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Thermochimica Acta 308 (1998) 23–34

thermochimica acta

Tripeptides in aqueous solution: Model compounds for the evaluation of the partial molar heat capacities of amino acid side-chains in proteins[1]

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Received 1 September 1996; accepted 18 December 1996

Abstract

The partial molar heat capacities of the tripeptides of sequence glycyl-X-glycine, where X is one of the amino acids glycine, alanine, valine, serine, tyrosine, lysine and aspartic acid, in aqueous solution have been determined over the 10–100°C range, using high sensitivity scanning microcalorimetry. Using these results, the partial molar heat capacities of the various amino acid side-chains were derived. The values obtained differ significantly from literature data that were derived using partial molar heat capacities for small organic compounds that model the various amino acid side-chains. A rationale has been proposed to account for the observed differences between the side-chain heat capacities derived using partial molar heat capacity data for the tripeptides and those derived using the partial molar heat capacities of the organic analogues. © 1998 Elsevier Science B.V.

1. Introduction

The thermodynamic stability of a protein in aqueous solution is defined by the differences in the Gibbs function between the native state, in which the protein is folded into its specific three-dimensional structure, and the denatured state in which the protein is in an unfolded form [1]. As the native state is more amenable to study than the denatured state through methods such as X-ray crystallography and NMR spectroscopy, discussion of the changes in protein stability that may occur, for example, following site-directed mutagensis, often has been focused on the native state alone [1,2]. This is understandable, since it is more likely that structural changes resulting from amino acid replacements cause more prominent changes in the interactions in the native state than in the unfolded state. It is, nevertheless, equally important to have a quantitatively characterized reference state for the calculation of stability differences [3].

The fully unfolded or random coil state of a globular protein in aqueous solution is generally used as the ideal references state in discussions on the thermodynamic stability of proteins [3,4]. This random coil state is not always experimentally accessible because, in the denatured state, proteins may still possess some residual folded structure [5]. Thermodynamic properties, such as heat capacity, of the random coil state can be estimated using a group additivity approach in which the partial molar heat capacity of the unfolded protein is obtained using a simple summation of the

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[1] Presented at the 14th IUPAC Conference on Chemical Thermodynamics, held in Osaka, Japan, 15–30 August, 1996.

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PII S0040-6031(97)00326-2

partial molar heat capacities of the constituent groups such as the amino acid side-chains, the peptide groups, and the ionic amino and carboxyl end-groups [6,7]. These group contributions are obtained using thermodynamic data for small solutes chosen to model the various constituent groups of a protein.

The amino acid side-chains of proteins can be modelled using tripeptides of sequence glycyl-X-glycine (gly-X-gly), where X is one of the amino acids [6,8–10]. In a previous work, we have determined the partial molar heat capacities of several of these peptides in aqueous solution at 25°C [8,9] and also over a broad temperature range for selected tripeptides [11,12]. In a comprehensive study to determine the partial molar heat capacities of all protein constituent groups over a wide temperature range, some small organic solutes were used as compounds to model some of the amino acid side-chains [6]. For several of these side-chains, the heat capacities obtained using the organic analogues differ significantly from the results obtained using the tripeptides [11,12]. In view of these discrepancies, we have determined the partial molar heat capacities of the tripeptides gly-X-gly, where X is one of the amino acids glycine alanine, valine, serine, tyrosine, lysine and aspartic acid, in aqueous solution over the 10–100°C range, using high sensitivity scanning microcalorimetry. The side-chain results reported herein are critically compared with those obtained using the organic analogues as model compounds [6].

2. Experimental

The glyglygly, glyalagly and glyvalgly used were samples remaining from earlier studies. The purification and analyses of these peptides have been described in detail elsewhere [8,12]. The sample of glysergly used was from a batch of material recovered from solutions used in a previous work [13]. The product was recrystallized from water-ethanol. The relative molar mass determined by alkalimetric titration [14,15] was 216.3 ± 2.2. This is slightly less than that expected for the anhydrous compound (219.20). Elemental analyses gave: C – 38.5, H – 6.1 and N – 19.0%, cf. calculated composition for $C_7H_{13}O_5N_3$; C – 38.4, H – 6.0 and N – 19.2%. The sample of glylysgly, as an acetate salt, was obtained from Bachem Feinchemikalien. A pH titration on a small amount of dried material indicated that the product contained some excess acetic acid. An attempted recrystallization of the material from the water–methanol–diethylether solvent mixture gave a gel-like product. This was removed by filtration, but on drying under vacuum to remove the trapped solvent, an oil rather than a solid was formed. On standing at room temperature for several days, a cream-coloured solid formed in the oil. Methanol was added to the oil + solid and the mixture was stirred at room temperature for two days. A creamy-white precipitate formed which was isolated by filtration, washed with methanol, and dried under vacuum to a constant weight. The peptide was chromatographically pure as determined by TLC. Alkalimetric titrimetry gave a relative molar mass of 326.4 ± 4.6 which is 1.9% higher than that for the anhydrous acetate salt (320.35). Elemental analyses gave: C – 43.6, H – 7.7 and N – 17.8%; cf. calculated for $C_{12}H_{24}O_6N_4$: C – 45.0, H – 7.6 and N – 17.5%. The glyaspgly sample, purchased from Bachem, was recrystallized from water + ethanol and dried under vacuum at room temperature for two days. The product was chromatographically pure as determined by TLC. Analysis by conventional pH titration gave a relative molar mass of 249.1 ± 1.7 which is almost in agreement, within the experimental uncertainty, with that for the anhydrous compound (247.21). Elemental analyses gave: C – 38.8, H – 5.3, N – 16.7%, cf. calculated for $C_8H_{13}O_6N_3$: C – 38.9, H – 5.3 and N – 17.0%. The sample of glytyrgly used was the solid recovered from solutions used in previous studies [16]. The material was recrystallized from water + ethanol. The relative molar mass determined by alkalimetric titrimetry was 298.3 ± 3.0 which is in agreement with that for the anhydrous compound (295.30). Elemental analyses gave: C – 53.0, H – 6.0 and N – 14.3%; cf. calculated for $C_{13}H_{17}O_4N_3$: C – 52.9, H – 5.8 and N – 14.2%.

