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LOW-TEMPERATURE HEAT CAPACITY OF DIGLYCYLGLYCINE
Some summaries and forecasts for the heat capacity of amino acids and peptides

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Heat capacity of tripeptide diglycylglycine was measured in a temperature range from 6.5 to 304 K. The results were compared with those for glycine and glycylglycine. Peptide bonding was found not to change $C_P(T)$ virtually above 70 K, where heat capacity does not obey the Debye model.

Comparison with literature data allows one to expect a significant difference in the heat capacity for enantiomorph and racemic species of valine and leucine, like it was found recently for *D*- and *DL*-serine.

Keywords: *adiabatic calorimetry, diglycylglycine, glycines, heat capacity, molecular crystals, peptide bond*

Introduction

Diglycylglycine, $C_6H_{11}N_3O_4$, is an amino acid with two peptide bonds. Glycine, glycylglycine, and diglycylglycine together form a series of molecular crystals with very similar intermolecular bonding and different molecules. Heat capacity of diglycylglycine was measured near the room temperature by using DSC and found to be of 216 J mol^{-1} K^{-1} at 298 K [1].

Recently we have investigated three polymorphs of glycine (α [2], β [3] and γ [2]), in order to define the difference in their heat capacities caused by the difference in their crystal structure formed by the same molecules.

Heat capacity of glycylglycine [4] was found to be very close to that of glycine polymorphs. The difference was the greatest at very low temperatures, where C_P of these compounds obeys the Debye model, with the whole molecule acting as an oscillator. It is very interesting to test crystalline diglycylglycine with the enlarger molecule as compared with glycine and glycylglycine.

The objective of this work was to measure low-temperature heat capacity of diglycylglycine and compare the results with the heat capacity of glycines and glycylglycine.

Experimental

Diglycylglycine of ICN Biomedicals Inc. (189.17 g mol^{-1}) was used as purchased. Its X-ray powder diffraction pattern is identical to that of diglycylglycine calculated after single-crystal X-ray diffraction structure solution [5]. The unit cell is triclinic (s.g. P-1) with a=1.1673(14), b=1.485(3), c=0.4816(11) nm, α=88.47(13), β=96.02(12) and γ= 105.40(10)°. The unit cell parameters were refined using software TOPAS after X-ray powder diffraction measurements on diffractometer D8-GADDS (Bruker).

Low-temperature calorimetric measurements were carried out using vacuum adiabatic calorimetric system working over the temperature range of 4.2–320 K, described elsewhere [2]. Sample mass in the calorimeter of 6 cm^3 was 3.86822 g. The calorimeter was evacuated at ambient temperature and filled with helium at a pressure of 30 mm Hg to improve the heat exchange inside the calorimeter.

Results and discussion

Low-temperature heat capacity of diglycylglycine was measured at 79 points over the temperature range of 6.5 to 304 K. Measured values are listed in Table 1 in time order. The standard deviation of the experimental points from the smoothed curve is 1.3% for T<10 K, 0.16% for 10<T<50 K and 0.03% for T>50 K. At very low temperatures (below 10 K) the heat capacity obeys the Debye model $C_P=aT^3$ with a=1.62 mJ mol^{-1} K^{-4}. The fitting of the experimental values to the cubic function is shown in Fig. 1. Heat capacity of diglycylglycine is a smooth function of temperature, without evident anomaly or phase transition. Smoothed values of heat capacity and derived enthalpy and entropy are listed in Table 2.

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Table 1 Experimental heat capacity of diglycylglycine, 189.17 g mol^{-1}

T/K	C_p/J mol^{-1} K^{-1}	T/K	C_p/J mol^{-1} K^{-1}	T/K	C_p/J mol^{-1} K^{-1}
Series 1		144.715	133.64	32.399	28.39
297.287	228.64	153.124	139.05	35.241	32.59
300.214	230.51	163.000	145.35	38.806	37.72
303.130	232.42	172.835	151.53	42.343	42.52
Series 2		182.648	157.63	45.902	47.31
174.824	152.79	Series 4		50.236	52.81
184.736	158.94	10.652	2.019	55.288	58.97
194.659	165.04	11.700	2.696	60.306	64.80
204.617	171.22	12.755	3.481	65.317	70.34
214.567	177.29	13.812	4.331	70.330	75.55
224.531	183.47	14.891	5.298	75.331	80.37
234.450	189.54	15.968	6.334	80.310	85.17
244.332	195.64	17.296	7.756	Series 7	
254.184	201.70	18.877	9.567	6.906	0.530
263.941	207.74	20.484	11.54	7.956	0.815
273.743	213.86	Series 5		9.047	1.218
283.654	219.99	8.006	0.8329	Series 8	
293.607	226.30	9.453	1.410	7.319	0.620
303.545	232.64	10.477	1.937	8.265	0.915
Series 3		11.527	2.585	9.383	1.366
83.692	88.17	12.580	3.341	Series 9	
88.715	92.57	Series 6		15.461	5.84
93.722	96.82	7.252	0.635	16.545	6.94
98.743	100.95	8.479	0.997	17.605	8.10
103.837	104.89	9.508	1.438	18.658	9.30
109.874	109.46	19.852	10.79	19.710	10.60
116.814	114.54	21.988	13.53	20.754	11.90
123.776	119.51	24.070	16.36	28.144	22.09
130.784	124.31	26.136	19.24		
137.758	129.01	30.311	25.33		

