The influence of different linker modifications on the catalytic activity and cellulose affinity of cellobiohydrolase Cel7A from Hypocrea jecorina

Various cellulases consist of a catalytic domain connected to a carbohydrate-binding module (CBM) by a flexible linker peptide. The linker if often strongly O-glycosylated and typically has a length of 20-50 amino acid residues. Functional roles, other than connecting the two folded domains, of the linker and its glycans, have been widely discussed, but experimental evidence remains sparse. One of the most studied cellulose degrading enzymes is the multi-domain cellobiohydrolase Cel7A from *Hypocrea jecorina*. Here, we designed variants of Cel7A with mutations in the linker region to elucidate the role of the linker. We found that moderate modification of the linker could result in significant changes in substrate affinity and catalytic efficacy. These changes were quite different for different linker variants. Thus, deletion of six residues near the catalytic domain had essentially no effects on enzyme function. Conversely, a substitution of four glycosylation sites near the middle of the linker reduced substrate affinity and increased maximal turnover. The observation of weaker binding provides some support of recent suggestions that linker glycans may be directly involved in substrate interactions. However, a variant with several inserted glycosylation sites near the CBM also showed lower affinity for the substrate compared to the wild-type, and we suggest that substrate interactions of the glycans depend on their exact location as well as other factors such as changes in structure and dynamics of the linker peptide.