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Partial molar heat capacities and volumes of Gly–X–Gly tripeptides in aqueous solution: model studies for the rationalization of thermodynamic parameters of proteins ☆

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Abstract

The thermodynamics of protein unfolding can be rationalized if the temperature dependence of the partial molar volumes and heat capacities of their constituent groups are known reliably. Despite many experimental and theoretical studies there are still several inconsistencies in the published thermodynamic data. We have investigated some of these inconsistencies by applying high sensitivity scanning densimetry and microcalorimetry to aqueous solutions of tripeptides of the structure Gly–X–Gly, where X is one of the amino acids Met, Asn, Gly and Ile. For these side-chains either no direct data have been determined or serious discrepancies exist between the values published by different laboratories. Partial molar heat capacities and volumes have been determined for the peptides in pure water, in water adjusted to pH = 4 and in 0.5 M sodium acetate buffer at pH = 4. The results obtained are critically compared with those in the literature.

Keywords: Tripeptides; Aqueous solution; Heat capacity; Increment system (proteins); Density; Partial molar volume

1. Introduction

The thermodynamic characterization of the unfolded state of proteins is essential, because it is generally used as the reference state for the quantitative description of protein stability parameters [1–3]. It has been common practice to assume additivity of partial molar heat capacities and to calculate the heat capacity of the unfolded chain by summing up the partial molar heat capacities of the constituent components such as the side-chains, the peptide groups and the amino and carboxyl terminal groups [4–8]. The excess heat capacity of these components is determined predominantly by their interaction with water, i.e. by their hydration properties. The heat capacity effects of the hydration of hydrophobic and hydrophilic groups contribute with opposite signs to the overall heat capacity of a solute [9]. Consequently, the choice of the model compounds from which the group parameters are delineated is of

Abbreviations: DSD, differential scanning densimetry; v_ϕ, apparent specific volume; V_ϕ, apparent molar volume; V^o, partial molar volume; DSC, differential scanning calorimetry; $C_{p,\phi}$, apparent molar heat capacity; $c_{p,\phi}$, apparent specific heat capacity; C_p^o, partial molar heat capacity; C_p^o (–R), partial molar heat capacity of the amino acid side-chain R in aqueous solution; NaAc, sodium acetate

☆ Dedicated to Prof. Dr. W. Müller-Warmuth on the occasion of his 65th birthday.

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paramount significance. Furthermore, medium effects may be important. The influence of charges, buffer components and pH all have to be properly taken into account.

One group of compounds that have been used as models for the amino acid side-chains in proteins are tripeptides of sequence Gly–X–Gly, where X is one of the amino acids [1,7,10,11]. The partial molar heat capacities and volumes for several of these peptides have been determined in pure water as solvent at 25°C [10,11]. In a comprehensive study to determine the partial molar heat capacities of protein constituent groups over a broad temperature range, some tripeptides of sequence Gly–X–Gly, as well as simple organic solutes, were used as model compounds [1,7]. For the tripeptides, the measurements were carried out using 0.5 M sodium acetate buffer at pH 4.0 as the solvent, rather than using pure water. A comparison of the results obtained with those in pure water indicates that there are several inconsistencies. In order to resolve whether or not these inconsistencies are due to medium effects, we have undertaken heat capacity studies on some selected tripeptides in pure water, in water adjusted to pH = 4.0 with HCl, and in the presence of 0.5 M sodium acetate buffer at pH = 4.0. The tripeptides used in this work are glycylisoleucylglycine (Gly**Ile**Gly), glycylasparagylglycine (Gly**Asn**Gly), glycylmethionylglycine (Gly**Met**Gly) and, as the reference compound, glycylglycylglycine (Gly**Gly**Gly).

Their structures are given in Fig. 1.

For a quantitative determination of heat capacity by constant volume scanning microcalorimetry, such as performed with a Privalov type instrument [12], the exact variations with temperature of the partial molar volumes $V^{\circ}(T)$ of the compounds under study are required. It is not sufficient to determine $V^{\circ}(T)$ from volume increments determined at 25°C. Along with the calorimetric measurements, we have determined the temperature dependencies of the partial molar volumes of the peptides by using high-sensitivity scanning densimetry.

