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The partial molar heat capacity and volume of the peptide backbone group of proteins in aqueous solution

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Abstract

The partial molar heat capacities have been determined for the series of peptides alanyl(glycyl)$_x$glycine, x = 1–3, and for the compounds *N*-acetylglycinamide and *N*-acetylglycylglycinamide in aqueous solution over the temperature range 10–100°C using high sensitivity scanning microcalorimetry. The partial molar volumes for these compounds have also been determined over the temperature range from 10 to 90°C using a scanning densimetric method. The results were used to derive the partial molar heat capacities and volumes of the glycyl group at temperatures in the range 10–100°C. The results obtained are critically compared with literature results derived using heat capacity and volume data for some oligoglycines.

Keywords: Partial molar heat capacity; Partial molar volume; Glycyl group; Model compound; Unfolded protein

1. Introduction

The partial molar heat capacities of the native and denatured states of proteins as functions of temperature are thermodynamic properties that are important in the quantitative determination of the stabilities of proteins in aqueous solution [1]. The heat capacity of the native state can be determined experimentally using high sensitivity differential scanning calorimetry (DSC) [1–3]. However, for many proteins the denatured or unfolded state does still possess some residual folded structure so in these cases the random coil state, which is used as the ideal reference state in discussions of the thermodynamic stability of proteins, is not experimentally accessible [1]. It is useful, therefore, to have a means of obtaining the heat capacity of the random coil form of a protein by some indirect method. One approach

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has been to use a group additivity scheme in which it is assumed that the partial molar heat capacity of the fully unfolded form of a protein is given by a simple summation of the partial molar heat capacities of the constituent groups [4,5]. These group contributions are obtained using heat capacity data for small solutes chosen to model the various constituent groups of a protein.

The partial molar heat capacity at 25°C of the backbone glycyl unit of a protein, $C_p(CH_2CONH)$, has been evaluated in several studies [4,6–9] using heat capacity data for a variety of model compounds. However, as indicated in the brief survey that follows, the results obtained in these studies are far from concordant. Jolicoeur and Boileau [6] derived a value for the heat capacity of the glycyl group using heat capacity data for the series of oligoglycines glycine to pentaglycine. For the three peptides tri-, tetra- and pentaglycine, the incremental contribution to the heat capacity reached a constant value leading to a glycyl unit contribution of 98 ± 5 J K^{-1} mol^{-1}. From an analysis of the partial molar heat capacities of some neutral cyclic compounds in aqueous solution at 25°C, Cabani et al. [7] reported a value for $C_p(CH_2CONH)$ of 75.3 J K^{-1} mol^{-1}. A group contribution analysis of the partial molar heat capacities of several simple amides and some *N*-acetyl amino acid and peptide amides in aqueous solution at 25°C gave a value for $C_p(CH_2CONH)$ of 77 J K^{-1} mol^{-1} [8]. The three peptides tri-, tetra- and pentaglycine were also used by Makhatadze and Privalov [4] to obtain values of the heat capacity of the glycyl group over the temperature range 5–125°C. The $C_p(CH_2CONH)$ value at 25°C reported by Makhatadze and Privalov is 93.2 ± 6.2 J K^{-1} mol^{-1}. The partial molar heat capacity of the glycyl group has also been estimated using heat capacity data for amino acids and dipeptides [9]. However, because of the influence of the charged amino and carboxyl groups in these molecules [8,10,11], the result obtained is not a reliable estimate of the heat capacity of a glycyl unit in unfolded polyglycines.

