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DIFFERENTIAL SCANNING MICROCALORIMETER SCAL-1

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Abstract

The paper describes the design and the principle of operation of the differential scanning microcalorimeter SCAL-1. This instrument serves to measure thermal parameters of protein or biopolymer solutions of low concentration (from 0.1 mg ml^{-1}) in the temperature range from –10 to 130°C using different rates. A distinctive feature of the microcalorimeter is that its calorimetric cell is made of glass, it is highly sensitive and measuring results can be easily and reliably reproduced.

Keywords: microcalorimetry, thermal parameters, thermodynamics

Introduction

Studies of protein stability are a fundamental direction in protein physics. Investigation of structure stability consists in the analysis of the denaturation process, i.e. destruction of its unique, native compact tertiary structure. The native structure can be disrupted by various actions on the molecule. However the energetics of such a process may be obtained only by temperature effect on the molecule. This destruction, accompanied by low heat absorption, is called heat denaturation of protein. In 1962 first measurements of heat denaturation of globular proteins were obtained on an absolute calorimeter specially improved for that [1]. The values of the absorbed heat, the denaturation enthalpy and temperature at which the structure is destroyed may be used to obtain thermodynamic parameters intrinsic to the system [2].

Scanning microcalorimetry has become a widely used and rapidly developing instrument for studying conformational transitions in proteins, lipids and biopolymers [3, 4]. The application of this technique has extended lately due to increased sensitivity of the instruments, high reproducibility of measurements and ability to study preparations in corrosive media.

Experimental

Structural scheme

Different designs of various microcalorimeters are described elsewhere [3–5]. A distinction of the differential scanning microcalorimeter described herein is that it is

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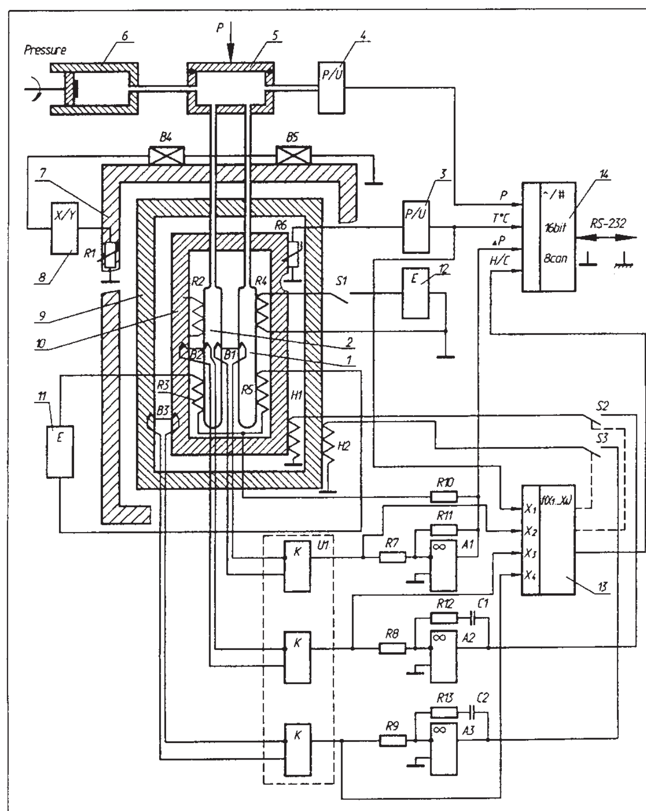

Fig. 1 Schematic design of the microcalorimeter. 1 – reference calorimetric cell, 2 – sample calorimetric cell, 3 – resistance-to-voltage converter, 4 – excess pressure-to-voltage converter, 5 – device for filling the cells, 6 – excess pressure pump, 7 – cold thermostat, 8 – temperature controller of the cold thermostat, 9 – external thermostatic shield, 10 – internal thermostatic shield, 11 – electric untied voltage source, 12 – reference voltage source, 13 – scheme of controlling heating and cooling, 14 – analog-to-digital converter, A1 – compensation controller, A2 – temperature controller of the internal thermostatic shield, A3 – temperature controller of the external thermostatic shield, C1, C2 – correction capacitors of the internal and external thermostatic shields, R1 – temperature sensor of the cold thermostat, R2-R5 – heating elements of the calorimetric cells, R6 – thermometer sensor of the cells, R7-R9, R11-R13 – correction resistors of the temperature controllers of the internal and external thermostatic shields, R10 – feedback resistor of the compensation controller, B1 – measuring thermopile, B2 – thermopile of temperature difference between the cell and the internal thermostatic shield, B3 – thermopile of temperature difference between the cell and the external thermostatic shield, B4, B5 – cooling device, H1, H2 – heating elements of the internal and external thermostatic shields, S1 – calibration power toggle switch, S2, S3 – control keys of the heating elements of the internal and external thermostatic shields, U1 – highly sensitive amplifier of direct current

based on novel principles of design of such instruments. Figure 1 shows a scheme of the SCAL-1 microcalorimeter.

