Crystal Structures of Decorated Xylooligosaccharides Bound to a Family 10 Xylanase from *Streptomyces olivaceoviridis* E-86*

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The family 10 xylanase from *Streptomyces olivaceoviridis* E-86 (SoXyn10A) consists of a GH10 catalytic domain, which is joined by a Gly/Pro-rich linker to a family 13 carbohydrate-binding module (CBM13) that interacts with xylan. To understand how GH10 xylanases and CBM13 recognize decorated xyloans, the crystal structure of SoXyn10A was determined in complex with α-L-arabinofuranosyl- and 4-O-methyl-α-D-glucuronosyl-xylooligosaccharides. The bound sugars were observed in the subsites of the catalytic cleft and also in subdomains α and γ of CBM13. The data reveal that the binding mode of the oligosaccharides in the active site of the catalytic domain is entirely consistent with the substrate specificity and, in conjunction with the accompanying paper (Pell, G., Taylor, E. J., Gloster, T. M., Turkenburg, J. P., Fontes, C. M. G. A., Ferreira, L. M. A., Nagy, T., Clark, S. J., Davies, G. J., and Gilbert, H. J. (2004) J. Biol. Chem. 279, 9597-9605), demonstrate that the accommodation of the side chains in decorated xylans is conserved in GH10 xylanases of SoXyn10A against arabinoglucuronoxylan. CBM13 was shown to bind xylose or xylooligosaccharides reversibly by using nonsymmetric sugars as the ligands. The independent multiple sites in CBM13 may increase the probability of substrate binding.

Carbohydrate-active enzymes display a modular structure featuring a catalytic domain and an ancillary noncatalytic module(s). The classification of the carbohydrate-active enzymes into specific families is based on amino acid sequence similarities, and currently there are more than 90 families of the catalytic modules of glycosyl hydrolases (GHs)† (afmb).

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The atomic coordinates and structure factors (codes 1v6u, 1v6v, 1v6w, 1v6x for the SoXyn10A Araf-X2, SoXyn10A Araf-X3, SoXyn10A MeGlcUA-X2, SoXyn10A MeGlcUA-X3 complexes, respectively) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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‡‡ The abbreviations used are: GH, glycosyl hydrolase; Araf, α-L-arabinofuranosyl residue; Araf-X2, 2^3-α-L-arabinofuranosyl-xyloligosaccharide; Araf-X3, 3^3-α-L-arabinofuranosyl-xyloligosaccharide; CBM, carbohydrate-binding module; CmXyn10B, family 10 xylanase from *Cellvibrio mixtus*; NCS, noncrystallographic symmetry; MeGlcUA, 4-O-methyl-α-D-glucuronosyl residue; MeGlcUA-X2, 2^3-4-O-methyl-α-D-glucuronosylxylooligosaccharide; MeGlcUA-X3, 3^3-4-O-methyl-α-D-glucuronosylxylooligosaccharide; SoCBM13, carbohydrate-binding module of family 10 xylanase from *Streptomyces olivaceoviridis* E-86; SoXyn10A, family 10 xylanase from *Streptomyces olivaceoviridis* E-86; X2, xylobiose; X3, xylotrioside.
xylooligosaccharides (X2) and xylotriose (X3) are located in the catalytic cleft. In SoCBM13 bound sugars in subdomains α and γ were identified as xylose, X2, X3, glucose, galactose, and lactose. Although SoCBM13 and ricin bind xylose polymers and lactose, respectively, at the same location, the mechanism by which xylooligosaccharides and galactose interact with these proteins is different. The O-1 and O-4 atoms of the bound xylose moiety were exposed on the surface area of the SoCBM13 so that the xylan chain could be extended from this xylose unit in both directions. In the SoXyn10A X2 and SoXyn10A X3 complexes, continuous electron densities were observed on both sides of the bound xylose at the O-1 and O-4 atoms, and the side chains can be accommodated. Currently there is a paucity of information on how proteins bind to decorated polysaccharides, although an understanding of these interactions is starting to emerge. Recently, the crystal structure of CBM27 from a Thermotoga maritima mannanase complexed with 5,4-O-d-galactosyl-mannopentaose has been reported, which represents the first structure of a CBM in complex with a branched plant cell wall polysaccharide (24). The crystal structure of a family 10 xylanase from Penicillium simplicissimum was solved in complex with a synthetic xylooligosaccharide that contained a 1,2-(4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid) side chain (25). In this paper, we present the crystal structure of SoXyn10A in complex with the arabinoxylooligosaccharides described above as ligands. The data demonstrate how these decorated ligands are accommodated in the binding site of SoCBM13 and, in conjunction with the accompanying paper (26), provide a generic understanding of how the substrate binding cleft of family 10 xylanases is able to interact with substituted xylooligosaccharides.

