechwood 4-*O*-methyl-D-glucuronoxylan (glucuronoxylan) was isolated from sawdust [10]. Aldopentaouronic acid MeGlcA^{3}Xyl$_4$ was produced by *Thermomyces lanuginosus* as described earlier [11]. Aldotetraouronic acid with MeGlcA attached to the non-reducing xylopyranosyl residue of xylotriose, MeGlcA^{3}Xyl$_3$, was obtained by hydrolysis of glucuronoxylan by endo-β-1,4-xylanase of *Thermoascus aurantiacus* [12]. Aldohexaouronic acid MeGlcA^{3}Xyl$_5$ was prepared by a partial hydrolysis of glucuronoxylan by endo-β-1,4-xylanase of *Trichoderma reesei* and separated by a paper chromatography [13]. Aldotetraouronic acid MeGlcA^{2}Xyl$_3$ and aldotriouronic acid MeGlcA^{2}Xyl$_2$ were prepared from aldopentaouronic acid MeGlcA^{3}Xyl$_4$ and aldotetraouronic acid MeGlcA^{3}Xyl$_3$, respectively, by the action of Xyn5A of *Erwinia chrysanthemi* [14]. Acidic oligosaccharides were purified from the reaction mixtures by applying on a Dowex 1 column (acetate form). Neutral saccharides were washed out from the column by water and acidic oligosaccharides were released by 3 M acetic acid. The acetic acid was eliminated by evaporation in vacuo. Reduced aldopentaouronic acid, MeGlcA^{3}Xyl$_3$-Xylitol, was prepared by $NaBH_4$ reduction. Aldopentaouronic acid (0.25 mmol) in 12 ml of 0.1 M NaOH was treated with 1 mmol of $NaBH_4$ overnight. After the solution was decationized on a Dowex 50 column (H^+ form) and evaporated three times with methanol to remove boric acid, the product was isolated using a Dowex 1 column (acetate form) as described above.

2.3. Activity assay

Specific activity was determined by measuring the liberation of MeGlcA from aldouronic acids (10 mM) or beechwood glucuronoxylan (2%). Released MeGlcA was quantified by the colorimetric method of Milner and Avigad [15]. Enzyme samples in 50 mM acetate buffer pH 5 (0.52 μg of *Ps*Agu115, 0.35 μg of *Sc*Agu115) were incubated for 5–30 min in 0.1 ml of the reaction mixture containing the substrate in 50 mM acetate buffer (pH 5). The effect of 20 mM xylotetraose (Megazyme, Bray, Ireland) on activity of *Ps*Agu115 and *Sc*Agu115 was examined on 10 mM MeGlcA^{3}Xyl$_4$ as substrate under similar conditions. The reaction was stopped by addition of 0.3 ml of the copper reagent and boiling for 10 min at 100 °C, followed by addition of 0.2 ml of the Nelson reagent. Absorbance was measured at 600 nm using calibration with GlcA. The measurements were made in triplicate with exception of aldotriouronic acid when only one measurement for each enzyme was done due to a lack of the substrate. One unit of α-glucuronidase activity is defined as the amount of enzyme producing 1 μmol of uronic acid in 1 min.

2.4. NMR experiment

Substrate and enzymes were lyophilized twice from D_2O before use. Reduced aldopentaouronic acid (MeGlcA^{3}Xyl$_3$-Xylitol, 2 mg) was dissolved in 600 μl of 0.1 M deuterated acetate buffer, pD 4.8, at 25 °C. The amount of enzymes assuring rapid substrate hydrolysis to avoid the effect of mutarotation on identification of the primary product of hydrolysis was established in pilot experiments evaluated by TLC. The load of *Ps*Agu115 (333 μg) had to be much higher in comparison with *Sc*Agu115 (5.5 μg) due to decrease in specific activity of *Ps*Agu115 during lyophilization from D_2O. The reaction was started in a NMR test tube by the addition of the enzyme solutions of α-glucuronidase *Sc*Agu115 or *Ps*Agu115. ^{1}H NMR spectra were recorded at different time intervals on a Varian VNMRS 600 (600 MHz) spectrometer. The assignment of important resonances was based on the literature data for D-glucuronic acid and for aldouronic acids derived from plant glucuronoxylans [11,16–23]. The numbering of xylopyranosyl residues is as indicated on Fig. 1F.

