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THREE GENERATIONS OF SCANNING MICROCALORIMETERS FOR LIQUIDS *

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ABSTRACT

Three distinct historical and technical stages in the development of sensitive, precise scanning microcalorimeters are critically reviewed. The practical and technical reasons which stimulated each developmental stage are described. The need for thermodynamic data on polymer solutions over a broad temperature range is discussed and presented as the justification for the development of highly sensitive, precise differential scanning calorimeters. Prospects are discussed for future progress in the design of scanning microcalorimeters.

THE FIRST SUPERSENSITIVE CALORIMETERS FOR STUDYING SMALL HEAT EFFECTS IN HEATED LIQUIDS

The year 1989 marks the 25th anniversary of the first publication describing a supersensitive instrument for measuring very small heat effects in heated liquids [1].

The immediate purpose for creating such an instrument was the need for quantitative information on the heat effects of the temperature-induced structural changes that occur in dissolved biopolymers. Two examples of such processes include the melting of the DNA double helix (the carriers of genetic information), which proceeds with a separation of its two strands, and the heat denaturation or disruption of the protein structure. The information gleaned from such measurements was required in order to understand the nature of the forces that control the formation and stabiliza-

* Dedicated to Professor Edgar F. Westrum, Jr., in honour of his contribution to calorimetry and thermal analysis, and on the occasion of his 70th birthday.

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tion of these unique three-dimensional macromolecules and, hence, their biological functions. The main technical challenge of such calorimetric measurements is that they must be conducted in highly dilute solutions where the intermolecular effects are sufficiently small to be neglected. For a biopolymer with a molecular mass in the order of 10^4, this requirement corresponds to concentrations of about 10^{-4} M (about 1 mg ml^{-1}). In such dilute solutions, the macromolecular contribution to the thermal properties of the entire sample will be generally small. The situation is aggravated by the fact that even dilute solutions of biopolymers are fairly viscous. Consequently, such solutions cannot be stirred to achieve rapid thermal equilibration upon heating, as is usually done in calorimetric studies.

The other important peculiarity of biological molecules is their poor availability and exceptionally high cost due to the difficulty of their isolation and purification. The amount of material generally available for studies on biological molecules does not usually exceed a few milligrams. Therefore, the experimental task of studying the heat effect of intramolecular temperature-induced processes in biopolymers involves the measurement of very small heat effects in a few millilitres of viscous solution, heated over a broad temperature range. None of the existing conventional heat capacity calorimeters could meet this experimental challenge. After many attempts, this challenge was successfully addressed in 1963 by the creation of a new type of instrument—the differential adiabatic scanning microcalorimeter (DASM) [1].

The new instrument incorporated a number of established techniques, each of which had been used in previously developed calorimetric instruments. Conventional heat capacity calorimeters measure the heat capacity in some temperature range in a discrete way, by using step-wise heating of the sample. In contrast, the new instrument measured the heat capacity continuously during the uninterrupted heating of a sample at a constant heating rate. Continuous heating has many advantages over discrete heating in that it provides much more detailed information on the temperature dependence of the heat effect under investigation, and is much more convenient for automatization. However, continuous heating also requires that the temperature-induced process is neither too sharp in temperature nor too slow in time. In addition, the instrument must be constructed so as to provide the minimal temperature gradient in the heated sample. This gradient should not change over the entire heating range.

Another specific feature of the new instrument was its differential design, measuring the difference in heat capacity between samples loaded in two identical calorimetric cells, rather than an absolute heat capacity (Fig. 1). The biopolymer solutions under study are loaded into one of the cells while the other cell is loaded with the solvent. With this arrangement, the measured differential heat capacity can be assigned to the solute in the solution, i.e. the biopolymer. Such a differential design automatically

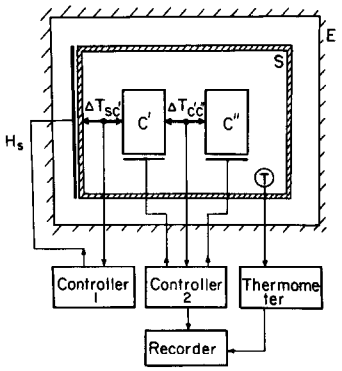

Fig. 1. The basic concept of the differential adiabatic scanning microcalorimeter: two identical cells (C′ and C″) heated at constant and identical rates inside the thermal shield under controlled temperature conditions. The difference in the heating power of the two cells is recorded as a function of temperature.

eliminates the large background of the solvent heat capacity, and the influence of the surroundings on the measured effect. In addition, the new instrument used a compensation method to measure the heat effect, by monitoring the heating power in one of the cells required to keep both cells at the same temperature. This compensation method enables not only a complete automatization of all the measurements, but also a significant improvement in the dynamic characteristics of the instrument as the heat balance in the cells is maintained by a feedback loop.

The complete adiabatization of the twin calorimetric cells represents another essential feature of the instrument design: the elimination of the influence of the surroundings on the cells and the provision of a constant heating rate in a broad temperature range. Adiabatization is achieved by a vacuum, the use of thermal shields, and a water jacket which surrounds both cells (Fig. 2). The temperature of the thermal shields and the jacket is monitored in such a way that it is always close to the temperature of the cells. The control of the temperature of the immediate environment of the cells is greatly facilitated by the fact that the cells are continuously heated at a constant (or almost constant) heating rate. This feature is very important in order to accurately adjust the temperature of the thermal shields and the jacket to the temperature of the cells. This adjustment is important in improving the adiabatization of the cells and, therefore, for increasing the accuracy of the instrument in the measurement of small differential heat effects.

The very first experiments with this instrument demonstrated its extreme efficiency in studying temperature-induced intramolecular processes in biopolymers. Figure 3 presents one of the first recordings of the heat effect observed upon heating a DNA solution. The calorimetric cell contained 640 mg of a 0.107% solution of DNA of T_2 phage (i.e. 0.685 mg of DNA).

