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Structural characterization of the family GH115 α-glucuronidase from *Amphibacillus xylanus* yields insight into its coordinated action with α-arabinofuranosidases

Ruoyu Yan a, Weijun Wang a, Thu V. Vuong a, Yang Xiu a, Tatiana Skarina a, Rosa Di Leo a, Paul Gatenholm b, Guillermo Toriz c, Maija Tenkanen d, Peter J. Stogios a, Emma R. Master a,*,*

a Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 3E5, Canada
b Department of Chemistry and Chemical Engineering, Wallenberg Wood Science Center and Biopolymer Technology, Chalmers University of Technology, Kernvägen 4, Gothenburg, 412 96, Sweden
c Department of Wood, Cellulose and Paper Research, University of Guadalajara, Guadalajara, 44100, Mexico
d Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, Helsinki, 00014, Finland
e Department of Bioproducts and Biosystems, Aalto University, FI-00076, Aalto, Kemistintie 1, Espoo, Finland

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ABSTRACT

The coordinated action of carbohydrate-active enzymes has mainly been evaluated for the purpose of complete saccharification of plant biomass (lignocellulose) to sugars. By contrast, the coordinated action of accessory hemicellulases on xylan debranching and recovery is less well characterized. Here, the activity of two family GH115 α-glucuronidases (SdeAgu115A from *Saccharophagus degradans*, and AxyAgu115A from *Amphibacillus xylanus*) on spruce arabinoglucuronoxylan (AGX) was evaluated in combination with an α-arabinofuranosidase from families GH51 (AniAbf51A, aka E-AFASE from *Aspergillus niger*) and GH62 (SthAbf62A from *Streptomyces thermoviolaceus*). The α-arabinofuranosidases boosted (methyl)-glucuronic acid release by SdeAgu115A by approximately 50% and 30%, respectively. The impact of the α-arabinofuranosidases on AxyAgu115A activity was comparatively low, motivating its structural characterization. The crystal structure of AxyAgu115A revealed increased length and flexibility of the active site loop compared to SdeAgu115A. This structural difference could explain the ability of AxyAgu115A to accommodate more highly substituted arabinoglucuronoxylan, and inform enzyme selections for improved AGX recovery and use.

Introduction

Xylans from coniferous wood and agricultural fibre comprise a β-(1→4)-linked D-xylopyranosyl (Xylp) backbone, partially substituted at O-2 positions with 4-O-(methyl)glucopyranosyluronic acid (MeGlcA) and at O-3 positions with arabinofuranosyl (Araf) residues [1,2]. These xylans differ in relative abundance of Araf and MeGlcA substituents, where arabinoglucuronoxylan from conifers typically contain higher levels of MeGlcA than Araf; the reverse is generally the case for glucuronoxarabinoxylans from agricultural fibre. Accordingly, the complete biological conversion of xylans to monosaccharides requires the action of multiple main-chain and side-group cleaving enzymes [3]. To date, the coordinated action of corresponding enzymes has been studied mainly to generate fermentable sugars used to produce biofuels and commodity chemicals [4–6]. Alternatively, accessory enzymes alone could be used to recover and tailor high molecular weight xylans for application in food and bio-based materials [7–13].

Examples of accessory hemicellulases relevant to xylan processing include α-arabinofuranosidases (3.2.1.55) and α-glucuronidases (3.2.1.131) that target side-group residues of arabinoglucuronoxylan (Fig. 1). Xylan-active α-arabinofuranosidases (i.e., arabinoxylan arabinofuranohydrolases; AXH) reported to date release α-(1→3)-L-Araf

Abbreviations: AFB, α-arabinofuranosidase; AniAbf51A, a GH51 α-arabinofuranosidase from *Aspergillus niger* (aka E-AFASE); AXG, arabinoglucuronoxylan; AXH, arabinoxylan arabinofuranohydrolase; AxyAgu115A, a GH115 α-glucuronidase from *Amphibacillus xylanus*; GH, glycoside hydrolase; SdeAgu115A, a GH115 α-glucuronidase from *Saccharophagus degradans*; SthAbf62A, a GH62 α-arabinofuranosidase from *Streptomyces thermoviolaceus*.  
* Corresponding author at: Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 3E5, Canada.

