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SPECIFIC HEAT OF POLYGLYCINE I AND II IN THE TEMPERATURE INTERVAL 150—375 K

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ABSTRACT

The specific heat, C, of polyglycine I (β-sheet) and II (3_1 helix) have been determined in the temperature interval 150—375 K by differential scanning calorimetry. The apparatus repeated National Bureau of Standards' adiabatic calorimetry specific heat measurements on sapphire to within 2% and on high density polyethylene to within 5%. The conformation of the lyophilized polyglycine samples was confirmed by IR spectroscopy. The specific heat of polyglycine is fitted by the following expressions to within ±7% experimentally:

polyglycine I: $C = 37.744 + 0.218T - 2.333 \times 10^{-6}\ T^2$ ($\sigma = 3.3$) J mole^{-1} K^{-1}

polyglycine II: $C = 57.598 + 0.05T + 2.357 \times 10^{-4}\ T^2$ ($\sigma = 2.5$) J mole^{-1} K^{-1}

The specific heat of polyglycine I and II differs markedly and unexpectedly from that of poly-L-alanine in the α-helix and β-sheet forms. It is hoped that these results will encourage theoretical calculations.

INTRODUCTION

Homopolypeptides have long been considered as suitable systems for models of proteins [1]. Solid-state and polymer physics have now advanced to the stage where the lattice dynamics of molecules of many atoms per unit cell can be confidently treated theoretically. The specific heat essentially depends on the density of vibrational states, i.e. how the vibrational modes are distributed over different frequencies. An experimental advantage to measuring the specific heat, compared with methods such as inelastic neutron scattering, is that large single crystals are not required. In this laboratory, as part of a program to study the simpler homopolypeptides, we have previously determined the low-temperature (2—20 K) specific heats of poly-L-alanine in the α-helix and β-sheet forms [2] and of polyglycine II (3_1 helix) [3], using adiabatic calorimetry. It is of interest to do the measurements at higher temperatures, to compare with theoretical predictions [4] and also to provide data in the biological temperature range. Like many structural

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studies on proteins, e.g. most X-ray diffraction, this work is not done under aqueous conditions existing in the cell. Nevertheless this approach can offer insight into structural fluctuations and the behavior of real proteins and enzymes [5,6].

In this paper we extend our previous low-temperature specific heat measurements [3] on the simplest homopolypeptide polyglycine (PG) to the high-temperature range 150—375 K using differential scanning calorimetry. We compare our results with existing data on homopolypeptides, i.e. with a theoretical calculation, and with experimental results on poly-L-alanine.

EXPERIMENTAL

Sample preparation

Polyglycine (PG), or poly -(COCHRNH)- with R = H, is dimorphic in the solid state [1], existing as a β-sheet structure (PG I) or as 3_1 helices (PG II). To prepare PG I, 100 mg of PG (Sigma Chemical Company, St. Louis, MO, PO254, "molecular weight 5000—10 000 Daltons", Lot No. C0012C0100) was completely dissolved in 50—100 ml of freshly distilled dichloroacetic acid (95%, Fisher Scientific, Pittsburgh, PA) at about 30°C using a magnetic stirrer for 2—3 h. PG I precipitated when excess distilled water (200—300 ml) was added to the above [7]. The solution was then centrifuged at 21 000 × g for 20 min at 7°C in 25 ml aliquots, the supernatant removed and the PG I resuspended in more water. This washing procedure, with a dilution factor of about 25, was repeated 5—6 times. The samples were then dried by lyophilization at room temperature, resulting in a yield of 90 mg of white, fluffy PG I. All sample operations, including weighing, sealing and storing were performed in a dry nitrogen gas atmosphere. (Drying at 100°C at 1 atm of air gave a slightly yellow, lumpy powder.) If the samples were then heated in dry nitrogen to 110°C then the resulting difference in sample mass was less than 0.1%. Formic (91.5%, Fisher Scientific, Fairlawn, NJ), instead of dichloracetic, acid was also used as a solvent in an identical procedure to obtain PG I. The specific heat of PG I prepared by these two methods was the same within ±4%.

