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Characteristics of an adiabatic differential scanning microcalorimeter *

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Abstract

An on-line system was designed for the acquisition and analysis of data from the differential adiabatic scanning microcalorimeter DASM-1M. The characteristics of the calorimeter, temperature dependence of the heating rates, calibration of the heat capacity and time constants of the apparatus at various heating rates were obtained. The heat capacity data of lysozyme measured with the DASM-1M were compared with those using a conductive type DSC instrument, the micro-DSC.

INTRODUCTION

Calorimetry is extremely important in studies of the structure of proteins because precise calorimetric measurements and data analysis provide thermodynamic information on the stability of the three-dimensional native structure [1,2]. From the calorimetric data the energetics of domain structure and subunit structure of proteins can also be elucidated by statistical thermodynamics [3–7].

Differential scanning calorimeters of the adiabatic type use an electrical calibration pulse [8] for converting the ordinate of the DSC curves to heat capacity. The temperature dependence of the heating rate affects the conversion coefficient. As Mayorga and Freire [9] pointed out, the precision calorimeter has a characteristic time constant which causes a distortion of the observed heat capacity curve. Analysis of the DSC data requires checking of these characteristics of the calorimeter and making corrections to the data.

In this paper, we report the characteristics of the differential adiabatic scanning microcalorimeter DASM-1M [8]: the temperature dependence of the heating rate and the time constant, which were obtained using the

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on-line system constructed for the instrument. The heat capacity data (DSC data) of lysozyme measured with the DASM-1M were compared with those obtained using a conductive type DSC instrument, the micro-DSC.

EXPERIMENTAL

Materials and DSC measurements

Six times recrystallized hen egg lysozyme (Seikagaku Co.) was used, and its solution was dialyzed for about 24 h against 50 mM glycine buffer of pH 2.5 or 3.5. The concentration of lysozyme (0.063–0.086%) was determined photometrically using the optical density [10] of 26.9 at 280 nm. The thermal transition of lysozyme was measured at pH 2.5 and 3.5 at a heating rate of $1\,^{\circ}\mathrm{C\ min^{-1}}$ with the differential adiabatic scanning microcalorimeter DASM-1M (Mashpriborintorg) and a differential scanning microcalorimeter of conductive type, the micro-DSC (Rigaku/Setaram). The volume of the enzyme solution was 1.0 ml and 0.9 ml with the DASM-1M and micro-DSC cells respectively.

On-line system and calibration of the time constant of DASM-1M

The on-line system of the DASM-1M is composed of a preamplifier (with amplification factors of 8, 40, 80, 160 and 400), AD converter (Canopus CAD 12-4), electrical isolator and PC 9801 microcomputer; about 5000 points of data on heat capacity and temperature were stored for each scan. The heating rate of the DASM-1M is set by a constant electric current to the heaters of the two cells, and the voltage is unchanged during heating. The heating rate may change, therefore, if the heat capacities of the cells containing the solutions change with temperature. The temperature dependence of the heating rate was measured at various scanning rates.

The calorimetric output response of heat capacity to a rectangular calibration input pulse has a shape showing instrumental distortion due to the time constant. The time constants at various heating rates were calculated by analyzing the DSC curve obtained with a calibration heater rated at 50 μW. The relationship between the measured calibration output ($C^{\mathrm{obs}}(t)$), which has a shape with instrumental distortion, and the rectangular input signal ($C^{\mathrm{true}}(t)$) is [3]

$$C^{\mathrm{obs}}(t) = \frac{1}{\tau}\int_{-\infty}^{t} \exp\left(-\frac{t-t'}{\tau}\right) C^{\mathrm{true}}(t')\,\mathrm{d}t' \tag{1}$$

We obtain eqn. (2) by Laplace transformation

$$C^{\mathrm{true}}(t) = C^{\mathrm{obs}}(t) + \tau\frac{\mathrm{d}}{\mathrm{d}t}C^{\mathrm{obs}}(t) \tag{2}$$

where τ is the time constant of the apparatus.