E-mail address: emma.master@utoronto.ca (E.R. Master).

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enzymes belonging to glycoside hydrolase (GH) family GH43 display activity from mono-substituted Xyl unit, n, is generally from 1 to 6 [36].

Fig. 1. Both α-arabinofuranosidases and α-glucuronidases are required to remove substituents from spruce arabinoglucuronoxylan. The number of D-xylopyranosyl substituents from di-substituted Xylp subunits along the xylan backbone (i.e., AXH-d3 activity), or release α-(1→3)-L-Araf and α-(1→2)-L-Araf from mono-substituted Xylp (i.e., AXH-m 2,3 activity). Whereas certain enzymes belonging to glycoside hydrolase (GH) family GH43 display AXH-d3 activity, AXH-m 2,3 activity is displayed by arabinoxylan arabinofuranohydrolases belonging to families GH43 and GH62 [14–21]. For example, our earlier study of the family GH62 α-arabinofuranosidase, StAbf62A from Streptomyces thermoviolaceus, confirmed its selective action towards L-Araf residues that are α-(1→2) and α-(1→3) linked to mono-substituted D-Xylp of wheat arabinoxylan [21]. Studies by other groups have reported GH51 and GH54 enzymes with AXH-m, d type activity towards the non-reducing end terminal Xylp of oligosaccharides; however, in these cases, only weak activity is detected towards internal Araf substitutions in polymeric arabinoxylans [22–25].

Xylan-active α-glucuronidases are currently classified into families GH67 and GH115. GH67 activity is restricted to MeGlcPA residues at the non-reducing end of the substrate; by contrast, xylan-active GH115 enzymes are able to remove MeGlcPA from both terminal and internal positions of the xylan backbone [26–28]. Our earlier studies of SdeAgu115A from Saccharophagus degradans and AxyAgu115A from Amphibacillus xylanus confirmed their action on a variety of xylan sources, including beechwood glucuronoxylan, spruce arabinoglucuronoxylan (AGX) and oat spelt glucuronoarabinoxylan, and also revealed the higher performance of AxyAgu115A on comparatively complex xylo-oligosaccharides; however, in these cases, only weak activity is detected towards internal Araf substitutions in polymeric arabinoxylans [29].

To delve deeper into structure-function relationships of family GH115 xylan-active α-glucuronidases, the potential of selected α-arabinofuranosidases to boost the performance of AxyAgu115A and SdeAgu115A action towards spruce arabinoglucuronoxylan was investigated. The x-ray structure of AxyAgu115A was also solved, which confirmed the increased length and flexibility of the active-site loop in AxyAgu115A compared to SdeAgu115A, potentially explaining the ability of AxyAgu115A to accommodate more highly substituted arabinoglucuronoxylan.

Materials and methods

Materials

Spruce arabinoglucuronoxylans (AGX) were isolated from a debarked spruce log (Picea abies), provided by the Wallenberg Wood Science Center (Stockholm, Sweden), based on a previously reported method [10], and were further purified by bleaching with hydrogen peroxide. Briefly, AGX (1 g) was dissolved in deionized water, pentetic acid (Sigma-Aldrich, St. Louis, MO, USA) was added (1.2 wt. % relative to the weight of xylan) to complex ions, and then left stirring overnight. Sodium silicate (Sigma-Aldrich) was then added (2.25 wt %), and the temperature was raised to 50 °C before adding hydrogen peroxide (600 μL; 50 %) (Sigma-Aldrich) and adjusting the pH to pH 11.0. After 4 h, the AGX was precipitated in acidic ethanol (4:1, v/v of ethanol to xylan), prepared with ethanol 96 % (Sigma-Aldrich) and glacial acetic acid (Sigma-Aldrich) (1:0.1, v/v). The solution was then dialyzed against deionized water for 3 d using a 1 kDa molecular weight cut-off dialysis membrane (Thermo Fisher Scientific, Waltham, MA, USA). GH51 α-L-arabinofuranosidase (AniAbf51A, aka E-AFASE from Aspergillus niger), as well as the D-glucuronic acid assay kit and L-arabinose assay kit, were from Megazyme (Bray, Ireland). Buffer exchange was performed using Amicon Ultra 0.5 mL centrifugal filters with molecular weight cut-off at 10 kDa (Millipore, Oakville, ON, Canada). SdeAgu115A from Saccharophagus degradans 2-40T [28], AxyAgu115A from Amphibacillus xylanus [29], and StAbf62A from Streptomyces thermoviolaceus [21] were produced in our laboratory based on previously published methods.