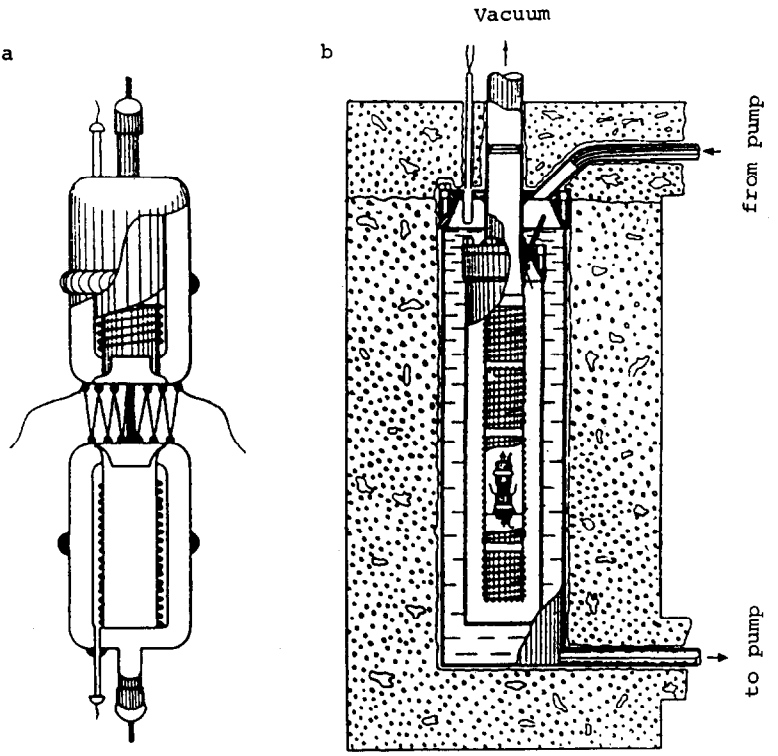

Fig. 2. The first differential scanning microcalorimeter for studying liquids. (A) A twin calorimetric cell with internal heaters and a thermopile between the cells. (B) The adiabatization system with thermal shields, vacuum and water jackets, and a jacket made of foamed plastics.

The heat absorption peak observed in the temperature range from 75 to 83 °C is caused by the melting of the DNA double helix. At 84 °C, there is a calibration mark corresponding to 21 μW, which permitted us to determine the area of the heat absorption peak in energy units at the known heating rate. The area shown corresponds to (32.2 ± 1.5) mJ. A recalculation per gram of dry weight of the preparation yielded a value of $\Delta H = (47 \pm 2.5)$ J g^{-1} for the specific enthalpy of melting of DNA at the given solvent condition (pH 7.0, 0.1 M NaCl).

At the same time (1964), Ackermann et al. [2] reported the measurements of the heat effect of DNA-melting using a conventional absolute heat capacity calorimeter with a highly improved accuracy, brought practically to the limit for this type of instrument. The volume of the sample used in their experiment was 280 ml and the concentration of the DNA solution was about 1%. Thus, for one measurement, 2.8 g of a pure DNA preparation was used. This amount represents 5000 times more than we used to measure the same effect with our scanning microcalorimeter. This example clearly shows that the appearance of the new scanning microcalorimeter was not a

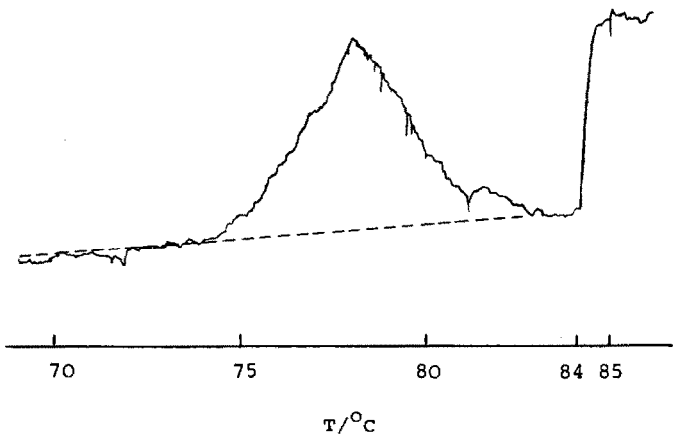

Fig. 3. One of the first experimental results obtained on a scanning microcalorimeter: the heat effect of melting of the DNA double helix (DNA of phage T_2 in 0.07 mM phosphate buffer, pH 7.0; 0.1 M NaCl). The DNA concentration is 0.107%, the amount of the solution in the calorimetric cell is 640 mg, and the amount of the DNA sample in the cell is 0.685 mg. At 84° C, a calibration mark is given corresponding to 21 μW [1].

quantitative but a qualitative step in the development of calorimetry, which introduced new possibilities for studying temperature-induced intramolecular processes in biopolymers.

After our first publication on the scanning microcalorimeter, several papers appeared describing the construction of supersensitive instruments for measuring small temperature-induced heat effects in liquids. Some of these papers were from our own laboratory [3,4], while some were from other laboratories [5,6]. All these supersensitive instruments incorporated the same three primary design features: continuous heating (scanning), differential cells and adiabaticity. The instruments differed only in their technical features, particularly the designs used to achieve adiabaticity. In the Gill [5] and Sturtevant [6] calorimeters, a temperature-controlled water jacket was used to create the adiabatic system, whereas we used the thermal shields and vacuum. To load the sample into these instruments, it was necessary to disassemble the adiabatic system and to remove the cells. The amount of material loaded was determined by weighing each cell on a precision balance.

At almost the same time, other instruments were developed which also could be classified as differential scanning calorimeters, although they were non-adiabatic. In these instruments, the constant heating rate was achieved by regulation of the heating power, thereby compensating for the increasing loss of energy upon heating [7,8]. The construction of these non-adiabatic differential scanning microcalorimeters (DSC) is essentially simpler than the construction of the corresponding adiabatic instruments (DASM). These non-adiabatic DSCs (e.g. Perkin–Elmer, Dupont, etc.) found broad applica-

tions, especially in industry and applied science. However, in these instruments, the calorimetric cells are not protected from the influence of the surroundings and the heating power is regulated over a broad range. Consequently, non-adiabatic DSC instruments have a much higher noise level which results in a lower sensitivity than the adiabatic scanning microcalorimeters. Usually non-adiabatic scanning calorimeters are not even regarded as microcalorimeters, and generally cannot be used for fundamental studies of intramolecular processes in biopolymers because of their lower sensitivity [9].