2. Materials and methods

Samples of the tripeptides GlyIleGly, GlyAsnGly and GlyMetGly used were from batches of material

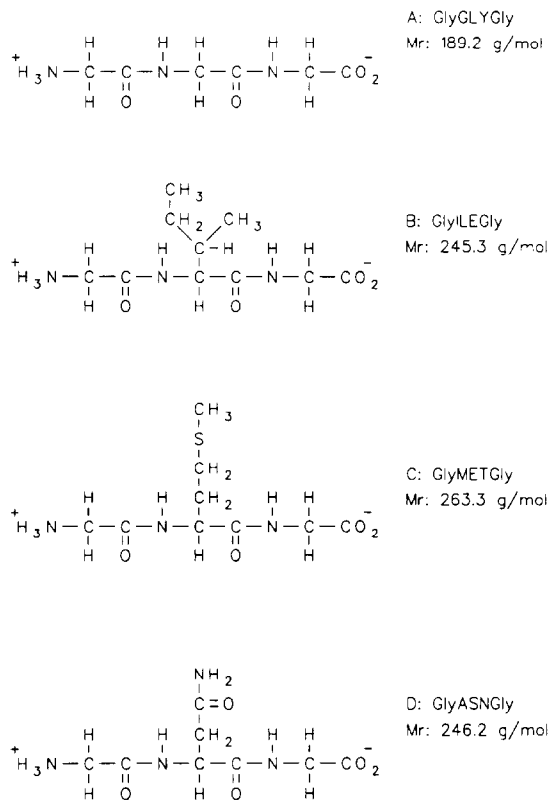

Fig. 1. Tripeptides employed in the present study: (A) Gly**Gly**Gly; (B) Gly**Ile**Gly; (C) Gly**Met**Gly; (D) Gly**Asn**Gly.

prepared in earlier studies. The preparation, purification and analyses of these peptides have been reported in detail elsewhere [11]. The tripeptide GlyGlyGly (Sigma Chemical Co.) was recrystallised from water–ethanol and dried under vacuum at room temperature. The purity was confirmed by alkalimetric titration [13,14] and by elemental analysis. Found: C 38.0, H 5.6, N 22.1; calculated for $C_6H_{11}N_3O_4$, C 38.1, H 5.9, N 22.2%. All the peptides were dried under vacuum at room temperature before use. The water used, both to prepare solutions and as the reference solvent, was deionized and glass-distilled.

Precise differential scanning density measurements (DSD) were performed using a Kratky–Paar densimetric system consisting of two matched DMA 602 HT external cells in combination with a DMA 60 measuring unit (Anton Paar, Graz, Austria) [15,16]. Temperature scans between 10 and 80°C were carried out using a Haake PD20 temperature controller and a Haake F3 thermostated bath. The

effective scan rate was 0.4 K min^{-1}. Temperature measurements were made using a Keithley 195 A multimeter and a platinum resistance thermometer placed in a capillary tube in the sample cell compartment. Data pairs of density and temperature were collected every 0.1 K employing a personal computer. The temperature scans were interrupted for the time of the density measurement to allow for temperature equilibration. Apparent specific volumes, v_ϕ, were calculated using the equation

$$v_\phi = \frac{1}{\rho_{bu}}\left(1 - \frac{\rho_{sol} - \rho_{bu}}{c_{tri}}\right) \quad (1)$$

where ρ_{bu} and ρ_{sol} are, respectively, the densities of the buffer and tripeptide solution and c_{tri} is the tripeptide concentration in mg ml^{-1}. Tripeptide concentrations between 6 and 15 mg ml^{-1} were used in the measurements. At all temperatures, the overall accuracy of the specific volume measurements is better than 0.003 ml g^{-1}. Partial molar volumes of the tripeptides, V^o, were calculated from the apparent molar volumes, V_ϕ, using the equation

$$V_\phi = V^o + S_v m \quad (2)$$

where S_v is the experimentally observed concentration dependence of V_ϕ at 25°C and m is the solution molality [11]. We assumed that these values are temperature invariant to a first approximation and used them for the extrapolation of V_ϕ to zero concentration at all temperatures [GlyIleGly $S_v = 2.7$ cm^3 kg mol^{-2}; GlyAsnGly $S_v = 8.0$ cm^3 kg mol^{-2}; GlyMetGly $S_v = 5.6$ cm^3 kg mol^{-2}; GlyGlyGly $S_v = 3.6$ cm^3 kg mol^{-2}].

Differential scanning heat capacity measurements (DSC) were performed using a DASM-1M calorimeter [12]. Runs were made in the temperature range of 10–100°C using tripeptide concentrations between 1.1 and 15 mg ml^{-1} and a heating rate of 1 K min^{-1}. Heat capacity and temperature data were recorded every 0.1 K using a computer. Each sample measurement was preceded by a calibration run with the buffer. The apparent molar heat capacities of the peptides were calculated using the equation

$$C_{p,\phi}(T) = M_r\left(\frac{c_{p,1}(T) \cdot v_\phi(T)}{v_1(T)} + \frac{\Delta c_{p,\phi}(T)}{m_P}\right) \quad (3)$$

where M_r is the solute molar mass, $c_{p,1}(T)$ and $v_1(T)$ are, respectively, the temperature dependent apparent specific heat capacity and volume of water (taken from ref. [17]) or buffer, m_P is the mass of the solute, $\Delta c_{p,\phi}(T)$ is the temperature dependent difference between the specific heat capacities of the solution and reference solvent, and $v_\phi(T)$ is the temperature dependent apparent specific volume of the tripeptide solution, as obtained from DSD measurements.

