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LOW-TEMPERATURE THERMODYNAMIC PROPERTIES OF *L*-CYSTEINE

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Heat capacity $C_p(T)$ of the orthorhombic polymorph of *L*-cysteine was measured in the temperature range 6–300 K by adiabatic calorimetry; thermodynamic functions were calculated based on these measurements. At 298.15 K the values of heat capacity, C_p; entropy, $S^0_m(T)–S^0_m(0)$; difference in the enthalpy, $H^0_m(T)–H^0_m(0)$, are equal, respectively, to 144.6±0.3 J K^{-1} mol^{-1}, 169.0±0.4 J K^{-1} mol^{-1} and 24960±50 J mol^{-1}. An anomaly of heat capacity near 70 K was registered as a small, 3–5% height, diffuse 'jump' accompanied by the substantial increase in the thermal relaxation time. The shape of the anomaly is sensitive to thermal pre-history of the sample.

Keywords: *amino acid crystal, heat capacity, low temperatures, order–disorder, phase transitions*

Introduction

Adiabatic calorimetry studies of crystalline amino acids provide important information on the intra- and intermolecular dynamics of these systems. They often help to detect dynamic transitions and phase transitions in these systems and to understand their nature. Early calorimetric studies did not reveal phase transitions in the crystalline amino acids at temperatures below or close to ambient, *L*-methionine (with a phase transition near 300 K) being considered as a rare exception [1]. More recently, the occurrence of low-temperature phase transitions in taurine [2] and in β-glycine [3] was confirmed by calorimetry. Several other crystalline amino acids were studied recently by adiabatic calorimetry in a wide temperature range, and, although no phase transitions were detected, the thermodynamic parameters could be calculated and compared for different systems, among which were different polymorphs of the same compound (α- [4], β- [3] and γ- [4] glycine), or chiral *vs*. racemic forms (*D*- and *DL*-serine [5]). The present contribution continues this series of studies and reports on the results of the adiabatic calorimetry measurements of the orthorhombic polymorph of *L*-cysteine, $C_3H_7NO_2S$, $^+NH_3–CH(CH_2SH)–COO^-$ (Fig. 1), a structural analogue of *L*-serine with heavier side chains, forming much weaker intermolecular SH…S and SH…O bonds, as compared to those OH…O in *L*-serine (Fig. 2) [6–8].

L-cysteine plays an important role in the biological systems. Cysteine thiol (also called sulfhydryl) –S–H groups are the most chemically reactive sites in proteins at physiological conditions [9]. The cysteine sulfhydryl may function as either a hydrogen bond donor (e.g. S–H…O) or acceptor (e.g. H…S–H) group. Sulfhydryl hydrogen bonding in proteins is not well understood, primarily because such interactions are difficult to detect experimentally due to their weakness.

The properties of the hydrogen bonded thiol-groups can be mimicked using *L*-cysteine in the crystalline state, which can exist as the orthorhombic [6–8] and the

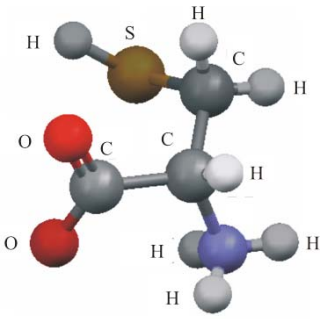

Fig. 1 A molecule of *L*-cysteine

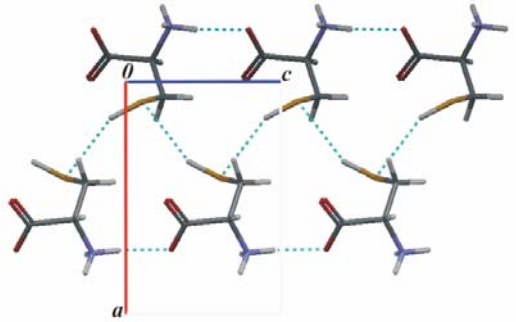

Fig. 2 A fragment of the crystal structure of the orthorhombic *L*-cysteine at 30 K [8]. Hydrogen bonds are shown by dashed lines

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monoclinic polymorphs [10, 11]. At ambient temperature, as evidenced by X-ray [6] and neutron [7] diffraction, the sulfur atoms of the thiol-groups in the orthorhombic form are disordered over two positions. The distances between the oxygen and the sulfur atoms of the neighboring molecules in the structure are consistent with the hypothesis on the formation of the two types of intermolecular hydrogen bonds, S–H...S and S–H...O [6, 7]. A recent structural study of the orthorhombic *L*-cysteine at 30 K has shown the sulfur atoms to be completely ordered and located at those positions, which correspond to the formation of the S–H...S hydrogen bonds [8]. Therefore, a disorder–order transition should occur at some points on cooling and manifest itself in the temperature dependence of heat capacity.

