Nanoscale Dynamics of Cellulase TrCel7A Digesting Cellulose

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https://sites.psu.edu/hancocklab/research/cellulase-motility/

Project Goals: To study the dynamics of TrCel7A as it actively hydrolyzes cellulose using a single-molecule approach. A custom built SCATTIRSTORM microscope was designed to allow sufficient temporal and spatial resolution to accurately study cellulases with nanometer resolution. The overall goal is to create a better understanding of the mechanisms involved when cellulases interact with cellulose by creating a model to characterize the various states the enzymes can enter when binding and degrading the substrate.

Abstract text:

Cellulose is an abundant polysaccharide found in plant cell walls that is digested to celllobiose subunits by cellulase enzymes. Optimizing the digestion of lignocellulose for biofuels applications is of great interest, motivating a need to better understand the molecular mechanism of cellulases, and is under constant investigation due to its ability to be hydrolyzed into glucose subunits. The addition of yeast allows these glucose molecules to be turned into useable biofuels, which can assist in the current energy crisis when performed at an industrial scale. Currently, this process is extremely costly and inefficient, since the energy used to purify the enzymes is only slightly less than the energy these enzymes can produce. Several glucoside hydrolases (GH) are used in this reaction to produce glucose, including the celllobiohydrolase Cel7ACel7A from Trichoderma reesei is a model which is an exoglucanase that degrades cellulose from the reducing-end of strands by cleaving individual celllobiose units as it processes along the cellulose surface. Despite TrCel7ACel7A being one of the most studied glucose celllobiohydrolases, the binding and hydrolysis mechanisms are still not fully understood. Here we took a single-molecule tracking approach to study the kinetics of quantum dot-labeled TrCel7ACel7A on immobilized acetobacter cellulose. 11,116 enzyme molecules were tracked with spatial precision of a few nanometers for hundreds of seconds as they bound to and moved processively along their cellulose substrate. During processive segments, enzymes moved at 3.2 nm/s on average for a distance of 39 nm. The static episodes preceding and following processive runs were of similar duration to one another and were similar to the duration of the static binding events that lacked any processive movement. Although transient jumps of >20 nm were observed, no diffusive behavior indicative of a diffusive search of the enzyme for a free end of a cellulose strand were observed. These data were integrated into a three state model, in which TrCel7A can bind from solution into either a static or a processive state, and the molecules switch between processive and static states.
before dissociating. From these data, we conclude that the rate limiting step for this cellulase is transition out of the static state either by dissociating from the cellulose surface or by initiating a processive run.

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