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MODULATION NANOCALORIMETER IN RESEARCH OF THERMAL DENATURATION OF PROTEINS

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The experimental model of the modulation nanocalorimeter has been developed. This nanocalorimeter has the following characteristics : the sensitivity is 50 nW; the base line stability for 3–5 hours is 50 nW; the fast-response is 60 s. In diluted protein solutions the precision measurements of heat flow have been performed with a modulated nanocalorimeter in the StepScan-modulation mode. This mode based on sequences of short intervals of heat (cooling) and isothermal intervals in a given temperature interval. Given functional scheme of the modulated nanocalorimeter is supplemented by an experimental estimation of the value of temperature difference along the length of the calorimetric chamber at its heating at a rate of 0.2 K/min. This estimation is made from the melting data of a test temperature sample in the calorimetric chamber. The test temperature sample is 1mg Gallium. It is shown, that the difference between temperatures of the chamber top and bottom does not exceed 0.02 K. Thus, it is experimentally confirmed that calorimetric chambers have equal temperature along the whole length, as the obtained temperature difference is less than the absolute error of a sample temperature measurement with modulated nanocalorimeter. All modern scanning microcalorimeters developed by foreign corporations have a disadvantage in comparison with modulated nanocalorimeter, as they do not provide operation in the modulation mode. Modulated nanocalorimeter is a computer aided instrument. For modulated nanocalorimeter we have developed the software, providing the calorimeter operation and calculation of a complete heat flow, and also kinetic and reversible parts of a complete heat flow, which is confirmed by the experimental data. The research of thermal denaturation of ovalbumin and of lysozyme was carried out. It is shown that the modulation nanocalorimetry gives an opportunity to discriminate qualitatively different mechanisms of thermal denaturation of proteins.

Keywords: thermal denaturation, kinetics, temperature-modulation differential scanning nanocalorimetry, capillary calorimetric chamber, stepscan modulation

INTRODUCTION

For the first time in the world's practice, in the frame of the research project "Study of physical mechanisms in the inactivation of enzymes and proteins for medical purposes by a method of temperature-modulation differential scanning nanocalorimetry" (Russian Foundation for Basic Research (RFBR) grant 10-08-00063-a), the Institute for Biological Instrumentation of the Russian Academy of Sciences (IBI RAS) built a modulation nanocalorimeter providing for measurements of kinetic parameters in a single experiment compared to measurements carried out by known methods (circular dichroism, evaluation of the decrease in the protein monomer form content after the system's cooling, i. e. not in the process of a protein denaturation) requiring a large number of experiments. This new instrument enables to discriminate the mechanisms of thermal denaturation of a protein on the basis of a lysozyme denaturation example where no kinetic process is observed, and of an ovalbumin denaturation where, on the contrary, only a kinetic process has been observed. These qualitatively new opportunities provided by the instrument are promising for its use in the study of protein conformation energetics [1–3]. Said works carried out in IBI RAS are based on the first national capillary adiabatic scanning microcalorimeter DASM-1 built in our Institute and on the studies [4–6] performed with said instrument. The modulation calorimetry progress disclosed in the publications [7–9] showed a need to build a modulation nanocalorimeter for studying low heat effects in diluted protein solutions.

BRIEF DESCRIPTION OF THE FUNCTIONAL STRUCTURE OF A MODULATION NANOCALORIMETER

Fig. 1 shows a functional diagram of a modulation nanocalorimeter comprising an operation chamber1 and a reference chamber 2 made as straight capillary tubes with 2 mm inner diameter. The calorimetric chamber whole volume is 268 μl, and its working volume is 156 μl. The calorimetric chamber working volume is that of the tube part situated between the chamber bottom and the boundary of the contact with a passive heat shunt 3. The capillary length of the