THE SECOND GENERATION OF SCANNING MICROCALORIMETERS—THE DISMOUNTABLE CALORIMETRIC BLOCK

The first scanning microcalorimeters were highly sensitive, thereby permitting studies on the heat effects of temperature-induced intramolecular processes in biopolymers. However, they were not precise instruments. Their main limitation was in the instability of the baseline, i.e. the calorimetric output obtained when both cells are filled with the same liquid, for example, the solvent. Generally, the baselines of these instruments were irreproducible during the second run of the experiment when both cells were filled with solvent. Therefore, these instruments could not be used to determine the absolute value of the heat capacity difference between the solution and the solvent, nor the partial heat capacity of the solute. They were used to register the sharp heat effects taking place in a small temperature range of the order of 10 °C. In many biopolymers, however, temperature-induced processes occur in a much wider temperature range. Therefore, for a detailed thermodynamic analysis of a process, one should know not only its heat effect (the enthalpy of the process), but also the accompanying heat capacity change which determines the temperature dependence of all the thermodynamic functions which characterize the process. In other words, it is necessary to know the partial heat capacity of dissolved molecules over a rather broad temperature range.

The problem of the baseline instability is closely connected with the problem of loading the samples into the cells. As noted above, sample loading in the first scanning microcalorimeters required one to dismount the adiabatic system and to extract the cells. But a microcalorimeter with extractable cells never gives reproducible results as each replacement of the cells results in a different slope and position of the baseline of the instrument. To measure heat capacities with an error of 10^{-5} J K^{-1}, the error in loading the cells with the sample should not exceed 10^{-6} g. However, it is practically impossible to load the calorimetric cells with such an accuracy by weighing the sample.

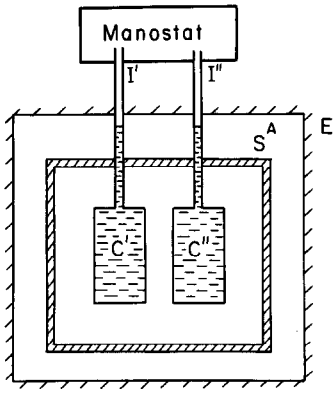

Fig. 4. The basic concept of the first undismountable calorimetric block with the cells completely filled with liquid samples through the capillary inlets I′ and I″. The operational volume of the cells is fixed by the point at which the adiabatic shield (S) contacts the capillary inlet and shunts the different heat effect. A manostat is used to apply excess pressure on the liquid in order to dissolve any bubbles which might be left in the cells and to prevent their reappearance upon heating.

The free volume in the partially filled cells also creates unacceptable difficulties. It is obvious that hermetic cells cannot be completely filled with a liquid sample, as the thermal expansions of the liquid and the cell are usually different. On the other hand, the free volume in the cell leads to numerous complications during heating caused by vaporization of the liquid. Although the vapour pressures of the solvent and the dilute solution do not differ greatly, the difference in the heat effect of their vaporization might be quite significant owing to the large specific heat of vaporization of the solvent.

The problems of sample loading and free volume were solved by replacing heat capacity measurements on a sample with a definite mass, by heat capacity measurements on a sample with a definite volume [10,11, see also 9]. The volume of the sample can be fixed by controlling the operational volume of the calorimetric cell. The main requirement which must be fulfilled is that the cell should be filled completely and include no microscopic bubbles. It is clear that this requirement can only be met by replacing the isolated hermetic cell, which has always been used in heat capacity calorimetry, by an open cell connected to an external vessel (Fig. 4). A thin capillary tube connects the cell with the external vessel so that the cell can be filled with the sample without removing it from the adiabatization system of thermal shells. This permits the liquid, which expands upon heating, to flow from the cell. The operational volume of the cell is determined by the thermal shell which is in thermal contact with the capillary tubes and which plays the role of a thermal shunt, preventing the influence of the external part of the capillary tube on the cell.

To exclude bubbles from the cells, an excess pressure is applied to the external ends of the capillary tubes by a manostat and all measurements are performed under this constant pressure. A few atmospheres are sufficient to compress and dissolve all the bubbles in the cells and to prevent their appearance during heating. Since the excess pressure raises the boiling temperature, this feature extends the operational temperature range of the instrument. An important feature of this construction is that the excess pressure is transferred into the cells through the liquid in the thin capillary tubes. In such a tube no convection can occur. Therefore, the gas from the manostat can penetrate into the cell only by diffusion which is slow. Thus, the capillary tube stops the penetration of gas from the outside into the cell, thereby preventing the liquid sample from becoming saturated with the gas.

When the cells are heated, the expanding liquid in the cells is removed through the capillary tubes, i.e. the mass of the liquid, which is in each cell, decreases with an increase of temperature. However, the flow of the liquid from both cells is almost identical when studying dilute solutions, since the thermal expansion coefficients of the dilute solution and the solvent are almost the same. Therefore, expansion of the liquid does not affect the measured heat capacity difference, although a decrease of the mass in the cells should be taken into account when calculating the specific heat capacity (see below).

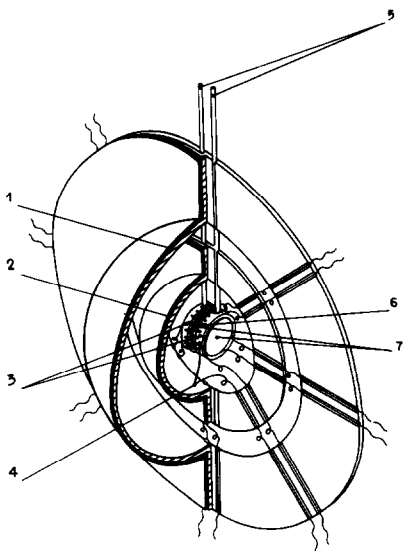

Fig. 5. The first undismountable calorimetric block of the scanning microcalorimeter DASM-1M: (1,2) internal and external adiabatic shields with heaters; (3) cells; (4) shield thermosensor; (5) capillary inlets; (6) thermopile and (7) cell heater.