3. Results

The unsmoothed experimental temperature scans in Fig. 2a and b show the quality of the DSC and DSD studies. Between 3 and 8 baselines and an equivalent number of sample scans were made with each compound in order to obtain high precision heat capacity data. Furthermore scans have been performed using different tripeptide concentrations. One example is given in Fig. 2a as a dashed $C_{p,\phi}$ curve. It refers to a GlyAsnGly concentration of 14.05 mg ml^{-1}, while the other curves refer to a concentration of 7.47 mg ml^{-1}. Shifts in the sample curve relative to the baseline that occur with repetitive scans will obviously perturb the precision of the derived apparent molar heat capacities to a much larger extent at low than at high concentrations. The precision of $C_{p,\phi}$, as expressed by the standard deviation (0.7%), is however quite satisfactory even at low oligopeptide concentration (1.1 mg ml^{-1}). The volumetric data are also of high precision. The effect of temperature fluctuations that alter solution densities is kept minimal because of the differential arrangement of the vibrating tubes. As a result of the large number of data in each DSD scan (every 0.1 or 0.2 K), an essentially smooth curve is obtained for the variation with temperature of the partial specific volume.

The dependence on temperature of the partial molar volumes, V^o, of the tripeptides in water at pH = 5.8 was analysed using a power series in temperature T (in °C) of the form

$$V^o(T) = V^o(0°C) + b \cdot T + c \cdot T^2 \quad (4)$$

where $V^o(0°C)$ is the partial molar volume of the peptide at $T = 0$°C and b and c are the fitted coefficients. The values of $V^o(0°C)$ and the coefficients

for the peptides in water and in buffer solution are given in Table 1.

The calorimetric measurements (Fig. 2a) indicated that there are small but significant concentration dependencies of the apparent molar heat capacities, $C_{p,\phi}$, of the tripeptides. To obtain values for the partial molar heat capacities, C_p^o, at each temperature the following procedure has been applied to the $C_{p,\phi}$ values. First the experimental $C_{p,\phi}$ functions shown in Fig. 2a have been fitted to a third order polynomial. Then the $C_{p,\phi}$ values at 25°C have been extrapolated to infinite dilution as illustrated in Fig. 3. Since the experimental $C_{p,\phi}$ functions at different concentrations are parallel to each other (Fig. 2a) we used the slopes obtained from Fig. 3 at 25°C for the extrapolation to infinite dilution at all temperatures and for all solvent systems. The partial molar heat capacity at 0°C, $C_p^o(0°C)$, and the coefficients for the heat capacity polynomial Eq. (5) that permits calculation of the partial molar heat capacity values at other temperatures are summarized in Table 2. For ease of use and comparison with literature data Table 3 provides numerical values of the partial molar heat capacities of the tripeptides determined in the present study for five selected temperatures. They have been calculated using Eq. (5) and the coefficients given in Table 2.

$$C_p^o = C_p^o(0°C) + b \cdot T + c \cdot T^2 + d \cdot T^3 \quad (5)$$

T refers to temperature in °C.

The V^o and C_p^o results at 25°C determined in this study are compared with some literature data in Table 4. Generally speaking, there is reasonable agreement between the V^o and C_p^o values determined at pH 5.8 in pure water and those obtained previously by one of us [11]. However, for the peptides GlyAsnGly and GlyMetGly the differences between the V^o values determined in the two studies are larger than expected. The comparison of heat capacities suggests that the DSC approach gives C_p^o data that compare favourably with those determined using more precise flow microcalorimetry [18]. Furthermore, it provides a reasonable basis for the assumption that the data in the high- and low-temperature ranges are also likely to be reliable.

