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Heat capacities of sixteen small peptides (*N*-acetyl-*N'*-methylamino acid amides) measured by differential scanning calorimetry

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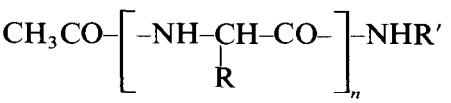
Abstract

The molar heat capacities at constant pressure of *N*-acetyl-*N'*-methylamides of glycine, L- and DL-alanine, DL-2-aminobutyric acid, L- and DL-proline, L- and DL-valine, DL-norvaline, L- and DL-leucine, DL-norleucine, L- and DL-isoleucine and L- and DL-phenylalanine have been measured by differential scanning calorimetry from 318 K to about 20 K below their melting points. Values at 298.15 K have been obtained by extrapolating the smoothed fitting equations.

The $C^*_{p,m}$ values at 298.15 K of both L- and DL-amino acid derivatives, including that of glycine, increase very linearly as a function of their molar masses. Slopes, giving 25 ± 1 J K^{-1} mol^{-1} per methylene group, are coincident with those of the corresponding amino acids. Isomeric compounds (iso- and noramino acid derivatives) show a small departure from linearity, whereas proline and phenylalanine derivatives present somewhat larger and decidedly strong deviations, respectively.

INTRODUCTION

Study of model compounds, such as amino acids [1], amides [2], and *N*-alkylamides [3], can give insight into the factors affecting the behaviour and stability of proteins in aqueous systems. Work by Lilley and his group in the early 80s [4] suggested the use of *N*-acetyl-substituted amino acids and peptides with the general formula



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in which R is the side chain of the amino acid and R′ is H or CH_3. These molecules have the advantage over amino acids and di- or tripeptides of being non-ionic; they are also called small peptides or terminally substituted peptides [5].

This study is one of our investigations into the thermodynamic properties of this class of model compounds in the form of pure solid substances [6–8]. We determined the $C^*_{p,m} = f(T)$ relations for the *N*-acetylamino acid derivatives with $n = 1$ and $R' = CH_3$ of glycine, L- and DL-alanine, DL-2-aminobutyric acid, L- and DL-proline, L- and DL-valine, DL-norvaline, L- and DL-leucine, DL-norleucine, L- and DL-isoleucine and L- and DL-phenylalanine. Measurements were made by differential scanning calorimetry (DSC) from ambient temperature to below the melting points. Because no solid–solid transitions occurred, we extrapolated the linear relations obtained at 298.15 K.

The aim of these determinations was to find a fundamental thermodynamic parameter that would also be useful in deriving enthalpies and entropies of sublimation [9], and, hence, the enthalpies of solvation [10], as well as the partial molar heat capacities of solution at infinite dilution [11].

EXPERIMENTAL

Materials

Indium (NIST-SRM 758), benzoic acid (NPL-SRM M16-06, 99.99% mole fraction) and urea (NIST-SRM 2152) were used without further purification.

Various *N*-acetyl-*N*′-methylamino acid amides were synthesized at the Vrije Universiteit in Amsterdam through acetylation of the amino acids and preparation of their methyl amides [12, 13]. Purification was performed by successive crystallizations from appropriate single or mixed solvent solutions, followed in some cases by sublimation. The compounds were characterized by their proton NMR spectra, melting temperatures and optical rotation [13]. In Table 1, the chemical structure of the compounds examined and their abbreviated symbols and molar masses [14] are given.

