Irreversible Thermal Denaturation of β-Hemocyanin of *Helix pomatia* and its Substructures Studied by Differential Scanning Calorimetry

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The thermal denaturation of β-hemocyanin from the gastropod *Helix pomatia* (β-HpH) at neutral pH was studied by means of differential scanning calorimetry (DSC). The denaturation was completely irreversible as judged by the absence of any endotherm on rescanning previously scanned samples. Two transitions, with apparent transition temperatures (T_m) of ca. 84 °C (main transition) and ca. 88 °C (minor transition), were detected by DSC in 20 mM MOPS buffer, containing 0.1 M NaCl, 5 mM $CaCl_2$ and 5 mM $MgCl_2$ at pH 7.2 (buffer A), using a heating rate of 1.0 K min^{-1}. Both T_m values were dependent on the scanning rate, suggesting that the thermal denaturation of β-HpH is a kinetically controlled process. The T_m and specific enthalpy values (ΔH_{cal}) for the thermal denaturation of β-HpH were found to be independent of the protein concentration, indicating that the dissociation of the protein into monomers does not take place before the rate-determining step of the process of thermal unfolding started. A successive annealing procedure was applied to obtain the experimental deconvolution of the irreversible thermal transitions. These transitions are tentatively attributed to the denaturation of, respectively, the wall (main transition) and the collar of the β-HpH molecule. The activation energies (E_A) of both transitions were found to be similar (about 500 kJ mol^{-1}).

In 130 mM glycine/NaOH buffer, pH 9.6 (buffer B), with β-HpH dissociated into subunits, the calorimetric profile had a more complex character. This could be ascribed to a different stability of the functional units (FUs) constituting the β-HpH subunit. FU *d*, which in the cylindrical didecameric β-HpH molecule is located in the wall, was markedly less stable than FU *g*, which belongs to the collar. The thermal denaturation of FUs *d* and *g* was described by the two-state irreversible model. On the basis of this model, the parameters of the Arrhenius equation were calculated.

Key words: Hemocyanin; *Helix pomatia*; Thermal Stability.