Design features

The reference and sample calorimetric cells are made of glass. The measuring part of the cell is a cylinder of 40 mm in length. The internal and external diameters of the cylinder are 3.2 mm and 3.7 mm, respectively. The volume of the measuring part of the cell is 0.32 cm^3. The inlet of the cell is a capillary of 70 mm in length whose internal diameter is 1 mm. Unique know-how are used to make heaters R7…R9, R11…R13 and heating thermopiles B1…B3 of the calorimetric cells. The internal and external thermostat shield as well as the shield of a cold thermostat have the shape of cylinders, are made of aluminium alloy and placed in a hermetic housing. Hot junctions of the cooling thermopiles B4 and B5 are cooled at the expense of ribs located on the external surface of the hermetic housing. The calorimetric block is connected to the electronic part of the microcalorimeter via hermetic wiring on its housing. Inlets of the capillaries serving to fill liquids in the cells are reinforced with a stainless washer and made hermetic due to rubber gaskets.

The calorimetric cells are filled by a syringe with a stainless needle of 120 mm long and a diameter of 0.7 mm.

Principle of operation

The calorimetric cells should be filled after switching the instrument and fixing the operating conditions of all devices (heating to the operation temperature). To this end, the pump piston for supplying excess pressure (7) is set in the left-side position. Then the cover of the block for filling the cells is opened to fill liquids of interest using the syringe. After that the cover is closed and the pump piston for supplying excess pressure is shifted in the right-side position (the liquid endures experiences pressure of about 2.5 atm).

When the temperature in the cells is about 6°C, the instrument switches automatically to controlled heating. Keys S2 and S3 become engaged. The internal and external thermostatic shields will have the same temperature equal to that of the cells. Then the reference voltage source (11) is connected to the heaters of cells R3 and R5 and sets the heating power of the cells whose value is proportional to the rate of their controlled heating. Because of the absence of heat exchange of the cells with the ambient temperature due to the two thermostatic shields, monitoring with a high accuracy the temperature in the cells, the rate of the controlled heating is linear through the whole temperature range.

The difference of temperatures in the cells controlled by the measuring thermopile B1 is proportional to the difference heat capacity and the compensation regulator distributes the current via the other thermopile so that the difference of temperature in the cells is minimal. The magnitude of the temperature or compensation current difference is determined as signal Y for further processing. Inasmuch as the cell temper-

ature is monitored with high accuracy by the internal and external thermostatic shields, one can judge about it having measured the temperature of the internal thermostatic shield. To measure the cell temperature, a copper thermometer R6 is fixed on the internal thermostatic shield. Its resistance being converted in voltage proportional to the measured temperature is supplied to an inlet of the analog-to-digital converter.

The calorimeter is adjusted by feeding a certain amount of electrical power to one cell. When the temperature in the cells reaches its preset value, heaters H1 and H2 are switched off and the instrument turns to uncontrolled cooling. The internal and external thermostatic shields and the cells are cooled to the temperature of the cold thermostat (7) that maintains the temperature of about 0°C. When the temperature of the internal thermostatic shield is 6°C, controlled heating of the cells is switched on again.

Signals 'Y' meaning heat capacity (ΔP), 'X' (T°C), the pressure in the cells (P) and signal of the heating/cooling state (H/C) of the instrument are accepted on the inlets of the analog-to-digital converter (ADC).

Program of calorimetric data accumulation and processing

The exchange of data from the computer to the microcalorimeter and back is realized via port COM1 or COM2 with the help of interface RS232. The microcalorimeter and the computer have no voltaic connection due to the use of optical electronic devices in interface ADC. A voltaic branching prevents a possible damage of inlet and outlet devices of interface RS232 both in the microcalorimeter and computer. The rate of data exchange is 9600 BOT. The computer accepts and processes 4 signals: signal X (temperature), signal Y (heat capacity), signal P (excess pressure in the cells), and signal of the instrument state H/C (controlled heating or uncontrolled cooling).

