Poster Presentation

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Low-resolution structure analysis of α-L-arabinofuranosidase (CtGH43) by SAXS

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Enzymatic hydrolysis of lignocellulose has attracted significant interest in the last few years for the viable production of bioethanol. Despite the recent advances in lignocellulose saccharification, the availability of effective enzymes is still a limiting factor in reducing the cost of second-generation fuels, bioethanol. Several enzymes involved in lignocellulose hydrolysis have a multi-domain structure, containing a catalytic module and one or more carbohydrate binding modules (CBM). Several studies have reported that CBMs facilitate substrate binding and access to the active site of the catalytic domain, promoting substrate specificity and stability of the enzyme-substrate complex. However, the conformational changes between the catalytic domain and the CBM in the presence or absence of natural substrates remains unknown and such knowledge is necessary to completely understand the complex mechanism of biomass hydrolysis by these multi-modular enzymes. The α-L-arabinofuranosidase from Clostridium thermocellum (CtGH43) is a thermostable modular enzyme having a catalytic module (CtAbf43) [1] at N-terminal and two carbohydrate binding modules (CtCBM6A and CtCBM6B) at the C-terminal [2,3]. CtGH43 catalyses the removal of arabinose side chains from hetero-polysaccharides, such as xylans and arabinan. In this study, CtGH43 was used to study the low-resolution model, overall dimensions and flexibility of this modular enzyme. Small-angle X-ray scattering (SAXS) combined with modelled three-dimensional structure of CtGH43 revealed its conformation. SAXS data for CtGH43 was collected at 1.18 mg/ml has a Radius of gyration (Rg) 4.08 nm for globular protein and Rc 1.36 nm for Rod Shape. CtGH43 possesses an elongated structure with an overall length 13.32 nm having two globular lobes connected via a linker. Kratky analysis suggested that CtGH43 is completely folded in solution state. Mw of CtGH43 calculated by Datmow Program was 67.54 kDa, suggesting its monomeric nature. CtGH43 solution structure was modelled by processing scattering profiles by ab initio approaches. Superposition of modelled CtGH43 structure with SAXS analysed low-resolution structure revealed close matching with two differences, (i) PT rich linker region connecting CtCBM6B with CtGH43-CtCBM6A is long and flexible, (ii) flexibility in linker region allows to CtCBM6B and CtGH43-CtCBM6A to position on the surface of substrate for catalysis.

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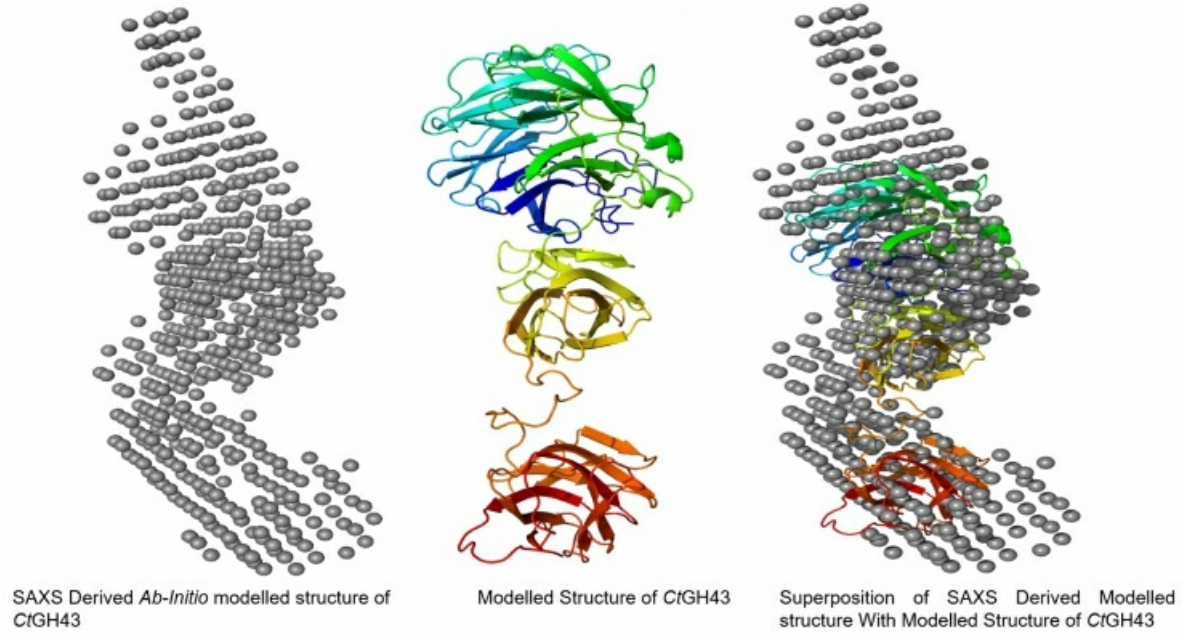

SAXS Derived *Ab-Initio* modelled structure of *Ct*GH43

Modelled Structure of *Ct*GH43

Superposition of SAXS Derived Modelled structure With Modelled Structure of *Ct*GH43

Keywords: CtGH43, α-L-arabinofuranosidase, SAXS