

Determination of the Ratio Between Hydrolysis and Transglycosylation Reactions Catalyzed by Beta-Glycosidases

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The reaction mechanism of β -glycosidases involves the formation of a covalent intermediate, which may follow two alternative routes. One of them involves the hydrolysis of the covalent intermediate, whereas in the other route the intermediate is attacked by a second substrate molecule. In this situation the reaction is called transglycosylation. In order to understand the factors determining the ratio between these two different catalytic routes, β -glycosidases from *Spodoptera frugiperda* (Sf β glu; AF052729) and *Tenebrio molitor* (Tm β glu; AF312017) were produced in *Pichia pastoris* and concentrated using 90% (w/v) ammonium sulfate and reverse dialysis in PEG 10.000. SDS-PAGE confirmed that Sf β glu and Tm β glu were pure after that procedure. The ratio between hydrolysis and transglycosylation reactions (H/T) was analyzed taking methylumbelliferyl β -glycoside (MU β glc 0.1 to 8 mM) and *p*-nitrophenyl β -glycoside (NP β glc 0.1 to 40 mM) as substrates. Tm β gly catalyzed transglycosylation reactions with both substrates, but H/T depended on the substrate concentration varying from $+\infty$ (H \gg T) to 1.5 for NP β glc and $+\infty$ to 1 for MU β glc. Conversely, Sf β gly did not catalyze transglycosylation reaction when MU β glc was the substrate. In addition to that, H/T for NP β glc was 2.3 and did not depend on the substrate concentration. The presence of 3.5, 7 and 82 M methanol decreased 7, 60 and 115 times, respectively, the ratio between hydrolysis and transglycosylation catalyzed by Tm β glu. Conversely, methanol had a more intense effect on reactions catalyzed by Sf β glu decreasing 80, 330 and 250 times the H/T. These data suggest that the binding site of the substrate and methanol in the transglycosylation reaction is different in these enzymes.

Palavras Chaves: *transglycosylation, beta-glycosidase and enzymes*

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