The partial molar heat capacities of the tripeptides can be used to obtain the group contributions of the side-chains that are relevant to heat capacity calculations of randomly coiled polypeptide chains [19]. The heat capacity contribution of an amino acid side-chain, $C_p^o(-R)$, can be calculated using the equation

$$C_p^o(-R) = C_p^o(Gly-X-Gly) - C_p^o(Gly-Gly-Gly) + C_p^o(-H) \quad (6)$$

Table 1
Summary of the temperature dependence of the partial molar volumes of the tripeptides. The coefficients refer to the quadratic equation $V^o(T) = V^o(0°C) + bT + cT^2$. T is in °C

Gly–X–Gly	$V^o(0°C)$/ml mol^{-1}	b/ml mol^{-1} K^{-1}	$c \times 10^{+4}$ ml mol^{-1} K^{-2}
Buffer H_2O, pH 5.8			
Gly	107.21	0.1862	−7.247
Asn	145.01	0.2248	−8.546
Ile	172.73	0.1839	−1.875
Met	172.02	0.2182	−4.025
Buffer H_2O, pH 4.0			
Gly	106.68	0.1965	−7.624
Asn	142.37	0.2589	−12.19
Ile	169.95	0.1874	−0.941
Met	168.71	0.2114	−1.483
Buffer 0.5 M NaAc, pH 4.0			
Gly	108.48	0.1728	−7.875
Asn	148.31	0.1531	−4.682
Ile	174.87	0.1036	+4.259
Met	173.06	0.1789	−2.044

Table 2
Coefficients for the heat capacity polynomial equation of the third degree $C_p^o = C_p^o(0°C) + bT + cT^2 + dT^3$, T refers to °C

Tripeptide	Parameter				
	$C_p^o(0°C)$/ J mol^{-1} K^{-1}	b/ J mol^{-1} K^{-2}	c/ J mol^{-1} K^{-3}	$d \times 10^{+4}$/ J mol^{-1} K^{-4}	r^2
Buffer H_2O, pH 5.8					
Gly**Asn**Gly	134.5	8.172	−0.1049	5.47	0.9996
Gly**Gly**Gly	57.1	6.678	−0.0798	3.99	0.9998
Gly**Ile**Gly	425.0	5.515	−0.0728	3.51	0.9985
Gly**Met**Gly	315.7	5.665	−0.0618	2.51	0.9991
Buffer H_2O, pH 4.0					
Gly**Asn**Gly	114.8	7.986	−0.1044	5.62	0.9983
Gly**Gly**Gly	23.0	6.893	−0.0869	4.35	0.9992
Gly**Ile**Gly	395.9	4.954	−0.0653	3.10	0.9937
Gly**Met**Gly	288.4	5.822	−0.0745	3.64	0.9953
Buffer 0.5 M NaAc, pH 4.0					
Gly**Asn**Gly	193.6	6.144	−0.0717	3.30	0.9990
Gly**Gly**Gly	48.2	6.005	−0.0655	2.65	0.9997
Gly**Ile**Gly	427.7	4.503	−0.0555	2.46	0.9980
Gly**Met**Gly	344.7	4.721	−0.0531	2.24	0.9986

where $C_p^o(-H)$ is the partial molar heat capacity of the hydrogen atom. For purposes of comparison, we have chosen to use the same values of $C_p^o(-H)$ as used in the earlier study by Makhatadze and Privalov [7]. At 25°C the value of $C_p^o(-H)$ is 78 J K^{-1} mol^{-1}. This value derived by Makhatadze and Privalov is the mean of four estimates taken from the literature [7]. The values of $C_p^o(-H)$ used at other temperatures were determined from the difference between the partial molar heat capacities of the $-CH_3$ and $-CH_2-$ groups [7]. The group parameters obtained by using Eq. (6) are given in Table 5 for a variety of temperatures and for the three buffer conditions used.

4. Discussion

An additivity scheme of heat capacity increments for constituent groups is a useful means to rationalize the heat capacity of unfolded proteins. For a general application to random coil polypeptides it would be desirable to have group parameters that are independent of the denaturation conditions. If this is not possible, then knowledge is required of the trends that can be expected at specific pH values or in specific buffer systems. The results obtained in this study provide information on the effects of pH and the presence of buffer salts on the partial molar volumes and heat capacities of peptides in aqueous solution.

The C_p^o results presented in Table 4 show that, at all temperatures, with water adjusted to pH = 4 as solvent the C_p^o values for the tripeptides are considerably smaller than those in pure water. This result is somewhat surprising. For peptides with neutral side-chains the zwitterionic form of the molecule is the predominant solute species in pure water as solvent. Although at pH = 4 this zwitterionic form will still be the major solute species, there will be a small contribution from the species with a protonated carboxylate group. The C_p^o values for unionized carboxylic acids [20] are greater than those for carboxylate ions [21]. Consequently, for tripeptides in water at pH = 4 the values of C_p^o should be greater than those in pure water. As this is not the case, perhaps the hydrogen ion concentration produces some changes in the microscopic environment of the tripeptides other than changing the degree of ionization that makes the observed contribution to the C_p^o values.