Table 1

General properties of the accessory xylan-acting enzymes selected for this study.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>GH family</th>
<th>Optimal pH</th>
<th>Optimal temperature</th>
<th>Enzyme specificity</th>
<th>Substrate (assay condition)</th>
<th>Specific activity (μmol product/min/mg protein)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SdeAgu115A</td>
<td>115</td>
<td>6.5</td>
<td>40 °C</td>
<td>Remove α-(1→2)-MeGlcPA substrates</td>
<td>Spruce arabinoglucuronoxylan (1.0 %, w/v, pH 6.5)</td>
<td>8.3 ± 0.1</td>
<td>Wang et al. [28]</td>
</tr>
<tr>
<td>AxyAgu115A</td>
<td>115</td>
<td>7.0</td>
<td>55 °C</td>
<td>Remove α-(1→2)-MeGlcPA substrates</td>
<td>Spruce arabinoglucuronoxylan (1.0 %, w/v, pH 7.0)</td>
<td>51.2 ± 0.6</td>
<td>Yan et al. [29]</td>
</tr>
<tr>
<td>StAbf62A</td>
<td>62</td>
<td>7.0</td>
<td>55 °C</td>
<td>Remove α-(1→2)-Araf and α-(1→3)-Araf mono-substituents</td>
<td>Wheat arabinoxylan (0.5 %, w/v, pH 6.5)</td>
<td>40 ± 3</td>
<td>Wang et al. [21]</td>
</tr>
<tr>
<td>AniAbf51A</td>
<td>51</td>
<td>4.0</td>
<td>50 °C</td>
<td>Remove α-(1→2)-Araf and α-(1→3)-Araf mono-substituents and Araf disubstitutions</td>
<td>Wheat arabinoxylan (1.0 %, w/v, pH 4.0)</td>
<td>1.2</td>
<td>McCleary et al. [24]</td>
</tr>
</tbody>
</table>

* All enzymes remained active at selected universal assay condition for synergistic study, namely 40 °C at pH 7.0.
mM universal buffer (0.067 M $H_3$BO$_3$, 0.067 M $H_3$PO$_4$, 0.067 M CH$_3$COOH, adjusted to pH 7.0 using a sodium hydroxide solution); each individual enzyme was validated under this condition (Suppl. Figure S1). Final concentrations of AxyAgu115A and SdeAgu115A in corresponding reaction mixtures were optimized to obtain initial rates of reaction, and were 9 ng/$\mu$L and 40 ng/$\mu$L, respectively. Three doses of SthAbf62A or AniAbf51A were used to supplement GH115 activity, where enzyme loadings were adjusted to achieve similar activity levels. Accordingly, the three SthAbf62A doses were 6 ng/$\mu$L, 12 ng/$\mu$L and 24 ng/$\mu$L, whereas the three AniAbf51A doses were 150 ng/$\mu$L, 300 ng/$\mu$L and 600 ng/$\mu$L. After 20 min, reactions were heated to 100 °C for 10 min to inactivate the enzyme activity. A typical enzymatic reaction mixture contained 10 μL of enzyme cocktail and 20 μL of 1 % (w/v) spruce AGX, representing a final substrate concentration at 0.67 % (w/v). The released MeGlc$_p$A and Ara$_f$ were quantified by D-glucuronic acid assay kit and L-arabinose assay kit, respectively.

