Discovery and characterization of surface binding sites in polysaccharide converting enzymes

Enzymes that act on various polysaccharides are widespread in any domain of life and they play a role in degradation, modification, and synthesis of carbohydrates.

These carbohydrate active enzymes interact with their substrate (the polysaccharide) at the active site and often at so-called subsites that are residues located on both sides of the active site within a few Ångstrøms from the active site. Further binding sites are often found on separate domains called carbohydrate binding modules (CBMs) and these binding sites have been shown in several cases to be important for both binding to and activity towards carbohydrates. Far from all carbohydrate active enzymes contain a CBM, but some of these enzymes contain binding sites on their catalytic domain outside the active site referred to as surface binding sites (SBSs). SBSs have been observed in several crystal structures; however, only in a few cases have these SBSs been characterized and shown to have an impact on the enzyme’s functionality. The understanding of these SBSs is currently at a level where their presence is difficult, if not impossible, to predict in the absence of structural evidence. The limited data on SBSs preclude a generalization and may mask the significance of these sites in catalysis.

GH62 α-L-arabinofuranosidase from Aspergillus nidulans FGSC A4 (AnAbf62A-m2,3) does not contain a CBM, however, AnAbf62A-m2,3 interacts strongly with wheat arabinoxylan, birchwood xylan and oatspelt xylan in affinity gel electrophoresis (AGE), which could indicate that binding outside the active site takes place. Mutation of Trp23 and Tyr44 in the active site area reduced specific activity for arabinoxyloligosaccharides (DP 3–5) to 3–25%, while modestly altered activity for wheat arabinoxylan and sugar beet arabinan reflected polysaccharide binding interactions to also occur at a distance from the active site. AnAbf62A-m2,3’s preferred substrate is wheat arabinoxylan having $k_{cat}$ and $K_M$ of $178 \pm 26$ s$^{-1}$ and $4.90 \pm 0.91$ mg ml$^{-1}$, respectively. While AnAbf62A-m2,3 from singly substituted xylose releases 1,2-linked at threefold higher rate than 1,3-linked arabinosyl residues, it has no activity towards doubly 1,2- and 1,3-arabinose substituted xylosyl residues. $^1$H NMR identified produced arabinose as the β-furanose form indicating AnAbf62A-m2,3 to have an inverting mechanism as also inferred from the similarity with GH43 that together with GH62 constitutes clan F of glycoside hydrolases. Sequence alignments and mutational analysis demonstrated the active site catalytic triad as Asp28 (general base), Glu188 (general acid) and Asp136 (pKa-modulator and general acid stabilizer).

Barley starch synthase I (HvSSI) has previously been shown to contain a SBS, which in the present thesis is shown to be responsible for HvSSIs affinity for β-cyclodextrin (β-CD) and maltooligosaccharides. HvSSI SBS mutant F538A lost the ability to bind β-CD and maltooligosaccharides. Using surface plasmon resonance (SPR) it was shown that wild-type Hv SSI has no detectable affinity for maltotriose and -tetraose, but clearly binds maltopentaose, -hexaose, -heptaose (M7) and β-cyclodextrin (β-CD) albeit with a measurable $K_D$ for only β-CD (0.94 ± 0.07 mM) and M7 (1.99 ± 0.10 mM). The plant phosphoglucan phosphatases Starch Excess 4 (SEX4) and Like Sex Four 2 (LSF2) have different affinity for amylopectin, $K_D$ being 0.030 ± 0.002 and 1.59 ± 0.08 mg ml$^{-1}$, respectively. Although corresponding $K_D$ values for β-CD of 1.69 ± 0.17 and 0.72 ± 0.06 mM are similar, SEX4 and LSF2 are suggested to have different binding modes and roles in starch dephosphorylation. While SEX4 contains a CBM48 SBSs in LSF2 are engaged in binding of amylopectin and β-CD as demonstrated by mutational analysis of SBS1 and SBS2 resulting in loss of β-CD and amylopectin binding. A crystal structure of β-agarase A (AgaA) from Zobellia galactanivorans in complex with dodecaagarooligosaccharide showed almost 10 years ago that a SBS was present on AgaA. AgaA E147S was subjected to a surface plasmon resonance (SPR) analysis and $K_D$ values were determined for deca-agarose (92.5 ± 9.8 μM), octa-agarose (128.0 ± 0.5 μM) and hexa-agarose (266.4 ± 7.2 μM). The three-fold difference in affinity between deca- and hexa-agarose suggests that residues interacting with reducing and non-reducing end of deca-agarose contribute significantly to the binding. It is clear though that the residues at the central part of the cleft shaped SBS are the most important ones when it comes to binding of agaro-oligosaccharides.

General information
Publication status: Published
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering
Contributors: Wilkens, C.
Number of pages: 279
Publication date: 2014

Publication information
Place of publication: Kgs. Lyngby
Publisher: Technical University of Denmark (DTU)
Original language: English