β-GLUCOSIDASES GH1 OF *Paenibacillus polymyxa*

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β-glucosidases GH1 (bglA) and (bglB) from *Paenibacillus polymyxa* have a (β/α)₈ barrel structure and high sequence identity (45%). In order to perform a comparative characterization of their structural stability, they were expressed in Nova Blue (DE3) bacteria using pLATE51 vector and purified by affinity chromatography (Ni-NTA resin), as checked by SDS-PAGE and western blotting. The enzymes showed a typical spectrum of folded proteins when characterized by circular dichroism. bglA secondary structure showed 47% of α-helix and 13% of β-sheets whereas bglB showed 61% of α-helix and 2.5% of β-sheets. Based on acrylamide quenching we estimated that the Stern-Volmer constant ($K_{SV}$), which represent the accessibility of the quencher to tryptophan residues, is higher for bglA (9.49) than for bglB (3.17). Transition temperatures ($T_m$) determined by DSF ("differential scanning fluorimetry" employing Sypro Orange as probe) were higher for bglB ($T_m = 43.8 \, ^\circ C$) than for bglA ($T_m = 35.2 \, ^\circ C$). In addition thermal inactivation kinetics at 47°C determined using relative remaining activity showed a rate constant $k_{inactivation}$ of 1.9 s⁻¹ for bglA and 31.3 s⁻¹ for bglB. The $k_{inactivation}$ at 37°C for bglB is 5.4 s⁻¹. Thereby bglB denaturation is faster. We also evaluated the chemical denaturation by urea which showed a similar midpoint of transition ($c_{50}$) for both enzymes, 7.9 mol·L⁻¹ for bglA and 7.1 mol·L⁻¹ for bglB. Regarding the effect of urea on their stability, the "m" parameter was 669 cal·mol⁻¹ for bglA and 860 cal·mol⁻¹ for bglB. Therefore, in spite of their homology and structural similarity, bglA and bglB showed different behavior in denaturing conditions. Supported by FAPESP and CAPES.

Keywords: beta-glucosidase; denaturation; stability