EXPERIMENTAL PROCEDURES

Crystallization and Data Collection—Recombinant full-length enzyme was expressed in Escherichia coli and purified by a modified method (3). Crystallization procedures were reported previously (27). The enzyme was crystallized by the hanging drop vapor diffusion method at room temperature using a 20 mg/ml protein solution and a reservoir solution composed of 27.5% ammonium sulfate and 2% Megazyme buffer (a mixture of 0.1 M citric acid and 0.2 M Na2HPO4, pH 5.7). After a week, rod-like crystals grew to ~1 mm long.

Sugars used in the experiments were 2-O-α-L-arabinofuranosyl-β-D-glucuronosyl-β-D-glucuronosyl-β-D-glucuronosyl-β-D-glucurono-xylobiose (Ara f-X2), 3-2-O-α-L-arabinofuranosyl-β-D-glucuronosyl-methyl-β-D-glucuronosyl-xylotriose (MeGlcUA-X3), and 3-4-O-methyl-α-L-arabinofuranosyl-β-D-glucuronosyl-β-D-glucuronosyl-β-D-glucuronosyl-β-D-glucurono-xylooligosaccharides (MeGlcUA-X2 and MeGlcUA-X3) were purchased from Megazyme under the names of aldotriouronic and aldotetrauronic acids. Arabinofuranosyl-xylooligosaccharides (Ara f-X2 and Ara f-X3) were prepared by the action of SoXyn10A on culms of Kumaizasa (Susa senensis Rehd) as reported previously (28). The decorated xylooligosaccharides were dissolved at a concentration of 25% in the reservoir solution and used as cryoprotectant when the protein crystals were

