Enzymes degrading cellulose: from molecular mechanisms to traffic simulator

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Cellulose is a major component of plant cell wall and the most abundant biomass on earth. Efficient degradation of cellulose makes it possible to produce fuels and chemicals from plant resources for the achievement of Bioeconomy, although biochemical conversion of cellulose by cellulase is quite slow and the reaction becomes a bottleneck of the process. Cellobiohydrolases (CBHs) are types of cellulases hydrolyzing crystalline cellulose to soluble oligosaccharides, and one of the key enzymes in the cellulose biorefinery. CBHs share a common two-domain structure, cellulose-binding domain and catalytic domain, and these domains cooperatively function for the effective hydrolysis of crystalline cellulose. Since the reaction is carried out at the surface of insoluble substrate, it is not straightforward to analyze the reaction at a solid/liquid interface\textsuperscript{1,2}.

We recently reported the real-time visualization of crystalline cellulose degradation by individual cellulase molecules using a high-speed atomic force microscopy, having sub-second time resolution and nanometer space resolution\textsuperscript{3-5}. Cellulose-degrading ascomycete \textit{Trichoderma reesei} cellobiohydrolase I (\textit{TrCel7A}) molecules were observed to slide unidirectionally along the crystalline cellulose surface\textsuperscript{3}, but at some points the movement of individual molecules was halted, leading to the appearance of traffic jams of enzyme molecules\textsuperscript{4}. From the comparison with GH family 6 cellobiohydrolase (\textit{TrCel6A})\textsuperscript{6}, the basidiomycete \textit{Phanerochaete chrysosporium} cellobiohydrolases (\textit{PcCel7C} and \textit{PcCel7D})\textsuperscript{7,8} and chitinases\textsuperscript{9}, we discuss possible molecular mechanisms of these processive enzymes and the natural degradation of crystalline cellulose.

We will also introduce neutron crystallography\textsuperscript{10} to clarify the detailed hydrolytic mechanisms of inverting cellulases and application of traffic simulator to the analysis of molecular behavior of cellulases to connect mesoscopic gap between biochemical and single molecular analysis.

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