Differential scanning calorimetry

A Mettler DSC 20 apparatus coupled with the Mettler TC 10A processor was used. The temperature and heat calibration procedures scheduled by the supplier were followed. The actual temperature of the sample at various scanning rates was established through a built-in program based on the melting points of indium (429.75 K), lead (600.55 K) and zinc (692.65 K). These values are given by Mettler [15], and are in excellent agreement with those recommended in a very recent paper concerning various DSC temper-

TABLE 1

List of the examined *N*-acetyl-*N'*-methylamino acid amides of general formula CH_3–CONH–CH(R)–COHN–CH_3, where R is the amino acid side chain, their adopted symbols, molar masses and fusion temperatures

Compound	–R	Symbol	M/g mol^{-1} [a]	T_{fus}/K [b]
N-acetyl-*N'*-methyl glycinamide	–H	NAGAMe	130.146	430.0 (429–430)
N-acetyl-*N'*-methyl-L-alaninamide	–CH_3	L-NAAAMe	144.173	454.8 (455–456)
N-acetyl-*N'*-methyl-DL-alaninamide	–CH_3	DL-NAAAMe	144.173	431.4 (430–431)
N-acetyl-*N'*-methyl-DL-2-butyramide	–CH_2CH_3	DL-NABAMe	158.175	434.8 (437–438)
N-acetyl-*N'*-methyl-L-prolinamide	–$(CH_2)_3$–	L-NAPAMe	170.211	376.2 (379)
N-acetyl-*N'*-methyl-DL-prolinamide	–$(CH_2)_3$–	DL-NAPAMe	170.211	378.4 (378–379)
N-acetyl-*N'*-methyl-L-valinamide	–$CH(CH_3)_2$	L-NAVAMe	172.227	532.7 –
N-acetyl-*N'*-methyl-DL-valinamide	–$CH(CH_3)_2$	DL-NAVAMe	172.227	496.3 –
N-acetyl-*N'*-methyl-DL-norvalinamide	–$(CH_2)_2CH_3$	DL-NAnVAMe	172.227	432.6 (435–436)
N-acetyl-*N'*-methyl-L-leucinamide	–$CH_2CH(CH_3)_2$	L-NALAMe	186.253	438.2 (439–440)
N-acetyl-*N'*-methyl-DL-leucinamide	–$CH_2CH(CH_3)_2$	DL-NALAMe	186.253	432.2 (425–426)
N-acetyl-*N'*-methyl-DL-norleucinamide	–$(CH_2)_3CH_3$	DL-NAnLAMe	186.253	444.1 (446–447)
N-acetyl-*N'*-methyl-L-isoleucinamide	–$CH(CH_3)CH_2CH_3$	L-NAiLAMe	186.253	525.2 –
N-acetyl-*N'*-methyl-DL-isoleucinamide	–$CH(CH_3)CH_2CH_3$	DL-NAiLAMe	186.253	480.5 (481)
N-acetyl-*N'*-methyl-L-phenylalaninamide	–$CH_2(C_6H_5)$	L-NAPhAAMe	220.271	478.5 (486)
N-acetyl-*N'*-methyl-DL-phenylalaninamide	–$CH_2(C_6H_5)$	DL-NAPhAAMe	220.271	452.6 (455–456)

[a] Based on the 1991 IUPAC Table of Standard Atomic Weights of the Elements [14]. [b] DSC onset points compared with values (in parentheses) obtained with a Reichert Thermovar 300409 [13].

ature calibration materials [16]. The standards used were the very-high-purity metals supplied by Mettler in a special sealed crucible.

The heat flow rate was calibrated through another built-in program based on the enthalpy of fusion of indium. We used our NIST product and the enthalpy-of-fusion reference value incorporated by Mettler (3.267 kJ mol^{-1}), which is in very good agreement with that of 3.271 ± 0.012 kJ mol^{-1} selected in the latest IUPAC compilation on reference materials for enthalpy measurements [17]. Moreover, several runs with fresh indium samples showed an agreement with the temperature and enthalpy standard values within 0.02% and 0.2%, respectively.

The experimental conditions for $C^*_{p,m}$ measurements with our DSC equipment were chosen after a preliminary study [18]. Thus, sample pellets of about 30 mg, exactly fitting into the aluminium calorimetric crucibles (total volume 40 μl), and a heating rate of 10 K min^{-1} in static air atmosphere were adopted. The calorimetric output was calibrated with benzoic acid, which is a recommended standard for heat capacity measurements of solid organic compounds [17]. However, for the sake of consistency, we selected a single set of experimental data, namely those of Arvidsson et al. [19].