### Table 1: Crystal parameters and refinement statistics of the SoXyn10A sugar complexes

| Ligand          | Cell parameter | Araf-X2        | Araf-X3        | MeGlcUA-X2 | MeGlcUA-X3 |
|-----------------|----------------|----------------|----------------|-------------|-------------|
|                 | (P2_12_1_2)    | a (Å)          | b (Å)          | c (Å)       | a (Å)       | b (Å)       | c (Å)       | a (Å)       | b (Å)       | c (Å)       |
|                  |                | 75.3           | 94.2           | 137.7       | 30-2.1      | 1.97-1.90   | 30-2.1      | 1.97-1.90   | 30-2.1      | 1.97-1.90   |
|                  |                | 75.3           | 94.2           | 137.7       | 30-2.1      | 1.97-1.90   | 30-2.1      | 1.97-1.90   | 30-2.1      | 1.97-1.90   |
| Experiment      |                |                |                |             | In-house    | PF6A        | In-house    | PF6B        | In-house    | PF6B        |
| Resolution (Å)  |                |                |                |             | 30-2.1      | 1.97-1.90   | 30-2.1      | 1.97-1.90   | 30-2.1      | 1.97-1.90   |
|                 |                |                |                |             | (2.18-2.10) | (2.07-2.0)  | (2.18-2.1) | (2.07-2.0)  | (2.18-2.1) | (2.07-2.0)  |
| No. of reflections |            | 56,504         | 80,951         | 68,082      | 56,625      |             |             |             |             |             |
| Completeness (%)| (5,425)        | 97.7           | 99.0           | 97.1        | 99.0        | 97.1        | 99.0        | 97.1        | 99.0        | 97.1        |
| (95.8)          |               | (99.8)         | (93.2)         | (98.6)      |             |             |             |             |             |             |
| R-factor (%)    |                | 22.0           | 18.6           | 17.4        | 19.4        | 22.0        | 19.2        | 23.0        | 22.0        | 19.2        |
| Rfree (%)       |                | (28.7)         | (23.2)         | (19.2)      | (23.0)      | (26.8)      | (25.1)      | (28.3)      | (25.1)      | (28.3)      |
| Average B-factor (Å²) |        | 32.1           | 21.9           | 27.9        | 21.4        | 32.1        | 21.9        | 27.9        | 21.4        | 21.9        |
| r.m.s.d. deviations |           |                |                |             |             |             |             |             |             |             |
| Bond (Å)        |                | 0.006          | 0.005          | 0.005       | 0.005       | 0.006       | 0.005       | 0.005       | 0.005       | 0.005       |
| Angle (°)       |                | 1.38           | 1.37           | 1.28        | 1.25        | 1.38        | 1.37        | 1.28        | 1.25        | 1.28        |

*R* free-factor was calculated using 10% of the unique reflections.

* Root mean square.

Family GH10 Xylanase with Decorated Xylooligosaccharides 9607

![Figure 1: Ribbon models of SoXyn10A:Araf-X3 (A) and SoXyn10A:MeGlcUA-X2 (B) complexes. α-helices and β-strands are drawn in red. Soaked sugars and disulfide bonds are indicated by ball-and-stick drawings in which the xylose, Araf, and MeGlcUA moieties and the cysteine residues are shown in green, orange, yellow, and light green, respectively. The figure was drawn with the MOLSCRIPT and Raster3D programs (36, 37).](image-url)
flash-frozen under a 100 K nitrogen stream. Diffraction experiments were conducted first on an imaging-plate detector RAXIS IV2 using Ultrax-18 CuKα x-ray generator (Rigaku). All crystals diffracted to ~2.0 Å resolution. The collected data sets were processed and scaled using program CRYSTALCLEAR (Rigaku). All of the crystals belonged to orthorhombic space group P2₁2₁2₁ and contained two molecules in an asymmetric unit. Further diffraction experiments were conducted at beamlines BL6A and BL6B, Photon Factory, Tsukuba, Japan. Diffraction data were collected using a Quantum 4 CCD x-ray detector (Area Detector Systems Corp.) at BL6A (λ = 0.978 Å) and an imaging plate detector, RAXIS IV2 (Rigaku), at BL6B (λ = 1.0 Å), in 1.0° oscillation steps over a range of 150°. The native data sets were processed and scaled using DFS/MOSFLM (29). Crystal parameters and structural refinement statistics are shown in Table I.

**Structure Determination**—Structural models were initially derived from the protein coordinates of the SoXyn10A structure in complex with X2 or X3 (19) (Protein Data Bank accession codes 1ISW and 1ISX). Rigid body refinement against the complex data using the program CNS (30) provided appropriate structure models and the resultant $F_{\text{obs}} - F_{\text{calc}}$ or $2F_{\text{obs}} - F_{\text{calc}}$ maps yielded an electron density corresponding to the soaked substrate. Sugar models were added carefully into the model, and successive manual model rebuilding was conducted with the program QUANTA (Accelrys). The model was then refined by several cycles of simulated annealing using the parameters of Engh and
Huber (31) and gradually adding water molecules. Five percent of the observed reflections was randomly removed for purposes of cross-validation (32). The stereochemistry of the models was analyzed with the programs PROCHECK (33) and WHATCHECK (34) (Table I).