Similar trends are also observed for the partial molar volumes. For the peptides GlyAsnGly, GlyIleGly and GlyMetGly the V^o values in water at

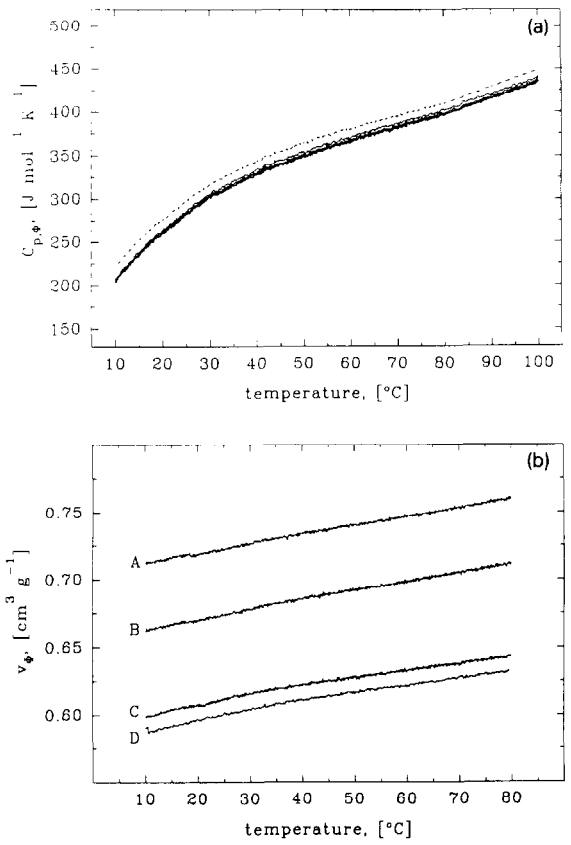

Fig. 2. (a) Temperature course of the apparent molar heat capacity of the tripeptide Gly**Asn**Gly in water of pH = 5.8; c_{tri} = 7.47 mg ml^{-1} (solid lines); c_{tri} = 14.05 mg ml^{-1} (dashed line); heating rate 1 K min^{-1}. The graph shows five independent measurements at c_{tri} = 7.47 mg ml^{-1} and one at c_{tri} = 14.05 mg ml^{-1}; the heat capacity curves provide the original unsmoothed data. (b) Temperature course of the apparent specific volume v_ϕ of (A) Gly**Ile**Gly, c_{tri} = 13.16 mg ml^{-1}; (B) Gly**Met**Gly, c_{tri} = 12.60 mg ml^{-1}; (C) Gly**Asn**Gly, c_{tri} = 14.05 mg ml^{-1}; (D) Gly**Gly**Gly, c_{tri} = 14.57 mg ml^{-1}. The compounds have been dissolved in water giving a pH of 5.8. Scan rate was 0.4 K min^{-1}.

pH = 4 are significantly smaller than those in pure water. The effect is less pronounced for the tripeptide GlyGlyGly. As the V° values for unionized carboxylic acids are greater than those for carboxylate ions [22,23], the differences between the V° values for the peptides in pure water and in water at pH 4 are opposite in sign to what might be expected.

The differences between the V° and C_p° results in H_2O at pH = 4 and those in 0.5 M sodium acetate

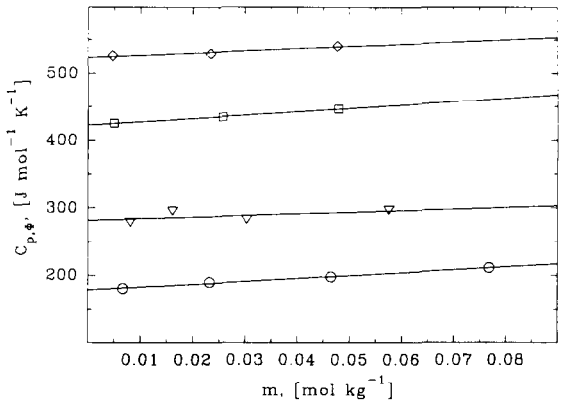

Fig. 3. Dependence of the apparent molar heat capacities $C_{p,\phi}$ of the tripeptides from the concentration. $C_{p,\phi}$ value vs. molal concentration of the tripeptides at 25°C. Gly**Ile**Gly (◇); Gly**Met**Gly (□); Gly**Asn**Gly (▽); Gly**Gly**Gly (○).

buffer at pH = 4 manifest the effect of buffer salts. The results in Table 4 show that the C_p° and V° values in acetate buffer at pH = 4 are significantly larger than those in water at the same pH. The volumes and heat capacities of transfer at 25°C of some amino acids, dipeptides and GlyGlyGly from water to aqueous sodium chloride are positive [24]. Similarly, the volumes of transfer of amino acids