Figure 5 shows a schematic diagram of the undismountable calorimetric block, in which the heat capacity measurements of liquid in a fixed volume were first made [11]. The calorimetric cells are made of flat hollow disks of gold (which is chemically inert and has a high thermal conductivity). The cells are positioned symmetrically with a small air-gap between the flat sides, facing each other. Electric heating elements are uniformly distributed in a thin layer on both flat surfaces of each cell.

Both cells are connected by a thermopile, the junctions of which are located at the cylindrical periphery of the cells (a 200 junction chromel-to-constantan thermopile). Such flat disk-like cells with large surface-to-volume ratios and with the heaters situated on the surfaces, provide a sufficiently uniform temperature field within each cell.

The inlet capillary tubes are made from platinum which has a low thermal conductivity and is chemically inert. The tubes are positioned vertically on the top of the cells.

The twin cells are inserted into a double adiabatic shell. Each of the closed adiabatic shells consists of two identical hemispherical shields with rims, resembling a sombrero hat. The rims are clamped to each other, to ensure thermal contact, and all connections (the wires to the cells and the capillary inlets) are laid in radial grooves between the rims. The shields are made of silver and have electric heaters which are uniformly distributed over the entire outside surface of each shield.

An essential feature of the design of the adiabatic system is its symmetry. The symmetry plane of both shields is orthogonal to the symmetry plane of the cells and these two planes are vertical. Such a configuration provides the most symmetric temperature field and the most identical conditions for both cells. The adiabatic shell design just described is named "sombrero" and has proved to be the most efficient. In fact, this design provides such a good adiabatization of the twin calorimetric cells that there has been no need for either a vacuum or a water jacket. This situation, in its turn, has led to a significant simplification of the whole calorimeter and to a decrease in its size. The scanning microcalorimeter with a "sombrero"-type adiabatization system permitted the construction of a compact instrument which was technologically suitable for serial production. In 1972, on the basis of the undismountable calorimetric block described above, the first serial scanning microcalorimeter (the DASM-1M) was produced by the Bureau of Biological Instrumentation of the Academy of Sciences of the U.S.S.R. (Fig. 6). For many years, it was the only precise scanning microcalorimeter which was commercially available. Consequently, many laboratories all over the world which conducted fundamental research on the physics of intramolecular transitions were equipped with this instrument (see ref. 9).

In subsequent years, the principle of a fixed volume in measuring the heat capacity of liquids, which enabled the construction of an undismountable calorimetric block, became conventional in the scanning microcalorimetry of

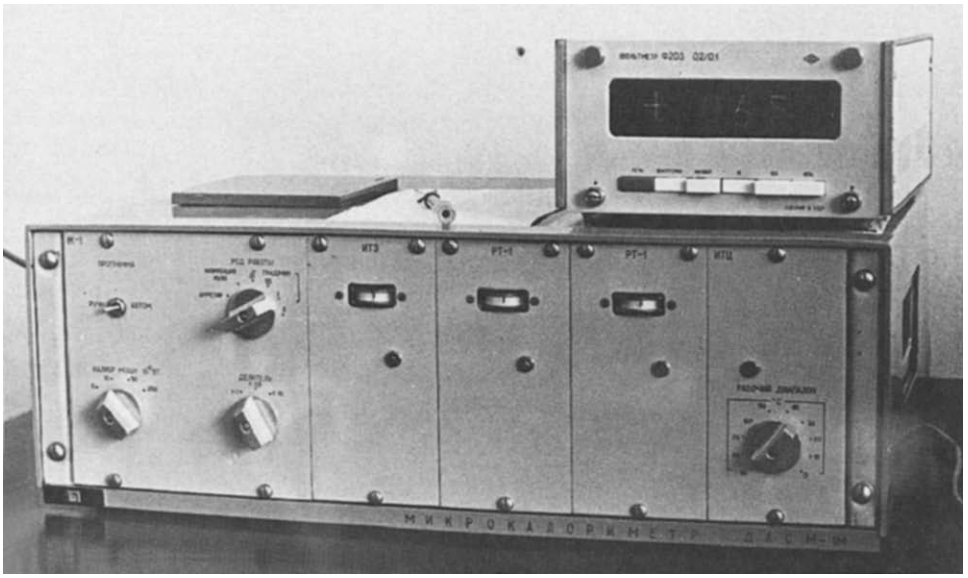

Fig. 6. The first commercial scanning microcalorimeter DASM-1M manufactured by the Bureau of Biological Instrumentation of the Academy of Sciences of the U.S.S.R.

liquids. All precise scanning microcalorimeters subsequently manufactured by the Bureau of Biological Instrumentation (DASM-4 and DASM-4A) and by other companies (e.g. the scanning microcalorimeter Biocal, manufactured by Seteram in France and the MC-2, manufactured by Microcal in the U.S.A.) followed the initial design of the DASM-1M. Unfortunately, there has been no publication describing the details of the calorimetric blocks of the latter two instruments. Therefore, we cannot discuss their technical advantages.

Heat capacity measurements on samples of definite volume rather than definite mass represented an essential step forward in the development of scanning microcalorimetry. Prior to this advance, scanning microcalorimeters were considered to be supersensitive instruments for measuring small heat effects. Following this innovation, it seems fair to describe these new instruments as superprecise DSCs for measuring heat capacity differences over a broad temperature range. This design improvement has opened new possibilities for the use of DSC in the determination of the absolute heat capacities of undiluted liquids and partial heat capacities of components in dilute solutions over the whole temperature range in which the studied sample exists as a liquid.