RESULTS AND DISCUSSION

Characteristics of the calorimeter DASM-1M

A slight linear increase in the heating rate with temperature was observed (Fig. 1). The relative increases between 0 and 100 °C calculated at various heating rates were less than 1.6% (Table 1). Correction of the temperature dependence of the heating rate in evaluating the heat capacity (expressed in $J\ K^{-1}\ g^{-1}$ or $J\ K^{-1}\ mol^{-1}$) over a wide temperature range is easily made by using the temperature coefficients of the heating rate.

The main factors which increase the heating rate with increasing temperature during measurements on samples of dilute aqueous solutions with the calorimeter DASM-1M are the isobaric specific heat C_p^w and the density d^w of water. The values of the heat capacity of water in the cell, C_p^w (cell), are

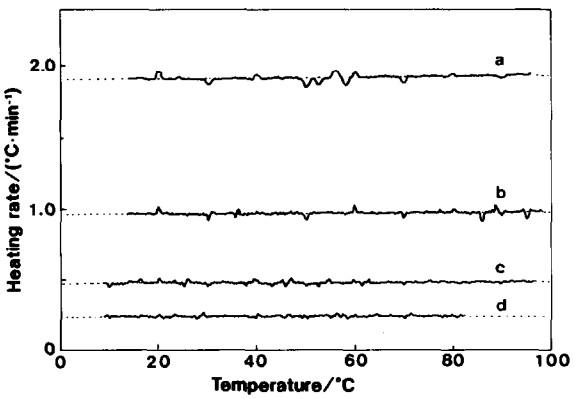

Fig. 1. Temperature dependence of the heating rates at various heating rates (°C min^{-1}): curve a, 2; curve b, 1; curve c, 0.5; curve d, 0.25.

TABLE 1

Characteristics of DASM-1M

Heating rate (°C min^{-1})	Temp. coeff. of heating rate [a] $\times 10^4$ (min^{-1})	Relative diff. [b] (%)	Time const. (s)	Scale of heat capacity (bit per 50 μW)
0.2393	0.313	1.3	40.4	3800
0.4757	0.769	1.6	30.0	3050
0.9670	1.33	1.4	27.2	2400
1.914	2.86	1.5	18.9	1800

[a] At 323 K.

[b] Relative difference $= \dfrac{[dT/dt(100\,°C) - dT/dt(0\,°C)]}{dT/dt(50\,°C)} \times 100.$

obtained by multiplying the values of C_p^w by d^w at various temperatures; the values of C_p^w are tabulated by Kell [11].

The value of C_p^w (cell) decreases linearly with temperature with a correlation coefficient of -0.9985 and the relative decrease between 0 and 100 °C is calculated to be 4.05%. The calorimeter contains about 14 g of gold, and by considering the heat capacity of cells made of gold (the weight of the cell is assumed to be approximately 7 g), the relative decrease in heat capacity becomes 2.9%, which leads to a still larger increase in heating rate than those obtained by experiment. The cubic coefficient of thermal expansion, β, of gold cells, which represents the relative increase in volume for a change in temperature of 1 °C at temperatures in the vicinity of 25 °C [12], is 4.3×10^{-5} K^{-1} and slightly decreases the heating rate increase. The above experimental values of the relative increases in the heating rates, which are less than 1.6%, result from the additional effects of other factors such as the increasing leakage of heat with increasing temperature.

Time constants at various heating rates were calculated using eqns. (1) and (2) and are listed in Table 1; the values increase with decreasing heating rate. The calibration heat output of the calorimeter and the corrected signals with an estimated time constant at various temperatures are shown in Fig. 2 for heating rates of 0.5, 1 and 2 °C min^{-1}. The figure also shows that the shift in heat capacity of DSC curves by the calibration heater (expressed in J

