Structural Characterization of a Xyloglucan-Specific β-1,4-endoglucanase (XEG-GH12) from Aspergillus niveus

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INTRODUCTION: The Aspergillus niveus xyloglucan-specific β-1,4-endoglucanase (AnXEG), belonging to GH12 family, is highly specific for cleavage of xyloglucan backbone and has great potential application in lignocellulosic biomass conversion. XEG is dimeric and less active at room temperature, while at 60ºC, its optimum temperature, the enzyme adopts a monomeric conformation. However, the relationship between dimerization and enzymatic activity is not well understood. Thus, the main goal of this work is to determine the three-dimensional structure of this XEG to elucidate this molecular event involved in the regulation of catalysis.

MATERIAL AND METHODS: AnXEG was crystallized by hanging-drop vapor-diffusion method in 20% PEG 400, 0.1 M sodium acetate pH 3.6 and 0.1 M cadmium chloride. X-ray data were collected at W01B-MX2 beamline and the structure was solved at 2.5 Å resolution.

RESULTS AND DISCUSSION: AnXEG displays a classical jelly-roll fold, composed by two bending β-sheets forming a concave face, where is located the substrate binding cleft. W26, W41, D119, D115, E133 and E219 residues form the active site and are conserved in GH12 members, including cellulases, while Y37 is present only in xyloglucan-specific enzymes. One dimer is present in the asymmetric unit and in this arrangement the substrate-binding cleft of one molecule is occluded by the same region of adjacent molecule, preventing the substrate binding in both cases.

CONCLUSIONS: The structure permitted to explain the low enzymatic activity of the dimeric form. Moreover, by structural comparisons it was possible to map the substrate binding site, indicating residues that are essential for enzyme activity and residues with key role in xyloglucan recognition.

Keywords: xyloglucan, GH12, XEG, crystal structure, lignocellulosic biomass conversion.

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