To prepare PG II, 100 mg of polyglycine (Sigma) was completely dissolved in 1 ml of a saturated solution (2.6 g in 1 ml water) of lithium bromide (anhydrous, 99+%, Alfa, Danvers, MA) at 35°C, and precipitated out by the addition of 100 ml excess water. (The initial eggshell-white cloudiness gradually thickens into a white precipitate.) After centrifugation (resistivity measurements of the final supernatant confirmed that the residual concentration of the LiBr was less than 1 ppm by weight) and drying, as for PG I, the yield was also 90 mg. Again samples dried in air atmosphere at 100°C were a yellowish lumpy powder. The PG II samples also lost less than 0.1% mass after heating to 110°C.

Only lyophilized samples of PG I and PG II were used for the quoted measurements of the specific heat. Effects of transitions were seen at temperatures above 130°C. Hence all our measurements were restricted to temperatures not exceeding 110°C.

Sample identification

Samples were prepared for IR analysis by mixing about 5 mg of PG with about 125 mg of KBr and compacting at some 10 MP (1 atm ~ 0.1 MP) for 5 min. Spectra were taken on a Beckman IR 457 and comparison of frequencies with those obtained by Small et al. [7] was made and a very good agreement obtained. The frequencies, f, which show that the samples are predominantly PG I are 1510, 1390, 1338, 1220, 1060, 1010, 905, 630, 614 cm^{-1} and predominantly PG II are 2995, 1545, 1425, and 1285 cm^{-1}. (Conservative errors for f are, for $f < 2500$, ±5 and for $2500 < f < 3000$, ±10 cm^{-1}).

Differential scanning calorimetry

The specific heat, C of a substance is the incremental heat $đQ$ supplied to unit mass of the substance to raise its temperature T by dT, i.e. $C = đQ/dT$. We have determined C using differential scanning calorimetry (DSC). Modern DSC instruments offer considerable advantages over adiabatic calorimeters in that small samples (down to mg quantities) can be measured very quickly, yet still with sufficient accuracy for much polymer work [8,9]. Our instrument (Mettler, model TA 2000B, Princeton, NJ) outputs an EMF proportional to the differential heat flow between the sample and a reference, while both are heated ("scanned") at a constant rate variable from 0.1 to 29.9 K min^{-1}, from 100 K to 400 K. The measuring sensor, located at the floor of the measuring cell, is a five-junction platinum—rhodium thermopile deposited on glass and is easily replaced when necessary; the aluminum sample and reference pans are positioned on the thermopile by centering pins at the bottom of the pans. The actual temperature of the cell is measured by a platinum resistance thermometer. A purge gas (dry nitrogen) continually flows past the sample.

The instrument is reproducible to ±2 mJ s^{-1} at 200 K, and ±4 mJ s^{-1} at 400 K with an 85 mg sample of powdered alumina (Mettler); and to ±4 mJ s^{-1} at 200 K, and ±8 mJ s^{-1} at 400 K with a 13 mg sample of PG I. If a sample is left in place, then scans will repeat to ±1 mJ s^{-1}, and the specific heat of 85 mg of alumina will repeat to ±4 mJ K^{-1} mg^{-1}.

"Baseline" scans, with no pans in sample or reference positions, are made every 5—10 scans, and at least once weekly, to check the stability etc. of the apparatus. The variation of baseline with time would contribute less than ±2% error to the quoted specific heat results.

The thermoelectric sensitivity, S (μV K^{-1}), the indium calibration constant E_T[μV (mJ s^{-1})$^{-1}$] and the instrument relaxation time are determined from thermograms of the melting of indium (Mettler). The sample and reference pans are of mass approximately 40 mg and within 0.05 mg mass of each other, and weighed on an electrobalance (model Gram, Cahn, Cerritos, CA) to ±0.02 mg. First a "reference" scan is made, i.e. a run with covered and sealed pans (both empty) in the sample and reference positions, respectively. Powdered alumina (Mettler), loaded into a pan identical in mass (±0.02 mg) and sealed in a dry nitrogen atmosphere, is then placed in the sample posi-

