Thermodynamic Stability and Volumetric Characterization of Molten Globule States of Cytochrome C Evaluated with Differential Scannning, Isothermal Acid-Titration, and Pressure Perturbation Calorimetry

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Cytochrome c is a small globular protein reported to show typical two-state thermal transition between the native (N) and denatured (D) states. In this study, the thermal transitions of this protein were thermodynamically evaluated by highly precise differential scanning calorimetry (DSC), VP-DSC (Microcal) in the pH range from 3.8 to 5.0. The heat capacity functions were analyzed with double-deconvolution and non-linear least-squares method. From the results, it was clearly shown that at least one stable intermediate state exists in the thermal transition where the mole fraction of the intermediate state shows the maximum value of 0.4 at around 340 K. The enthalpy change between N and the intermediate state is 141 kJ/mol at pH 4.1 and 343 K. This value is much smaller than the enthalpy change between N and D state, 498 kJ/mol at the same condition. The structure of this intermediate state was detected by circular dichroism (CD) experiment, and it was found that the intermediate has the properties of the molten globule (MG) state which possesses the native-like alpha-helix content and disordered tertiary structure. As far as we know, this is the first report where the MG state of cytochrome c under the "normal" solution condition: not acidic pH and without concentrated anions. Also the volumetric characterization of the MG state is evaluated by pressure perturbation calorimetry (PPC). The partial volume change on the transition from N to the MG state under the "normal" solution condition is positive and that on the transition from the MG to D state is also positive. On a "special" solution condition, at low pH and with the high anion concentration, where the MG state has been extensively studied traditionally, the partial volume of this state shows higher value than those of N and D state. It suggests that the change of the hydration of the protein molecules and the ions affects the partial volume of the MG state.