High-sensitivity differential scanning calorimetry: basic principles and main applications in protein science

V.Ya. Grinberg and T.V. Burova *(Institute of Biochemical Physics, Russian Academy of Sciences, Vavilov St. 28, 119991 Moscow, Russian Federation)*

High-sensitivity differential scanning calorimetry (HS-DSC) is a modern powerful method of evaluation of protein energetics, structure and interactions. This technique has been developed in the Former Soviet Union to study co-operative conformational transitions of biopolymers in extremely dilute solutions. An instrumental basis of the method is provided by Privalov-type adiabatic differential scanning microcalorimeters DASM-4 ("Biopribor", Russia), MC-2 and VP-1 ("MicroCal Inc.", USA), Scal-1 ("Scal Co., Ltd.", Russia), Nano-DSC ("Setaram", France) with sensitivity and precision exceeding most other commercial scanning calorimeters. A specific property of these instruments is using of built-in calorimetric cells and relatively high heating rates (1-2 K/min). Such a design of the calorimetric cells provides high stability of a base line of the instrument that is a key factor for determination of the partial heat capacity of proteins. A further benefit of HS-DSC is rather short measurement time and modest sample consumption.

In the majority of cases, the heating rates used in HS-DSC give a possibility to carry out experiments on protein denaturation in the quasistatic mode for a reasonable time (less than 2 hours). In the course of the scanning the equilibrium distribution of protein molecules between the native and denatured states is achieved at each temperature. This allows one to use formalism of thermodynamics for interpretation of HS-DSC data.

The partial heat capacity of a protein can be determined as a function of temperature with high precision at a very low protein concentration (down to 0.02-0.03 mg/mL). Analysis of this function within the protein denaturation range enables to determine the denaturation temperature, enthalpy and heat capacity increment of a protein in a single experiment. These data can be readily converted into temperature dependences of the denaturation free energy, entropy, and enthalpy by standard thermodynamic relations. Using independent data on changes in the accessible surface area of amino acid residues in result of the denaturation, it is possible to make deconvolution of the thermodynamic functions of denaturation into contributions of various factors, of hydrophobic effect, in particular. Very often this approach results in a paradoxical conclusion that the conformational stability of globular proteins is mainly defined by cooperative hydrogen or Van-der-Waals bonds contrary to a widespread opinion on prevailing role of hydrophobic effect.

Additional structural information can be extracted from HS-DSC data on protein denaturation by analysis of the profile of denaturation transition. In this way a number of structural domains in protein molecule can be evaluated. In many cases these estimates are in close agreement with the three-dimensional structure of proteins.

As a rule, interactions of proteins with low-molecular weight ligands change significantly the relative occupancy of the native and denatured states. This opens up great possibilities for investigation of these reactions by HS-DSC. One of such approaches is based on calorimetric data obtained at different ligand concentrations. For each ligand concentration, one can determine the temperature dependence of denaturation free energy. A section of the set of these dependences at a reference temperature gives the excess free energy of denaturation as a function of ligand concentration. The excess free energy of denaturation is directly related to energetics and stoichiometry of the binding for the native and denatured states. This gives a valuable information on conformational mechanisms of binding.