A number of scannings from about 295 to 373 K were carried out with the NIST benzoic acid and its molar heat capacity was measured at 5 K intervals through the built-in Mettler heat capacity determination program [15]. The averaged values in all intervals fell within the 1.5% uncertainty range, and linear smoothing gave a value at 298.15 K of 146.45 ± 0.62 J K^{-1} mol^{-1}, in good agreement with our reference value (146.79 J K^{-1} mol^{-1} [17]). Moreover, we calculated an appropriate slope conversion factor [20] to fully superimpose our smoothed straight line on that of Arvidsson et al. [19].

Our calibration procedure was further checked by determining the heat capacity of urea in the same temperature range. The following linear equation was obtained

$$C^*_{p,m}/(\text{J K}^{-1}\,\text{mol}^{-1}) = (19.52 \pm 1.31) + (0.244 \pm 0.007)(T/\text{K})$$

which corresponds to a satisfactory average of the literature data, as shown in Fig. 1.

The experimental determinations on our compounds, like those made with benzoic acid and urea, were performed from ambient temperature to well below the melting points, following the blank method. A preliminary run is made with an empty sealed crucible in the place of the sample, very similar to the reference. This output is stored in the built-in program for heat capacity determinations, and subtracted from those recorded during the runs with samples. At least 5 scans were made for each compound. Experimental data were taken in 5 K intervals, starting only from 318 K, because of the time needed for the calorimetric baseline to reach stability. For proline derivatives, which melt at lower temperatures (375–

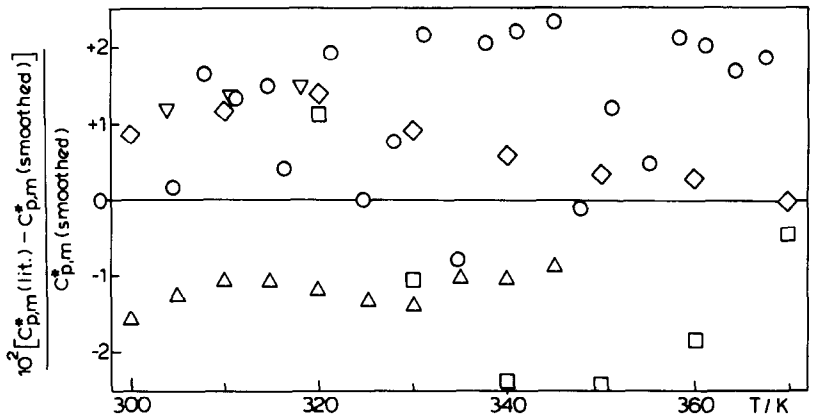

Fig. 1. Deviation of literature values for the molar heat capacity of urea from our smooth curve as a function of temperature: horizontal line, this work; ▽, ref. 21; ◇, ref. 22; ○, ref. 23; □, ref. 24; △, ref. 25.

380 K), intervals of only 2 K were used. Experimental data were corrected using the slope conversion factor.

For all the compounds, one scan was continued to melting, so as to determine their temperatures of fusion by DSC using the onset point method. Table 1 (column 5) compares our results with those obtained in the Amsterdam laboratory with a Reichert Thermovar 300409 [13].

TABLE 2

Molar heat capacities $C^*_{p,m}/(J\,mol^{-1}\,K^{-1})$ for glycine and L-amino acid derivatives at selected temperatures

T/K	NAGAMe	L-NAAMe	L-NAVAMe	L-NALAMe	L-NAiLAMe	L-NAPhAAMe
318	191.87	212.45	259.85	298.54	286.95	292.60
323	195.32	214.82	263.39	303.84	291.56	294.37
328	198.78	218.37	267.87	305.03	295.70	300.38
333	199.47	220.57	270.04	310.33	298.35	303.66
338	204.37	223.42	274.06	314.27	301.54	309.91
343	207.00	226.03	278.98	318.99	306.10	311.21
348	210.50	228.83	282.48	322.33	310.29	317.96
353	212.32	231.07	284.46	326.99	314.41	318.52
358	215.95	233.97	288.62	331.04	317.82	322.15
363	220.08	236.49	292.77	335.21	320.34	323.74
368	224.65	240.05	296.39	338.75	323.59	327.58
373	299.65	243.48	299.33	344.19	329.45	332.61
378			305.08	348.58		336.98
383			307.39			342.95
388			312.04			345.67
393			313.88			349.21
398			319.14			355.14
403			321.49			