The disparity among these estimates of the heat capacity of the glycyl group at 25°C is of some concern because of the significant contribution that this group makes to the total heat capacity of an unfolded protein. Moreover, in the one study [4] to determine the temperature dependence of $C_p(CH_2CONH)$, data are available at only five temperatures in the range 5–100°C. Although the method to determine $C_p(CH_2CONH)$ using the oligoglycines as model compounds is sound in principle, there are some practical difficulties. The higher members of the series, e.g. tetra-, penta- and hexaglycine, are not very soluble in water which means the uncertainties in the partial molar heat capacities of these compounds are large. This in turn leads to a high uncertainty in the estimated value of $C_p(CH_2CONH)$. These difficulties can be overcome by using an alternative homologous series in which the peptides are more soluble in water than the corresponding members of the oligoglycines. In the homologous peptide series of sequence alanyl$(glycyl)_x$glycine, x = 1–3, the tetra- and pentapeptides are significantly more soluble in water than tetra- and pentaglycine. This paper reports the partial molar heat capacities of the three peptides alanylglycylglycine (ala$(gly)_2$) alanylglycylglycylglycine (ala$(gly)_3$) and alanylglycylglycylglycylglycine (ala$(gly)_4$) in aqueous solution determined at 25°C using flow microcalorimetry and over the temperature range 10–100°C using high sensitivity scanning microcalorimetry (DSC).

An estimate of the partial molar heat capacity of the glycyl group can also be obtained from the difference between the partial molar heat capacities of the two neutral amino acid and peptide derivatives *N*-acetylglycinamide ($CH_3CONHCH_2CONH_2$, AcglyNH$_2$) and *N*-acetylglycylglycinamide ($CH_3CONHCH_2CONHCH_2CONH_2$, AcglyglyNH$_2$). The partial molar heat capacities of these neutral solutes have also been determined over the temperature range 10–100°C.

The determination of partial molar heat capacities by DSC requires that the solution densities are known over the temperature range of interest. These densities, which were determined using differential scanning densimetry (DSD), have been used to calculate the partial molar volumes of the oligoglycines and the two acetyl amides over the temperature range 10–90°C. These results were

used to derive the partial molar volume of the glycyl group over the temperature range 10–90°C.

In addition to the studies of these new model compounds, we have, for the purposes of comparison, determined the partial molar volumes of tetra- and pentaglycine and the partial molar heat capacity of tetraglycine at the single temperature of 25°C.

2. Materials and methods

The sample of DL-alanylglycylglycine dihydrate used was from a batch of material prepared for earlier studies [12,13]. Details of the purification and analysis of the peptide have been reported elsewhere [13]. Samples of the two peptides ala(gly)$_3$ and ala(gly)$_4$ (Bachem Feinchemikalien) were recrystallized from water + ethanol and dried under vacuum at room temperature. Alkalimetric titrimetry [14,15] and elemental analyses indicated that both compounds were anhydrous and of high purity. The AcglyNH$_2$ and AcglyglyNH$_2$ used were samples remaining from a previous study [8]. Pentaglycine (Sigma) was used without further purification. The sample was dried under vacuum at room temperature for 30 h and stored in a desiccator. Analysis by alkalimetric titration gave a relative molar mass of 302.3 ± 2.4 which is in good agreement with that expected for an anhydrous compound (303.28). Elemental analyses were not in agreement with those expected for an anhydrous compound. Found: C, 38.7%; H, 6.4%; N, 21.4%; cf. calculated for $C_{10}H_{17}O_6N_5$: C, 39.6%; H, 5.7%; N, 23.1%. These results suggest that, at the time of the analysis, the material was a hemi-hydrate. However, as the solid sample was routinely vacuum dried before the preparation of any solutions, the molar mass used for the calculation of solution molalities was that of the anhydrous compound. Attempts were made to purify samples of pentaglycine, obtained from both Sigma and Bachem Feinchemikalien, by recrystallization from water + ethanol. However, the material produced was, rather surprisingly, very insoluble in water and so could not be used in this work.

The tetraglycine (Bachem Feinchemikalien) used was recrystallized from water + ethanol and dried under vacuum for 2 days at room temperature. The product was chromatographically pure as determined by TLC. Elemental analyses gave: C, 39.0%; H, 5.5%; N, 22.6%; cf. calculated for $C_8H_{14}O_5N_4$: C, 39.0%; H, 5.7%; N, 22.8%.

The water used to prepare solutions and as the reference solvent was deionized, twice glass-distilled and thoroughly degassed immediately prior to use. All solutions were prepared by mass. TLC measurements were used to check for possible hydrolysis products in the peptide solutions following the experimental measurements at high temperatures.

