An efficient arabinoxylan-debranching α-L-arabinofuranosidase of family GH62 from Aspergillus nidulans contains a secondary carbohydrate binding site - DTU Orbit
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An efficient arabinoxylan-debranching α-L-arabinofuranosidase of family GH62 from Aspergillus nidulans FGSC A4 (AnAbf62A-m2,3) has an unusually high activity towards wheat arabinoxylan (WAX) (67 U/mg; \( k_{\text{cat}} = 178/s \), \( K_m = 4.90 \text{ mg/ml} \)) and arabinoxylooligosaccharides (AXOS) with degrees of polymerisation (DP) 3–5 (37–80 U/mg), but about 50 times lower activity for sugar beet arabinan and 4-nitrophenyl-α-L-arabinofuranoside. α-1,2- and α-1,3-linked arabinofuranoses are released from monosubstituted, but not from disubstituted, xylose in WAX and different AXOS as demonstrated by NMR and polysaccharide analysis by carbohydrate gel electrophoresis (PACE). Mutants of the predicted general acid (Glu188) and base (Asp28) catalysts, and the general acid pK_a modulator (Asp136) lost 1700-, 165- and 130-fold activities for WAX. WAX, oat spelt xylan, birchwood xylan and barley β-glucan retarded migration of AnAbf62A-m2,3 in affinity electrophoresis (AE) although the latter two are neither substrates nor inhibitors. Trp^23 and Tyr^44, situated about 30 Å from the catalytic site as seen in an AnAbf62A-m2,3 homology model generated using Streptomyces thermoviolaceus SthAbf62A as template, participate in carbohydrate binding. Compared to wild-type, W23A and W23A/Y44A mutants are less retarded in AE, maintain about 70% activity towards WAX with \( K_i \) of WAX substrate inhibition increasing 4–7-folds, but lost 77–96% activity for the AXOS. The Y44A single mutant had less effect, suggesting Trp^23 is a key determinant. AnAbf62A-m2,3 seems to apply different polysaccharide-dependent binding modes, and Trp^23 and Tyr^44 belong to a putative surface binding site which is situated at a distance of the active site and has to be occupied to achieve full activity.

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