L- Thermal and Chemical Structural Stability of a Thermophilic Beta-Glucosidase GH1

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Proteins from thermophilic microorganisms are of major interest to industrial biotechnology because they are usually more stable than their analogues from mesophilic organisms whilst they retain the folding patterns of GH1 family. Thus we aim to characterize the stability of thermophilic b-glucosidase GH1 against the denaturants as urea, guanidinium hydrochloride (GndHCl) and high temperature. The b-glucosidase (bglThm) from thermophile *Thermotoga maritima* was expressed as recombinant protein in NovaBlue (DE3) using pLATE51 vector and purified with Ni-NTA agarose resin. To evaluate this stability, we followed the transitions between the native and unfolded states using tryptophan fluorescence, enzymatic activity and differential scanning fluorimetry (DSF). The recombinant bglThm, which presents a $(b/a)_8$ barrel folding as shown in crystal structures, exhibited typical tryptophan fluorescence spectra of a folded protein. Circular dichroism analysis in the far-UV region (190 nm to 240 nm) confirmed that and showed a secondary structure composition of 30% of a-helix and 20% of b-sheets. The average degree of accessibility of the tryptophan residues was evaluated using acrylamide quenching and revealed a Stern-Volmer constant (K_{sv}) of 3.84. In addition, bglThm was stable at 47°C and also up to 4 h at 80°C. Interestingly its thermal inactivation kinetics at 95°C was a two-step process, which exhibited an initial fast step ($k_{inativation}$ of 2.9 s^{-1}) followed by a slow step ($k_{inativation}$ of 0.2 s^{-1}). We also evaluated the bglThm chemical denaturation by urea, which indeed bglThm was stable up to 9 $mol \cdot L^{-1}$ urea. Conversely the denaturation by GndHCl showed a transition midpoint (c50) at 5.4 ± 0.006 $mol \cdot L^{-1}$. The *"m"* parameter, representing the denaturant effect on protein stability, is 1,690 $cal \cdot mol^{-1}$. In conclusion, bglThm showed a high thermal and chemical stability.

Keywords: beta-glucosidase, protein stability, thermophilic

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