Let us consider the determination of the partial heat capacity of a protein in solution. Figure 7 shows a calorimetric recording of the differential heat capacity of a 0.182% solution of lysozyme relative to the solvent (4 mM aqueous solution of glycine, pH 2.55). The calorimetric recording of the solvent against the solvent (the baseline) is also shown. The distance

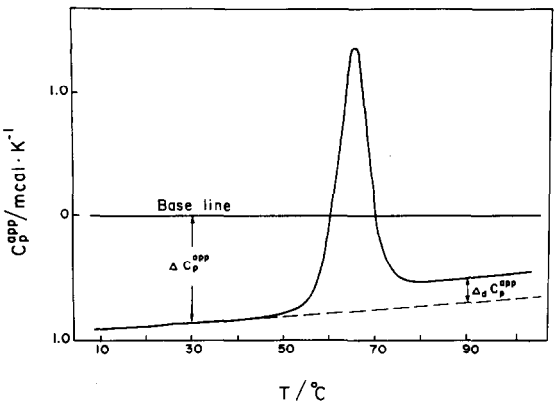

Fig. 7. A microcalorimetric recording of the differential heat capacity of 0.182% lysozyme solution against the solvent, 40 mM glycine buffer, pH 2.5. The baseline was obtained by filling both cells with the solvent (for details see ref. 12).

between these two recordings ΔC_p^{app} corresponds to the difference in the heat capacity of the same volume, V, of the solution and the solvent at corresponding temperatures, where V is the operational volume of the calorimetric cell. Since the heat capacity of the solution is smaller than the heat capacity of the same volume of the solvent, it is clear that the partial specific heat capacity of protein in solution, $C_{p,pr}$, is lower than the specific heat capacity of solvent, $C_{p,solv}$. For the differential heat capacity, ΔC_p^{app} we can write

$$\Delta C_p^{app} = C_{p,pr} m_{pr} - C_{p,solv}\, \Delta m_{solv} \tag{1}$$

where m_{pr} is the mass of protein in the calorimetric cell, which is cV, and Δm_{solv} is the mass of the solvent which is replaced by the protein in the cell

$$\Delta m_{solv} = m_{pr}\left(\frac{V_{pr}}{V_{solv}}\right) \tag{2}$$

Here V_{pr} is the partial specific volume of the protein and V_{solv} is the specific volume of the solvent. From eqns. (1) and (2), we obtain an expression for the partial heat capacity of the protein

$$C_{p,pr} = C_{p,solv}\left(\frac{V_{pr}}{V_{solv}} - \frac{\Delta C_p^{app}}{m_{pr}}\right) \tag{3}$$

Thus, to use scanning microcalorimetry to determine the partial specific heat capacity of a solute at some temperature, one must know the partial specific volume of the solute and the specific volume of the solvent at a corresponding temperature. Both these quantities can be determined with sufficient accuracy by precise vibration densitometers. Therefore, the accuracy of the

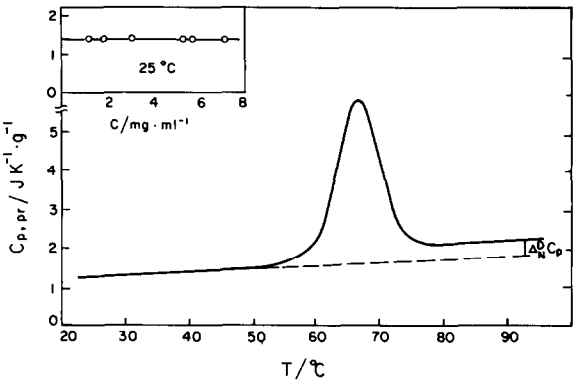

Fig. 8. Partial specific heat capacity of lysozyme in 40 mM glycine buffer, pH 2.5 as a function of temperature. The inset shows the concentration dependence of the partial specific heat capacity of lysozyme at 25 °C.

determination of the partial heat capacity of a solute is determined mainly by the accuracy in measuring the concentration of the solute in solution c at a corresponding temperature, and by the accuracy in measuring the apparent heat capacity difference ΔC_p^{app}. The latter depends completely on the stability of the baseline observed after multiple fillings of the calorimetric cells (for details see ref. 13).

For the scanning microcalorimeter DASM-1M with operational cell volumes of 1 ml, the reproducibility of the baseline upon refilling is about 3×10^{-4} J K^{-1} [9]. Therefore, at a solution concentration of about 3 mg ml^{-1} (or 0.3 wt.%), the partial specific heat capacity of the solute can be determined with an accuracy of 0.1 J K^{-1} g^{-1}. This corresponds to about 8% of the value of the partial heat capacity of the protein.

The partial heat capacity of lysozyme determined from the recording in Fig. 7 is shown in Fig. 8. It should be noted that at the concentrations used for its determination, there is no dependence of the partial heat capacity on the concentration (see the insertion in Fig. 8). Therefore, the value of the partial heat capacity so obtained can be regarded as corresponding to an infinitely dilute solution, i.e. $C_{p,pr} = C_{p,pr}^{\infty}$. References 9 and 13 provide more detailed descriptions of the determination of the partial heat capacity of solutes.

As seen from Fig. 8, the partial heat capacity of native lysozyme increases linearly with the temperature to about 50 °C. In the temperature range from 50 to 60 °C, an extensive heat absorption peak is observed, which corresponds to the denaturation heat effect. After denaturation, the heat capacity of the denatured protein is significantly higher than the heat capacity of the native protein. However, the heat capacity of the denatured protein continues to increase with increasing temperature with almost the same slope as

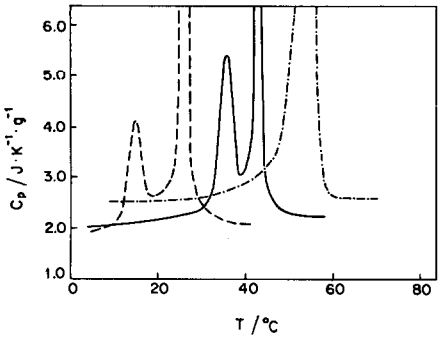

Fig. 9. Partial specific heat capacity of various phospholipid membranes in the temperature range of conformational transition: (— — —) DMPC; (———) DPPC; (·–·–·) DSPC (for details see ref. 15).

the heat capacity of the native protein. (See ref. 14 for a more complete discussion on the thermodynamics of globular proteins.)