The water used, to prepare solutions and as reference solvent, was deionized glass-distilled and thoroughly degassed immediately prior to use. The solutions of the peptides were prepared by mass. All the peptides, except glyvalgly monohydrate, were dried under vacuum at room temperature before use.

Heat capacity measurements were carried out using a DASM-1M differential scanning calorimetry (DSC) which has twin gold cells with a fixed operational

volume of 1 cm^3 [17]. Scans were made over the 10–100°C range at a scan rate of 1 K min^{-1} and at an operating pressure of 2 bar. Measurements were recorded every 0.1 K using a Keithley 192 programmable DMM connected to a personal computer. A baseline scan with water in both calorimeter cells was recorded prior to the measurement of each sample solution. For each peptide, measurements were made on a minimum of four solutions over a molality range of typically 0.02 to 0.06 mol kg^{-1} (ca. 5–15 mg cm^{-3}). In order to maximise the precision of the measurements, five or six scans were made for each sample solution. On completion of the measurements, the peptide solution was monitored for possible decomposition products using TLC.

Densities of solutions over the 10–85°C range were measured using a differential scanning densimetric system, comprising two matched Anton Paar DMA 602 HT cells coupled to a DMA 60 measuring unit. Details of the apparatus and operational procedures used have been described previously [11,18].

3. Results

The difference between the heat capacities of the solution and the solvent at a given temperature T, $\Delta c_p(T)$ ($\Delta c_p(T) = c_p(\text{solution}) - c_p(\text{solvent})$) is the quantity that is obtained from measurements made using the DSC instrument. The apparent molar heat capacity of each peptide at a temperature T, $C_{p,\phi}(T)$ was calculated from the $\Delta c_p(T)$ value using the equation [19]

$$C_{p,\phi}(T) = c_{p,1}(T)V_\phi(T)/\nu_1(T) + \Delta c_p(T)/n_2 \quad (1)$$

where $c_{p,1}(T)$ and $\nu_1(T)$ are, respectively, the specific heat capacity and specific volume of the solvent, $V_\phi(T)$ is the apparent molar volume of the solute, and n_2 is the number of moles of solute in the calorimeter cell. Values of the specific volume of water over the 10–100°C range were calculated using the equation given by Kell [20] which expresses the density as a power series in temperature. The specific heat capacity of water at a given temperature was calculated using an equation derived by fitting the heat capacity data, available in literature [21], to a fourth-order polynomial in temperature.

The apparent molar volumes of the peptides were obtained from the solution densities using the following equation

$$V_\phi = M_2/d - (d - d_0)/mdd_0 \quad (2)$$

where M_2 is the solute molar mass, m is the solution molality, and d and d_0 are, respectively, the densities of the solution and the solvent. The temperature dependence of V_ϕ was obtained by fitting the V_ϕ data to a power series in temperature of the form

$$V_\phi(T) = p_1 + p_2(T - 273.15) + p_3(T - 273.15)^2 \quad (3)$$

where p_1, p_2 and p_3 are the fitted coefficients. The apparent molar volumes required for the calculation of the apparent molar heat capacities were obtained using Eq. (3). The uncertainty in V_ϕ was estimated to be ± 0.5 cm^3 mol^{-1}. A summary of the molar volume data for the peptides is presented in Table 1. For the peptides glyglygly, glyalagly, glyvalgly and glysergly, a concentration dependence of V_ϕ was observed. As the $V_\phi(T)$ curves were approximately parallel, V_ϕ values at the selected temperatures of 25, 50 and

Table 1
The coefficients of Eq. (3) and the S_v values for the tripeptides

Peptide	p_1/ (cm^3 mol^{-1})	p_2/ (cm^3 mol^{-1} K^{-1})	10^4 p_3/ (cm^3 mol^{-1} K^{-2})	S_v/ (cm^3 kg mol^{-2})
glyglygly	108.4	0.167	−8.68	3.2
glyalagly	127.8	0.125	−4.28	6.0
glyvalgly	156.5	0.165	−5.36	15
glysergly	126.1	0.182	−9.87	17
glytyrgly	189.6	0.235	−8.19	[a]
glyaspgly	138.8	0.225	−10.9	[a]
$glylysgly^+$ Ac^- [b]	212.3	0.234	−10.6	[a]

[a] No concentration dependence of V_ϕ was observed.
[b] Ac^- is the acetate ion.