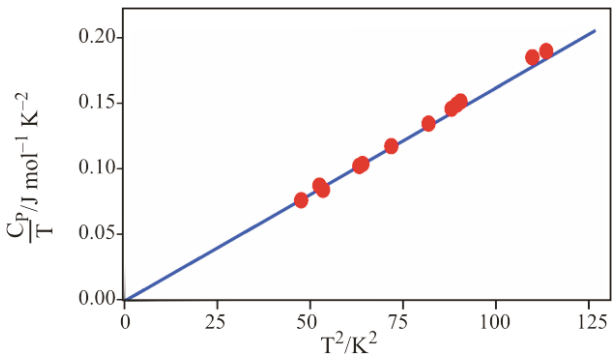

Fig. 1 Low-temperature heat capacity of diglycylglycine: experimental data (filled circles) and fitting cubic function $C_p=aT^3$ (solid line)

Comparison with glycine and glycylglycine

Lattice molar heat capacity of diglycylglycine is greater than that of glycylglycine because it contains the greater number of oscillators (atoms) in its mole. Similarly, heat capacity of glycylglycine is greater than that of glycine (Fig. 2a). When normalized to the number of atoms (average gram-atom=mole/the number of atoms in the molecule), heat capacities of three substances turn out to be very close to each other, especially above 70 K (Fig. 2b). The greatest difference is at low temperatures, when the heat capacity obeys the Debye model with the whole molecule acting as an oscillator. It was discussed in the previous report with the heat capacity of α-glycylglycine [4]. The ratio of molecule masses for the pair of glyglygly-glygly is less than that for glygly-gly, and the differ-

Table 2 Smoothed heat capacity and derived thermodynamic functions for diglycylglycine, 189.17 g mol^{-1}

T/K	C_P/ J mol^{-1} K^{-1}	$H(T)$–$H(0)$/ J mol^{-1}	S/ J mol^{-1} K^{-1}
(5)	(0.202)	(0.253)	(0.067)
10	1.677	4.096	0.545
15	5.397	20.91	1.858
20	10.95	61.12	4.133
25	17.63	132.3	7.281
30	24.86	238.4	11.13
35	32.20	381.1	15.51
40	39.34	560.1	20.28
45	46.11	773.8	25.31
50	52.54	1021	30.51
60	64.46	1607	41.16
70	75.18	2306	51.91
80	84.83	3107	62.59
90	93.69	4000	73.10
100	101.89	4978	83.40
110	109.57	6036	93.48
120	116.83	7168	103.33
130	123.79	8372	112.95
140	130.51	9643	122.38
150	137.04	10981	131.60
160	143.45	12384	140.65
180	156.00	15379	158.28
200	168.35	18622	175.35
220	180.66	22112	191.98
240	192.96	25849	208.22
260	205.30	29831	224.15
280	217.72	34061	239.82
298.15	229.19	38117	253.85
300	230.38	38542	255.27

ence in the Debye temperatures is less for the first pair as compared to the second pair. By this reason, heat capacities differ from one another less in the first pair than in the second one. One can deduce that the heat capacity of a polypeptide tends to an asymptotic function of $C_P(T)$. Detail analysis of this point is beyond the scope of this report. Here we can only emphasize that the peptide bonding does not change the general function of $C_P(T)$ for an amino acid above the temperature range where the Debye model with the whole molecule acting as an oscillator is valid. This result may be very useful in the unification of thermodynamic approaches used in the calorimetry of peptides [6] and polymers [7].

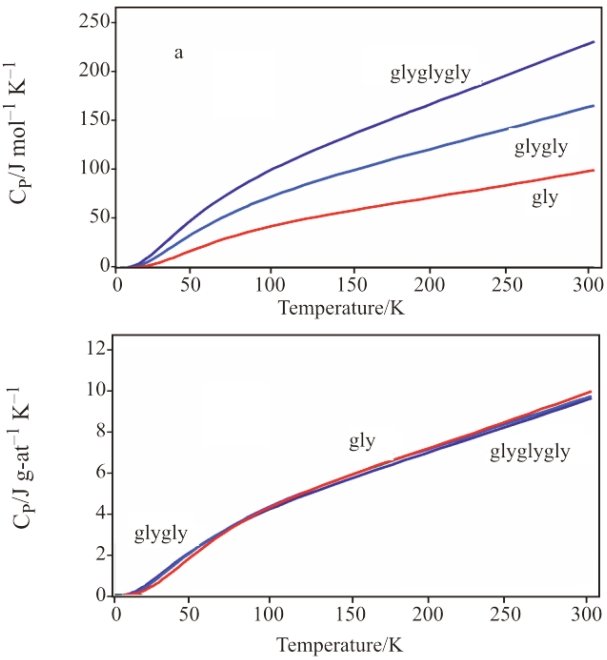

Fig. 2 Heat capacity of glycine (gly), glycylglycine (glygly), and diglycylglycine (glyglygly): a – heat capacity per mole, b – heat capacity per average gram-atom (per mole divided by the number of atoms in the molecule)

