ADDITIVITY OF MUTATIONAL EFFECTS ON THE CATALYTIC ACTIVITY OF A b-GLYCOSIDASE

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The manner in which the effects of simultaneous mutations on the enzymatic activity are combined (additivity) is a significant factor for the comprehension of enzymes activity and specificity.

In order to characterize the additivity of the mutations of residues Q39 and E451, which are essential to the specificity of a β-glycosidase from *Spodoptera frugiperda* (Sfβgly50), three double mutants (E39A451, E39A451 and N39S451) were produced as recombinant proteins and purified. The k_{cat}/K_m for hydrolysis of p-nitrophenyl β-fucoside and p-nitrophenyl β-galactoside were used to estimate changes in the $ES^{\ddagger}$ energy resulting from double ($\Delta\Delta G^{\ddagger}_{xy}$) and single ($\Delta\Delta G^{\ddagger}_{x}$ and $\Delta\Delta G^{\ddagger}_{y}$) mutations. Finally, the coupling energy between residues 451 and 39 ($\Delta G^{\ddagger}_{I}$) was determined by comparing these data.

$\Delta G^{\ddagger}_{I}$ is negative for all double-mutants, except for S451N39, which has $\Delta G^{\ddagger}_{I} = 0$ when p-nitrophenyl β-fucoside is used as substrate. Negative $\Delta G^{\ddagger}_{I}$ indicates that double mutations are less damaging to the k_{cat}/K_m than the sum of the single mutations that compose them. Moreover, negative $\Delta G^{\ddagger}_{I}$ suggests the presence of an interaction between residues 451 and 39, which affects their bonds with the substrate and favors the stabilization of $ES^{\ddagger}$.

The interaction between residues 39 and 451 may result from the formation of a bidentate hydrogen bond involving them and the same substrate hydroxyl. As similar bonds are usual among β-glycosidases, interaction between residues that bind the substrate may be a general feature of these enzymes.