At the initial start of the program of accumulation and processing of the calorimetric data, port COM2 or COM1 is installed via which data will be sent from the microcalorimeter to the computer. Then the temperature settings on the microcalorimeter and the computer are adjusted, during which the program creates a new graduation file and saves it, suggesting the operator to utilize it at the following usage of the program. The 'NEW SCAN' option allows the user to set new scanning parameters (temperature range, controlled heating rate, etc.), information about the preparation studied and the file name that will save the experimental data. Therefore if the user does not stop the program and switch off the microcalorimeter, all experimental results will be saved in one document.

The program for accumulation and processing of calorimetric data includes a possibility of preliminary assessment of the experimental data (the 'Preparation' option serves to this end). Using the 'Preparation' option it is possible to load documents, import or export curves and perform their computations (summation, extraction, averaging, smoothing and differentiation). It is also possible to shift the curves along the Y and X axes, to change the slope of the curves, to rotate them around the point of interest, to change the scale of a curve along the X axis and to save all these

changes in the document. The 'Export' option allows saving the curves chosen for further processing on a disc in a standard format compatible with widely used graphic programs.

The program for accumulation and processing of calorimetric data works in Windows 3.1 and following versions.

Basic parameters

Since the microcalorimeter is designed for studying liquids of different chemical composition, the cells should be inert to the action of various chemicals. Glass has high chemical resistance to the majority of chemicals. It can be readily treated on a burner, is strong, durable and smooth. Therefore microcalorimeter SCAL-1 is the first among the instruments of such a type where its calorimetric cells are made of glass. The smooth inner surface of the cells enables easy removal of the substance remnants of the previous experiment, and the cells are readily washed. This also allows the user to obtain reproducible results upon refilling of the cells due to lesser probability of bubble formation in the measuring part of the cells. The cells can be made thin-walled due to glass strength. The walls in the measuring part of a cell are from 0.2 to 0.3 mm thick. This only slightly effects the time constant of the calorimetric cells that is about 20 s. The cells withstand the pressure of about 90 atm.

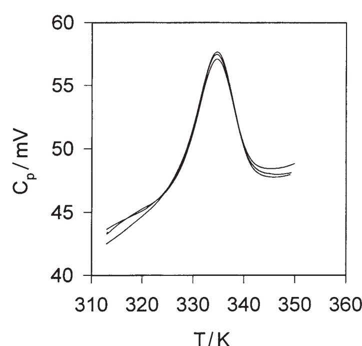

Fig. 2 Partial heat capacity of lysozyme solution at the concentration of 0.29 mg ml^{-1}. Three curves were obtained at 3 refillings of the calorimetric cell

Inasmuch as the preparations studied are low-concentrated solutions of proteins from 0.1 to 10 mg ml^{-1}, the heat effect of such amounts is quite low (1–100 μw) which corresponds to the temperature of $1\cdot10^{-5}$ to $1\cdot10^{-6}$ °C. Usually a considerable number of consecutively connected thermopiles are employed to detect such slight changes in the temperature. The SCAL firm has designed a construction of a measuring thermopile made of constantan wire of 0.06 mm in diameter, covering it with a layer of copper 20 μm thick. Such a thermopile has about 200 copper-constantan junctions. The gain of this thermopile is nearly 8 mV $°C^{-1}$, and the resistance is about

1 kOhm. To obtain a low noise level of 0.26 μw, it is necessary to use a highly sensitive direct-current amplifier with the sensitivity of 5–15 nV in the range from 0 to 1 Hz. The SCAL firm has constructed such an amplifier. It has three identical channels. To minimize temperature drifts, the preamplifier board is placed in an active thermostat adjusted for the temperature of 40–45°C.

The microcalorimeter is connected to the external computer by an 8-channel ADC with 16-bit capacity. The converter is built-in in the microcalorimeter and a cable of 2.5–3 m long provides the connection.

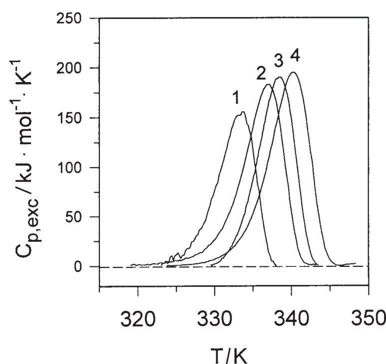

Fig. 3 Dependence of the excess partial heat capacity of δ-endotoxin on temperature at pH 3.0 and different heating rates: 1 – 0.125 K min^{-1}; 2 – 0.5 K min^{-1}; 3 – 1.0 K min^{-1}; 4 – 2.0 K min^{-1}

The development of microcalorimeter SCAL-1 was carried out by the firm under the scientific supervision of the Institute of Protein Research, Russian Academy of Sciences (Pushchino). Three years ago a prototype of the microcalorimeter was installed in its Laboratory of Protein Thermodynamics. The firm has manufactured several stock-produced instruments SCAL-1.