Comparison with literature data

Each time when we publish heat capacity of amino acids or peptides we compare our results with those published earlier, if any, and discuss why they differ from one another, if differ. Several times, we discussed the difference between our data and results of Badelin *et al.* [1]. Here we can explain the main reason of the differences.

Article of Badelin *et al.* is based on the other work, Kulikov *et al.* [8]. Heat capacity for selected amino acids and peptides from these reports are listed in Table 3, together with the relevant data published in other reports. Values in [8] rounded off to an integer are equal to those in [1]. One can see that the values from [1, 8] are the least for every substance as compared with other references. The difference ranges within the limits of 5–7%. It exceeds the experimental error of 0.01 J g^{-1} K^{-1} as indicated in [1]. For example, molecular mass of alanine is 89.09 g mol^{-1}, and the error in its heat capacity is 0.9 J g^{-1} K^{-1}. The difference in C_P for alanine in Table 3 is about 7 J g^{-1} K^{-1}. Most likely, the calibration coefficient of the DSC in [8] was incorrect, about 6% less than its actual value. We recalculated the values of C_P in [8], multiplying them by 1.06. The results are shown in Table 3 in the last column. Now the difference is close to the experimental error in accurate DSC measurements (±1%), except for glycylglycine.

Table 3 Heat capacities at 298.15 K for several amino acids and peptides (in J mol^{-1} K^{-1})

	References		References		
	[8]	[1]	Other ref.		[8] corrected
glycine	95.1	95	100.5	[11]	100.8
			99.2	[12]	
			99.3	[13]	
			99.23 (α)	[2]	
			98.69 (β)	[3]	
			96.00 (γ)	[2]	
β-alanine	109.3	109	116.4	[14]	115.9
glycylglycine	149.0	149	162	[9]	157.9
			163.97	[10]	
			164.33	[4]	
diglycylglycine	215.5	216	229.20	this work	228.4
alanine (*D*)			120.8	[15]	
alanine (*L*)	115.2	115	122.26	[12]	122.1
alanine (*DL*)	113.8	114	121.71	[16]	120.6
			121.6	[13]	
valine (*D*)	158.2	158			167.7
valine (*L*)			168.82	[17]	
			168.5	[13]	
valine (*DL*)	164.5	165			174.4
leucine (*L*)	190.6	191	200.96	[17]	202.0
			201.4	[13]	
leucine (*DL*)			194.3	[16]	

Recently we reported heat capacity of α-glycylglycine [4] and found it being close to the values published in [9, 10]. There are several facts indicating that too low value in C_P of glycylglycine in [1, 8] after the correction can be the result of polymorphism in this peptide. This is the subject of our future work. As for the diglycylglycine, our value of C_P(298.15) agrees well with the corrected value in [8].

Very interesting are the results for enantiomorphic and racemic species of amino acids in Table 3. Heat capacities for *L*-, *D*- and *DL*-alanine in Table 3 are very similar. Recently we have found out that the C_P differs significantly (up to 12%) at low temperatures for *D*- and *DL*-serine [18]. It was an unexpected result, that could be interpreted later, after the dynamics of the two forms was studied by Raman spectroscopy and inelastic neutron scattering [19, 20]. The difference exceeds that caused by the 'conventional' polymorphism in α-, β-, and γ-glycines. At 298.15 K, enantiomorphic and racemic serines differ by 3.5%. We may predict a large difference in the low-temperature heat capacity values for those racemic/chiral pairs, which show the difference of 2–3% already at ambient temperature. For example, one can expect this large difference for valine and for leucine: C_P of *DL*-valine is about 2.5% greater than that of *D*- and *L*-valine; C_P of *DL*-leucine is about 2.5% less than that of *L*-leucine.

Conclusions

Low-temperature heat capacity of diglycylglycine was measured, and its thermodynamic functions were derived. C_P per average gram-atom for glycine, glycylglycine and diglycylglycine are very close to each other above 70 K, but differ at very low temperatures, where they obey the Debye model. The mass of a molecule in a crystal increases when the peptide bond forms, decreasing its characteristic Debye temperature.

Our values of C_P(298) were found to be significantly greater than those reported by Badelin *et al.* Most likely, DSC in that report was calibrated incorrectly, with the 6% reduce in the calibration coefficient. Nevertheless, their data are very useful.

Together with the data of other authors, they allow us to expect that the racemic and enantiomorphic species of valine and leucine differ from one another in low-temperature heat capacity, as it was found recently for *D*- and *DL*-serine.

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