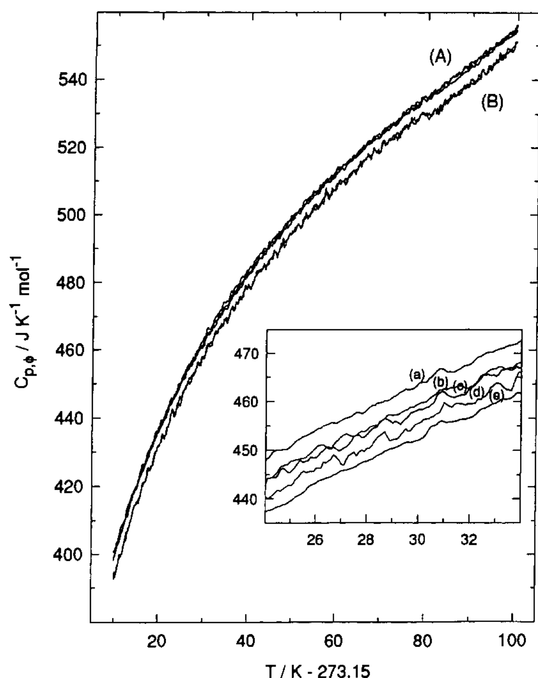

Fig. 1. Temperature dependence of the apparent molar heat capacity of the tripeptide glyvalgly in aqueous solution: curve (A), $m = 0.06340$ mol kg^{-1}, three successive scans are shown; curve (B), $m = 0.03010$ mol kg^{-1}, two scans are shown. The insert shows a portion of a single scan for solutions at the molalities (a) – 0.06340 mol kg^{-1}, (b) – 0.04707 mol kg^{-1}, (c) – 0.03010 mol kg^{-1} and (d) – 0.02316 mol kg^{-1}. Curve (e) is a portion of the plot of $C^0_{p,2}$ for glyvalgly against temperature.

75°C were analysed by linear least-squares using the equation

$$V_\phi = V_2^0 + S_v m \quad (4)$$

to obtain an average value for the experimental slope, S_v. Using the average S_v, the temperature dependences of the partial molar volumes of the peptides at infinite dilution, V_2^0, were determined. These results are given in Table 1. For the remaining peptides, a concentration dependence of V_ϕ was not discernable. The results summarized in Table 1 were obtained by fitting the V_ϕ data at all concentrations to Eq. (3).

Some experimental $C_{p,\phi}(T)$ data for the peptide glyvalgly are shown in Fig. 1. The excellent reproducibility of successive scans is illustrated for both curves displayed in the figure. There are three scans shown for curve (a) (0.06340 mol kg^{-1}, 14.47 mg cm^{-3}) and two for curve (b) (0.03010 mol kg^{-1}, 6.91 mg cm^{-3}). The data presented also indicate that, for the peptide glyvalgly, a concentration dependence of $C_{p,\phi}$ was observed. In the interest of clarity the complete curves of all four solutions studied are not given. The figure insert using an expanded scale illustrates a small portion of the $C_{p,\phi}$ data for all solutions and also for the curve for $C^0_{p,2}$ (see in the following).

The procedure used to obtain the temperature dependence of the partial molar heat capacity of the peptide at infinite dilution is as follows. The $C_{p,\phi}$ data obtained at each peptide concentration were fitted to a polynomial of the form

$$C_{p,\phi} = a + b(T - 273.15) + c(T - 273.15)^2 + d(T - 273.15)^3 \quad (5)$$

where a, b, c and d are the fitted coefficients. This cubic function is the lowest order polynomial that gave a good representation of the experimental heat capacity data over the temperature range of interest. It should be mentioned that the coefficients obtained have no theoretical meaning. They are merely the quantities that enable $C_{p,\phi}$ to be derived at any given temperature within the 10–100°C range. As the $C_{p,\phi}(T)$ curves at the various peptide concentrations were approximately parallel, $C_{p,\phi}$ values at each of the three selected temperatures, 25, 50 and 75°C, were fitted to the equation

$$C_{p,\phi} = C^0_{p,2} + S_c m \quad (6)$$

where $C^0_{p,2}$ is the partial molar heat capacity of the solute at infinite dilution and S_c is the experimental slope. The mean S_c value was then used to convert $C_{p,\phi}$ to $C^0_{p,2}$ values at all temperatures using Eq. (6). The $C^0_{p,2}$ data obtained were analysed using a polynomial in temperature analogous to Eq. (5) but with the $C_{p,\phi}$ term replaced by $C^0_{p,2}$. The coefficients obtained are given in Table 2. Concentration dependences of $C_{p,\phi}$ were also observed for the tripeptides glyglygly, glyalagly and glysergly, but not for the other peptides studied. For the peptides glytyrgly and glyaspgly, the concentration ranges that could be used were limited (0.017–0.032 and 0.014–0.018 mol kg^{-1} respectively) because of the low solubility of these compounds in water. For these peptides and for the acetate salt of glylysgly, the coefficients given in Table 2 were obtained by fitting all the $C_{p,\phi}$ data to the polynomial

Table 2
Coefficients from the fitting of experimental heat capacities to Eq. (5), and the S_c values

Peptide	a/ ($J K^{-1} mol^{-1}$)	b/ ($J K^{-2} mol^{-1}$)	c/ ($J K^{-3} mol^{-1}$)	$10^4 d$/ ($J K^{-4} moJ^{-1}$)	S_c/ ($J kg K^{-1} mol^{-2}$)	m [a]/ ($mol kg^{-1}$)
glyglygly	71.2	6.16	−0.0671	3.23	23 (15) [e]	0.035–0.081
glyalagly	192.1	5.39	−0.059	2.98	54 (28)	0.027–0.079
glyvalgly	347.7	4.74	−0.0482	2.06	120 (24)	0.023–0.063
glysergly	139.4	5.80	−0.0562	2.35	110 (12)	0.027–0.072
glytyrgly	324.5	6.63	−0.0712	3.3	[b]	0.017–0.032
glyaspgly [c]	145.6	6.92	−0.0780	3.4	[b]	0.014–0.018
glylysgly$^+$ Ac$^-$ [d]	319.5	7.58	−0.0603	2.75	[b]	0.023–0.048

[a] Molality range used.
[b] No concentration dependence of $C_{p,\phi}$ was observed.
[c] Coefficients apply to the 10–80°C range.
[d] Ac^- is the acetate ion.
[e] Standard deviations are in parentheses.

in temperature using Eq. (5). The uncertainties in the partial molar heat capacities were estimated by the application of propagation of errors to Eq. (1) with the assumption that the uncertainties in the specific volume and heat capacity of the solvent make a negligible contribution to the error in $C_{p,\phi}$.