Heat capacity and density measurements at 25°C were made at Massey University using a Picker differential flow microcalorimeter and an Anton Paar digital density meter (model DMA 60/602), respectively. Details of the apparatus and procedures used have been described in previous work [11,13]. For tetraglycine, some of the solution density measurements were made by one of us (GRH) at the University of Bergen using an Anton Paar digital density meter, details of which have been described previously [16].

Heat capacity measurements over the temperature range 10–100°C were carried out at Westfälische Wilhelms-Universität Münster using a DASM-1M DSC. The procedures used have been described elsewhere [17,18]. Densities of solutions over the temperature range 10–90°C were measured using a differential scanning densimetric system (DSD) comprising of 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 [17–19].

3. Results

Densities of aqueous solutions of *N*-acetylglycinamide and the oligoglycines determined at 25°C are given in Tables 1 and 2. These data were used to calculate the apparent molar volumes of the solutes, V_ϕ, using the equation

$$V_\phi = M_2/\rho - (\rho - \rho_0)/m\rho\rho_0 \quad (1)$$

where M_2 is the solute molar mass, m is the

solution molality and ρ and ρ_0 are, respectively, the densities of the solution and pure solvent (0.997047 g cm^{-3} at 25°C [20]). For each of the solutes $AcglyNH_2$, $ala(gly)_3$ and $ala(gly)_4$, the apparent molar volume was found to be a linear function of molality over the range studied. The partial molar volume of each solute at infinite dilution, V_2^0, was obtained from a weighted least-squares analysis of the V_ϕ data using the equation

$$V_\phi = V_2^0 + S_V m \quad (2)$$

where S_V is the experimental slope. The weighting factors for the V_ϕ data were calculated using the procedures outlined earlier [13] with the uncertainty in an experimental density measurement taken as 3×10^{-6} g cm^{-3}. Values of V_2^0 and S_V together with their standard deviations are given in Table 3. For $(gly)_4$ and $(gly)_5$ the molality range available for study is restricted to below approx. 0.012 mol kg^{-1} and 0.0033 mol kg^{-1}, respectively, because of the low solubility of these compounds in pure water. At these low molalities the uncertainties in the V_ϕ values conceal any concentration dependence of V_ϕ. The V_2^0 values for $(gly)_4$ and $(gly)_5$ given in Table 3 are actually the mean values of the V_ϕ data over the molality range studied.

Specific heat capacities, c_p, of solutions of the peptides tetraglycine, $ala(gly)_3$ and $ala(gly)_4$ determined using the Picker calorimeter were converted to the apparent molar heat capacities of the peptides, $C_{p,\phi}$, using the equation

$$C_{p,\phi} = M_2 c_p + \left(c_p - c_p^0\right)/m \quad (3)$$

where c_p^0 is the specific heat capacity of water (4.1793 J K^{-1} mol^{-1} at 25°C) [21] and the remaining symbols are as defined for Eq. (1). The $C_{p,\phi}$ results with their experimental uncertainties, determined as described in previous work [13], are given in Table 2. For tetraglycine, specific heat capacity measurements were made on some solutions for which the density was not measured. The densities of these solutions, which are required to convert volumetric heat capacities into specific heat capacities, were calculated using a power series in solution molality of the form

$$\rho = \rho_0 + a_1 m + a_2 m^2 \quad (4)$$

where a_1 and a_2 are coefficients determined by least-squares fitting to the density data given in Table 2 (10^{-3} $a_1 = 0.0981 \pm 0.0002$ g^2 cm^{-3} mol^{-1}, 10^{-6} $a_2 = -0.078 \pm 0.02$ g^3 cm^{-3} mol^{-2}).

