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Research review paper

GH62 arabinofuranosidases: Structure, function and applications

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ABSTRACT

Motivated by industrial demands and ongoing scientific discoveries continuous efforts are made to identify and create improved biocatalysts dedicated to plant biomass conversion. α-1,2 and α-1,3 arabinofuranosyl specific α-L-arabinofuranosidases (EC 3.2.1.55) are debranching enzymes catalyzing hydrolytic release of α-L-arabinofuranosyl residues, which decorate xylan or arabinan backbones in lignocellulosic and pectin constituents of plant cell walls. The CAZy database classifies α-L-arabinofuranosidases in Glycoside Hydrolase (GH) families GH2, GH3, GH43, GH51, GH54 and GH62. Only GH62 contains exclusively α-L-arabinofuranosidases and these are of fungal and bacterial origin. Twenty-two GH62 enzymes out of 223 entries in the CAZy database have been characterized and very recently new knowledge was acquired with regard to crystal structures, substrate specificities, and phylogenetics, which overall provides novel insights into structure/function relationships of GH62. Overall GH62 α-L-arabinofuranosidases are believed to play important roles in nature by acting in synergy with several cell wall degrading enzymes and members of GH62 represent promising candidates for biotechnological improvements of biofuel production and in various biorefinery applications.

1. Introduction

Sustainable advances in plant cell wall degradation profoundly rely on carbohydrate active enzymes. Several such enzyme activities actually lag behind with regard to insight into structure/function relationships as well as biological sources of enzymes with prominent application potential. The enzymes of glycoside hydrolase family 62 (GH62), all so far being α-L-arabinofuranosidases (ABFs) (EC 3.2.1.55), belong to this category. A family GH62 enzyme was first reported in 1990 as purified from *Pseudomonas fluorescens* subsp. *cellulosa* designated xylanase C (XYLC) which released only arabinose from oat spelt xylan and is an arabinofuranosidase (Kellett et al., 1990). The specific removal by GH62 of decorations on xylan and arabinan backbones in hemicelluloses and pectins make them key for complete degradation of these polysaccharides in combination with enzymes of complementary specificities, *e.g.* xylanases (EC 3.2.1.8), β-xylosidases (EC 3.2.1.37), ferulic acid esterases (EC 3.1.1.73) and acetyl xylan esterases (EC 3.1.1.72). Recent progress including the first crystal structures of GH62 (see Section 5) call for an overview of current knowledge, adding importantly to the brief coverage of GH62 in the review by Lagaert et al. (2014) on β-xylosidases and ABFs accessory enzymes for arabinoxylan degradation. Clearly GH62 enzymes can increase efficacy of plant biomass conversions as a component in enzyme cocktails to be discussed at the end of the present review (see Section 6).

1.1. Natural substrates of GH62 α-L-arabinofuranosidases

Plant cell walls contain substantial amounts of arabinose (Ara*f*) in

Abbreviations: ABF, α-L-arabinofuranosidase; AOS, arabinooligosaccharides; Ara*f*, α-L-arabinofuranosyl; AX, arabinoxylan; AXOS, arabinoxylan-oligosaccharides; CAZy, Carbohydrate Active enZymes database; DP, degree of polymerization; GH, glycoside hydrolase; HPLC, high performance liquid chromatography; OSX, oat spelt xylan; *p*NPA, *para*-nitrophenyl α-L-arabinofuranoside; *p*NPX, *para*-nitrophenyl β-D-xylopyranoside; RAX, rye arabinoxylan; WAX, wheat arabinoxylan; WAX-HV, high viscosity wheat arabinoxylan; WAX-LV, low viscosity wheat arabinoxylan; Xyl*p*, β-D-1,4-xylose