RESULTS AND DISCUSSION

The molar heat capacities obtained by DSC for our sixteen *N*-acetyl-*N'*-methylamino acid amides are reported in Tables 2–4. Tables 2 and 3 present average $C^*_{p,m}$ values taken at 5 K intervals for L-derivatives (including non-chiral NAGAMe) and DL-derivatives, respectively. Table 4 presents results for the L- and DL-proline derivatives, taken at 2 K intervals.

The linear equations obtained by a least-squares treatment are shown in Table 5. Column 2 reports the experimental temperature ranges and column 3 gives the number of temperature intervals, which also represents the number of points used for the least-squares treatment. Columns 4, 5 and 6 give the intercepts at 298.15 K (*A*), slopes (*B*) and the correlation coefficients (*r*), respectively. The validity of these relations, however, should be limited to our experimental temperature ranges, starting in all cases from 318 K. In reality, our scans began at ambient temperature, and no compound displayed any evidence of solid–solid transitions up to 318 K. Because of this and the short interval of temperature, our linear equations can be reliably extrapolated to obtain $C^*_{p,m}$ values at 298.15 K.

The molar heat capacities at 298.15 K regularly increase as a function of the molar masses, as represented in Figs. 2 and 3, referring to the L- and

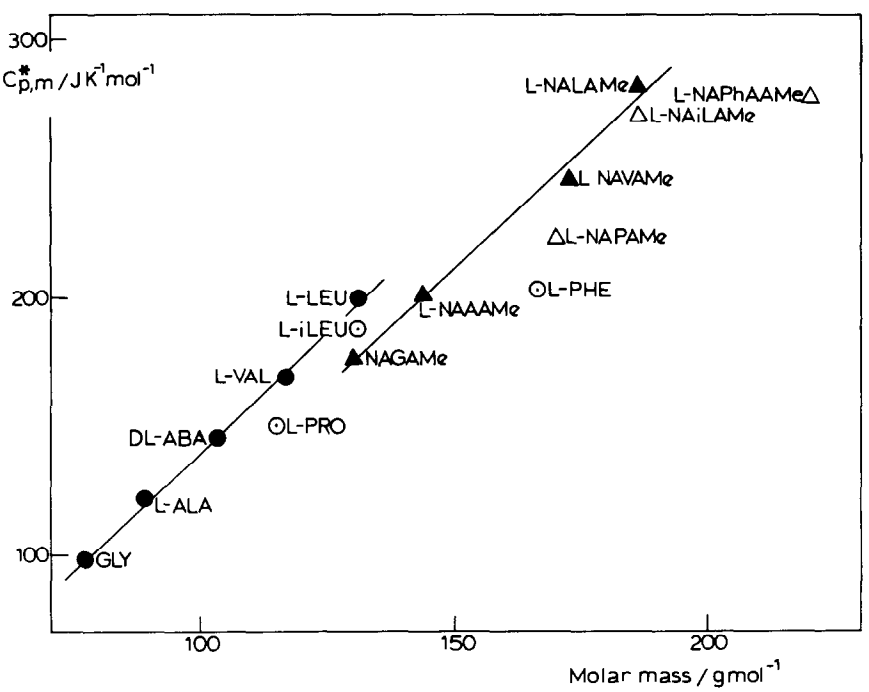

Fig. 2. Molar heat capacities of the L-*N*-acetyl-*N'*-methylamino acid amides (▲, △) as a function of their molar masses compared with the corresponding amino acids (●, ⊙) (refs. 1 and 26–29). The lines were drawn using the solid symbol values only.