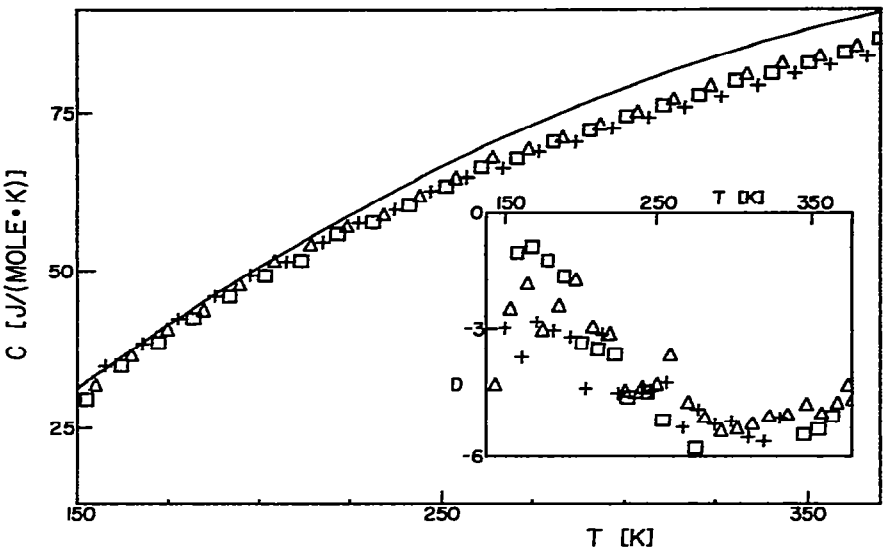

Fig. 1. Specific heat of powdered alumina (85 mg) vs. temperature (+). The insert shows the deviation $D = (C - C')/(C'$ vs. temperature, where C is the measured specific heat and C' is given by a fit to published data [10] vs. T (continuous curve). The scan rates (K min^{-1}) are +, 10; Δ, 15; and □, 20. The standard deviation would be shown as a vertical bar less than one of the symbols in height.

tion, and a "sample" scan is made. Empty and sample scans are always made alternately. At a given temperature, T, the difference $\Delta V(\mu V)$ between the recordings for the reference and sample runs gives the specific heat, $C = \Delta V/[m(dT/dt)_p E_T]$, where m is the sample mass, $(dT/dt)_p$ is the programmed heating rate, and E_T is the calibration constant (evaluated from the indium

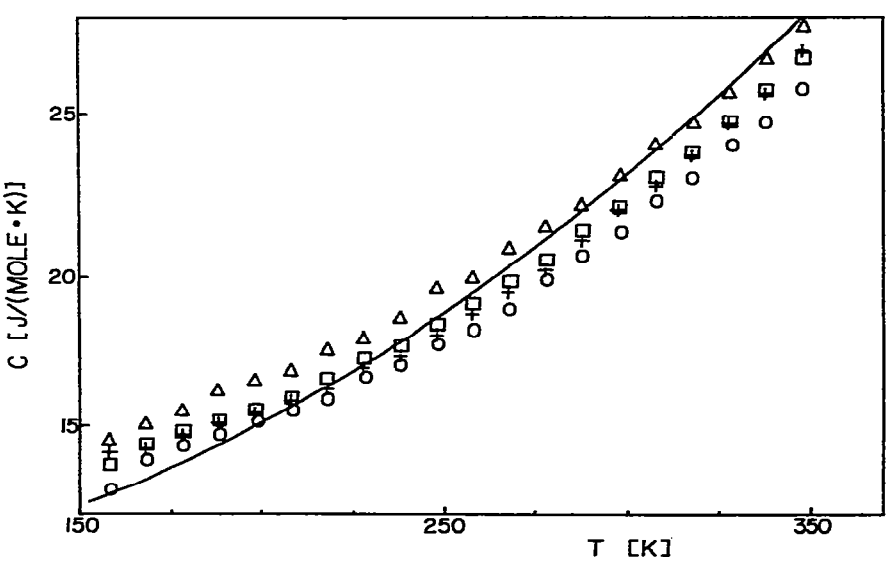

Fig. 2. Specific heat of polyethylene (pressure crystallized, high density [11]) (27 mg) vs. temperature. The continuous curve is from smoothed published data [11]. Four runs are shown (see Procedure). The standard deviation would be shown as a vertical bar less than one of the symbols in height.