Successive calorimetric scans for solutions of the peptide glyaspgly were not coincident as observed for the other peptides. There was a trend towards a smaller solution heat capacity with increasing scan number. Thin-layer chromatograms of the solutions following several calorimetric scans over the 10–100°C range indicated that peptide hydrolysis had occurred. There was also evidence of some hydrolysis in a single run using the differential densimetric system up to 90°C. However, for a single scan from 10° to 80°C using both the DSC and the densimetric system, TLC measurements indicated that the extent of hydrolysis was negligible up to this temperature. The calorimetric data used in the analysis were taken from the first scan over the 10–80°C range.

It should be stressed that the $C_{p,\phi}$ results obtained for the peptide glyaspgly do not only pertain to the unionized form of the side-chain carboxylic acid group. As the side-chain ionizes to a small extent, there are contributions to the measured apparent molar heat capacity of the peptide from both, the unionized and ionized forms of the side-chain. In order to obtain the partial molar heat capacity of the peptide with an unionized side-chain, a correction has to be made for the degree of ionization of the acidic side-chain. For this analysis, values of the acid dissociation constant over the temperature range of interest are required but, to-date, these data have not been determined. It is likely that the differences between the partial molar heat capacities of the peptide with, and without, the ionization corrections will be similar to those for the amino acid *L*-aspartic acid. Hakin et al. [22] recently reported the partial molar heat capacities of *L*-aspartic acid that were corrected for ionization effects. From the analysis of their results obtained over the 15–55°C range, the value of the partial molar heat capacity of the amino acid with an unionized side-chain is about 11% higher than that obtained if no corrections were made [22].

For the peptide glyglygly, the partial molar heat capacities over wide temperature ranges have been determined in previous studies using both DSC [6] and differential flow calorimetric [12] methods. A comparison of these results with those obtained in this work is shown in Fig. 2. In general, there is good agreement among the $C^0_{p,2}$ results for glyglygly in water obtained in the various studies. A comparison of the partial molar heat capacities of glyalagly, determined in this work, with values determined at specific temperatures in the previous study [12] using flow calorimetry is also shown in Fig. 2. The agreement between the results obtained in these two studies is good.

The partial molar heat capacities at infinite dilution and at the single temperature of 25°C have been reported previously [6,8,16,23,24] for all the peptides in this study. Table 3 gives a comparison of these results with those calculated using Eq. (5) and the

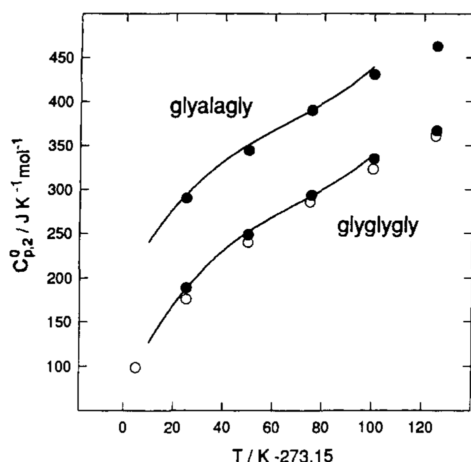

Fig. 2. Comparisons of the partial molar heat capacities of glyglygly and glyalagly data available in literature. The curves have been drawn using Eq. (5) and the parameters given in Table 2: (●) – from Ref. [12] and (○) – from Ref. [6].

Table 3
A comparison of the partial molar heat capacities of the tripeptides in aqueous solution with some data at 25°C available in literature

Tripeptide	$C^0_{p,2}$/(J K^{-1} mol^{-1})	
	this work	literature
glyglygly	188 (3) [a]	188.3 (0.7) [b], 185.9 (0.9) [c], 187 (1) [d], 175 (5) [e]
glyalagly	295 (4)	289.8 (0.3) [b]
glyvalgly	439 (5)	443.6 (0.7) [f]
glysergly	253 (3)	263 (1) [f]
glytyrgly	451 (4)	449 (2) [d]
glyaspgly	275 (4)	283 (4) [d]
glylysgly$^+$ Ac$^-$ [g]	476 (5)	495 (3) [d]

[a] Standard deviations are in parentheses.
[b] From Ref. [23].
[c] From Ref. [24].
[d] From Ref. [16].
[e] From Ref. [6].
[f] From Ref. [8].
[g] Ac$^-$ is the acetate ion.

polynomial parameters given in Table 2. The agreement between the $C^0_{p,2}$ results for the tripeptides determined using DSC measurements and those using the Picker flow calorimeter [8,16,23,24] are within the combined estimated uncertainties, with the exception of the peptides glysergly and the acetate salt of glylysgly. Even for these two peptides, the differences between the $C^0_{p,2}$ values are not large (ca. 5%).