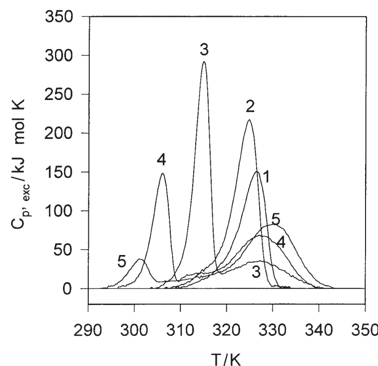

Fig. 4 Dependence of partial heat capacity of δ-endotoxin on temperature at different methanol concentrations. Curves 1-5 correspond to 0, 10, 20, 25 and 28% of methanol, respectively

Application of scanning microcalorimeters

With a scanning microcalorimeter it is possible to obtain temperature dependence of the heat capacity of the studied preparation rather than its enthalpy (ΔH), heat capacity being a derivative of enthalpy. Thus all thermodynamic parameters can be obtained from direct heat capacity measurements of calorimetric heat absorption curves. In many cases it is necessary to work with quite diluted solutions. However the lower is the preparation concentration, the higher should be the sensitivity of the instrument, so that reliable data on temperature-dependent changes in heat capacity could be obtained. Figure 2 shows results of heating lysozyme, a small globular protein, at a low concentration. Three curves were obtained at 3 refillings of the calorimetric cell.

As seen, the temperature heat absorption curves are compatible. The accuracy of such experiments is especially important for studying the concentration dependence to determine the order of the denaturation process.

Figure 3 shows a plot of temperature dependence of the excess heat capacity of Cry3A δ-endotoxin at different heating rates. The high class of the instrument has

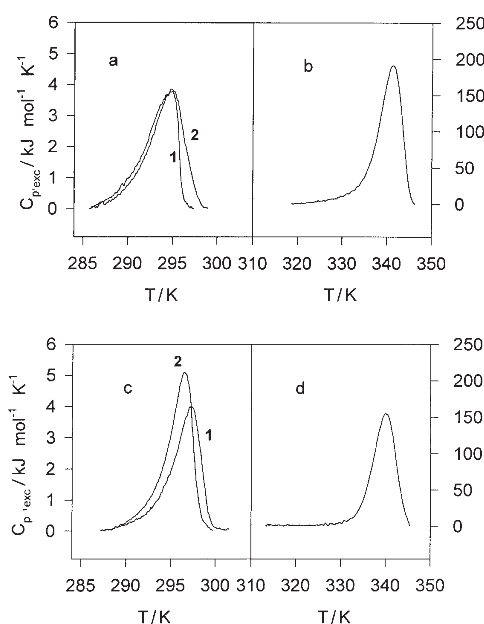

Fig. 5 Dependence of excess partial molar heat capacity of δ-endotoxin with phospholipids on temperature at pH 3.5. a – Phospholipids POPE:POPC before 1 – and after 2 – interaction with the protein. b – δ-Endotoxin with phospholipids POPE:POPC, the toxin-to-vesicle ratio is 1:100. c – Phospholipids POPE:POPG before 1 – and after 2 – interaction with the protein. d – δ-Endotoxin with phospholipids POPE:POPG, the toxin-to-vesicle ratio is 1:100

permitted conclusion on a kinetically controlled model of one-stage heat denaturation of a protein molecule [6].

Results of melting Cry3A δ-endotoxin in methanol solution of increasing concentration (up to 50%) are given in Fig. 4. The curves clearly reflect all the changes caused by the high concentration of methanol. It is seen that at the beginning the stability of protein molecule drops, but starting from a certain methanol concentration (~25%) the second peak appears and grows with high temperature stability. The emergence of another tertiary structure, differing from the native one that is formed at high concentrations of methanol has been demonstrated and then supported by other methods [7, 8].

Calorimetric studies of biological lipids and model membranes attract special interest. To study temperature transitions from gel to liquid crystals in these compounds, scanning microcalorimetry has been increasingly employed. This transition is highly cooperative and proceeds in a narrow temperature range. Hence demands claimed to calorimetry concerning relaxation time of the cell and temperature resolution of the instrument become higher. Figure 5 shows the results of the gel-to-liquid crystal transition of POPE:POPC (3:1) and POPE:POPG (3:1) vesicles and curves of excess partial heat capacity Cry3A δ-endotoxin after interaction with the vesicles.

The examples given demonstrate wide possibilities of the scanning microcalorimetry technique in various fields of biology.

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