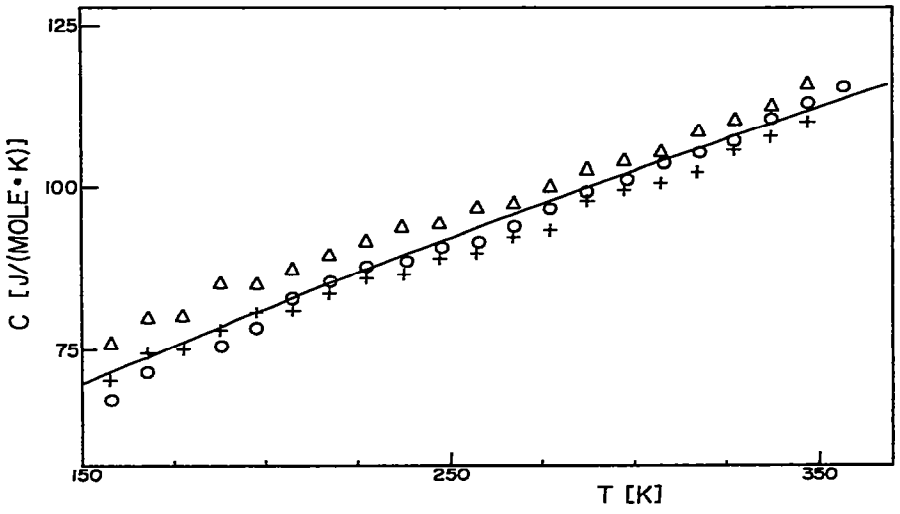

Fig. 3. Specific heat of polyglycine I (13 mg) vs. temperature. For clarity of illustration, three runs are shown (see Procedure) which cover the maximum variation shown in all our runs; the samples were prepared with dichloroacetic acid. The continuous curve represents a fit to seven runs, of $C = 37.744 + 0.218T - 2.333 \times 10^{-6}\ T^2$ ($\sigma = 3.3$) J mole^{-1} K^{-1}. The standard deviation would be shown as a vertical bar less than one of the symbols in height.

calibration constant and the thermometric sensitivity at T).

Specific heat values for 85 mg of powdered alumina (Mettler) then agree with recommended values [10] from adiabatic calorimetry to within 6%. The deviation, as shown in Fig. 1, is smooth and consistent. Specific heat values for 85 mg of sapphire (Du Pont specific heat standard) similarly agree to within 2%. The variation of measured specific heat with mass for alumina (Mettler) is less than ±1% from 65 mg to 85 mg. The specific heat measurements repeat to 3% on 85 mg of alumina, to 5% on 27 mg of polyethylene (PE) [pressure crystallized, high density; National Bureau of Standards, Washington DC, SRM 1475 (ref. 11, p. 132)] (Fig. 2), and to ±8% on 7 mg of PG I (Fig. 3).

Sample thermal diffusivity

PG may be expected to have a more similar thermal diffusivity to PE than to alumina: specific heat measurements on 27 mg of PE (SRM 1475) agreed with published data [11] to within ±5%. This reassures us that the DSC measurements are close to adiabatic calorimetry measurements on specimens that have poor thermal diffusivity.

PROCEDURE

Our procedure is to (i) measure the specific heat of a sample based on the thermoelectric sensitivity, S, and the indium calibration constant E_T at a scan rate of 10 K min^{-1}; (ii) then apply a correction (of less than 5%) based on the mean of the deviations of our measured specific heat runs of PE from

that of Chang [11]. The specific heat, C, was then given by $[C - C(\text{measured})]/C(\text{measured}) = 0.4713 - 0.00303T + 4.56 \times 10^{-6}\ T^2$ (Fig. 2). The procedure of Naas and Mraw [12], which includes the idea of having a known calibration sample of thermal properties as close as possible to that of the unknown, would be approached more closely if powdered PE were used. Their procedure of alternately measuring the calibration and unknown samples presents advantages.