The denaturation heat capacity increment, which determines the temperature dependence of the denaturation enthalpy and entropy, is one of the most important thermodynamic parameters. It is especially large for proteins and is almost unnoticeable for nucleic acids and membranes. Figure 9

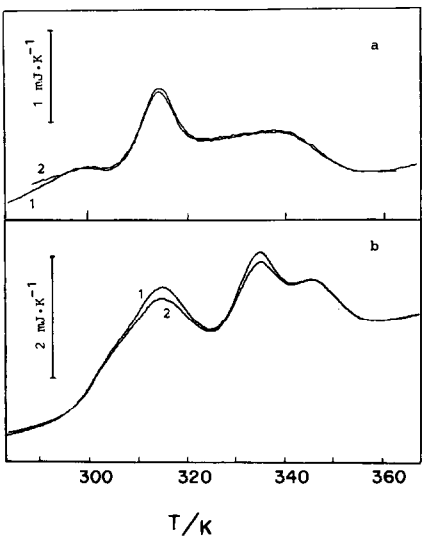

Fig. 10. A microcalorimetric recording showing two consecutive runs of a sample of: (a) transfer ribonucleic acid (valine-specific) in salt-free solution with 5 mM phosphate buffer, pH 7.0, the concentration of the tRNA being 0.75 mg ml^{-1} [16]; (b) multidomain protein plasminogen in 50 mM glycine solution, pH 3.4, the concentration of the protein being 3.0 mg ml^{-1} [17].

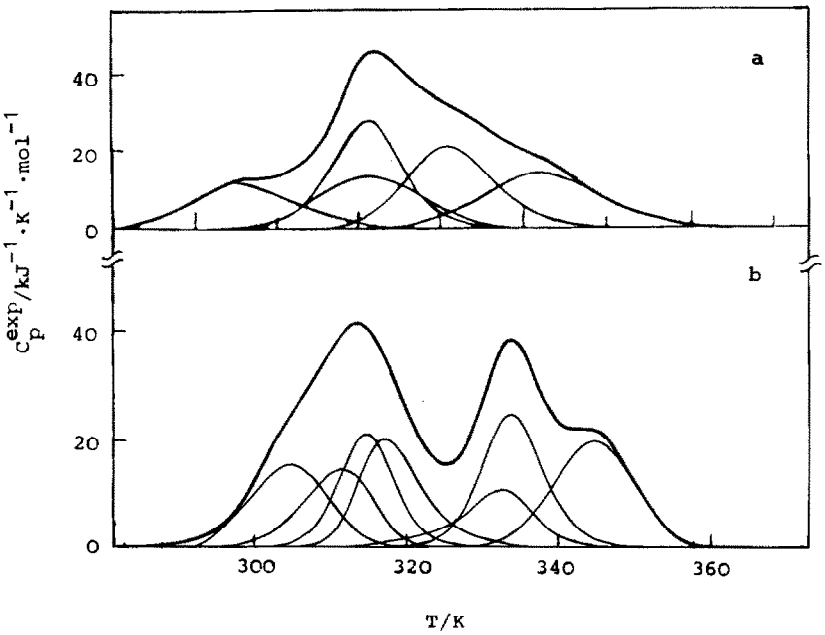

Fig. 11. Deconvolution of the excess heat capacity of $tRNA^{val}$ (a) and plasminogen (b) into a set of two-state transitions (for details, see refs. 17 and 19).

shows that the melting of membranes differs significantly from the intramolecular melting of proteins [15].

It should be noted that because of its baseline stability, the precise scanning microcalorimeter has opened up new possibilities in studying the complicated intramolecular processes which are observed in multidomain proteins and nucleic acids. Heating of such biopolymers frequently yields complicated melting profiles, as is demonstrated in Fig. 10 for a $tRNA^{val}$ molecule and for plasminogen. A repeat heating of the same samples after cooling shows that the observed complicated melting curves are quite reproducible. The reproducibility of the recordings shows both the high precision of the instrument and the reversibility of the observed process. The latter feature is very important for a thermodynamic analysis of the observed effect.

The use of rigorous statistical thermodynamics permits us to deconvolute the observed complicated heat absorption curves into simple constituents, each corresponding to two-state transitions. The results of the deconvolution of the melting curves of $tRNA^{val}$ and plasminogen are shown in Figs. 11(a) and (b). The details of these studies are described in refs. 17 and 18, while the deconvolution analysis is discussed in ref. 13. A review of the denaturation of multidomain proteins is presented in ref. 19.

THE THIRD GENERATION OF SCANNING MICROCALORIMETERS—CALORIMETERS WITH CAPILLARY CALORIMETRIC CELLS

The appearance of the undismountable calorimetric block was a significant step in the development of scanning microcalorimeters. However, this instrument still had a number of shortcomings. The major shortcoming was two-fold: the difficulty of washing the calorimetric cells through a thin capillary tube and of refilling the sample without leaving a bubble inside the cell. Although small bubbles can be squeezed and dissolved by applying an excess pressure, this procedure can lead to a change in the solution composition. These changes are rather small, but they can prove to be a problem for determining the absolute values of differential heat capacities and they can influence the accuracy of the partial heat capacity determination.

Another complication could arise from the cylindrical design of the cells, in which some layers of liquid are not in contact with the surface of the cell which serves as a heater. In the DASM-1M scanning microcalorimeter, each cell is 3 mm thick. Consequently, the distance to the heated cell wall is 1.5 mm for the most distant layer of the liquid inside the cell. As a result, a significant temperature gradient is set up inside the cell upon heating. The gradient increases with increasing heating rate, and, in cylindrical cells with significant vertical dimensions, it induces a convectional circulation of the liquid, which, in turn, affects the gradient in a manner that depends on the viscosity of the liquid. Therefore, the viscosity of the liquid influences the temperature field inside the cell and this field changes with variations in the viscosity of the liquid. All these effects are recorded as a change in the heat capacity. This anomaly can be decreased either by decreasing the heating rate or by decreasing the maximal distances of the liquid layer to the heating surfaces within the cell. The latter can be accomplished by increasing the surface : volume ratio for the cell and decreasing the vertical dimensions of the cell. Decreasing the heating rate is unacceptable because it results in a decrease in the sensitivity of the intrument for heat capacity measurements. Decreasing the maximal distances between the liquid layer in the cell and the heating surfaces, raises the problem of the most optimal shape of the cell, i.e. its construction.