RESULTS AND DISCUSSION

Overall Structure—Fig. 1 shows the crystal structures of Araf and MeGlcUA-xylotriose in complex with SoXyn10A. All crystals contained two noncrystallographic symmetry (NCS)-related molecules, A and B. The root-mean-square differences between molecules A and B were less than 1.0 Å for all atom pairs. Crystal parameters and structural refinement statistics are shown in Table I. The overall structure of SoXyn10A in all complexes was almost identical to that of the uncomplexed enzyme. The root-mean-square differences between the complexed and uncomplexed structures were less than 1.0 Å. The model of SoXyn10A consisted of an N-terminal 301-residue (β/α)8-barrel motif, which comprises the catalytic domain, a 123-residue ricin-type lectin domain of SoCBM13, with an 11-residue Gly/Pro-rich linker region between the two domains. The electron density for the linker region was obscured, suggesting that this region is disordered.

The electron densities corresponding to the soaked side-chain-substituted xylooligosaccharides were found in the catalytic cleft of the catalytic domain and in subdomains α and γ of the SoCBM13, as previously observed in xylooligosaccharide soaking experiments (19) (Figs. 1 and 2). The electron density for the soaked sugar was not observed in subdomain β, because this site was involved in the crystal packing interface with neighboring molecules. The results of the soaking experiments are summarized in Fig. 3.

Side-chain-substituted Xylooligosaccharides Bound in the Catalytic Cleft—The structures of the bound sugars were almost identical between the NCS-related molecules A and B. Therefore only molecule A is analyzed in detail, as its refined B-factors are relatively low and the electron density is clearer than in molecule B. The active site is positioned at the bottom of the cleft that runs along the C-terminal side of the β-strands of the (β/α)8-barrel. Subsites at the catalytic cleft of GHs are defined as +1, +2... in the direction of the reducing end and as −1, −2... toward the nonreducing end of the oligosaccharide with the cleavage site located between −1 and +1 (35). In the structure of the SoXyn10A-Araf-X3 complex (Fig. 2A), three xylose units were observed at subsites −1 to −3. The substi-
tuted Ara/ residue was linked α-1,3 to the xylose at subsite −2, and the O-3 hydrogen-bonded to the main-chain carbonyl oxygen atom of Asp-272 and the Oe1 atom of Glu-44. In contrast, only two xylose units were observed at subsites +1 and +2. Although the density corresponding to the Ara/ moiety was not observed in the structure of the SoXyn10A:Ara/X2 complex, two X2 moieties were apparent in subsites −1 and −2 and at subsites +1 and +2. In both complexes there were numerous hydrogen bonds between the enzyme and the xylose moieties at subsites −1 and −2, as observed in the previous study (19). Subsites −1 and −2 were thus considered to have high affinities for xylooligosaccharides. Similar to the accompanying paper (26), in the SoXyn10A:Ara/X3 complex, the Ara/ moiety was held in a rigid conformation as it was sandwiched between the xylose at subsite −3 and the protein, although in solution the α-1,3-linkage might be rather flexible. This is likely, as the Ara/ moiety was not observed in the SoXyn10A:Ara/X2 complex and might therefore be disordered. Only two xylose moi-
eties, however, were observed in the (+) subsites of the cleft, and the Araf residue could not be identified because of the unclear electron density. The Araf residue should have been linked to xylose at +1 when the soaked sugar was Soxyn10A:Araf-X2 and to xylose at +2 when the soaked sugar was Soxyn10A:Araf-X3. Although O-3 of the xylose at subsite +2 was close to Arg-275, the xylose molecule at subsite +2 might contain the Araf side chain at O-3, as the substrate was slightly displaced from the +2 binding site, which may obscure the electron density. These binding modes are consistent with the observation that Soxyn10A cannot cleave substrates that require the arabinose side chain to be positioned in the −1 subsite, where the O-2 and O-3 of xylose is completely buried but is able to accommodate this side chain at the +1 and +2 subsites (4). It is interesting to note that in the accompanying paper arabinose is observed at the −2 subsite but is not accommodated at −1, and no decoration is apparent when Araf-X3 is bound at +1 to +3, although the biochemical analysis of the family 10 xylanase from Cellvibrio mixtus (CmXyn10B) indicates that arabinose can be positioned at the +1 but not at the +2 subsite (26). Thus, both Soxyn10A and CmXyn10B can accommodate arabinose side chains linked to O-3 at −2, −3, and +1, but only the Streptomyces can tolerate Araf at +2.