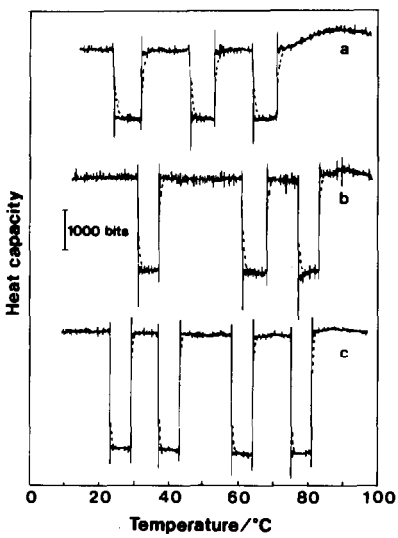

Fig. 2. The calibration heat output of the calorimeter (dotted lines) and the corrected signal with estimated time constant (solid lines) at various temperatures and heating rates (°C min^{-1}): curve a, 2; curve b, 1; curve c, 0.5. The scale in the figure corresponds to 1000 bits on the PC 9801 microcomputer. The duration of the power input to the calibration heater was the time which corresponds to the temperature range 6–8 °C.

s^{-1} ml^{-1}) can, for practical purposes, be considered not to depend on temperature.

DSC curves for lysozyme solutions

The DSC curves obtained for lysozyme solutions at pH 2.5 and 3.5 with the DASM-1M at a heating rate of 1° C min^{-1} were compared with those obtained with a conductive type calorimeter, a micro-DSC, at the same heating rate; the experimental DSC curves corrected for baseline are shown in Fig. 3. To obtain a reproducible baseline with the micro-DSC, care was needed in precisely equalizing solution weights in the sample and reference cells. The curvature of the DSC curves for a buffer–buffer solution recorded at very high sensitivity with the conductive type calorimeter often differed from that for a buffer–protein solution; therefore only the data over a limited temperature range are shown in Fig. 3B. The solid lines in the figure denote experimental curves, and the dotted curves correspond to those corrected for the calorimeter time constant. The time constants of 27.2 s and 60 s were used to correct DASM-1M and micro-DSC data respectively. The figure shows that the difference between the corrected DSC curve and the experimental DSC curve for the thermal transition of lysozyme measured at a heating rate of 1° C min^{-1} is very small for the DASM-1M, and still small but a little larger for the micro-DSC. In Table 2, the thermodynamic parameters which were calculated by non-linear least-squares fitting with the program SALS [13] using a two-state model from the experimental and corrected DSC data are tabulated. The values of the standard deviation in the table signify the deviation between the fitted values and data for C_p. The differences between the corrected and experimental data for the denaturation temperature T_d with the DASM-1M and the micro-DSC are 0.4–0.5 ° C

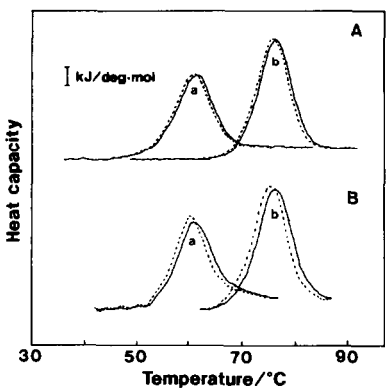

Fig. 3. DSC curves of lysozyme at pH 2.5 (a) and 3.47 (b) with DASM-1M (A) and micro-DSC (B). Solid and dotted lines denote experimental and corrected data for time constants of the apparatus respectively.

TABLE 2

Thermodynamic parameters for lysozyme obtained with DASM-1M and micro-DSC

Calorimeter	pH	T_d (°C)	$\Delta H(T_d)$ (kJ mol^{-1})	Std. dev. (J K^{-1} mol^{-1})
DASM-1M	2.5 Expt.	60.7	389.5	1.7
	Corr.	60.2	390.4	1.8
	3.47 Expt.	76.0	502.2	1.6
	Corr.	75.6	503.1	1.9
Micro-DSC	2.5 Expt.	60.8	398.9	2.2
	Corr.	59.9	406.8	2.9
	3.47 Expt.	76.1	501.9	2.7
	Corr.	75.3	511.8	3.2

and 0.8–0.9 °C respectively, and those for denaturation enthalpies at T_d are 0.2% and 2.0% respectively.

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