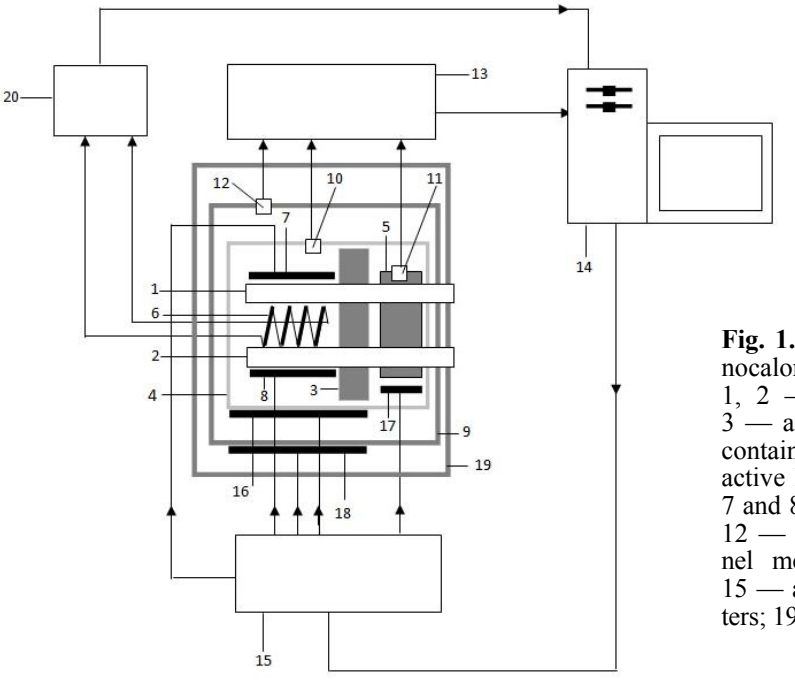

Fig. 1. Functional diagram of a modulation nanocalorimeter.
1, 2 — reference and calorimetric chambers; 3 — a passive heat shunt; 4 — the first shield containing the calorimetric chambers; 5 — an active heat shunt; 6 — a measuring thermopile; 7 and 8 — heaters; 9 — a second shield; 10, 11, 12 — temperature detectors; 13— a multichannel measuring amplifier; 14 — a computer; 15 — a power amplifier unit; 16, 17, 18 — heaters; 19 — a cooling zone; 20 — a nanovoltmeter.

working volume in the chambers is 50 mm. The calorimetric chambers are enclosed in a first shield 4. On the outlet capillary ends of the calorimetric chambers, an active heat shunt 5 is positioned that prevents any heat exchange of the calorimetric chambers and of the passive heat shunt with the environment via the outlet part of the capillary tubes. The calorimetric chambers are provided with a measuring thermopile 6 and with heaters 7 and 8. Said chambers enclosed in the first shield 4 are positioned inside a second shield 9. The first shield comprises a temperature detector 10, the active shunt holds a temperature detector 11, and the second shield comprises a temperature detector 12. The temperature detectors 10, 11 and 12 are connected, via a multichannel measuring amplifier 13, to a computer 14 having a multichannel analog-digital converter and a PC-TIO-10 module both containing timers. The control outputs of the computer 14 are connected to a power amplifier unit 15 linked to a first shield heater 16, an active shunt heater 17 and a second shield heater 18 fitted in a cooling shield 19. The calorimeter is provided with a heat process power meter containing the thermopile 6 an output of which is connected to the input of a nanovoltmeter 20 bound to the computer 14 via a RS-232 interface. The heaters 7 and 8 of the chambers are connected to the power amplifier unit 15. A complete operation of the modulation nanocalorimeter is disclosed in the publications [3–8].

The StepScan-modulation mode is implemented in the present instrument. The StepScan mode temperature modulation is based on a sequence of short intervals of heating and isothermal intervals in a given temperature interval. The main equation of the StepScan mode is expressed by:

$$\text{Heat flow } P = C_p(\mathrm{d}T/\mathrm{d}t) + f(T,t),$$

where C_p — is the heat capacity of a sample, $\mathrm{d}T/\mathrm{d}t$ — is the heating rate, $f(T,t)$ — is the kinetic contribution of the heat process. The use of a sinusoidal signal in the modulation is less advantageous since in this mode, $C_p(\mathrm{d}T/\mathrm{d}t)$ permanently changes whereas in the StepScan mode, this component has a fixed value. Thus, the StepScan method provides for a fast and clear result of measurements [10].

In addition to the theoretical substantiation of the method of calorimetric chamber linear heating and cooling [2], an experimental evaluation of temperature gradients along the length of the calorimetric chamber heated at a rate of 0.2 K/min for melting a temperature reference sample (1 mg Gallium, 29.9 °C) in the calorimetric chambers. The peaks of the reference sample melting while positioned on the chamber bottom as well as in the upper part of the chamber are recorded by the modulation nanocalorimeter at a temperature of 29.9 °C. Thus, the temperature gradients along the length of the chambers does not exceed 0.1 K, which is consistent with

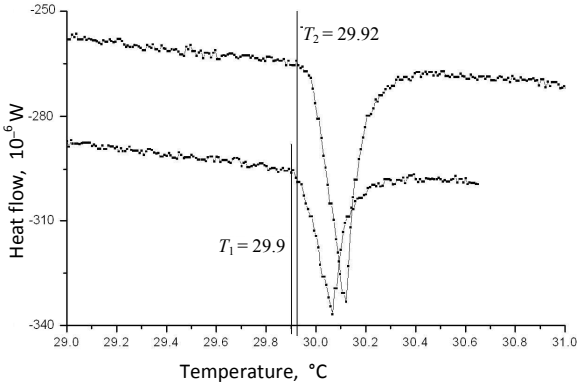

Fig. 2. A thermogram of heating a temperature reference sample (1 mg Gallium, 29.9 °C) in the calorimetric chambers at a rate 0.2 K/min