The data on the heat capacity measurements for *L*-cysteine in a rather wide temperature range, 85–298 K, were reported by Huffman and Ellis in 1935 [12]. No anomalies were observed. The aim of the present communication, therefore, was to extend the temperature range for measuring the heat capacity down to lower temperatures, in order to find the point of a phase transition. Going to lower temperatures was important also for calculating the values of the thermodynamic functions: since the lowest temperature in the temperature range in the study [12] was rather high, the values of the thermodynamic functions calculated from these data are not very reliable. Part of the results (related to the phase transition) was described briefly in a preliminary communication [13].

Experimental

Sample

A sample of *L*-cysteine, $C_3H_7NO_2S$, molecular mass 121.16 g mol^{-1} from Fluka, BioChemica (99.5% chemical purity) was used as purchased. According to the data of X-ray powder diffraction analysis (Bruker GADDS), only a pure orthorhombic polymorph of *L*-cysteine was present in the sample.

Methods

The low-temperature heat capacity was measured using an automatic vacuum adiabatic calorimeter as described elsewhere [14, 15]. The reliability of the measurements was confirmed by test measurements of the heat capacity of benzoic acid as a calibrant in the temperature range 6–300 K. The accuracy of the measurements was equal to 2% at 6 K, 0.3% at 15–40 K and 0.1% at 40–300 K. A 3.9597 g sample of *L*-cysteine was put in a copper calorimeter (20.4 g, 5.7 cm^3). The heat capacity of *L*-cysteine was measured in 90 experiments in the temperature range 5.7–304.8 K. After the sample was loaded, the calorimeter was filled with gaseous helium at the pressure $1\cdot10^4$ Pa, to improve the heat exchange. A correction for the excess heat capacity of helium in a filled calorimeter as compared to the calibration measurement with empty ampoule was introduced and turned out to be significant (~2%) only at very low temperatures, decreasing rapidly with increasing temperature (0.1% at 15 K).

Heat capacity of *L*-cysteine was measured in pulse and in continuous modes. The pulse technique is the basic method for getting precise values of regular heat capacity of compounds (in the temperature range not characterized by any anomalies). During a measurement in pulse technique, a fixed amount of heat is input into the calorimeter, the temperature of the calorimeter changes from temperature T_1 to T_2 and the measured value of the heat capacity is the average in the temperature range $\Delta T=T_2-T_1$. The value of ΔT, depending on the temperature of the measurement, ranges usually from 1 to 10 K. After heating, the system is let to equilibrate until the temperatures of the sample and of the calorimeter become equal. It is very important that the new temperature T_2 really corresponds to an equilibrium, otherwise the results of the calculations of average heat capacity can be erroneous.

The continuous heating measurements are commonly used for a more detailed analysis of the $C_p(T)$ functions, especially in the regions of the anomalies [16, 17]. The continuous heating mode allows one to achieve a much smaller temperature interval between the experimental points (down to 0.01 K, as compared to 1–10 K in the pulse mode), thus increasing the sensitivity of the measurements to any anomalies in the $C_p(T)$ function. Heat capacity can be calculated from the power input and from time dependence of temperature for as small ΔT values as desirable and the limit of the calculated values of C_p with $\Delta T\rightarrow0$ approaches the value of the real heat capacity dH/dT.

Results and discussion

Experimental data on the heat capacity of the orthorhombic *L*-cysteine measured by pulse technique are plotted in Fig. 3. The primary experimental data are given in Table 1, the smoothed values of heat capacity and the thermodynamic functions calculated from these values – entropy and enthalpy – are summarized in Table 2. The extrapolation of $C_p(T)$ below 5.7 K to zero temperature was made using $C_p\sim T^3$ function. The inaccuracies of standard values of the thermodynamic functions at 298.15 K shown in the Table 2 were estimated taking into account the dispersion of the experimental data, the results of the cali-

Table 1 Experimental values of heat capacity C_p^0 of *L*-cysteine (J K^{-1} mol^{-1})