In the structure of the Soxyn10A:MeGlcUA-X3 complex,
MeGlcUA-X3 was observed at subsites $-1$ to $-3$ in the active site, and only two xylose units were observed at subsites $+1$ and $+2$, similar to the SoXyn10A-Araf-X3 complex (Fig. 2B). The substituted MeGlcUA residue was linked $\alpha$-$1,2$ to the xylose at subsite $-3$ and was oriented toward the solvent making no direct contact with the protein. The pyranose ring of MeGlcUA had a normal chair conformation, and 2-hydroxyl, 3-hydroxy, 4-methoxy, and 5-carboxyl groups were all in equatorial positions. In the accompanying paper (26), MeGlcUA was also observed at the $-3$ subsite of CmXyn10B. However, in contrast to SoXyn10A, the uronic acid was clearly visible at the $+1$ subsite of the Cellulibrio enzyme, suggesting differences in the affinity for substrate in the ($+$) subsite region of the active site of the two xylanases. The structure of the SoXyn10A-MeGlcUA-X2 complex was different between the NCS molecules A and B. In molecule A, no sugars were identified in the catalytic cleft; but in molecule B, two xylose units were observed at subsites $-2$ and $-3$, and it is assumed that the MeGlcUA residue is linked to the pyranose sugar located at subsite $-3$. As seen in Fig. 2B, the O-2 of xylose at subsite $-2$ makes a hydrogen bond with the Ne atom of Trp-266, and thus the aromatic residue would prevent occupancy of a xylose decorated at the O-2 position.

Fig. 4 shows a surface potential model of the catalytic cleft of SoXyn10A with a model of Araf-MeGlcUA-X3. Trp-274 and Gln-88 form a gate to the ($-$) subsite of the cleft, and the O-2 and O-3 atoms of xylose at subsite $-1$ and the O-2 atom of xylose at subsite $-2$ are pointing into the cleft, whereas the O-3 atom of xylose at subsite $-2$ and the O-2 and O-3 atoms of xylose at subsite $-3$ are pointing into solvent. Open space is seen to spread from the gate, and the Araf residue linked to xylose at subsite $-2$ is accommodated in the space between Trp-274 and xylose at subsite $-3$. In the ($+$) subsite, possibly because of weak affinities, the substituted sugar residues appear disordered and could not be observed. It appears that most of the substituted xyooligosaccharides, however, could be accommodated in the ($+$) subsite of the cleft, because the O-2 and O-3 atoms of xylose at subsite $+1$ point into solvent, whereas the xylose at subsite $+2$ is positioned almost outside of the cleft.

