Data supporting the hypothesis that contact pathways modulate the substrate specificity of a β-glucosidase

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Introduction and aims: A previous study had showed that the substrate specificity of a β-glucosidase (Sfβgly) might be modulated by residues which are not in the active site through contacts pathways. Additional data are presented here to support this hypothesis. Materials and methods: A total of 46 Sfβgly with mutations in different contact pathways previously identified on the Sfβgly structure were expressed in bacteria and purified by affinity chromatography. Following that, Sterner-Volmer constants ($K_{sv}$) for the fluorescence quenching by acrilamide of wild-type and Sfβgly mutants were determined. These enzymes were also incubated at 50 °C for different times and their rate constants of thermal inactivation ($k_{obs}$) were determined by plotting the logarithm of their relative remaining activity versus time of incubation at 50 °C. Results and discussion: The average values of $K_{sv}$ values and $k_{obs}$ for the Sfβgly containing mutations in different pathways contacts tend to be more distinct than for mutations grouped in the same pathway. These data suggest that mutations placed on the same contact pathway propagate throughout the same region causing similar structural effects, which could indicate that a contact pathway works as a unit. Therefore, these data support the previous hypothesis that the Sfβgly catalytic specificity may be modulated through contact pathways linking distant residues to the active site.

Keywords: contact pathways, β-glycosidase, glycoside hydrolase, substrate specificity

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