T/K	C_p^0	*T*/K	C_p^0	*T*/K	C_p^0	*T*/K	C_p^0
Series 1		248.49	126.9	7.23	0.3088	70.30	52.99
296.01	143.7	255.95	129.4	8.90	0.6442	76.26	58.24
299.04	144.8	260.86	131.5	10.47	1.131	82.25	61.71
302.02	145.9	265.74	133.5	12.06	1.804	Series 6	
Series 2		270.58	134.8	13.60	2.636	67.01	49.16
83.93	62.66	275.45	136.2	15.17	3.630	71.02	54.01
88.98	65.31	280.36	138.0	16.73	4.771	75.02	57.43
94.02	67.83	285.23	139.8	18.31	6.017	79.01	59.91
98.99	70.21	290.14	141.6	19.87	7.370	Series 7	
104.90	72.84	295.07	143.5	21.75	9.098	61.27	44.65
111.86	75.82	299.97	145.3	23.85	11.12	64.29	46.96
118.88	78.75	304.83	147.2	25.94	13.17	78.90	59.86
125.88	81.59	Series 3		28.04	15.20	81.91	61.56
132.90	84.36	6.09	0.1724	30.10	17.33	Series 8	
139.86	87.00	7.68	0.3781	32.14	19.40	67.69	49.59
148.33	90.22	9.46	0.7965	34.98	22.25	68.72	50.64
158.31	93.94	11.01	1.333	38.53	25.84	69.76	52.17
168.30	97.59	12.61	2.096	42.08	29.04	70.78	54.23
178.25	101.2	14.18	2.992	46.90	32.92	71.79	55.00
188.19	104.8	15.74	4.043	49.99	35.76	72.82	55.83
198.15	108.3	17.29	5.216	54.52	39.35	73.83	56.67
208.12	111.9	18.83	6.445	60.18	43.86	74.82	57.28
218.11	115.4	20.38	7.813	Series 5		75.80	57.95
228.07	119.1	Series 4		60.32	43.78		
238.49	122.9	5.66	0.1341	64.33	46.86		

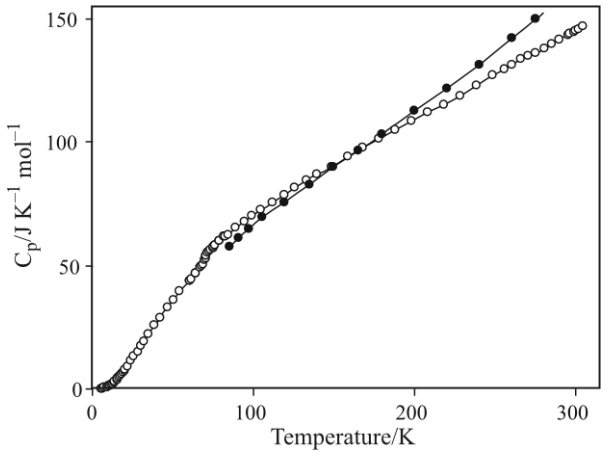

Fig. 3 Heat capacity, C_p, of the orthorhombic *L*-cysteine *vs*. temperature ○ – measured in pulse mode in a wide temperature range; for a comparison, ● – the data from [12] are also plotted

bration measurements of benzoic acid and the chemical purity of the sample.

An anomaly in the $C_p(T)$ curve was observed in the temperature range 67–75 K as a small, ~3–4% height

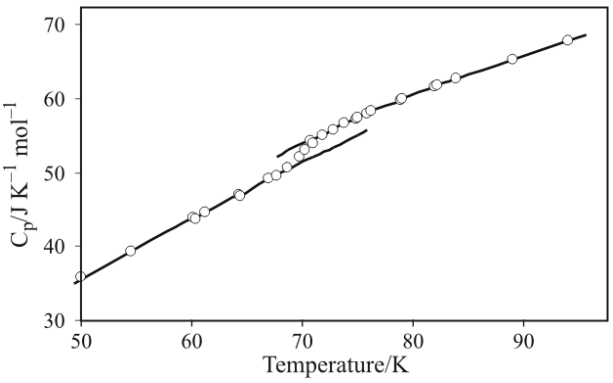

Fig. 4 An anomaly in the $C_p(T)$ of *L*-cysteine near 70 K

diffuse 'jump' (Fig. 4). When measuring heat capacity of *L*-cysteine by pulse technique, at temperatures close to 71 K we have observed an unusually long time of thermal equilibration after heat was input into the calorimeter. The way, how the sample temperature changed at the initial stage of heating also indicated at a slow relaxation of the system.