Table 3
Partial molar heat capacities, C_p°, [J mol^{-1} K^{-1}], of the tripeptides at different temperatures in water and in 0.5 M sodium acetate buffer. The values have been calculated with Eq. (5) using the parameters in Table 2

Temperature, °C	10	25	50	75	100
Buffer H_2O, pH 5.8					
Gly**Asn**Gly	206.3	281.8	349.2	388.1	449.7
Gly**Gly**Gly	116.3	180.4	241.4	277.4	325.9
Gly**Ile**Gly	473.2	522.8	562.7	577.2	599.5
Gly**Met**Gly	366.4	422.6	475.8	498.9	515.2
Buffer H_2O, pH 4.0					
Gly**Asn**Gly	184.8	258	323.2	363.6	431.4
Gly**Gly**Gly	83.7	147.8	204.8	234.7	278.3
Gly**Ile**Gly	439.2	483.8	519.1	531.0	548.4
Gly**Met**Gly	339.5	393.1	438.9	459.6	489.6
Buffer 0.5 M NaAc, pH 4.0					
Gly**Asn**Gly	248.2	307.5	362.8	390.4	421.0
Gly**Gly**Gly	101.9	161.5	217.8	241.9	258.7
Gly**Ile**Gly	467.4	509.4	544.8	557.0	569.0
Gly**Met**Gly	386.9	433.0	476.0	494.6	509.8

Table 4
A comparison of the partial molar volumes and heat capacities of the tripeptides with some literature data at 25°C

Tripeptide	V°/cm^3 mol^{-1}					C_p°/J K^{-1} mol^{-1}				
	H_2O, pH 5.8			H_2O, pH 4	0.5 M NaAc, pH 4	H_2O, pH 5.8			H_2O, pH 4	0.5 M NaAc, pH 4
Gly**Gly**Gly	111.4	111.9 [a]	112.9 [b]	111.1	112.3 115.5 [b]	180.4	188.3 [c]	175.3 [d]	147.8	161.5 194.6 [d]
Gly**Asn**Gly	150.1	147.4 [a]		148.11	51.9 –	281.8	279.2 [a]		258.0	307.5 –
Gly**Ile**Gly	177.2	177.2 [a]		174.61	77.7 171.3 [b]	522.8	530.4 [a]		483.8	509.4 487.8 [c]
Gly**Met**Gly	177.2	175.1 [a]		173.9	177.4 177.3 [b]	422.6	429.9 [a]		393.1	433.0 292.5 [d]

[a] From ref. [11].
[b] From ref. [27].
[c] From ref. [18].
[d] From ref. [7].
[e] From ref. [1].

from water to aqueous solutions of other salts (ammonium chloride and potassium thiocyanate) are also positive [25,26]. As it is reasonable to expect that the heat capacities and volumes of transfer of peptides from water to aqueous sodium acetate will also be positive, the C_p° and V° values of the tripeptides in 0.5 M sodium acetate at pH = 4 should be larger than those in water at the same pH, as has been observed.

There are significant differences between some of the C_p° values determined in this study and those reported by Makhatadze and Privalov [7]. For the tripeptide GlyMetGly the C_p° value in 0.5 M sodium acetate buffer, 25°C, determined by Makhatadze and Privalov is 292.5 J K^{-1} mol^{-1} which is significantly lower than that determined in this work (433.0 J K^{-1} mol^{-1}). This difference is surprising, particularly, since almost identical partial molar volumes in the presence of buffer have been obtained by the two groups. However, even if there were an error in V° as large as 3 cm^3 mol^{-1}, the approximate error introduced into C_p° would be, according to Eq. (3), of the order of only 10 J K^{-1} mol^{-1}. There is no way a difference in C_p° of more than 130 J K^{-1} mol^{-1} can be explained by differences in the partial molar volumes.

On the other hand, there is fair agreement between the C_p° value for GlyIleGly in 0.5 M sodium acetate, 25°C, determined in this study (509.4 J K^{-1} mol^{-1}) and that reported previously [1] (487.8 J K^{-1} mol^{-1}). The small discrepancy could be accounted for by the difference between the V° values used in the derivation of the heat capacities. The V° value of 171.3 cm^3 mol^{-1} reported earlier [27] is low compared with that determined in this work. Furthermore, this V° result for GlyIleGly differs significantly from that of the isomeric peptide GlyLeuGly (V° = 182.8 cm^3 mol^{-1}) reported in the same study [27]. With pure water as solvent, negligible differences between the V° values for these two isomeric peptides have been reported [11]. Similar values of V° for the Ile and Leu containing tripeptides would also be expected in 0.5 M sodium acetate buffer as solvent.

