Analysis of Surface Binding Sites (SBS) within GH62, GH13, and GH77 - DTU Orbit (11/11/2019)

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Certain interactions between carbohydrate active enzymes and polysaccharides involve surface binding sites (SBS) situated on catalytic domains outside of the active site. We recently undertook to develop a toolbox for SBS identification and characterization. In affinity gel electrophoresis (AGE) SBS containing proteins are migrating slower in native polyacrylamide electrophoresis gels cast with polysaccharide versus without polysaccharide. Amylolytic enzymes from GH13 and GH77 and xylanases from GH10 and GH11 are the best studied GH families with respect to SBS, presenting about half of the reported SBSs. In GH13 SBSs have been seen in 17 subfamilies including SBSs with highly diverse functions in the same enzyme. Circumstantial evidence is provided for an SBS in the GH77 MalQ from Escherichia coli, the bacterial orthologue of Arabidopsis DPE2 involved in starch metabolism. Furthermore, Aspergillus nidulans α-L-arabinofuranosidase AnAbf62A-m2,3 of GH62 that has very high activity on wheat arabinoxylan (WAX) shows an unusually strongly retarded migration by WAX during AGE analysis. Using a recent GH62 crystal structure as template, Trp23 and Tyr44 in an AnAbf62A-m2,3 model are proposed to form an SBS situated about 30 Å from the catalytic site. Compared to wild-type, W23A/Y44A AnAbf62A-m2,3 retained 45% activity on WAX and was less retarded in AGE by WAX as well as by barley β-glucan and birchwood xylan, which are neither hydrolysed nor inhibiting activity towards WAX. The presence of a functional SBS agrees with W23A/Y44A AnAbf62A-m2,3 retaining only 3-25% activity for arabinoxyloligosaccharides (AXOS) of DP 3-5 possibly reflecting allosteric activation of wild-type through SBS occupation by AXOS.

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