Molecular characterization of a trimodular family 5 glycoside hydrolase reveals a unique conformational selection mechanism

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Introduction: Cellulases and hemicellulases are glycoside hydrolases (GHs) found in all kingdoms of life. These enzymes cleave glycosidic bonds of complex carbohydrates and play fundamental roles in the decomposition of lignocellulosic biomaterials. Fungal and bacterial GHs have numerous applications in the food, paper, and biofuel industries. Most GHs are modular proteins comprising a catalytic core domain (CCD) and one or more carbohydrate-binding modules (CBMs), which enhance CCD activity by holding the catalytic domains in close proximity to their substrates. **Objective:** Elucidate how the accessory modules of a trimodular celullase from *Bacillus licheniformis* (*Bl*Cel5B) influence its catalytic properties. **Material and Methods:** Cloning of *Bl*Cel5B wildtype, mutants and deletions; protein expression in *Escherichia coli* (BL21); affinity and size exclusion chromatography; colorimetric activity assay, X-ray crystallography, small-angle X-ray scattering and molecular dynamics simulations. **Results and Discussion:** Here, we report the full-length structure of *Bl*Cel5B, a GH5 subfamily 4 member that is entirely dependent on its immunoglobulin-like module (CBM_X2) and CBM46 for catalytic activity. Full-length *Bl*Cel5B efficiently cleaves beta-glucan, xyloglucan, lichenan, and carboxymethylcellulose; however, deletion of the carboxi-terminal CBM46 abrogates enzymatic activity on these substrates. We demonstrate that the distal C-terminal CBM46 caps the N-terminal CCD to establish a fully functional substrate binding site via a large-scale multidomain conformational selection mechanisms. The CBM_X2, whose function is not known, connects CCD to CBM46 and is shown to be essential for pivoting the packing and unpacking motions of modules in the assembly of the binding site. **Conclusions:** The CCD of *Bl*Cel5B is completely dependent of its accessory modules for activity. Primary sequence alignments and phylogenetic mapping indicate that the multidomain architecture and molecular conformational selection mechanism proposed herein are not specific to this particular enzyme but may apply to various members of the GH5_4 subfamily.

Keywords: Cellulase; carbohydrate-binding module; multidomain conformational selection.

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