Table 5
Partial molar heat capacities, C_p°, [J mol^{-1} K^{-1}], of the side chains of the amino acids at different temperatures in water and in 0.5 M sodium acetate buffer. The values have been calculated with Eq. (5) using the parameters in Table 2. The values of the partial molar heat capacity of the proton, C_p°(–H), at different temperatures are from Table 5 of ref. [7] and were used for all buffer systems and pH values

Temperature, °C	10	25	50	75	100
Buffer H_2O, pH 5.8					
Asn	171.1	179.4	179.6	177.1	183.5
Ile	438.1	420.5	392.9	366.2	333.3
Met	331.3	320.2	306.2	287.8	249.0
Buffer H_2O, pH 4.0					
Asn	182.3	188.2	190.3	195.3	212.8
Ile	436.7	414.0	386.0	362.6	329.7
Met	337.1	323.3	305.7	291.3	271.0
Buffer 0.5 M NaAc, pH 4.0					
Asn	227.4	224.0	216.7	214.8	222.0
Ile	446.7	425.9	398.7	381.5	370.0
Met	366.1	349.5	329.9	319.1	310.8
H[a]	81.2	78.0	71.7	66.4	59.7

[a] Data from ref. [7].

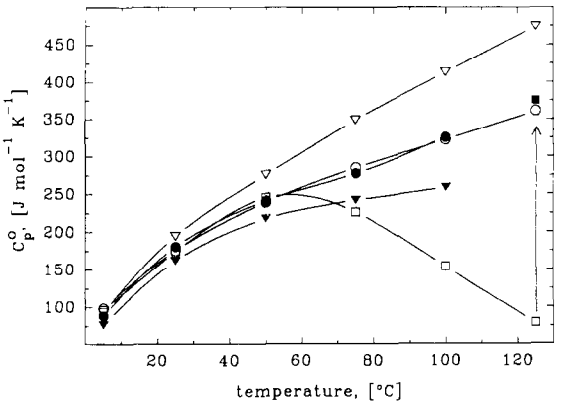

Fig. 4. Experimental and calculated heat capacity functions for triglycine. Open symbols refer to data from ref. [7], closed symbols to values from the present study. (▽) 0.5 M sodium acetate, pH = 4.0 (Table 4, ref. [7]); (○) H_2O, pH = 5.8 (Table 4, ref. [7]); (□) calculated C_p^o values using the parameters of Table 5 in ref. [7]; (▼) 0.5 M sodium acetate, pH = 4.0; (●) H_2O, pH = 5.8; (■) corrected C_p^o value at 125°C as described in the discussion. The arrow indicates the change in the parameters necessary to obtain agreement between experiment and calculated C_p^o values of GlyGlyGly. The calculation is described in the text.

The accuracy of the $C_p^o(T)$ values for the side-chains depends, in part, on the accuracy of the heat capacity data of the reference compound GlyGlyGly. Fig. 4 gives a comparison of the experimental partial molar heat capacity of triglycine determined by Makhatadze and Privalov [7] and in the present study. Inspection of the graph shows there is good agreement over the whole temperature range between the $C_p^o(T)$ curves of triglycine measured in water at pH 5.8. However, the differences between the $C_p^o(T)$ curves in 0.5 M sodium acetate buffer are quite marked. The temperature dependence of C_p^o obtained by Makhatadze and Privalov [7] is much greater than that observed in the present study. Although at 25°C the difference between the C_p^o values is not too great, at 100°C it amounts to about 60%. Similar effects are also observed for the peptides GlyMetGly and GlyILEGly. For GlyMETGly the literature C_p^o value [7] at 100°C (503.5 J K^{-1} mol^{-1}) is close to that obtained in this work whereas, as mentioned above, the literature value at 25°C is significantly lower than that found in the present study. For GlyILEGly the literature value at 100°C (681.4 J K^{-1} mol^{-1}) is about 20% greater than that found in this work.

While no explanation can be given at present for these differences, it seems significant that for GlyGlyGly the present and literature temperature dependent C_p^o curves measured in 0.5 M acetate buffer lie, respectively, below and above the same function for water at pH 5.8.