The $C_{p,\phi}$ data for the peptide $ala(gly)_3$ were analysed by weighted least-squares using the equation

$$C_{p,\phi} = C_{p,2}^0 + S_c m \quad (5)$$

where $C_{p,2}^0$ is the partial molar heat capacity of the solute at infinite dilution and S_c is the experimental slope. Values of $C_{p,2}^0$ and S_c, along with

Table 1
Densities of aqueous solutions of *N*-acetylglycinamide and pentaglycine at 25°C

m/mol kg^{-1}	ρ/g cm^{-3}	m/mol kg^{-1}	ρ/g cm^{-3}	m/mol kg^{-1}	ρ/g cm^{-3}
N-Acetylglycinamide ($AcglyNH_2$)					
0.24951	1.003227	0.12033	1.000057	0.05033	0.998317
0.19924	1.002004	0.09931	0.999534	0.03898	0.998029
0.16006	1.001043	0.08019	0.999064	0.02501	0.997678
0.13970	1.000539	0.06172	0.998603		
Pentaglycine ($(gly)_5$)					
0.003303	0.997433	0.002842	0.997378	0.002341	0.997321
0.003178	0.997421	0.002640	0.997354	0.002295	0.997314
0.003094	0.997409	0.002501	0.997338	0.002241	0.997307
0.002997	0.997397				

Table 2
Densities and apparent molar heat capacities of aqueous solutions of tetraglycine, ala(gly)$_3$ and ala(gly)$_4$ at 25°C

m/mol kg^{-1}	ρ/g cm^{-3}	$C_{p,\phi}$/J K^{-1} mol^{-1}	m/mol kg^{-1}	ρ/g cm^{-3}	$C_{p,\phi}$/J K^{-1} mol^{-1}
Tetraglycine ((gly)$_4$)					
0.012191	–	276.2 (9.1)[a]	0.010118	0.998031	–
0.012068	–	277.7 (11.6)	0.010062	0.998027	265.7 (12.0)
0.012049	0.998216	266.6 (12.5)	0.009937	0.998015	275.7 (11.1)
0.011890	0.998202	–	0.009600	0.997983	268.9 (8.4)
0.011785	–	266.2 (9.4)	0.009368	0.997959	–
0.011486	0.998161	264.5 (9.6)	0.009366	0.997958	–
0.011485	0.998162	–	0.009301	0.997952	272.5 (8.6)
0.011466	–	271.0 (9.6)	0.008996	0.997923	271.8 (20.0)
0.011295	–	268.5 (9.8)	0.008798	–	279.7 (9.1)
0.011211	0.998137	274.1 (11.6)	0.008399	0.997865	–
0.010966	0.998114	274.1 (16.5)	0.008120	0.997840	–
0.010840	–	269.7 (9.2)	0.007778	0.997802	–
0.010529	–	264.5 (8.6)	0.007223	0.997751	–
0.010435	0.998060	275.2 (10.6)	0.007189	0.997748	–
0.010297	0.998051	–	0.006579	0.997687	–
Alanylglycylglycylglycine (ala(gly)$_3$)					
0.05785	1.002392	379.0 (3.9)	0.03481	1.000287	375.7 (4.1)
0.05493	1.002126	374.9 (4.1)	0.03249	1.000067	372.2 (4.3)
0.05162	1.001826	374.2 (4.1)	0.02979	0.999821	376.3 (4.4)
0.04941	–	372.5 (4.1)	0.02496	0.999375	378.8 (5.2)
0.04578	1.001293	376.1 (3.5)	0.02249	0.999144	369.8 (5.4)
0.04154	1.000905	375.4 (4.6)	0.01797	0.998725	372.1 (6.7)
0.03712	1.000492	374.2 (3.8)	0.01479	0.998428	–
Alanylglycylglycylglycylglycine (ala(gly)$_4$)					
0.04114	1.001720	455.1 (5.2)	0.02752	1.000184	455.2 (7.0)
0.03757	1.001317	453.7 (7.0)	0.02455	0.999850	453.0 (5.3)
0.03495	1.001024	456.8 (5.8)	0.02197	0.999555	456.6 (11.0)
0.03242	1.000735	452.9 (6.2)	0.01805	0.999111	458.0 (7.2)
0.03032	1.000502	459.3 (6.7)	0.01504	0.998770	456.3 (12.7)

[a] The estimated uncertainties are in parentheses.

their standard deviations are given in Table 3. For ala(gly)$_4$ the value of S_c obtained in a least-squares analysis using Eq. (5) was -23 ± 126 J kg K^{-1} mol^{-2}, which is not statistically different from zero. The value of $C^0_{p,2}$ for this compound was taken as the mean of the $C_{p,\phi}$ data. A molality dependence of $C_{p,\phi}$ for tetraglycine was unable to be detected for the reason outlined above for V_ϕ. The $C^0_{p,2}$ value given in Table 3 is actually the mean value of all the $C_{p,\phi}$ data.

