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Carbohydrate binding module of family 48 enable ferulic acid esterases action on polymeric arabinoxylan

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Arabinoxylans (AXs) are a major component of hemicelluloses, which is widely distributed in secondary cell walls of plants. Their backbone is composed of β-1,4-linked xylopyranose residues that are single substituted with α-L-1,3-arabinofuranose (Ara*f*) or double substituted with both α-L-1,2- and α-L-1,3-Ara*f*, which can be further substituted with by 5-*O*-ferulic acid and other hydroxycinnamic acids [1]. Ferulic acid esterases (FAEs) catalyze the hydrolysis of ester bonds between hydroxycinnamic acids and Ara*f*, however, most characterized FAEs do not display a preference for polymeric substrates [2,3].
Recently, a number of carbohydrate esterase family 1 (CE1) identified in metagenomic studies was shown to have a carbohydrate binding module of family 48 (CBM48) appended [4,5], a family associated with starch binding [6]. Our phylogenetic analysis demonstrated that these are in fact CBM48s suggesting that CBM48s is a polyspecific family since CE1s do not target starch. Adsorption assays with two CE1-CBM48 enzymes demonstrated binding to AXs, but not to starch, which was supported by a surface plasmon resonance analysis showing no binding to β-cyclodextrin or maltohexaose. Binding was detected to arabino- and xylooligosaccharides and interestingly also to maltotetraose. The two CE1-CBM48 enzymes released FA from AXs, while the CE1 domain on its own only released FA from oligosaccharides and unlike the full-length enzymes the CE1 domain was unable to bind to AXs. Crystal structures of the two CE1-CBM48s revealed two integrally folded units and multiple structurally conserved hydrogen bonds fix the CBM48's position relative to the CE1 domains. Molecular dynamics simulations confirmed that the two domains form a rigid structure. Docking studies suggest that the xylan main chain is accommodated in the cleft formed at the interface between the CE1 and CBM48 domains.

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