4. Discussion

The partial molar heat capacities of the tripeptides of sequence glycyl-X-glycine, $C^0_{p,2}$ (gly-X-gly), along with the partial molar heat capacity of triglycine, $C^0_{p,2}$ (glyglygly), can be used to estimate the heat capacities of the side-chains of amino acid X. In some of our earlier work [8,9], the side-chain contribution to the heat capacity was derived by taking the difference between the heat capacity for the peptide gly-X-gly and that for triglycine,

$$C^0_p[(\mathrm{R})-(\mathrm{H})] = C^0_p(\text{gly-X-gly}) - C^0_p(\text{glyglygly}) \quad (7)$$

It should be stressed that the quantity $C^0_p[(\mathrm{R}) - (\mathrm{H})]$ is not the absolute heat capacity of the side-chain *R*, but gives the contribution to the heat capacity on replacing a C–H group by a C–R group. These $C^0_p[(\mathrm{R}) - (\mathrm{H})]$ values for the various amino acid side-chains, along with estimates of the heat capacities of the glycyl unit, -CH$_2$CONH-, and the ionic end groups of a polypeptide are the quantities that are required to estimate the heat capacity of an unfolded protein in aqueous solution. Rather than calculating $C^0_p[(\mathrm{R}) - (\mathrm{H})]$ values, an alternative approach that can be used is to estimate the partial molar heat capacity of the hydrogen atom, $C^0_p(\mathrm{H})$, and thereby calculate the absolute value of the heat capacity contribution of a side chain, $C^0_p(\mathrm{R})$ using the equation

$$C^0_p(\mathrm{R}) = C^0_{p,2}(\text{gly-X-gly}) - C^0_{p,2}(\text{glyglygly}) + C^0_p(\mathrm{H}) \quad (8)$$

These $C^0_p(\mathrm{R})$ values can be combined with the heat capacities of the -CHCONH- unit of the polypeptide and the ionic end groups to estimate the heat capacity of an unfolded protein. In some of our recent studies [11,12], we have also used Eq. (8) to calculate side-chain heat capacities. One advantage of this latter approach is that it enables direct comparisons to be made with side-chain heat capacities estimated using the partial molar heat capacities of other compounds chosen to model the amino acid side-chains [6]. The side-chain heat capacities reported in this work were derived using Eq. (8).

In the study by Makhatadze and Privalov [6], various small organic solutes were chosen as compounds to model the side-chains of many of the

naturally occurring amino acids. For example, the side-chains of the amino acids, ala, val, ser, try, asp, lys^+, which are those relevant to this study, were modelled using the organic compounds methane, propane, methanol, 4-methylphenol, acetic acid and n-butanamine, respectively. The value of each side-chain heat capacity was obtained by subtracting from the partial molar heat capacity of the organic analogue, an estimated value of the heat capacity of the hydrogen atom. At 25°C, the estimated value for $C_p^0(\mathrm{H})$ was taken as 78 J K^{-1} mol^{-1}. This result is the mean of four estimates of the heat capacity of the hydrogen atom taken from the literature [6]. At other temperatures, the value of $C_p^0(\mathrm{H})$ was obtained from the difference between the partial molar heat capacities of the CH_3 and CH_2 groups [6].

For the purpose of comparison, we have chosen, firstly, to use the same values of $C_p^0(\mathrm{H})$ as used by Makhatadze and Privalov. The values given [6] for 5°, 25°, 50°, 75°, 100° and 125°C were fitted with unit weighting to a third-order polynomial in temperature to give

$$C_p^0(\mathrm{H}) = 83.41 - 0.215(T - 273.15) - 3.00 \times 10^{-4}(T - 273.15)^2 + 1.05 \times 10^{-6}(T - 273.15)^3 \quad (9)$$

with a correlation coefficient of 0.9994. This expression, along with those for the peptides gly-X-gly and for triglycine given in Table 2, were used to calculate the side-chain heat capacities over the 10–100°C range, using Eq. (8). The results obtained are displayed in Figs. 3–5.

A comparison is given in Fig. 3(a) of the results for the alanyl side-chain with those derived previously using $C_{p,2}^0$ data for methane [6] and the tripeptides [12]. There is good agreement, within the combined experimental uncertainties, between the $C_p^0(\mathrm{ala})$ results obtained in this work and those derived using tripeptide $C_{p,2}^0$ data that were obtain using flow calorimetry [12]. The $C_p^0(\mathrm{ala})$ results obtained using the $C_{p,2}^0$ data for methane are smaller than those calculated from $C_{p,2}^0$ data for the tripeptides. The $C_{p,2}^0$ data for methane were derived using $C_p^0(\mathrm{g})$ values and heat capacity of solution data determined over the 0–50°C range. It should be stressed that the results above 50°C are based on the assumption that the temperature dependence of $C_{p,2}^0$ is the same as that below 50°C [6].

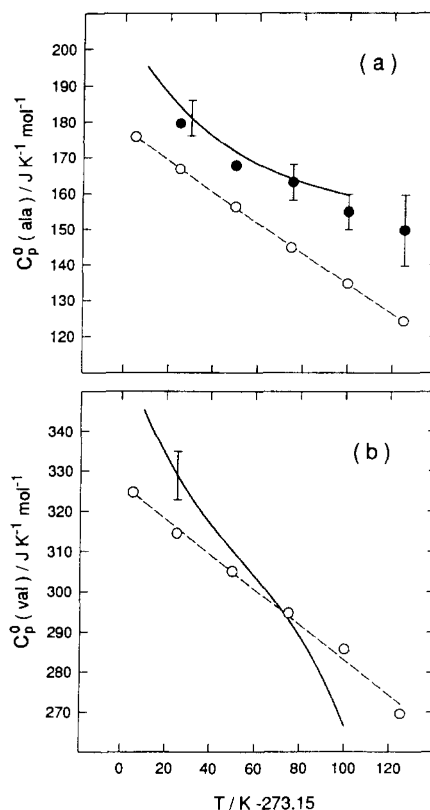

Fig. 3. Partial molar heat capacities of the alanyl and valyl side-chains: (a) – alanyl side-chain, (———) this work; (●) from Ref. [12]; (– ○ –) from Ref. [6]. The C_p^0 values were derived from $C_{p,2}^0$ data for methane: (b) – valyl side-chain, (———) this work; (– ○ –) from Ref. [6]. The C_p^0 values were derived from $C_{p,2}^0$ data for propane.

