

Effect of pH and temperature on the global compactness, structure and activity of cellobiohydrolase 1 from *Trichoderma harzianum* IOC 3844

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Cellulases produced by filamentous fungi are widely used in biotechnological applications, including biomass depolymerization and second generation bioethanol production. However, stability of cellulases in defined pH and temperature ranges sets limits to their industrial utilizations. Here, we examine the effects of pH and temperature on the secondary and tertiary structures, compactness and enzymatic activity of a filamentous fungus *T. harzianum* cellobiohydrolase 1 IOC-3844 (*ThCBH1*) using a number of biophysical and biochemical techniques. Our results show that pH and temperature perturbations affect *ThCBH1* stability by two different mechanisms. Variations of pH modify protonation of the enzyme residues, directly affecting its activity, but leading to structural destabilization only at extreme pH limits. Temperature, on the other hand, has direct influence on mobility, fold and compactness of the enzyme, causing irreversible unfolding of *ThCBH1* just above optimum temperature limit. Finally, we demonstrate that incubation with the product of reaction and competitive inhibitor, cellobiose, increase significantly thermal stability of *ThCBH1*. Thus, our studies might provide insights into understanding, at molecular level, of the interplay between structure and activity of *ThCBH1* at different pH and temperature conditions, which can be useful for possible biotechnological applications of the enzyme as bioethanol production.

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