To evaluate the impact of α-arabinofuranosidases on the extent of MeGlc$_p$A released by AxyAgu115A and SdeAgu115A, reaction mixtures were prepared as described above and contained 0.6 μg/$\mu$L GH115 α-glucuronidase (SdeAgu115A or AxyAgu115A) and 0.6 μg/$\mu$L α-arabinofuranosidase (AniAbf51A or SthAbf62A). Following 24 h of incubation at 25 °C, MeGlpC and Ara$\beta$ released were quantified using a D-glucuronic acid assay kit and L-arabinose assay kit, respectively.

**Protein crystallization, data collection, and structure determination**

The *E. coli* codon-optimized aaxygu115A gene (locus tag AXY_23000 from *Amphibacillus xylanus* NBRC 15112) (Suppl. Figure S2) was purchased from Genscript (Piscataway, NJ, USA) and subcloned into the p15-Tv-LIC vector (The Structural Genomics Consortium, Toronto, ON, Canada), coding for a fusion protein with N-terminal His6-tag and TEV protease cleavage site. As this improved crystal quality according to the surface entropy reduction approach and the SerP server [30], the triple mutant K79A, K80A and E81A of AxyAgu115A enzyme was cloned using a Quikchange mutagenesis kit (Agilent Technologies, Santa Clara, CA, USA). The AxyAgu115A enzyme was purified as previously described.
[29]. Crystals of AxyAgu115A were grown using the sitting-drop method with 0.5 μL of 20 mg/mL protein solution with 0.5 μL of reservoir solution 20% (w/v) PEG 3350, 0.2 M diammonium hydrogen citrate and 10 mM D-glucuronic acid (Sigma-Aldrich, St. Louis, MO, USA). Crystals were cryoprotected in paratone oil (Hampton Research, Aliso Viejo, CA, USA) before flash-freezing in liquid nitrogen. X-ray diffraction data was collected at 100 K at beamline 21-ID-D at the Life Sciences Collaborative Access Team, Advanced Photon Source, Argonne National Laboratory (Lemont, IL, USA). Diffraction data was processed by XDS [31] and Aimless [32]. The structure was solved by Molecular Replacement with Phenix.phaser [33] using models of AxyAgu115A generated by the Phyre2 server [34] and the three N-terminal domains of SdeAgu115A [28] and BoAgu115A [27]. Automated model building from the initial MR solution was performed using Phenix.autobuild. Manual model building and refinement were performed using Coot [35] and Phenix.refine with TLS parameterization, and all B-factors were refined as isotropic. Electron density in the active site was modeled as glycerol molecules. Structure figures were prepared using PyMol (Schrödinger LLC, New York, NY, USA).

Docking analysis

The PDB IDs of SdeAgu115A and SthAraf62A are 4ZMH and 4O8N, respectively, while the X-ray structure of AxyAgu115A was solved in this study (PDB ID: 6NPS). The dimeric structures of SdeAgu115A and AxyAgu115A were used for computational docking. The structural model of AniAbf51A was built based on a Thermobacillus xylanilyticus GH51 arabinofuranosidase (PDB ID: 2VRQ) by the Phyre2 server [34]. Based on the mass spectrometry MS/MS analysis of spruce AGX [36], three xylohexaoses with a different substitution pattern were used: (1) one MeGlcA α-(1→2)-linked to Xylp in the -2 position (XXXXU4m2X), (2) an MeGlcA α-(1→2)-linked to Xylp in the -2 position and an Araf α-(1→3)-linked to a Xylp in the -4 position (XXA3XU4m2X); and (3) two MeGlcA substitutions α-(1→2)-linked to consecutive Xylp residues in the -2 and -3 positions (XXXU4m2X4m2X). The 3D structures of these oligosaccharide ligands were drawn by ChemBioDraw v11.0.2 (PerkinElmer, Waltham, MA, USA).

