Molecular Insights into GH6 Family Cellobiohydrolase Activity on Lignocellulosic Substrates

Shabir Ahmad*, Munir S. Skaf
Department of Chemistry, UNICAMP, Brazil.
*E-mail: shabirjan427@gmail.com

ABSTRACT

Introduction: The catalytic mechanism of inverting glycoside hydrolases (GHs), particularly within family 6 (GH6), remains an area of active investigation (Varrot *et al*. 2003). GH6 enzymes are key components of cellulase cocktails, crucial for the efficient depolymerization of cellulose, a major component of plant biomass. These enzymes, specifically cellobiohydrolases (CBHs) or exoglucanases, are characterized by their ability to processively hydrolyze the 1,4-β-d- glycosidic bonds at the reducing or non-reducing ends of cellulose chains, yielding cellobiose and glucose. While CBHs are found across several GH families (5, 6, 7, 9, 48, and 74), the GH6 family, exemplified by Cel6A from Humicola insolens, presents a unique challenge due to the elusive identity of its catalytic base (Varrot *et al*. 2003). Cel6A's active site is a characteristic tunnel structure, facilitating processive degradation of the polysaccharide substrate. This tunnel architecture is defined by two flexible loops, crucial for substrate binding and progression through the active site; these loops exhibit dynamic conformational changes, including opening and closing movements during catalysis.

Objective: The objective of the current study is to elucidate the role of specific residues in this dynamic process and to identify the roles of aspartate as a catalytic base using molecular dynamics (MD) simulations.

Computational Methodology: The crystallographic structure of wild-type Cel6A (PDB: 1OCB) and two mutants, PDB D405N (PDB: 1OC5) and D416A (PDB: 1OCJ), was investigated. Enzyme structures were prepared using CHARMM-GUI (Huang *et al*. 2017; Jurrus *et al*. 2018), with missing glucose residues in the active site tunnel (subsites -4 to +4) modeled based on a homologous crystal structure to complete the typical eight-sugar-unit substrate binding site of GH6 CBHs. Each system (wild-type and two mutants) underwent seven steps of energy minimization, followed by ten steps of equilibration. During equilibration, restraints were gradually reduced while increasing temperature to achieve a stable, equilibrated system (Case *et al*. 2020). Finally, each system was subjected to triplicate 1 microsecond MD simulations.

Result: Analysis of the simulation trajectories revealed critical insights into Cel6A's catalytic mechanism. The simulations highlighted the importance of a hydrophobic tryptophan residue sheath in maintaining flexibility of the active site tunnel surfaces and facilitating substrate movement through solvent-mediated interactions. Significantly, the wild-type Cel6A displayed substantially greater loop conformational dynamics (opening and closing movements) compared to the mutants. This observation indicates that the aspartate residues (Asp405 and Asp416) are essential for the dynamic behavior of the active site loops required for effective substrate processing. The reduced loop mobility noted in the D405N and D416A mutants suggests that these aspartate residues are crucial, likely by affecting the electrostatic conditions within the active site. The conservative mutation of Asp405 to asparagine (D405N) and the more significant alteration of Asp416 to alanine (D416A) both led to diminished catalytic activity, corroborating the hypothesis that these residues are directly or indirectly implicated in catalysis.

Conclusion: These findings highlight the significance of the aspartate residues and the intrinsic flexibility of the Cel6A active site tunnel in the catalytic mechanism. Additional investigations are required to conclusively identify the catalytic base; however, these findings offer compelling evidence implicating Asp405 and Asp416 in critical components of the GH6 catalytic cycle.

Keywords: GH6, Cellobiohydrolases, Molecular Dynamics.

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