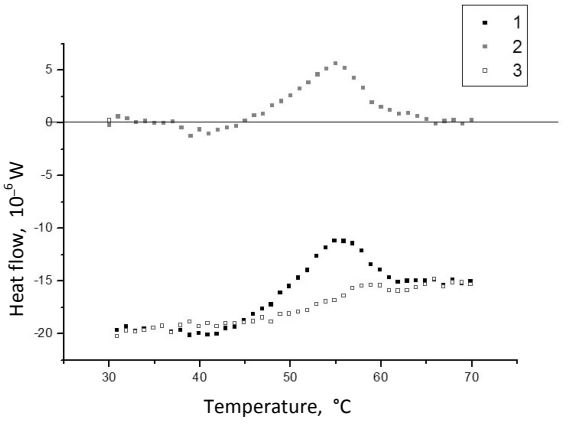

Fig. 3. Heat denaturation of ovalbumin in the temperature-modulation mode StepScan.
1 — P, 2 — $f(T,t)$ 3 — $C_p(\mathrm{d}T/\mathrm{d}t)$ for the process under study

the theoretical evaluation according to which the gradient value is 0.033 K at the rate of 0.2 K/min [2].

The results of the experimental evaluation of the temperature gradient are illustrated in fig. 2. The temperature scale of the figure is taken for a narrow temperature range, which enables to evaluate the temperature difference between the upper and lower parts of the chamber on the order of 0.02 K. In this way, it is experimentally confirmed that the used system of heating the calorimetric chambers provides for their uniform heating in their whole length, since the resulting temperature difference is lower than the absolute error of calorimetric measurements of a sample temperature.

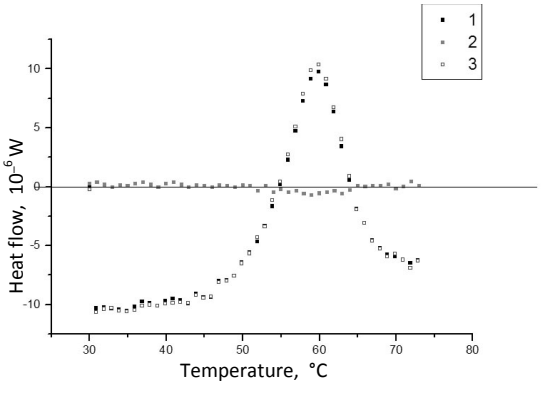

Fig. 4. Heat denaturation of lysozyme in the temperature-modulation mode StepScan.
1 — P, 2 — $f(T,t)$, 3 — $C_p(\mathrm{d}T/\mathrm{d}t)$ for the process under study.

The StepScan modulation mode is carried out the modulation nanocalorimeter by the modification of the first shield temperature according to a required law and by repeating said temperature modification law in the second shield, while the law of temperature modification in the first shield is reproduced in gold calorimetric chambers bound to the first shield with a high heat transfer [2]. The modulation nanocalorimeter is a computer aided instrument. A software has been developed for the last, providing the calorimeter operation for a whole extent of requirements, as well as the calculation of the whole heat flow together with the kinetic and reversible parts of a complete heat flow. The experimental model of the modulation nanocalorimeter provides for the following characteristics:

operation mode: isothermal;
linear heating at a rate up to 0.5 K/min;
linear cooling at a rate up to 0.5 K/min;
StepScan mode;
measuring volume of the calorimetric chambers of 156 μl;
the calorimetric chamber are made of gold;
the temperature scanning range from 20 to 70 °C;
sensitivity of 50 nW.

EXPERIMENTAL RESULTS

The experimental research work carried out on the modulation nanocalorimeter to study the ovalbumin denaturation showed that in the area of the conformation transition, the thermogram records only the peak of a kinetic process whereas the peak of the heat ca-

pacity modification is absent (Fig. 3). This circumstance fundamentally distinguishes our modulation nanocalorimeter from non-modulated scanning calorimeters where the peak of a kinetic process is recorded erroneously as a heat process of the sample heat capacity modification. In this case, the heat denaturation of lysozyme is recorded only as a peak of a sample heat capacity modification (Fig. 4), which corresponds to literature data [11]. The studied samples of ovalbumin and lysozyme were prepared in conformity with the methods presented in [12] and [13], respectively.

CONCLUSIONS

The experimental studies carried out on Kunitz trypsin inhibitor [1], ovalbumin and lysozyme with the modulation nanocalorimeter showed that this instrument allows one to experimentally discriminate the protein denaturation mechanisms, such as thermodynamic, kinetic and mixed on the basis of one experiment. This experimental instrument can be used as a base for the design of a competitive instrument the most important technical solutions of which can be protected by patents.

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