Side-chain-substituted Xylooligosaccharides Bound in the SoCBM13—Although bound ligands were observed in subdomains $\alpha$ and $\gamma$ in both the SoXyn10A-Araf-X2 and SoXyn10A-Araf-X3 complexes, the Araf side chain was evident only in subdomain $\gamma$ (Fig. 3). The binding mode of linear (19) and decorated xylooligosaccharides (this study) were very similar. Each binding site in SoCBM13 interacted mainly with a single xylose moiety of the xylooligosaccharides (Fig. 5). The O-2 and O-3 atoms of the xylose at the binding site were positioned in the inner part of the site, forming hydrogen bonds with the side chains of the conserved Asp and Asn residues. The O-1 or O-4 atoms made hydrogen bonds with Gln-338 in subdomain $\alpha$ or Glu-421 in subdomain $\gamma$ and were located at the SoCBM13 surface. The O-2 or O-3 atom of the bound xylose had other hydrogen bonding interactions with main-chain carbonyl of Pro-327 in subdomain $\alpha$ or the carbonyl of Val-410 in subdomain $\gamma$, and the side chain of His-343 in subdomain $\alpha$. The aromatic rings of Tyr-340 in subdomain $\alpha$ and Tyr-423 in subdomain $\gamma$ stacked against the pyranose rings of the xylose sugars of the xylose sugars. The adjacent xylose units were linked via $\beta$-$1,4$-glycosyl linkages in either direction. In the subdomain $\gamma$ of the SoXyn10A-Araf-X2 complex, the xylose at the reducing end was located in the binding site, and thus the xylose containing the Araf residue made weak interactions with SoCBM13 (Fig. 5A). To discuss the mechanism of ligand binding in SoCBM13, the sugar residues in the oligosaccharides need to be defined. The xylose residues adjacent to the sugar located at the $\gamma$ and binding sites were defined by conserved amino acids on either side of the bound pentaose. One site was designated as $\gamma_{Q1}$ ($\alpha_{Q1}$), because it is close to the conserved residue Gln-421 in subdomain $\gamma$ (Gln-338 in subdomain $\alpha$), whereas the opposite site was designated as $\gamma_{Q2}$ ($\alpha_{Q2}$), because the conserved residue Asn-430 in subdomain $\gamma$ (Asn-347 in subdomain $\alpha$) is located on this side. The continuous sugar residues were designated as $\gamma_{N1}$, $\gamma_{N2}$, etc. In the SoXyn10A-Araf-X2 complex, the Araf-substituted xylose was

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**Fig. 6. Stereo views of Araf-X3.** A, Araf-X3 bound in the catalytic cleft; B, Araf-X3 bound in subdomain $\gamma$ of SoCBM13, with the $F_{calc}$ $-$ $F_{obs}$ omit electron density maps contoured at 2.5 $\sigma$. The intramolecular hydrogen bond is shown as a broken line.
bound in the γ1 site in subdomain γ. In contrast, in the SoXyn10A-Araf-X3 complex, the xylose bound in the γ binding site was at the nonreducing end of the oligosaccharide, and the Araf residue was linked α-1,3 to the adjacent middle xylose at the γ1 site (Fig. 5B). Thus, in the SoXyn10A-Araf-X2 and SoXyn10A-Araf-X3 complexes, the Araf residue is positioned at the opposite side of the γ binding site (Fig. 3), and SoCBM13 seems to be able to accommodate Araf in arabinopyranosyl at either γ1 or γ1. Fig. 6 shows the \( F_{o} - F_{c} \) electron density maps around the bound Araf in the catalytic cleft and in subdomain γ of SoCBM13. The only difference was in the puckering of the furanose ring, which had a considerable influence on the direction of the hydroxyl and hydroxymethyl groups of the sugar. Thus, the conformation of the furanose ring of the Araf residue is influenced by the network of hydrogen bonds. In the catalytic domain, the O-2 of the Araf residue hydrogen bonds to the main-chain carbonyl oxygen atom of Asep-272 and the Oε1 of Glu-44, as shown in Fig. 2A. In the subdomain γ of SoCBM13, the Araf residue points into solvent and makes no direct contact with the protein. However, the O-3 atom of the Araf residue makes an intramolecular hydrogen bond to the endocyclic oxygen of the xylose at the γ binding site.