Table 2 Heat capacity and thermodynamic functions of orthorhombic *L*-cysteine

T/K	$C_p^0(T)$/ J K^{-1} mol^{-1}	$S_m^0(T)-S_m^0(0)$/ J K^{-1} mol^{-1}	$H_m^0(T)-H_m^0(0)$/ J mol^{-1}
5.66	0.1342	0.0447	0.1901
6	0.1630	0.0533	0.2397
10	0.9659	0.2830	2.170
15	3.521	1.099	12.67
20	7.479	2.629	39.72
25	12.23	4.800	88.82
30	17.22	7.468	162.4
35	22.29	10.50	261.2
40	27.12	13.80	384.9
45	31.50	17.25	531.6
50	35.67	20.79	699.5
60	43.64	28.00	1097
70	52.64	35.33	1573
80	60.48	42.95	2145
90	65.84	50.39	2777
100	70.66	57.58	3460
120	79.22	71.24	4960
140	87.07	84.04	6624
160	94.56	96.16	8441
180	101.8	107.7	10405
200	109.0	118.8	12510
220	116.1	129.5	14760
240	123.5	140.0	17160
260	131.1	150.2	19710
280	138.0	160.1	22400
298.15	144.6±0.3	169.0±0.4	24960±50
300	145.3	169.9	25230
304.83	147.1	172.2	25940

Additional measurements were carried out in the temperature range 68–76 K (close to the temperature of the anomaly) by continuous heating technique (curves 1–3). The occurrence of the anomaly was confirmed and the size of the effect was higher during continuous heating, than when measured on pulse heating (Fig. 5). Curve 1 was carried out using liquid nitrogen at reduced pressure as a cooling agent. Before starting continuous heating, the calorimeter was cooled down to ~60 K. Curve 2 was obtained using liquid helium as a cooling agent: the calorimeter was cooled down to 4 K and then heated up to 68 K, after what the detailed measurements started. A very unusual observation is that although curves 1 and 2 were measured at the same heating rate, the anomaly near 70 K was much more pronounced, when cooling with liquid helium. A reason can be sought in the different temperature of cooling prior to heating start (4 K for curve 2 and 60 K for curve 1), which has resulted in a different completeness of the transformation into the low-temperature phase. A test curve (3) made in the temperature range outside the anomaly region gave exactly the same C_p values as obtained on pulse heating (Fig. 3).

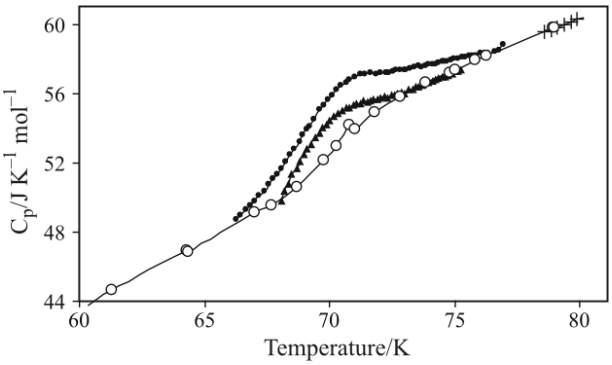

Fig. 5 Heat capacity, C_p , *vs*. temperature measured in more details in the region of an anomaly: ○ – measured in pulse mode, ▲ – a continuous mode, cooling with solid nitrogen, ● – a continuous mode, cooling with liquid helium; crosses + – a test measurement outside the anomaly region

We have compared our results of measuring the heat capacity of *L*-cysteine with data reported in 1935 by Huffman and Ellis [12] (Fig. 1). The striking discrepancy is clearly seen, especially at temperatures close to room temperature, when the difference reaches 12%. The curves $C_p(T)$ measured in our experiments and reported in [12] cross each other at 158 K; at higher temperatures the values reported in [12] are higher and at lower temperatures – lower, than obtained in our experiments. At 85 K the difference in the heat capacity values is about ~8%. In our opinion, the reason of such a discrepancy should be sought in different composition of the samples. It is difficult to interpret the data from [12] reliably, since the characteristics of the sample (chemical and phase composition) were not reported. We can suppose, that either monoclinic *L*-cysteine, or *DL*-cysteine, or both could be present in the sample as impurities in considerable amounts. To test this hypothesis, measurements of the heat capacity of *DL*-cysteine and of the monoclinic form of *L*-cysteine are required.