In Fig. 3(b), the heat capacity of the valyl side-chain derived using peptide data is compared with results based on propane as the model compound. The $C_{p,2}^0$ data for propane were derived using the same procedure and assumption as outlined above for methane. As the source of the $C_p^0(\mathrm{g})$ data used was not given by Makhatadze and Privalov [6], the uncertainties in the $C_p^0(\mathrm{val})$ values derived using the propane heat capacities cannot be assessed. The results presented in Fig. 3(b) indicate that although the differences, between $C_p^0(\mathrm{val})$ values at any given temperature, are not excessively large, there is a significant difference between the temperature dependence of $C_p^0(\mathrm{val})$ obtained using tripeptide data and that derived using heat capacity data for propane.

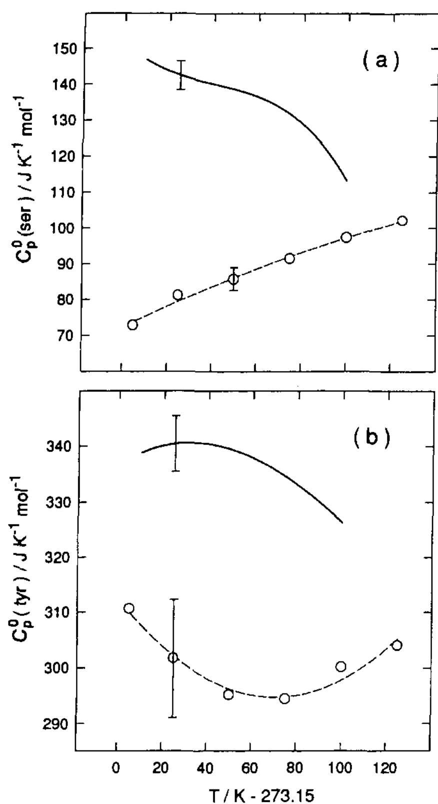

Fig. 4. Partial molar heat capacities of the seryl and tyrosyl side-chains: (a) – seryl side-chain, (———) this work; (– o –) from Ref. [6]. The C_p^0 values were derived from $C_{p,2}^0$ data for methanol: (b) – tyrosyl side-chain, (———) this work; (– o –) from Ref. [6]. The C_p^0 values were derived from $C_{p,2}^0$ data for 4-methylphenol.

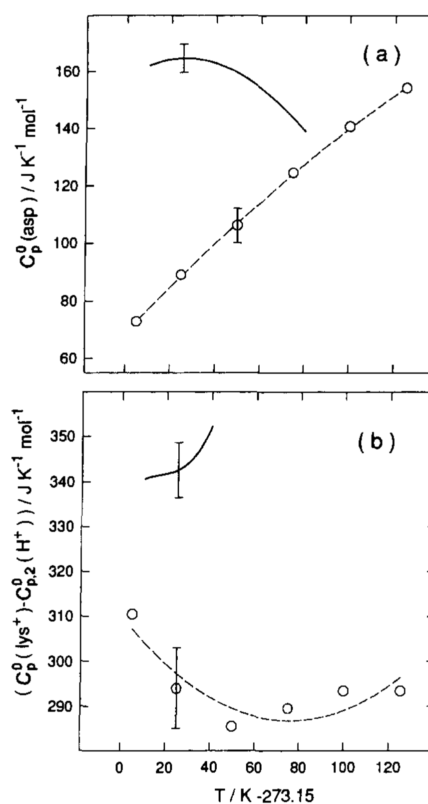

Fig. 5. Partial molar heat capacities of the aspartyl and lysyl side-chains: (a) – aspartyl side-chain, (———) this work; (– o –) from Ref. [6]. The C_p^0 values were derived from $C_{p,2}^0$ data for acetic acid: (b) – lysyl side-chain. The quantity determined is $C_p^0(\mathrm{lsy}^+)$-$C_{p,2}^0(\mathrm{H}^+)$, see text. (———) this work; (– o –) based on data from Ref. [6].

The results shown in Fig. 4(a) indicate that there are very significant differences between the heat capacity of the seryl side-chain derived using methanol as a model compound and that based on the tripeptides. At 25°C, the difference between the C_p^0(ser) values is 64 J K^{-1} mol^{-1}, which is 44% of the value determined using tripeptide $C_{p,2}^0$ data. Furthermore, the temperature dependence of C_p^0(ser), determined using methanol as a model compound, has the opposite sign to that determined using the tripeptides. A similar effect is observed for the tyrosyl side-chain as shown in Fig. 4(b). The C_p^0(tyr) values determined in this study are ca. 30–40 J K^{-1} mol^{-1} larger than those derived using $C_{p,2}^0$ data for 4-methylphenol. The large experimental uncertainties associated with the C_p^0(tyr) values obtained by Makhatadze and Privalov [6] negate a meaningful comparison of the observed temperature dependence with that obtained in this study.

In Fig. 5(a), a comparison is given between the heat capacities of the aspartyl side-chain derived using the tripeptides and acetic acid as model compounds. The results obtained are similar to those shown in Fig. 4(a) for the polar seryl side-chain. The C_p^0(asp) values obtained using the organic analogue acetic acid increase with an increase in temperature, whereas the results based on the tripeptides show a more complex temperature dependence. Over the 30–70°C range, the C_p^0(asp) values decrease with an increase in temperature, in marked contrast to the results based on acetic acid.

The tripeptide glylysgly used in this study was obtained as an acetate salt. In order to obtain the

partial molar heat capacity of the cationic peptide, a scale of single-ion heat capacities, based on some suitable extrathermodynamic assumption, is required. Single-ion heat capacities have been derived [25], but only at 25°C. Consequently, it is not possible to obtain, using Eq. (8), the absolute value of the heat capacity of the protonated lysine side-chain over a wide temperature range.