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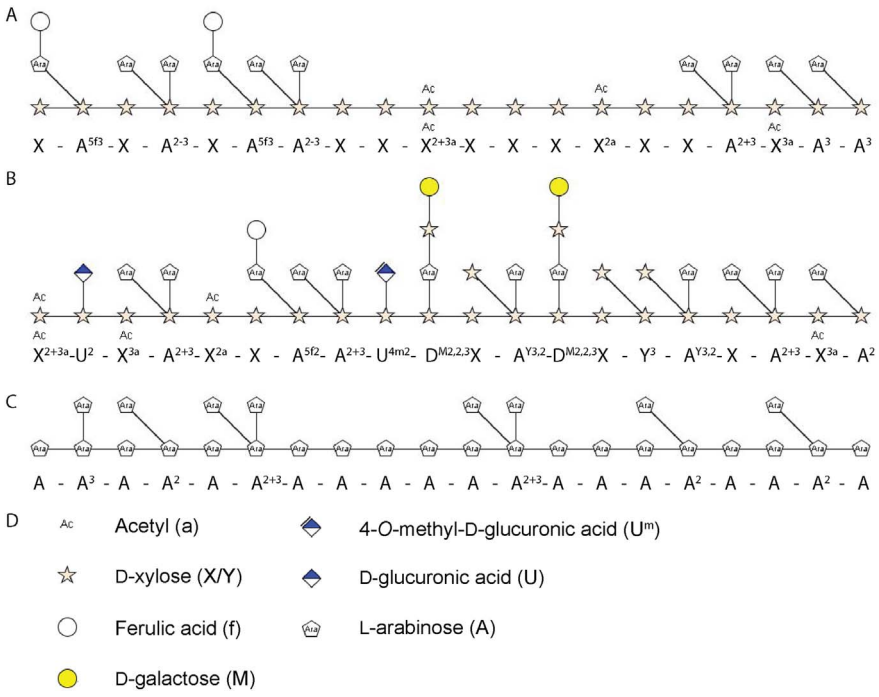

Fig. 1. Schematic structures of A) arabinoxylan, B) corn glucuronoarabinoxylan, C) L-arabinan and D) nomenclature as used for these in the text. The one letter code system developed by Faure et al. (2009) is used to describe the arabinoxylan and corn glucuronoarabinoxylan, and has been expanded to the L-arabinan.

the hemicelluloses arabinoxylans (AXs) and in L-arabinan side chains of pectins (Caffall and Mohnen, 2009; Scheller and Ulvskov, 2010). AXs and L-arabinan are proven as the main targets of different ABFs of GH62 (Siguier et al., 2014; Wang et al., 2014; Wilkens et al., 2016).

AXs consist of a backbone of β-D-1,4-linked xylosyl residues (Xyl*p*) singly or doubly substituted with α-L-1,2- and/or α-L-1,3-Ara*f* that can be further substituted by β-D-1,2-Xyl*p* (Bowman et al., 2014) or 5-*O*-ferulic acid (Scheller and Ulvskov, 2010) (Fig. 1A). In more complex xylans like glucuronoarabinoxylan from corn, the β-D-1,4-Xyl*p* backbone in addition to the above mentioned substituents is decorated by α-L-galactose and α-D-1,2-(4-*O*-methyl)-glucuronic acid (Rogowski et al., 2015; Scheller and Ulvskov, 2010) (Fig. 1B). By contrast, L-arabinan has an α-L-1,5-Ara*f* backbone that is hydrolyzed by other ABFs than of GH62, but which similarly to the AXs is substituted singly with α-L-1,3- as well as doubly with both α-L-1,2-Ara*f* and α-L-1,3-Ara*f*, hydrolyzed off by some GH62 enzymes (Fig. 1C) (Caffall and Mohnen, 2009).