AutodockTools v1.5.2 on Python v2.5 (http://autodock.scripps.edu) was then used to prepare the proteins and ligands, and to setup grid boxes. Docking simulation was conducted using Autodock Vina v1.1.2.
Results and discussion

Comparison of AxyAgu115A and SdeAgu115A activity on spruce arabinogluconoxylan in the presence of selected α-arabinofuranosidases

It has been shown previously that in spruce arabinogluconoxylans (AGX), Araf and MeGlcpA are evenly spaced and closely positioned, and that two MeGlcpA can occupy adjacent positions along the xylan backbone [36]. This close proximity of Araf and MeGlcpA residues could impact α-glucuronidase and arabinofuranosidase activities on AGX (Fig. 1).

To investigate potential differences in AxyAgu115A and SdeAgu115A activity on complex xylans like AGX, corresponding enzyme activities were measured using spruce AGX in the presence or absence of α-arabinofuranosidases, specifically AniAbf51A from family GH51 and StAbf62A from family GH62 (Table 1). As mentioned above, AniAbf51A displays dual ABF-m/d activity, and targets α-(1→2)-Araf and α-(1→3)-Araf mono-substituents, whereas AniAbf51A releases by both StAbf62A and AxyAgu115A act on complex xylans like AGX, corresponding enzyme activities were measured using spruce AGX in the presence or absence of α-arabinofuranosidases, specifically AniAbf51A from family GH51 and StAbf62A from family GH62 (Table 1). As mentioned above, AniAbf51A displays dual ABF-m/d activity, and targets α-(1→2)-Araf and α-(1→3)-Araf mono-substituents, whereas StAbf62A and AxyAgu115A act on complex xylans like AGX, corresponding enzyme activities were measured using spruce AGX in the presence or absence of α-arabinofuranosidases. AniAbf51A is distinguished from StAbf62A by its ability to release by both StAbf62A and AxyAgu115A also increased by approxi mately 15 % in reactions containing StAbf62A (Fig. 2C). Although small, this reproducible impact on MeGlcpA release by both α-glucuronidases showed that steric hindrance caused by Araf can be quickly alleviated with addition of StAbf62A, but that once this constraint is overcome, reduced substrate solubility resulting from Araf removal from AGX can hinder α-glucuronidase performance [37,38].

In contrast to StAbf62A, the impact of AniAbf51A on α-glucuronidase activity was enzyme dependent, where AniAbf51A increased SdeAgu115A activity by 48 %, but only by 15 % for AxyAgu115A (Fig. 2B). AniAbf51A is distinguished from StAbf62A by its ability to target Araf disubstitutions [24]. The lower impact of AniAbf51A on AxyAgu115A compared to SdeAgu115A, therefore, suggests that AxyAgu115A activity is less hindered by nearby di-substitutions of the xylan backbone [29].

StAbf62A activity and performance was not significantly impacted by the presence of AxyAgu115A or SdeAgu115A (Fig. 3A, C). Conversely, rates of Araf release by AniAbf51A increased by approximately 300 % in the presence of either α-glucuronidase (Fig. 3B); the extent of Araf release by AniAbf51A also increased by more than 200 % in reactions supplemented with either α-glucuronidase (Fig. 3C). To investigate further the structural basis for the lower impact of AniAbf51A on AxyAgu115A activity and performance, the crystal structure of AxyAgu115A was solved to permit direct comparisons with the previously reported structure for SdeAgu115A [28].

AxyAgu115A adopts a 5-domain dimeric structure, similar to SdeAgu115A

Since efforts to crystallize the wild-type enzyme were unsuccessful, a triple mutant of AxyAgu115A, AxyAgu115A^K79AAS80AES81A, designed using the surface entropy reduction approach, was crystalized [30]. These mutations were not expected to affect enzyme activity, being on the surface and distant from the active site (Suppl. Figure S3); we therefore use the name of the wild-type enzyme, AxyAgu115A, when referring to the corresponding structure. The structure was solved by molecular replacement using the structure of SdeAgu115A [28]. All crystallographic data and refinement statistics are summarized in Table 2.