The organic analogue used by Makhatadze and Privalov [6] to model the lysine side-chain was the nitrate salt of *n*-butanamine. The results obtained for this compound can be compared with those in this study using a method that enables the contributions from the anions to be eliminated. Since the standard state partial molar heat capacities of cations and anions are additive, $C^0_{p,2}$ data for the strong electrolytes HCl, NaCl and sodium acetate, NaAc, were combined with the $C^0_{p,2}$ results for the tripeptide to obtain the difference between the partial molar heat capacities of the tripeptide cation glylysgly$^+$ and the proton using the equation

$$C^0_{p,2}(\text{glylysgly}^+) - C^0_{p,2}(\text{H}^+) = C^0_{p,2}(\text{glylysglyAc}) - C^0_{p,2}(\text{NaAc}) + C^0_{p,2}(\text{NaCl}) - C^0_{p,2}(\text{HCl}) \quad (10)$$

Values of $C^0_{p,2}$ for the electrolytes NaCl and HCl were obtained by fitting selected literature data to polynomials in temperature of the form

$$C^0_{p,2} = a + b(T - 273.15) + c(T - 273.15)^2 + d(T - 273.15)^3 + e(T - 273.15)^4 \quad (11)$$

The values of the fitted coefficients are given in Table 4. Unfortunately, for the electrolyte NaAc, $C^0_{p,2}$ data obtained using flow microcalorimetry are available only over the narrow 10–40°C range [33]. The coefficients obtained by fitting the data to a second-order polynomial in temperature are also given in Table 4. The values for the quantity $C^0_{p,2}(\text{glylysgly}^+) - C^0_{p,2}(\text{H}^+)$ over the 10–40°C range were combined with $C^0_{p,2}$(glyglygly) and C^0_p(H) data to obtain, using an expression analogous to Eq. (8), the quantity $C^0_p(\text{lys}^+) - C^0_{p,2}(\text{H}^+)$, where $C^0_p(\text{lys}^+)$ is the partial molar heat capacity of the protonated lysine side-chain. These results are displayed in Fig. 5(b). Also shown in Fig. 5(b) are values of $C^0_p(\text{lys}^+) - C^0_{p,2}(\text{H}^+)$ obtained by subtracting from the $C^0_{p,2}$ value for *n*-butanamine nitrate, the $C^0_{p,2}$ value for HNO_3 and the partial molar heat capacity of the hydrogen atom C^0_p(H) [6]. Although the comparison is restricted to a narrow temperature range, it is clear that the heat capacity of the lys$^+$ side-chain estimated using the tripeptides is ca. 40 J K^{-1} mol^{-1} larger than that obtained using the organic anaologues.

A perusal of the result displayed in Figs. 3–5 indicates that although the agreement between the heat capacities of the hydrophobic side-chains derived using the different model compounds is not good, there are at least some similarities – which is certainly not the case for the hydrophilic side-chains. These differences for the polar side-chains are perhaps surprising in view of the results of some comparisons that can be made among the $C^0_{p,2}$ data for the organic analogues and the tripeptides. Some of these comparisons are given in Table 5. The differences between the partial molar heat capacities of pairs of organic analogues and pairs of tripeptides that model the same amino acid side-chains are very similar, as shown in columns 4 and 7 of Table 5. These comparisons suggest that the origin of the differences between

Table 4
Coefficients for the calculation of the $C^0_{p,2}$ values of electrolytes as functions of temperature using Eq. (11)

Electrolyte	*a*/ (J K^{-1} mol^{-1})	*b*/ (J K^{-2} mol^{-1})	*c*/ (J K^{-3} mol^{-1})	*d*/ (J K^{-4} mol^{-1})	*e*/ (J K^{-5} mol^{-1})
NaCl [a]	−180.89	6.762	−0.1394	1.25×10^{-3}	-4.27×10^{-6}
HCl [b]	−169.87	2.495	−0.0322	8.54×10^{-5}	3.06×10^{-7}
NaAc [c,d]	−15.24	4.63	−0.0524		

[a] $C^0_{p,2}$ data over the 1.5–100°C range were taken from Refs. [26–30].
[b] $C^0_{p,2}$ data over the 10 to 102.6°C range were taken from Refs. [29,31,32].
[c] Ac is the acetate ion.
[d] $C^0_{p,2}$ data over the 10–40°C range were taken from Ref. [33].

Table 5
A comparison of the differences between the partial molar heat capacities of pairs of compounds in aqueous solution

$T/K-273.15/(°C)$	Organic analogue	$C^0_{p,2}$ [a]/ ($J\ K^{-1}\ mol^{-1}$)	$\Delta C^0_{p,2}$ [b]/ ($J\ K^{-1}\ mol^{-1}$)	Peptide	$C^0_{p,2}$/ ($J\ K^{-1}\ mol^{-1}$)	$\Delta C^0_{p,2}$/ ($J\ K^{-1}\ mol^{-1}$)
25	CH_3COOH	167 (6) [c]		glyaspgly	275 (4)	
			0 (9)			−7 (5)
25	CH_3CONH_2	167 (6)		glyasngly	282 (2) [d]	
50	CH_3COOH	178 (6)		glyaspgly	339 (4)	
			−4 (9)			−10 (5)
50	CH_3COHN_2	182 (6)		glyasngly	349 (3) [d]	
25	CH_3COOH	167 (6)		glyaspgly	275 (4)	
			9 (7)			20 (5)
25	CH_3OH	158 (3)		glysergly	255 (3)	
50	CH_3COOH	178 (6)		glyaspgly	339 (4)	
			19 (7)			22 (5)
50	CH_3OH	159 (3)		glysergly	317 (3)	

[a] From Ref. [6].
[b] $\Delta C^0_{p,2} = C^0_{p,2}$ (line 1)–$C^0_{p,2}$ (line 2).
[c] Standard deviations are in parentheses.
[d] From Ref. [11].

the heat capacities of hydrophilic side-chains derived using tripeptides and small organic solutes is unlikely to arise from errors in the $C^0_{p,2}$ data. It follows then that a closer examination of the C^0_p(H) values used in the calculation of side-chain heat capacities is warranted.