These problems were solved by replacing the cylindrical cells which have a rather small surface : volume ratio with capillary tubes which have a very large surface : volume ratio. The capillary tubes can be washed by the flow of an appropriate solvent and can easily be filled with any liquid of interest, even if it includes volatile components, such as gases. The capillary tubes can also have the desired configuration; namely, one with small vertical dimensions which promotes convection of the liquid when heated.

The use of a capillary cell has opened a number of new prospects; it can withstand a much larger internal pressure than cells of any other shape having the same wall thickness. Therefore, a capillary cell enables the excess

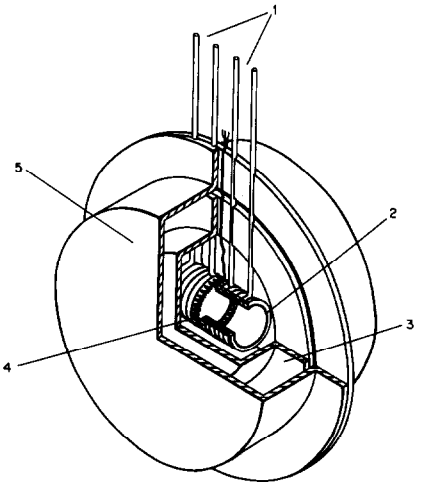

Fig. 12. The undismountable calorimetric block with the capillary cells and a sombrero-type double thermal shield: (1) external shield; (2) internal shield; (3) capillary cells.

pressure of the liquid to be significantly raised. Thus, the upper limit of the operational temperature or the concentration of the dissolved gases in the sample (if gases are studied in the experiment) can both be increased.

Success in the use of the capillary cell design depended completely on the search for the optimal configuration of the entire calorimetric unit, which includes two calorimetric cells, the heater and the thermosensor. This search was a difficult one, involving many technological problems. Thus, although the idea of the use of capillary cells in scanning microcalorimetry appeared at the end of the sixties [20,21], the optimal construction of the measuring cell was not achieved until ten years later, after many versions had been tried (see ref. 9). The design currently used is shown in Fig. 12. The capillary tubes are wound on hollow metal cylinders with helical grooves. The heaters are uniformly distributed on the inner surface of the cylinders, and the junctions of the thermopile are fixed on their peripheral rims. The thermopile is used as a sensor of the temperature difference between the two cells. The cells are locked in a sombrero-type double adiabatic shell.

This design of the calorimetric block with capillary cells was used in the construction of the DASM-4 and DASM-4A which were manufactured by the Bureau of Biological Instrumentation of the Academy of Sciences of the U.S.S.R. in 1980 and 1986, respectively. The main difference between these two instruments is in the cell volumes and the material used to manufacture the calorimetric cells. In the DASM-4, the cells are of a single platinum tube and have an operational volume of 0.5 ml. In the DASM-4A the measuring part of the cells are made of a gold capillary tube and the exterior inlet parts are made of platinum, and the operational volume of the cell is 1.0 ml. The calorimetric block of both instruments is equipped with a semiconductor

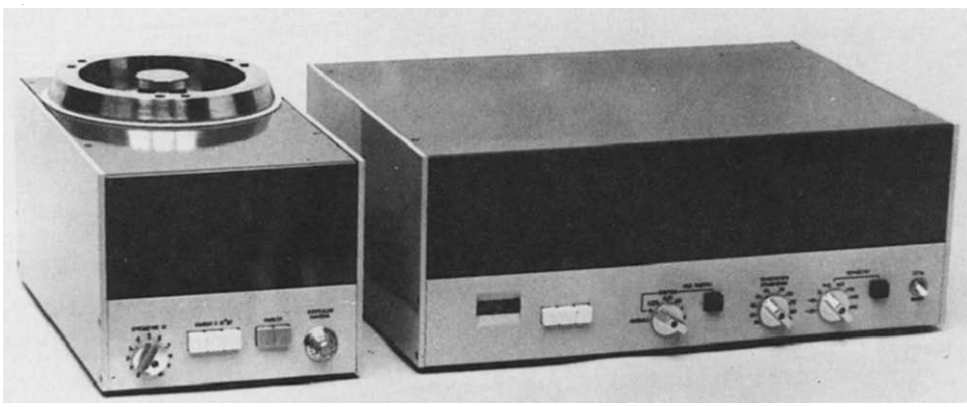

Fig. 13. Photograph of the commercial scanning microcalorimeter DASM-4, produced by the Bureau of Biological Instrumentation of the Academy of Sciences of the U.S.S.R. since 1984.

cooling device which expands the lower limit of their operational temperature range to $-10\,^{\circ}C$.

An essential feature of the DASM-4 and DASM-4A instruments is that they can scan at varying constant rates, not only up the temperature scale, but also down it, thereby measuring the heat effects which occur during cooling of the sample. The cooling of cells at a constant rate is achieved by controlling the temperature of the adiabatic shell.

Another difference between the DASM-4 and DASM-4A instruments is in the electronics and the control system. In DASM-4A, the control is performed by a microcomputer of the IBM-XT class, which also processes all the experimental data (e.g. memorizes the baseline and records each experiment, calculates the partial heat capacity and the enthalpy as a

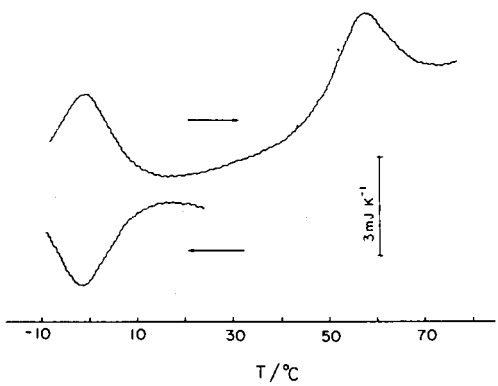

Fig. 14. An original recording of the scanning microcalorimeter DASM-4 showing the heat effect observed at cooling and consecutive heating of an aqueous solution of apomyoglobin. The amount of sample in the cell is 4.7 ml and the scanning rate is 0.5 K min^{-1} (for details see ref. 23).