TABLE 3

Molar heat capacities $C^*_{p,m}$/(J mol $^{-1}$ K^{-1}) for DL-amino acid derivatives at selected temperatures

T/K	DL-NAAAMe	DL-NABAMe	DL-NAVAMe	DL-NAnVAMe	DL-NALAMe	DL-NAnLAMe	DL-NAiLAMe	L-NAPhAAMe
318	208.35	242.97	272.33	255.49	293.20	302.97	285.27	295.91
323	211.58	246.31	273.99	262.79	298.28	307.91	289.07	300.52
328	215.23	250.14	275.52	267.26	302.15	312.25	293.34	304.98
333	218.04	252.88	280.93	270.44	308.12	314.81	296.44	309.59
338	220.68	258.28	282.84	273.66	312.25	318.34	300.48	313.10
343	224.45	261.45	286.24	277.12	317.97	321.35	305.98	319.46
348	228.17	264.44	289.67	283.64	323.12	325.98	309.00	324.67
353	229.93	266.40	293.50	288.44	328.88	329.48	311.98	325.44
358	233.38	270.65	297.72	292.70	333.87	331.81	317.46	333.45
363	236.13	274.92	302.04	298.63	339.28	336.29	320.49	337.09
368	238.81	278.60	305.12	303.86	343.84	341.66	324.79	338.91
373	243.83	281.90	306.65	390.17	350.91	345.99	328.98	344.23
378			312.45			351.11		350.57
383			315.46			354.04		353.28
388			318.05					358.90
393			321.23					365.54
398			324.41					368.96
403			327.19					371.90
408			332.04					

TABLE 4

Molar heat capacities $C^*_{p,m}/(J\,mol^{-1}\,K^{-1})$ for L- and DL-proline derivatives at selected temperatures

T/K	L-NAPAMe	DL-NAPAMe	T/K	L-NAPAMe	DL-NAPAMe
319	243.71	251.13	333	259.42	262.02
321	245.85	253.11	335	261.10	263.82
323	249.83	254.15	337	263.87	266.16
325	251.47	255.92	339	265.62	267.94
327	253.09	257.63	341	268.71	270.07
329	255.05	259.79	343	270.85	271.96
331	257.23	261.07			

TABLE 5

Molar heat capacities as a function of temperature from the equation $C^*_{p,m}/(J\,K^{-1}\,mol^{-1}) = A + B(T/K - 298.15\,K)$, where $A = C^*_{p,m}(298.15\,K)$

Compound	T/K	N [a]	A [b]	B	r [c]
NAGAMe	318–373	12	178.0 ± 2.5	0.6562	0.99447
L-NAAAMe	318–373	12	201.3 ± 0.8	0.5510	0.99909
DL-NAAAMe	318–373	12	196.2 ± 1.1	0.6223	0.99872
DLNABAMe	318–373	12	228.9 ± 1.3	0.7052	0.99866
L-NAPAMe	319–343	13	221.3 ± 1.6	1.0931	0.99797
DL-NAPAMe	319–343	13	233.0 ± 1.2	0.8524	0.99792
L-NAVAMe	318–403	18	245.1 ± 1.0	0.7329	0.99934
DL-NAVAMe	318–408	19	256.6 ± 1.3	0.6814	0.99867
DL-NAnVAMe	318–373	12	237.4 ± 2.6	0.9384	0.99693
L-NALAMe	318–378	13	281.5 ± 1.4	0.8275	0.99892
DL-NALAMe	318–373	12	271.5 ± 1.3	1.0409	0.99937
DL-NAnLAMe	318–383	14	287.6 ± 1.8	0.7716	0.99775
L-NAiLAMe	318–373	12	272.5 ± 1.4	0.7471	0.99857
DL-NAiLAMe	318–373	12	269.2 ± 1.1	0.7937	0.99931
L-NAPhAAMe	318–398	17	277.1 ± 2.3	0.7595	0.99649
DL-NAPhAAMe	318–403	18	277.9 ± 1.6	0.9018	0.99876

[a] Number of points used in the least-squares treatment. [b] Uncertainties at 5% confidence limits. [c] Product-moment correlation coefficient.