3. Results and discussion

3.1. Substrate preference

Specific activities of the α-glucuronidases *Sc*Agu115 and *Ps*Agu115 were measured on a series of aldouronic acids – aldotriouronic acid MeGlcA^{2}Xyl$_2$, aldotetraouronic acids MeGlcA^{3}Xyl$_3$ and MeGlcA^{2}Xyl$_3$, aldopentaouronic acid MeGlcA^{3}Xyl$_4$ and aldohexaouronic acid MeGlcA^{3}Xyl$_5$ (Fig. 1) as well as on a beechwood glucuronoxylan. Both enzymes were active on all substrates (Fig. 2). Specific activity of *Sc*Agu115 increased with the length of

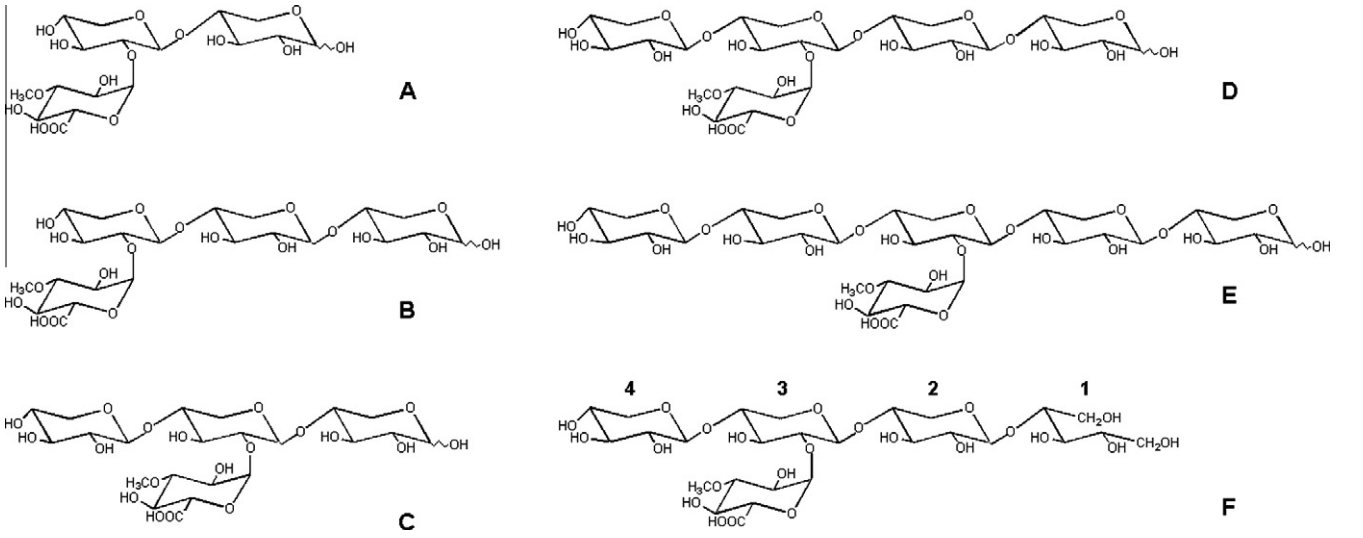

Fig. 1. Compounds used as the substrates for α-glucuronidases. (A) Aldotriouronic acid MeGlcA^{2}Xyl$_2$, (B) aldotetraouronic acid MeGlcA^{3}Xyl$_3$, (C) aldotetraouronic acid MeGlcA^{2}Xyl$_3$, (D) aldopentaouronic acid MeGlcA^{3}Xyl$_4$, (E) aldohexaouronic acid MeGlcA^{3}Xyl$_5$, (F) reduced aldopentaouronic acid MeGlcA^{3}Xyl$_3$-Xylitol.