Table 2

| X-ray diffraction data collection and refinement statistics. |
|-----------------------------|-----------------------------|
| PDB code                    | AxyAgu115A^K79AAS80AES81A   |
| Space group                 | P 1                         |
| Unit cell                   |                             |
| a, b, c (Å)                 | 62.08, 96.21, 111.75        |
| α, β, γ (°)                 | 84.37, 78.37, 83.32         |
| Resolution, Å               | 47.64 – 1.99                |
| Rmerge = Σ|Fobs| - |Fcalc|/|Σ|Fobs|               |
| Rmerge = Σ|Fobs| - |Fcalc|/|Σ|Fobs|               |
| Rmerge = Σ|Fobs| - |Fcalc|/|Σ|Fobs|               |
| Completeness, %             | 94.6 (90.8)                 |
| Redundancy                  | 11.9 (3.9)                  |

Refinement

| Resolution, Å               | 47.64 – 1.99                |
| No. unique reflections:     | 165435, 8156                |
| R-factor/free R-factor      | 15.0/19.0 (24.5/26.6)       |
| No. refined, molecules      | 15552, 2                    |
| Solvent                     | 123                         |
| Water                       | 1878                        |
| B-factors                   | 45.1                        |
| Protein                     | 86.2                        |
| r.m.s.d.                    | 55.8                        |
| Bond lengths, Å             | 0.012                       |
| Bond angles, °              | 1.133                       |

α NPS

| a Rmerge = Σ|I|/|Σ|I|               |
| b Rmerge = Σ|I|/|Σ|I|               |
| c All values in brackets refer to the highest resolution shell. |

The AxyAgu115A crystal contained two chains in its asymmetric unit, each possessing the five-domain architecture originally observed in SdeAgu115A [28] (domains A: residues 1–165, B: residues 166–477, C: residues 478–656, D: residues 657–775, D: residues 776–966, Fig. 4A and Suppl. Fig. S2A); the pairwise RMSD is 2.8 Å across all five domains of each of the two enzymes. The two AxyAgu115A chains in the asymmetric unit were related by non-crystallographic symmetry and interacted along an interface that buried 1,460 Å2. This extent of buried surface as well as the fact that the packing arrangement of the two chains closely resembled that of the dimeric SdeAgu115A enzyme [28] are closely resembled that of the dimeric SdeAgu115A enzyme [28] are closely resembled that of the dimeric SdeAgu115A enzyme [28] are closely resembled that of the dimeric SdeAgu115A enzyme [28]

The AxyAgu115A catalytic center is covered by a comparatively long and flexible loop

Similar to other GH115 crystal structures, the active site of AxyAgu115A was localized to the central cavity of domain B, a (β/α)8 TIM barrel fold (Fig. 4B and Suppl. Fig. S4C) (pairwise RMSD values with SdeAgu115A, BoGH115A and BoAgu115A are 1.5–1.3 Å, across 388–292 matching Ca atoms of domain B) [39,27,28]. Amino acids making up this putative active site were mainly found on loops of domain B; moreover, the presumed catalytic center was covered by a prominent loop from domain B (residues 301–313) (Fig. 4B). The electron density for this loop was interpretable only for one chain in the dimer (shown in Fig. 4B) while the second was more disordered and accordingly not all residues could be fully modeled. In the resolved loop, Asp305 was 4.3 Å from a trapped glycerol molecule; this residue also formed an
electrostatic interaction with Arg301. Notably, mutation of the residues equivalent to Arg301 in SdeAGU115A (Arg331) and BoAGU115A (Arg328) were both shown to seriously impact catalysis by those enzymes [27,28]. Beyond the conservation of this arginine residue, however, the corresponding loop differs dramatically between structurally characterized GH115 enzymes. For instance, this loop in AxyAGU115A is notably longer than for SdeAGU115A (13 residues versus 8), which is consistent with the previous structural model of AxyAGU115A [29].

Secondly, the position of Asp305 in AxyAGU115A is not structurally conserved in SdeAGU115A, and the position of Asp335 from SdeAGU115A, which was shown to be essential for catalysis, is not structurally conserved in AxyAGU115A. Thirdly, there is considerable sequence diversity in this loop region across GH115 enzymes (Suppl. Fig. S5). Collectively, these observations imply that the flexibility and sequence composition of this loop is important for recognition of substrates and determination of substrate specificity across the GH115 family.