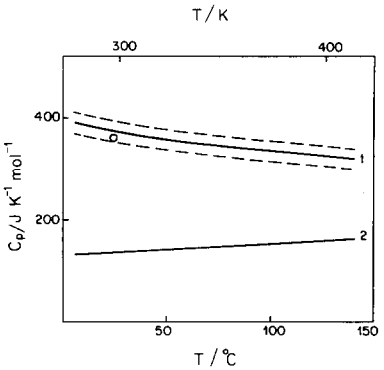

Fig. 15. Partial heat capacity of benzene in water as a function of temperature, obtained by the scanning microcalorimeter DASM-4 (1). Circles show the values obtained from the temperature-dependence of the enthalpy of dissolution of benzene in water by Gill et al. [25]. (2) Specific heat capacity of pure benzene in a liquid state.

function of temperature, and deconvolutes the complex heat capacity function into its constituent two-state transitions). Figure 13 shows a photograph of the scanning microcalorimeter DASM-4, with which many laboratories all over the world have been equipped. Figures 14–16 present some examples which demonstrate the possibilities of capillary scanning microcalorimeters.

Figure 14 shows an original calorimetric recording of the heat effect observed upon cooling and subsequent heating of an aqueous solution of apomyoglobin in 10 mM acetate buffer, pH 4.0. The protein solution was cooled at a rate of 0.5 K min^{-1} from 25 to -7°C. At temperatures below

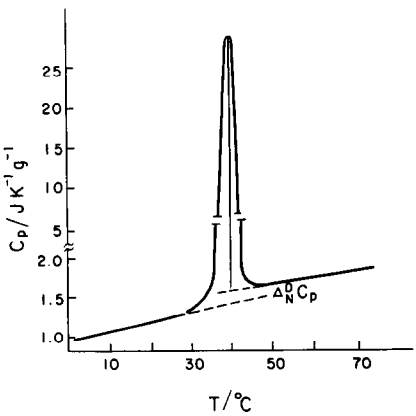

Fig. 16. Temperature dependence of the partial heat capacity of collagen (rat skin) in an aqueous solution. The intense heat absorption peak is caused by denaturation of collagen. Denaturation leads to an increase in the heat capacity by 0.2 J K^{-1} g^{-1} (for details see ref. 26).

0 °C, it was in a supercooled state but did not freeze because all the dust particles which usually serve as a nucleus for the crystallization of ice had been removed from the solution. In the temperature region from 10 to −5 °C, the peak of heat evolution is observed, caused by the cold denaturation of the protein. Upon subsequent heating in the same temperature range, one observes the heat absorption peak caused by renaturation of protein. The processes of cold denaturation and renaturation of the protein are completely reversible and the corresponding heat effect appears as a mirror image on the calorimetric recording. Further heating of the protein solution above 25 °C provides a second peak of heat absorption caused by heat denaturation of the protein. Cold and heat denaturation of proteins is discussed in greater detail in refs. 22 and 23.

As illustrated by the example described above, the cooling scan capability greatly extends the possibilities for studies using scanning microcalorimeters. Specifically, one can now not only observe the processes induced by cooling, such as cold denaturation, but also study an aqueous solution in a supercooled state (in the temperature range below 0 °C).

Let us now consider the use of scanning microcalorimetry in the determination of the partial heat capacity of poorly soluble solutes, such as an aqueous solution of benzene. The solubility of benzene in water is very low, 1.71 mg ml^{-1} at 15 °C. Owing to this low solubility, the direct measurement of the partial heat capacity of benzene, or of other non-polar solutes, in water has been impossible; this is serious because such information is important in order to understand the nature of the hydrophobic interactions which stabilize many biopolymers. The partial heat capacity of benzene in water has been determined indirectly at 25 °C by using a reaction microcalorimeter to measure the temperature dependence of the heat of benzene dissolution in water [24]. Capillary scanning microcalorimeters now make it possible to determine the heat of benzene dissolution in the temperature range from 5 to 140 °C under the excess pressure of 5 atm (Fig. 15). For further details see ref. 25.

Scanning microcalorimeters can also be used to study liquids with high and variable viscosity. One such liquid is a solution of collagen, a fibrillar rod-like protein. Solutions of this protein are very viscous (even at 0.1% concentration) and their viscosity drops dramatically as collagen denatures. For mammalian collagens, this denaturation occurs at about 40 °C. The change in viscosity upon heating induces anomalies in the heat capacity measurement using scanning microcalorimetry. Because of this fact, until now, nobody has successfully determined the heat capacity change of collagen denaturation. Such information is important in order to understand the nature of the molecular forces that stabilize its structure. The requisite measurement on collagen can now be made using the capillary scanning microcalorimeter which is insensitive to the changes in viscosity (Fig. 16). For details the reader is directed to ref. 26.

PROSPECTS FOR FURTHER DEVELOPMENT OF SCANNING MICROCALORIMETERS

It is impossible to predict future developments in scanning microcalorimeters as qualitative technical changes usually occur as a result of unpredictable reflections. Therefore, we only can discuss the probable directions of development for these instruments based on the requirements of contemporary science.

The sensitivity of the existing scanning microcalorimeters is so high that one can hardly expect this sensitivity to improve significantly. As for the precision (reprodicibility of the baseline upon refilling the cells), one can expect progress to derive from mainly technological improvements (e.g. a better selection of materials). Significant changes should be expected also in the extension of the operational temperature range of the instrument. In DASM-4A, this range varies from -10 to $130\,^{\circ}C$. In the near future, models with an operational range from -50 to $150\,^{\circ}C$ are expected. Essential improvements are also expected in the control and data processing provided by the incorporation of micro-processors and also by the computerization of all the microcalorimetric measurements and calculations.

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