The structural elements of the above mentioned xylans are described by a one letter code system developed Faure et al. (2009) where *e.g.* X designates an unsubstituted or terminal Xyl*p* and A a Xyl*p* substituted with an Ara*f*. The positions of the substitutions are indicated by superscript numbers. A plus symbol indicates that the Xyl*p* is double substituted. Accordingly, $A^{3}X$ describes α-L-Ara*f*-(1 → 3)-β-D-Xyl*p*-(1 → 4)-D-Xyl*p* and $A^{2\ +\ 3}X$ describes [α-L-Ara*f*-(1 → 2)]-[α-L-Ara*f*-(1 → 3)]-β-D-Xyl*p*-(1 → 4)-β-D-Xyl*p*. If Ara*f* is further substituted this is indicated by a superscript number that indicates the position of the substitution and a letter indicating the type of substitution, which precedes the number indicating the position of the Ara*f*. Lower case letters are used to designate non-glycosidic substituents. Accordingly A^{5f2} describes [5-*O*-Feruloyl]-[α-L-Ara*f*-(1 → 2)]-β-D-Xyl*p*-(1 → 4). More complex side chains are described by a combination of composite letters formed from single letters and superscript letters and numbers as above. *E.g.* $D^{M2,2,3}$ describes α-D-galactopyranosyl-(1 → 2)-α-D-xylopyranosyl-(1 → 2)-β-D-Xyl*p*-(1 → 2)-α-L-Ara*f*-(1 → 3) - where D designates the β-D-Xyl*p*-(1 → ?)-α-L-Ara*f*-(1 → ?) - motif that is linked to the xylan backbone and M the α-D-galactopyranosyl residue. The motifs or residues are separated by commas from the non-reducing terminal end. More examples are shown in Fig. 1.

GH62 ABFs also show limited activity on other Ara*f* containing polysaccharides such as gum arabic, which has a β-1,3-D-galactopyranose backbone carrying β-1,6-linked galactopyranose branch chains and is substituted with α-L-1,3-linked Ara*f* (Street and Anderson, 1983), and pectic arabinogalactan type I that has a β-1,4-D-galactopyranose backbone substituted by α-L-1,3-linked Ara*f*, while type II is composed of short β-1,3- and β-1,6-D-galactopyranose chains linked to one another *via* 1,3 or 1,6 branch points and substituted with α-L-1,3- or 1,6-linked Ara*f* (Carpita and Gibeaut, 1993). Rhamnogalacturonan is a heterogeneous part of pectin where Ara*f* is present as L-arabinan branches (Nakamura et al., 2001).

1.2. Classification of α-L-arabinofuranosidases

Carbohydrate active enzymes early on were classified according to reaction mechanism and substrate specificity and given an EC number by the Enzyme Commission of the International Union of Biochemistry and Molecular Biology (IUBMB). However, in the 90's, the Carbohydrate Active enZyme database (CAZy) (www.cazy.org) started to categorize amino acid sequences of these enzymes, which as of today resulted in 145 glycoside hydrolase (GH) families, within each of which the members share structures and catalytic mechanism. Notably, enzymes in a given GH family can have different substrate specificities, and *vice versa*, the same substrate specificity can be represented in different GH families. ABFs are currently found in six GH families: GH2, GH3, GH43, GH51, GH54 and GH62 of which GH43 and GH62 share tertiary fold and constitute clan GH-F (Lombard et al., 2014).

ABFs catalyze hydrolysis of Ara*f* from non-reducing ends of different Ara*f* containing poly- and oligosaccharides (Margolles-Clark et al., 1996; Tagawa and Kaji, 1969). Type A ABFs act on arabinoxylooligosaccharides (AXOS) and *para*-nitrophyl-α-L-arabinofuranoside (*p*NPA), while type B in addition degrades polysaccharides (Vincent et al., 1997). All GH62s are active on polysaccharides, hence they are type B ABFs (Table 1). ABFs are further distinguished by their ability to release Ara*f* from singly and doubly substituted Xyl*p* residues (Van Laere et al., 1999). Some ABFs specifically release both 1,2- and 1,3-linked Ara*f* from singly substituted Xyl*p*, which are given the suffix m2,3 (Lange et al., 2006; McCleary et al., 2015; Siguier et al., 2014; Van Laere et al., 1999; Wilkens et al., 2016). ABFs that release Ara*f* from doubly substituted Xyl*p* are specifically releasing either 1,2- or 1,3-linked Ara*f* and are given the suffix d2 or d3, respectively (Cartmell