At 25°C, the value of C^0_p(H) suggested by Makhatadze and Privalov [6] is 78 $J\ K^{-1}\ mol^{-1}$. This value seems to be a reasonable estimate of the heat capacity contribution of a hydrogen atom attached to a carbon atom when part of a hydrocarbon chain. The question that arises is whether or not this is an appropriate value to use when the H group is adjacent to a polar group or, in the case of the tripeptides, flanked by two polar peptide groups. As part of a study of some *N*-acetyl amino acid and peptide amides [34] in aqueous solution at 25°C, the partial molar heat capacities of these peptide derivatives, along with those of some simple amides, were analysed using a group-additivity approach based on the equation

$$C^0_{p,2} = \sum C^0_p(X_i)n_i \quad (12)$$

In Eq. (12), $C^0_p(X_i)$ is the contribution to the partial molar heat capacity of the solute from a group of type X_i and n_i is the number of such groups. The value obtained for the heat capacity of the hydrogen group was 45 $J\ K^{-1}\ mol^{-1}$ which is significantly smaller than the value of 67 $J\ K^{-1}\ mol^{-1}$, obtained from an earlier analysis of just the simple amides using the same set of groups [35]. Differences were also observed in the values of other group contributions, in particular that of the peptide group [34]. These results clearly demonstrated that the set of group coefficients obtained from the simple amides [35] are of limited predictive utility for more complex molecules. As the *N*-acetyl amino acid and peptide amides have structural similarities to the tripeptides used in this study, a C^0_p(H) value of 45 $J\ K^{-1}\ mol^{-1}$ is probably a better estimate to use in the calculation of side-chain heat capacities at a temperature of 25°C. The side-chain heat capacities calculated using Eq. (8) and a C^0_p(H) value of 45 $J\ K^{-1}\ mol^{-1}$ are given in Table 6 along with the results reported by Makhatadze and Privalov [6]. The overall agreement between the side-chain heat capacities derived using the organic analogues and those based on the tripeptides with a C^0_p(H) value of 45 $J\ K^{-1}\ mol^{-1}$ (column B of Table 6) is better than when the C^0_p(H) value suggested by Makhatadze and Privalov is used (column A). Furthermore, as acetamide was an amide included in the group contribution analysis discussed above, a C^0_p(H) value of 45 $J\ K^{-1}\ mol^{-1}$ is perhaps more appropriate to use in deriving the heat capacity of the asparagyl side-chain from the $C^0_{p,2}$ value of acetamide. The result obtained is in closer agreement to that derived using the tripeptide model compounds.

For the lowest members of some homologous series of compounds such as the diols or diamines, there are substantial deviations from linearity in the plots of

Table 6
The side-chain heat capacities in aqueous solution at 25°C calculated using different C_p^0(H) values

Side-chain (R)	C_p^0(R)/(J K^{-1} mol^{-1})			
	tripeptide		analogue [a]	
	A [b]	B [c]	A [b]	B [c]
ala	185	152	167	—
val	329	296	314	—
leu	416 [d]	383 [d]	382	—
ile	421 [d]	388 [d]	402	—
ser	143	110	81	—
thr	229 [d]	196 [d]	185	—
asn	169 [d]	136 [d]	89	122
asp	165	132	89	—
tyr	341	308	302	—

[a] From Ref. [6].
[b] Derived using a C_p^0(H) value of 78 J K^{-1}mol^{-1}.
[c] Derived using a C_p^0(H) value of 45 J K^{-1} mol^{-1}.
[d] Derived using $C_{p,2}^0$ data from Ref. [9].

$C_{p,2}^0$ against the number of methylene groups in the series [36]. These deviations presumably arise because of the mutual interactions between the polar functional groups and the intervening methylene groups. Based on these observations, it could be argued that a C_p^0(H) value of 78 J K^{-1} mol^{-1} is also inappropriate for the model compounds methanol and acetic acid because the hydrogen group is adjacent to the polar functional groups. A reduction in this C_p^0(H) value would also lead to better agreement with the results based on the tripeptide model compounds.

We have been able to rationalize, with some degree of success, the differences between the side-chain heat capacities at 25°C derived using tripeptide and organic analogues. However, as the temperature dependence of the C_p^0(H) group derived using the series of simple amides and amide derivatives has not been determined, we are unable to extend the analysis to other temperatures.

In summary, we have demonstrated that there are significant differences between the side-chain heat capacities derived using $C_{p,2}^0$ data for tripeptides and those for simple organic side-chain analogues. Some of the observed differences at 25°C can be rationalized if a C_p^0(H) value of 45 J K^{-1} mol^{-1} is used in the derivation of the side-chain heat capacities using the tripeptide model compounds. As the side-chain in peptides of sequence gly-X-gly are structurally the same as those found in proteins, heat capacities derived using these model compounds should give a good representation of the side-chain heat capacities in an unfolded protein in aqueous solution.

Acknowledgements

We are grateful for the financial assistance from the NZ/FRG Scientific and Technological Cooperation Agreement and from the University of Münster.

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