In general, the polymers (PG, PE) exhibited greater scatter (±8%, ±5%) in the specific heat data than metals (AG: 20 mg; Au: 40 mg; β-brass: 30 mg) (±2%) and sapphire (±2%). The scatter could therefore easily be attributed to the smaller diffusivity of the polymer samples. The slightly better consistency (±5%) obtained with polycrystalline PE samples also could be due to their crystalline nature whereas our experimental PG samples were amorphous powders. Also, the PG powders were so fluffy that reasonable pellets could not be obtained even by compressing the powder up to 1 MP, and so only relatively small (13—15 mg) samples of powder could be packed into the sample pans, which are 40 μl in capacity. Another technique to increase the thermal diffusivity of a polymer is to mix a known amount of the polymer with known amount of fine copper powder and to compress the mixture [13]. Although found to be extremely useful at very low temperatures, this technique was of little help in the present measurements. The measured specific heat is independent (±2%) of the heating rate (10, 15, 20 K min^{-1}), is independent (±4%) of the two ways in which the PG I samples are prepared, and is also independent (±2%) of the mass (5.9, 7.3 mg) of the sample.

RESULTS AND DISCUSSION

The specific heat of the simplest synthetic homopolypeptide, polyglycine (PG), has been determined by differential scanning calorimetry in the range 150—375 K. Previous measurements [3] were below 20 K. Commercial PG was prepared as PG I (β-sheet) and PG II (3_1 helix), as described under "Sample preparation".

The results for PG I and PG II (molar mass: 57.05) are shown in Figs. 3 and 4, respectively, and are typical polymers. Polyethylene (PE) is shown in Fig. 2. The specific heat difference between PG I and PG II (Fig. 5) is outside experimental uncertainties.

A non-parametric test (Wilcoxon's [14] signed rank sum on paired data) was applied to pairs of PG I and PG II data at the same temperatures. That the equations given in the captions to Figs. 3 and 4 are indeed different is significant at better than the 0.05% level. (Even for the worst imaginable case of the lowest PG I results being compared with the highest PG I results, the Wilcoxon test shows that the difference is significant at the 1% level.) An application of the conventional normal distribution shows that the results for PG I and PG II are different at better than 0.3% significance. That systematic errors are made small is shown by the above-mentioned comparisons of our DSC measurements with adiabatic calorimetry measurements on identical samples.

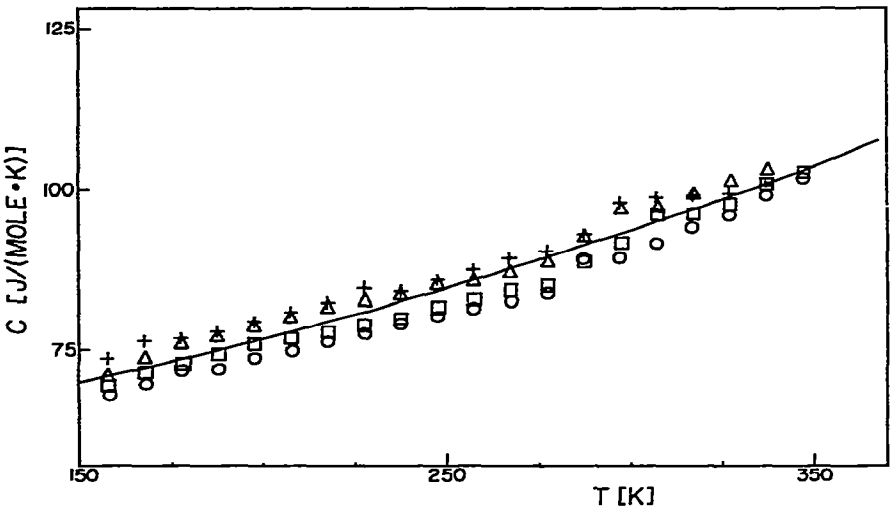

Fig. 4. Specific heat of polyglycine II (10 mg) vs. temperature. For clarity of illustration, four runs are shown which cover the maximum variation shown in all our runs (see Procedure). The continuous curve represents a fit to five runs, of $C = 57.598 + 0.05T + 2.357 \times 10^{-4}\ T^2$ ($\sigma = 2.5$) J mole^{-1} K^{-1}. The standard deviation would be shown as a vertical bar less than one of the symbols in height.