Starting from this localization of the presumed catalytic center, the putative glucuronoxylan binding surface of AxyAGU115A was traced. As was observed for SdeAGU115A, domain C+ from the partner chain in the AxyAGU115A dimer contributed to sharing the broader active site cleft (Fig. 4C, Suppl. Fig. S4C). Notably, Trp680 from the partner subunit of the dimer pointed directly into the presumed glucuronoxylan binding site. Trp177 and Trp216 from domain B and Trp643 from domain C were also prominently displayed near the catalytic center. Furthermore, electron density features near Trp177, Trp643, His642 and His681 were modeled as four glycerol molecules (Suppl. Fig. S4C).

Interestingly, these residues from domains C (His642, Trp643) and C+ (Trp680, and His681) colocalize to residues in SdeAGU115A that were shown to play a role in substrate binding (W773, W689, and F696) [28].

Binding of xylo-oligosaccharides from spruce AGX [36] to AxyAGU115A, as well as SdeAGU115A, was subsequently investigated using computational docking (Fig. 5). Among three substituted xylo-hexaoses: XXXU4m2X, XXA3XU4m2X, and XXXU4m2U4m2X, AxyAGU115A showed the most negative docking energy to XXXU4m2U4m2X (Fig. 5A, B, C), indicating particularly favorable interactions between AxyAGU115A and MeGlcA subunits that are consecutively attached to neighboring Xylp residues. Three additional H-bonds to the second MeGlcA unit were revealed by docking (Suppl. Figure S6). By contrast, SdeAGU115A was predicted to interact most favorably with XXXXU4m2X (Fig. 5D, E, F). Interestingly, the binding modes of all ligands were flipped 180° between AxyAGU115A and SdeAGU115A (Fig. 5), likely due to the presence of the distinct long loop in AxyAGU115A, which is

![Fig. 4. Crystal structure of AxyAGU115A. (A) Overall structure of dimeric AxyAGU115A with each of the 5 domains coloured separately and labelled A, B, C, C+ and D and with apostrophes for the partner subunit. The two catalytic sites are shown with arrows. Both catalytic sites are formed primarily by domain B/B’ with contributions from C+/C+ (left) and without surface (right). Glycerol bound in the catalytic center is shown as grey sticks and the loop 301-313 that forms a cap over the active site is labeled. Dashes indicate hydrogen or electrostatic interactions between active site residues or with the bound glycerol molecule. (B) Catalytic center of AxyAGU115A with enzyme shown in solvent accessible surface representation (left) and without surface (right). (C) Comparison of catalytic centers of AxyAGU115A (green) and SdeAGU115A (grey).](image-url)
located at the center of the active site (Fig. 5), separating two MeGlcP units (Suppl. Fig. S6). Notably, SthAbf62A was predicted to bind XXA3XU 4m2 X better than AniAbf51A (Suppl. Figure S7), which is in agreement with the lower impact of α-glucuronidases on SthAbf62A performance (Fig. 3A, C).

Conclusions

The current study has investigated the coordinated action of accessory hemicellulases that release Araf and MeGlcpA substituents from spruce arabinoglucuronoxylan (AGX). In addition to uncovering enzyme combinations that can facilitate AGX recovery and use, the comparison of AxyAgu115A and SdeAgu115A action in the presence of α-arabinofuranosidases underscored the lower susceptibility of AxyAgu115A to steric hindrance compared to SdeAgu115A. The structural characterization of AxyAgu115A revealed a prominent, flexible loop in the active site that might contribute to the binding modes and recognition of xylo-oligosaccharides. The diversity in sequence and length of this loop region amongst family GH115 α-glucuronidases invites further studies to unravel the functional implications of its composition. Such efforts could inform GH115 selections to achieve distinct xylan forms and properties.

Authors’ contributions

RY, WW, TVV, PJS and ERM conceived and designed research. RY, TVV, YX, TS, RDL and PJS conducted experiments. PG, GT and MT prepared materials. RY, PJS, TVV and ERM analyzed data and wrote the manuscript. All authors read and approved the manuscript.

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Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

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