$C_p(T)$ dependence measured for the orthorhombic *L*-cysteine was compared with those previously reported for *L*-serine [18] and *D*-serine [5]. The heat capacity of the orthorhombic *L*-cysteine is higher, than those of *L*- and *D*-serine, in accordance with a higher molecular mass of *L*-cysteine. The curves were brought to the same scale taking into account the difference in

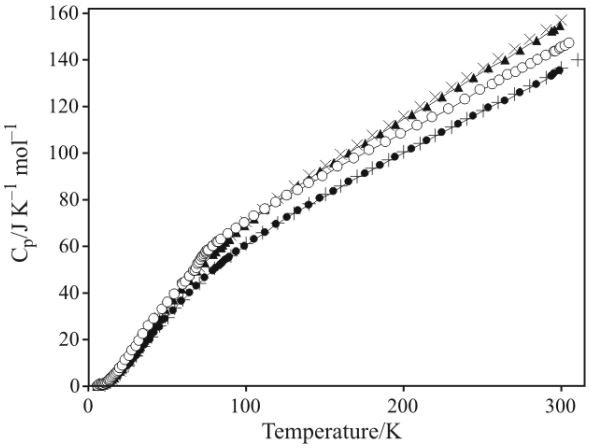

Fig. 6 Comparison of the $C_p(T)$ functions for *L*-cysteine, *L*- and *D*-serine: ○ – data for *L*-cysteine (this study), + – data for *L*-serine [18], ● – data for *D*-serine [5], x – data for *L*-serine scaled to the molecular mass of *L*-cysteine, ▲ – data for *D*-serine scaled to the molecular mass of *L*-cysteine

the molecular masses – the C_p values for serines were divided by the molar mass of serine and multiplied by the molar mass of cysteine. After that, the curves $C_p(T)$ for *L*-serine and orthorhombic *L*-cysteine look similar in the low-temperature range, the Debye temperature being lower for *L*-cysteine, (i.e. the lattice modes in *L*-cysteine correspond to lower frequencies, as should be expected from a larger molecular mass). The obvious discrepancy of the curves in the phase transition region is natural. With increasing temperature, the scaled C_p values for *L*- and *D*-serine deviate significantly from $C_p(T)$ of orthorhombic *L*-cysteine, becoming higher as compared to the latter (Fig. 6). This may seem strange, since the SH…O and SH…S hydrogen bonds of the $-CH_2SH$ side groups in *L*-cysteine are weaker than the OH…O hydrogen bonds of the side $-CH_2OH$ groups in *L*-serine, as follows from the X-ray diffraction and vibrational spectroscopy data [19]. Some higher-frequency modes seem to be activated in *L*-serine as compared to *L*-cysteine at temperatures $T>\sim150$ K. Further studies, possibly using Raman spectroscopy, are required, to interpret this fact, taking into account all types of interactions in the crystals of *L*-cysteine and *L*-serine.

The anomaly observed at about 70 K can be interpreted as a manifestation of a dynamic transition, related to the ordering of the thiol-groups on cooling [13]. The ordering of the thiol groups due to the formation of the S–H…S hydrogen bonds was observed earlier by single-crystal X-ray diffraction at 30 K [8]. It can play an important role also in the pressure-induced phase transitions in *L*-cysteine at ambient temperature, although at 0.5 GPa the thiol groups still remain disordered [20]. It is remarkable, that the pressure-induced transitions show pronounced kinetic effects in the changes in the orientation of the thiol groups: an intermediate high-pressure phase was described as a structure with alternating thin domains with different orientations of thiol groups typical for another high-pressure phase and the ambient-pressure phase. The $C_p(T)$ dependence obtained for *L*-cysteine near 70 K is characteristic not for a sharp disorder–order transition, but for a transformation extended over a wide temperature range and depending on the thermal pre-history of the sample, that can be interpreted as non-simultaneous changes in the dynamics and the orientation of the numerous thiol groups in the structure. The measurements of the cell parameters and cell volume (STOE STADI-4, Oxford Cryostreams) on cooling down to 100 K (the lowest temperature accessible) suggest that a discontinuity in their values over the transition point exists: the points reported for 30 K [8] obviously deviate from the extrapolated curves [13]. Further diffraction and NMR studies in the temperature range of the heat capacity anomaly will give a better insight in the nature of the observed anomaly.

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