

UNFOLDING STUDIES OF THE CYSTEINEPEPTIDASE BAUPAIN: EFFECT OF pH, GUANIDINE HYDROCHLORIDE AND TEMPERATURE

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The unfolding properties of baupain, a cysteinepeptidase present in *Bauhinia forficata* leaves was studied by using CD and fluorescence. Baupain belongs to the $\alpha+\beta$ class of proteins with 44% α -helix, 16% β -sheet and 12% β -turn, and fluorescence emission maximum at 343 nm. The structural transition induced by pH is noncooperative and shows a biphasic nature. No important differences were observed at pH 2.5 to 10.5 but, at pH 2 it retains substantial non-native structure with strong ANS binding. GuHCl-induced unfolding did not change significantly its structure until 4.0 M GuHCl indicating the high rigidity of the molecule. The unfolding is cooperative showing by the sigmoidal transitions curves with midpoints at 4.7 ± 0.2 M and 5.0 ± 0.2 M GuHCl, measured by CD and fluorescence, respectively. A red shift of 7 nm of intrinsic fluorescence was observed with 6 M GuHCl. Temperature-induced unfolding of baupain was incomplete and at least 35% of the native structure of the protein is retained even at a higher temperature (90°C). Baupain shows characteristics of molten globule due to the ANS preferentially binds to the enzyme at pH 2.0 in comparison to native (pH 7.0) and completely unfolded (6 M GuHCl) states. These result, in addition to the N-terminal sequence similarity, allowed us to include baupain in a member of papain superfamily. FAPESP, CNPq, SPDM and CAPES/DAAD).