STRUCTURE AND FUNCTION OF ENZYMES AND AUXILIARY PROTEINS FROM TRICHODERMA, ACTIVE IN CELL-WALL HYDROLYSIS

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Lignocellulosic biomass, such as sugarcane bagasse, holds a promise of environmentally friendly bioenergy production in Brazil. However, enzymatic hydrolysis, currently considered a method of choice in biomass saccharification, is hampered by considerable cell-wall recalcitrance. To make this technology sustainable and cost effective, our comprehension of cellulose enzymatic hydrolysis should be significantly improved. Here we propose to conduct systematic structure-functional studies of *Trichoderma cellulases* and auxiliary proteins active in cell-wall degradation using a combination of X-ray protein crystallography, biophysical and biochemical studies, molecular dynamics simulations, statistical coupling analysis aligned with the site-directed mutagenesis and enzymatic assays aiming to obtain in-depth comprehension of cellulose hydrolysis. We plan to contribute toward structural analysis of *Trichoderma reesei* endoglucanases by solving a crystal structure of endoglucanase II (Cel5A), main enzymatically active, but structurally uncharacterized endoglucanase of this important industrial fungus. Moreover, we will contribute toward our knowledge of *Trichoderma cellulases* molecular organization by solving X-ray structures of main *Trichoderma harzianum* endo- and exoglucanases (primarily focusing on Cel7A and Cel5A) and by comparing them with the correspondent *T. reesei* enzymes. We also aim to structurally characterize swollenins, non-hydrolytic proteins, shown to enhance cellulose hydrolysis catalyzed by celulases, and to study thermodynamically its interactions with cellulose. In addition, we will construct chimeric enzymes by fusing of swollenin with the cellulases and will study enzymatic properties of such chimeras. Furthermore, we will conduct systematic molecular dynamics studies of the cellulases and swollenin, and investigate their flexibility by hydrogen deuterium exchange followed by mass-spectrometry. Finally, we will use all these acquired knowledge to modify the proteins using site-directed mutagenesis aiming to better comprehend molecular basis of their function and to produce enzymes and their mixtures with enhanced hydrolytic properties.

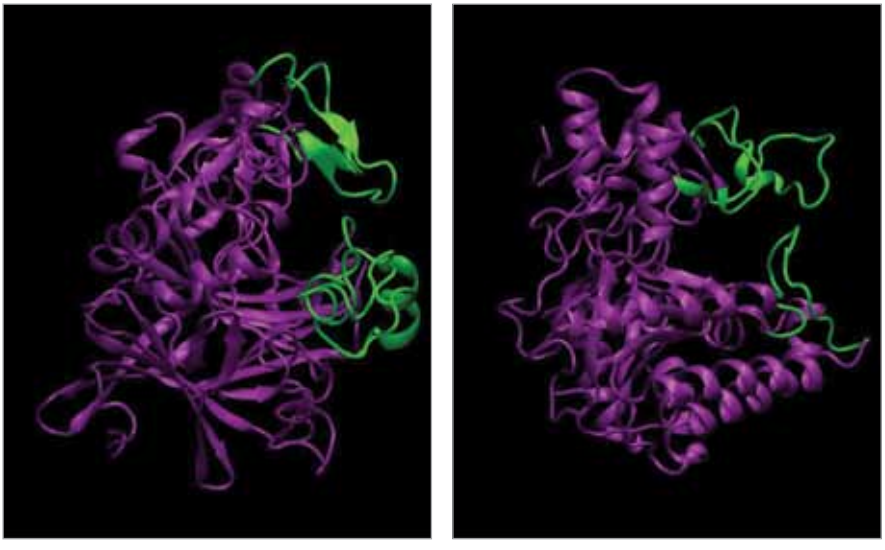

Figure 1. Catalytic core domains of T. reesei *CBH I (Cel7A, left) and CBH II (Cel6A, right). Loops in green highlight tunnel roofs. Tunnel lengths are 50 Å and 20 Å long (out of the plane of the paper) in CBH I (Cel7A) and CBH II (Cel6A), respectivelly*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Enzymatic hydrolysis is one of the crucial steps in cellulosic ethanol production, for example from sugarcane bagasse or eucalyptus tree wood. The importance of this process steams from the considerable recalcitrance of biomass to saccharification procedures. To optimize cellulosic bioethanol production and to turn it cost-effective, we need to comprehend a process of enzymatic hydrolysis on the molecular level and therefore, to decipher structures and functions of the enzymes that participate in this process and to understand how main enzymatic components interact with each other during hydrolysis of biomass. As a first step in this direction we advanced with fermentation, purification and characterization of cellulases from the filamentous fungi *Trichoderma harzianum*, *Trichoderma reesei* and *Aspergilus niger* and their structural and enzymatic studies, as well as with structural studies of other hydrolytic enzymes, such as (*R. marinus laminarinase*, *T. reesei* beta-mannosidase, *Xantamonas citri* endoglucanase and lignine oxidases, among others). Our aims is to proceed with the structure-funcional studies of glicosyl hydrolases, to improve our understanding of their concerted action during the process of enzymatic hydrolysis and to contribute to the development of enzymatic blends with improved hydrolytic properties, particularly as applied to sugarcane bagasse and eucalyptus tree biomass.

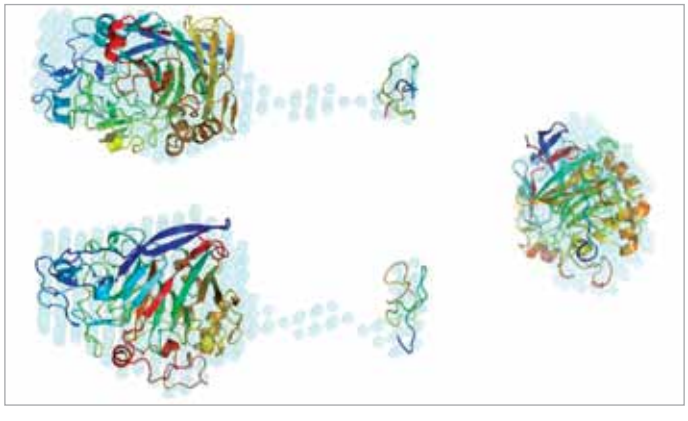

Figure 2. Superposition of small-angle X-ray scattering (SAXS) derived low-resolution envelope of T. harzianum *CBHI with two separate high-resolution structures: of the catalytic domain of CBHI from* T. reesei *and of its cellulose-binding module (CBM). Three orthogonal views are given*

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