The general polypeptide is poly -(CO CHR NH)-; if R = H the polypeptide is PG. If R = CH_3 (methyl group) then the polypeptide is polyalanine, which can exist as optical isomers L and D. The specific heat of poly-L-alanine (PLA) has been measured [15] by adiabatic calorimetry 1—250 K in the α-helix and β-sheet structures, and is also shown in Fig. 5. For both PG and PLA the β-sheet conformation ("two-dimensional") has a higher specific heat than has the helical conformation ("one-dimensional"). The specific heat of

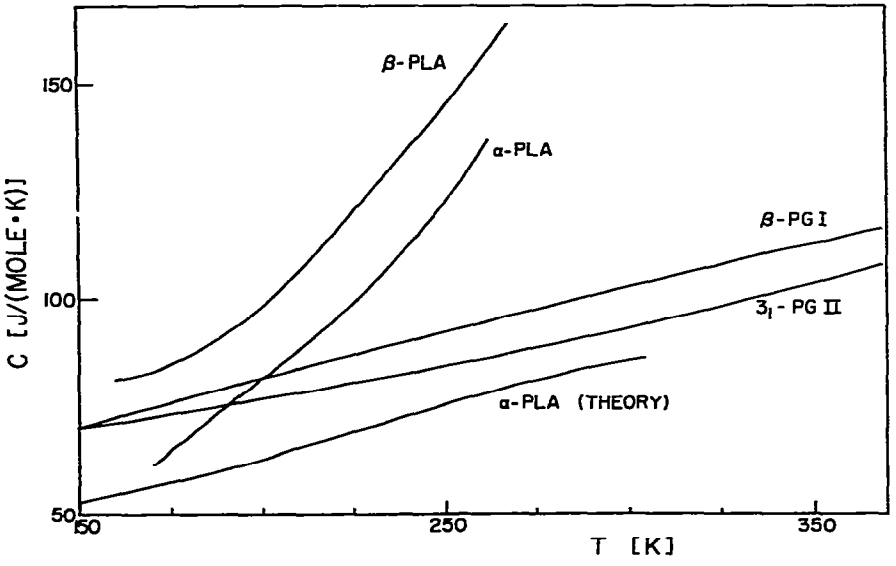

Fig. 5. Specific heat of polyglycine I and II (experiment, this work) of α- and β-poly-L-alanine (experiment [15]) and α-poly-L-alanine (theory [18]).

PLA rises with increasing temperatures much faster than that for PG. From a survey of many linear high polymers [16], the empirical specific heat contribution for CH_2 group which is part of a methyl group is expected to be 19.3 J mole^{-1} K^{-1} (ref. 16: Table 3, III) at 240 K. But a comparison of β-PLA with β-PG (PG I) shows a specific heat difference of some 46 J mole^{-1} K^{-1} (Fig. 5). However, since the additive concept is strictly applicable only to polymers of similar crystal structure [17], this comparison remains speculative.

The samples of PLA [15] were presumably dried. In preliminary measurements on undried PG (as received) we saw specific heat peaks over the complete temperature range 280—400 K and peaking at around 340 K. These peaks were not present in lyophilized samples kept under dry nitrogen gas. If attributed to water, the peaks corresponded to 0.6 wt.% of water.

Figure 5 also shows early theoretical results for α-PLA [18], which appear to be closer to the PG results. Recent refined vibrational analyses of polypeptides are now available, from Krimm and co-workers [19], and it should be a relatively straightforward task to generate the specific heat of PG and PLA.

CONCLUSIONS

A comparison of the specific heat measurements reported here on PG I and PG II with previous measurements [15] on α- and β-PLA and theoretical results on α-PLA [18] show that:

(1) the temperature range 150—375 K exhibits interesting variation;

(2) our theoretical colleagues should be encouraged to refine specific heat calculations.

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