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GH30 Glucuronoxylan-Specific Xylanase from *Streptomyces turgidiscabies* C56

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ABSTRACT

Endoxylanases are important enzymes in bioenergy research because they specifically hydrolyze xylan, the predominant polysaccharide in the hemicellulose fraction of lignocellulosic biomass. For effective biomass utilization, it is important to understand the mechanism of substrate recognition by these enzymes. Recent studies have shown that the substrate specificities of bacterial and fungal endoxylanases classified into glycoside hydrolase family 30 (GH30) were quite different. While the functional differences have been described, the mechanism of substrate recognition is still unknown. Therefore, a gene encoding a putative GH30 endoxylanase was cloned from Streptomyces turgidiscabies C56, and the recombinant enzyme was purified and characterized. GH30 glucuronoxylan-specific xylanase A of Streptomyces turgidiscabies (*St*Xyn30A) showed hydrolytic activity with xylans containing both glucuronic acid and the more common 4-*O*-methyl-glucuronic acid side-chain substitutions but not on linear xylooligosaccharides, suggesting that this enzyme requires the recognition of glucuronic acid side chains for hydrolysis. The *St*Xyn30A limit product structure was analyzed following a secondary β-xylosidase treatment by thin-layer chromatography and mass spectrometry analysis. The hydrolysis products from both glucuronoxylan and 4-*O*-methylglucuronoxylan by *St*Xyn30A have these main-chain substitutions on the second xylopyranosyl residue from the reducing end. Because previous structural studies of bacterial GH30 enzymes and molecular modeling of *St*Xyn30A suggested that a conserved arginine residue (Arg296) interacts with the glucuronic acid side-chain carboxyl group, we focused on this residue, which is conserved at subsite −2 of bacterial but not fungal GH30 endoxylanases. To help gain an understanding of the mechanism of how *St*Xyn30A recognizes glucuronic acid substitutions, Arg296 mutant enzymes were studied. The glucuronoxylan hydrolytic activities of Arg296 mutants were significantly reduced in comparison to those of the wild-type enzyme. Furthermore, limit products other than aldotriouronic acid were observed for these Arg296 mutants upon secondary β-xylosidase treatment. These results indicate that a disruption of the highly conserved Arg296 interaction leads to a decrease of functional specificity in *St*Xyn30A, as indicated by the detection of alternative hydrolysis products. Our studies allow a better understanding of the mechanism of glucuronoxylan recognition and enzyme specificity by bacterial GH30 endoxylanases and provide further definition of these unique enzymes for their potential application in industry.