

Sets of Co-Variant Positions in β -glucosidases Are Involved in Modulating the Activity and the Thermal Stability

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Introduction: A Statistical Coupling Analysis (SCA) of 768 β -glucosidase sequences revealed 23 positions which have coupling among its amino acid frequencies. **Objectives:** The role of 19 of these co-variants positions on the β -glucosidases properties were investigated using the β -glucosidase from the fallworm *Spodoptera frugiperda* (Sf β gly) as study model. **Material and Methods:** Employing single site-directed mutagenesis, we introduced alanine residues at each of 19 studied positions in Sf β gly. k_{cat}/K_m for purified recombinant mutants Sf β gly was determined for the hydrolysis of at least 10 different concentrations of three different *p*-nitrophenyl β -glycosides, and the mutational effect on the transition temperature (T_m) were determined using Differential Scanning Fluorimetry (DSF). Sf β gly tridimensional structure was modeled by homology. **Results and Discussion:** The mutational effect of fifteen co-variant positions presented decreases on the k_{cat}/K_m ratio (mutant/wt) in the range of 0 to 0.15, demonstrating that those positions are related to the enzymatic activity. Structural comparison evidenced that 11 of these co-variant residues form a layer surrounding the active site of the β -glucosidases, indicating that residues at coupled positions modulate the catalysis and substrate binding through their direct contacts to the active site. Moreover, mutations in nine coupled positions that do not contact each other produced variations higher than 2,5 K when compared to wild-type Sf β gly (decreases from 2.8 to 12.2 K and increases from 3.2 to 6.0), indicating that these positions, preferentially occupied by prolines and concentrated at loop segments, are related to Sf β gly thermal stability. **Conclusions:** β -glucosidases present 2 sets of co-variant positions, each set having jointly positions involved in modulating the activity or the thermal stability.

Keywords: β -glucosidase; SCA; Activity modulation; Thermal Stability.

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