DL-amino acid derivatives, respectively. Non-chiral NAGAMe is present in both figures. A comparison is made with the $C^*_{p,m}$ values at 298.15 K of the corresponding amino acids by using the only comprehensive set of experimental data reported in the literature [1]. A few values for other amino acids obtained by other authors [26–29] are also included. The following comments can be made on these figures.

(i) The $C^*_{p,m}$ dependence on molar mass for both the L and the DL series of compounds, as well as for the corresponding amino acids, shows a fairly

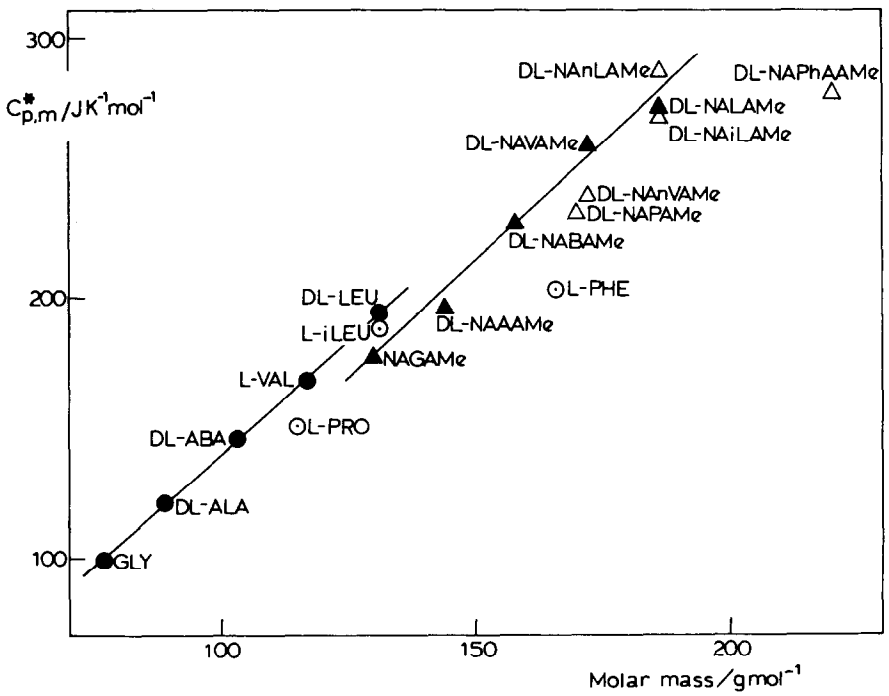

Fig. 3. Molar heat capacities of the DL-*N*-acetyl-*N'*-methylamino acid amides (▲, △) as a function of their molar masses compared with the corresponding amino acids (●, ⊙) (refs. 1 and 26–29). The lines were drawn using the solid symbol values only.

good linearity (lines with solid symbols), with exactly the same slope. Deviating compounds (open symbols) were not taken into account when drawing these lines.

(ii) Both proline derivatives and L-proline itself, whose chemical structures include an aliphatic ring, show $C^*_{p,m}$ values rather below the corresponding straight lines, with differences roughly proportional to their molar masses. More marked deviations can be observed for both the phenylalanine derivatives and for L-phenylalanine, probably owing to the presence of an aromatic ring.

(iii) In the same way, the minor departures from linearity observed for the iso- and nor-isomers (valine and leucine derivatives) may be related to the different structure of their side chains. The heat capacities of isomeric compounds, therefore, appear to be influenced by their molecular volumes.

(iv) The slopes for both series of L- and DL-amino acid derivatives and amino acids were practically the same, showing an increment of (25 ± 1) J K^{-1} mol^{-1} per methylene group. As a consequence, the observed linear trends appear to confirm the validity of the various group additivity schemes for $C^*_{p,m}$, at least with respect to methylene increments, even for compounds in the solid state [30].

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