For the MeGlcUA-xylooligosaccharides, the bound ligands were clearly observed in subdomains α and γ in both the SoXyn10A-MeGlcUA-X2 and SoXyn10A-MeGlcUA-X3 complexes, except for the xylose residue at the γ1 site in subdomain γ in the SoXyn10A-MeGlcUA-X3 complex. In the SoXyn10A-MeGlcUA-X2 complex, the MeGlcUA-X2 displayed the same binding mode in subdomains α and γ. The reducing end xylose was bound in the α and γ binding sites, respectively, and the MeGlcUA residue was bound in either the αN or γN1 site through an α-1,2 linkage (Fig. 5C). The MeGlcUA residue pointed into solvent and did not interact with the protein, similar to Araf. In the SoXyn10A-MeGlcUA-X3 complex, the middle xylose was bound in the α binding site, and xylose linked to the MeGlcUA residue was located at the αN1 site, similar to the SoXyn10A-MeGlcUA-X2 complex (Fig. 5D), whereas in subdomain γ, the reducing end xylose was bound in the γ binding site. The distal xylose in the αN2 site and the MeGlcUA residue were not clearly visible.

In both the SoXyn10A-Araf-X2 and the SoXyn10A-MeGlcUA-X2 complexes (Figs. 3 and 5, A and C), subdomain γ recognized the reducing end xylose, with the adjacent substituted xylose positioned on the opposite side of the binding site at γ1 and γN1, respectively. In previous studies, the O-1–O-4 direction of the xylose unit could not be determined by analyzing 2-Å resolution electron density maps because of the highly symmetrical structure of the xylose moieties in xylooligosaccharides (19). The determination of the probable direction of the bound xylose was therefore guided by the orientation of the glucose moiety in the SoXyn10A-glucose complex, as the hexose sugar has a nonsymmetrical structure; it contains an extra hydroxymethyl group at C-5 (compared with xylose), which provided clear electron densities enabling the orientation of the sugar to be determined. In decorated xylooligosaccharides, the presence of the side chains makes these ligands nonsymmetrical, enabling the orientation of the xylose polymer to be determined. Even though the electron densities were not clear for some sugar side chains, the continuous density from the hydroxyl group of a xylose molecule sometimes allowed identification of the sugar decoration. The confirmed directions from the nonreducing end to the reducing end of the bound xylooligosaccharides are indicated in Fig. 3 by arrow.

In the MeGlcUA-xylooligosaccharide complexes, MeGlcUA residues were placed on the same side of the SoCBM13 binding site, and the orientation of the bound xylooligosaccharides was reversed. Because the O-2 of the adjacent nonreducing end xylose at the γ1 site points at the protein (Fig. 5A), the MeGlcUA residue linked α-1,2 could not be accommodated at this site. In subdomain α of the SoXyn10A-Araf-X2 complex, the xylooligosaccharide bound in both directions.

These results demonstrate that SoCBM13 can bind the xylan backbone in either direction. The results also show that SoCBM13 can accommodate the side chains in various ways, interacting with both the adjacent and decorated xylose and the attached Araf and MeGlcUA xyans in the reverse direction. Because the role of SoCBM13 is considered only to bind xylan and not to direct the substrate into the active site of the enzyme, the capacity of the protein module to display multiple binding modes for its polymeric ligands may increase the probability of the catalytic domain hydrolyzing decorated xylans.

In conclusion, a crystallographic study of Araf- and MeGlcUA-decorated xylooligosaccharides in complex with the family GH10 xylanase SoXyn10A has revealed details of the mechanism by which these side chains are accommodated in both the active site and in CBM13 and how these complex carbohydrates are hydrolyzed by the catalytic domain. The sugar-binding modes in the catalytic cleft were consistent with the previously reported substrate specificity of SoXyn10A toward arabinoglucuronoxylan and, in conjunction with the accompanying paper (26), demonstrate that the mechanism by which sugar side chains are accommodated in family 10 xylanas is highly conserved. Using nonsymmetric sugars as ligands, CBM13 was shown to bind xylose and xylooligosaccharides reversibly. This flexible binding mode displayed by SoCBM13 increases the probability of the enzyme interacting with its substrate and thus catalyzing glycosidic bond cleavage.

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