There is a further discrepancy that requires explanation. The partial molar heat capacity of triglycine was calculated using the residue increments given in ref. [7]. This resulted in the C_p^o values given by the open squares in Fig. 4. There is an obvious downshift deviation of the calculated heat capacity function above 50°C from the C_p^o curves for water. This is probably a consequence of typographical errors in Table 5 of ref. [7]. Based on the C_p^o measurements of oligoglycines (Table 4 in ref. [7]) an average C_p^o value at 125°C of 79.9 J K^{-1} mol^{-1} for the glycyl, $-CH_2-CO-NH-$, group can be obtained by subtraction (pentaglycine (520.4 J K^{-1} mol^{-1}) − tetraglycine (438.5 J K^{-1} mol^{-1}) = 81.9 J K^{-1} mol^{-1}; tetraglycine (438.5 J K^{-1} mol^{-1}) − triglycine (360.6 J K^{-1} mol^{-1}) = 77.9 J K^{-1} mol^{-1}; the average value is 79.9 J K^{-1} mol^{-1}). This quantity is used to calculate the heat capacity increment of the ($-NH_2$ + $-CH_2-COOH$) group from the C_p^o values of the oligoglycines (pentaglycine (520.4 J K^{-1} mol^{-1}) -4×79.9 J K^{-1} mol^{-1} = 200.8 J K^{-1} mol^{-1}; tetraglycine (438.5 J K^{-1} mol^{-1}) -3×79.9 J K^{-1} mol^{-1} = 198.8 J K^{-1} mol^{-1}; triglycine (360.6 J K^{-1} mol^{-1}) -2×79.9 J K^{-1} mol^{-1} = 200.8 J K^{-1} mol^{-1}; the average value is 200.1 J K^{-1} mol^{-1}). Finally, evaluation of the heat capacity increment of the end groups of proteins and oligopeptides ($-NH_2$ + $-CH-COOH$) requires subtraction of the heat capacity of the hydrogen atom, C_p^o(H). At 125°C this value is 53.9 J K^{-1} mol^{-1} (ref. [7]), which leads to a C_p^o value of the end groups of +146.2 J K^{-1} mol^{-1}. The difference between this value and that listed in Table 5 of ref. [7] C_p^o(125°C) is 296.2 J K^{-1} mol^{-1} (+146.2 J K^{-1} mol^{-1} −(−150 J K^{-1} mol^{-1})). If this value of 296.2 J K^{-1} mol^{-1} is used to correct the calculated C_p^o value at 125°C, shown in Fig. 4 by the open square, a heat capacity (solid square) is obtained (the arrow indicates the correction) that is in good agreement with the experimental curve and leads to an excellent agreement between the calculated and experimental heat capacity functions. It is evident, therefore, that

the heat capacity increments of the end group ($-NH_2$ + $-CH-COOH$) listed as negative by Makhatadze and Privalov [7] for 75, 100 and 125°C should, in fact, be positive. It should be mentioned in this context that in heat capacity calculations for proteins this error in the end group heat capacity increment results in negligibly small errors in the C_p^o value of the whole polypeptide because it is counted only once. However, for oligopeptides, use of the uncorrected parameters given in ref. [7] can lead to seriously incorrect values of the $C_p^o(T)$ function.

There is another factor that contributes to the discrepancy between the heat capacity results in this study and those determined in earlier work [1,7]. As shown in Fig. 3 we observe a concentration dependence of the apparent molar heat capacities that renders it necessary to extrapolate to infinite dilution in order to determine the partial molar heat capacity. Although the concentration ranges used in the earlier studies (3 to 15 mg ml^{-1} [1] and 3 to 9 mg ml^{-1} [7]) are similar to those used in this work, no concentration dependence of $C_{p,\phi}$ was reported. Concentration dependencies of $C_{p,\phi}$ for tripeptides in water have been observed previously [10,11], although the slopes of $C_{p,\phi}$ vs. molality are considerably smaller than those observed in the present study.

The partial molar heat capacity of the Asn side-chain, C_p^o(Asn), in water at 25°C is 179.4 J K^{-1} mol^{-1}. In the study by Makhatadze and Privalov [7] the small solute acetamide was used as the analogue for the side-chain of the amino acid Asn. The side-chain heat capacity was derived from the C_p^o for acetamide by subtracting the heat capacity for the H atom resulting in a C_p^o(Asn) value at 25°C in water of 88.8 J K^{-1} mol^{-1} which, is considerably smaller than that determined in this work. Furthermore, the results given in Table 5 show that C_p^o(Asn) is more or less independent of temperature over the range 10–100°C. In contrast, the C_p^o(Asn) values obtained using acetamide to model the side-chain increase with an increase in temperature [7] ($dC_p^o(Asn)/dT$ ca. 0.67 J K^{-2} mol^{-1}). As the side-chain in the peptide GlyAsnGly is adjacent to two peptide groups, which is structurally analogous to that in proteins, the side-chain heat capacity for Asn obtained in this study is likely to represent better that in a random-coil polypeptide than is that obtained using acetamide as a model compound.

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