Deleting α-helix of CtAbf43A for enhancing affinity by in-silico approach

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Amalgamation of X-ray crystallography studies, computational biology and protein engineering has proven to be successful for generation of tailor made enzymes for efficient degradation of plant polysaccharides3. Such tailor made enzymes generated from existing recombinant enzymes are more stable, have enhanced specific activity and higher tolerance against physical and chemical environments1. C.thermocellum genome encodes a multi-modular family 43 glycoside hydrolase (GH43) comprising an N-terminal signal peptide a GH43 catalytic module, followed by two tandemly repeating carbohydrate binding modules (CBM6-1 and CBM6-2) and a C-terminal dockerin2. This study explores: (a) comparative structural analysis of CtAbf43A (an α-L-arabinofuranosidase belonging to family 43 glycoside hydrolase) and other enzymes responsible for removal of arabinose side chain of xylan (b) structural variation of the 3D-structures, 5A8C and 5A8D of CtAbf43A (c) binding analysis of 5A8C and 5A8D with arabinoxylotriose through molecular docking studies and (d) in-silico analysis of effect of mutations in CtAbf43A on binding with arabinoxylotriose. CtAbf43A structure comprises 5-bladed β-propeller fold typical of GH43 enzymes. Crystal structures of CtAbf43A were solved by molecular replacement at 0.97 Å for monoclinic form (PDB id: 5A8C) and 1.65 Å for cubic form (PDB id: 5A8D)2. In 5A8C structure, the N- and C-terminal β-strands are in close association, providing additional stability to the structures. The full length CtAbf43A enzyme contains catalytic module and two CBM6s at C-terminal providing additional stabilization to the structure. 5A8C structure of CtAbf43A shows the conformation that is expected in full length protein. In absence of both CBM6s in CtAbf43A (5A8D), the terminal amino acids from 29 to 42 make a 180º turn in the direction of catalytic cleft, whereas this turn is not present in 5A8C structure. Therefore, the catalytic cleft in 5A8D may lead to a significant compensatory movement of some loops around the carbohydrate binding cleft. The structural superimposition of both 5A8C and 5A8D structures showed an identical core with a significant difference at N- and C-terminal as well as in the loops responsible for carbohydrate binding. N-terminal residues, 29 to 42 in case of CtAbf43A (5A8D) form a helical structure over the catalytic cleft. An insilico deletion of these N-terminal residues of CtAbf43A (5A8D) using Pymol molecular graphic system was done and a model was generated. The mutant 5A8D structure and the native structure of CtAbf43A were used for comparative docking study using arabinosylxylotriose as ligand. The native structure 5A8D of CtAbf43A showed no binding with arabinosylxylotriose (ΔG, +121 kcal/mol) whereas, a stronger binding (ΔG, -2.46 kcal/mol) in case of mutant CtAbf43A (5A8D) was found. This indicated that the mutated 5A8D structure of CtAbf43A showed higher binding affinity for arabinosylxylotriose when compared with the native structure 5A8D. Thus, CtAbf43A will be engineered for increasing its substrate affinity for enhanced activity using the information from in-silico study.


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