Rate-limiting step and substrate accessibility of cellobiohydrolase Cel6A from Trichoderma reesei

The cellobiohydrolase (CBH) Cel6A is an important component of enzyme cocktails for industrial degradation of lignocellulosic biomass. However, the kinetics of this enzyme acting on its natural, insoluble substrate remains sparsely investigated. Here, we studied Cel6A from Trichoderma reesei with respect to adsorption, processivity, and kinetics both in the steady-state and pre-steady-state regimes, on microcrystalline and amorphous cellulose. We found that slow dissociation ($k_{off}$) was limiting the overall reaction rate, and we suggest that this leads to an accumulation of catalytically inactive complexes in front of obstacles and irregularities on the cellulose surface. The processivity number of Cel6A was low on both investigated substrates (5-10), and this suggested a rugged surface with short obstacle-free path lengths. The turnover of the inner catalytic cycle (the reactions of catalysis in one processive step) was too fast to be fully resolved, but a minimum value of about 20 s$^{-1}$ could be established. This is among the highest values reported hitherto for a cellulase, and it underscores the catalytic efficiency of Cel6A. Conversely, we found that Cel6A had a poor ability to recognize attack sites on the cellulose surface. On amorphous cellulose, for example, Cel6A was only able to initiate hydrolysis on about 4% of the sites to which it could adsorb. This probably reflects high requirements of Cel6A to the architecture of the site. We conclude that compared to the other CBH, Cel7A, secreted by T. reesei, Cel6A is catalytically more efficient but less capable of attacking a broad range of structurally distinct sites on the cellulose surface.

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