The partial molar heat capacity of AcglyNH$_2$ at 25°C was determined in previous work [8]. The experimental heat capacities have been reanalysed using the new density data obtained in this study to give the $C^0_{p,2}$ and S_c values shown in Table 3. The $C^0_{p,2}$ value is slightly larger than that obtained in the earlier work (238.4 ± 0.5 J K^{-1} mol^{-1} [8]).

Density data determined over the temperature range 10–90°C by DSD were analysed using Eq. (1) to obtain the apparent molar volumes. For each solute, density-temperature scans were carried out on a minimum of four solutions with molalities spanning the narrow range shown in Table 4. As density measurements made using the DSD method are not as precise as those determined isothermally, the concentration dependence of V_ϕ for a solute cannot always be de-

Table 3
Partial molar volumes and heat capacities of the peptides in aqueous solution at 25°C

Peptide	V_2^0/cm^3 mol^{-1}	S_v/cm^3 kg mol^{-2}	$C_{p,2}^0$/J K^{-1} mol^{-1}	S_c/J kg K^{-1} mol^{-2}
ala(gly)$_2$	130.16 (0.01)[a,b]	2.29 (0.08)[a]	286.2 (0.4)[a]	32 (3)[a]
	129.6 (0.5)[c]		281 (4)[d]	
ala(gly)$_3$	166.62 (0.06)	9.6 (1.4)	372.1 (2.5)	69 (58)
	166.3 (0.5)[c]		371 (4)[d]	
ala(gly)$_4$	202.59 (0.08)	11.6 (2.3)	455.7 (2.1)	[g]
	202.2 (0.5)[c]		455 (5)[d]	
(gly)$_3$	111.92 (0.03)[a]	3.6 (0.2)[a]	188.3 (0.7)[a]	52 (6)[a]
(gly)$_4$	149.0 (0.2)	[g]	271 (5)	[g]
(gly)$_5$	186.7 (0.5)	[g]		
AcglyNH$_2$	91.02 (0.02)	−0.14 (0.09)	240.6 (0.6)[e]	2.8 (2.6)[e]
	91.2 (0.5)[c]		249 (3)[d]	
AcglyglyNH$_2$	127.37 (0.05)[f]	−0.54 (0.08)[f]	322.1 (0.8)[f]	17 (4)[f]
	127.0 (0.5)[c]		331 (4)[d]	

[a] From [13].
[b] Standard deviations are in parantheses.
[c] Calculated using Eq. (6) and the polynomial coefficients given in Table 4.
[d] Calculated using Eq. (8) and the polynomial coefficients given in Table 5.
[e] Obtained from a reanalysis of earlier heat capacity data. See text.
[f] From [8].
[g] See text.

tected. For the solutes in this study no concentration dependence of V_ϕ was discernable over the molality ranges chosen. The temperature dependence of V_ϕ for each solute was obtained by fitting all the V_ϕ data to a power series in temperature, T, of the form

$$V_\phi = p_1 + p_2(T - 273.15) + p_3(T - 273.15)^2 \quad (6)$$

where p_1, p_2 and p_3 are the fitted coefficients. Values of the coefficients are given in Table 4.

The uncertainty in V_ϕ determined using the DSD method was estimated to be ± 0.5 cm^3 mol^{-1}. For the purposes of comparison, the V_ϕ values at 25°C calculated using Eq. (6) are given in Table 3. There is good agreement between the V_ϕ values derived from density data measured by DSD and those determined isothermally.

The quantity obtained from DSC measurements is the difference $\Delta(T)$[J/K] between the heat capacities of a solution and solvent at any given temperature T. The apparent molar heat capacity of each solute at a temperature T, $C_{\rho,\phi}(T)$, was calculated from $\Delta(T)$ using the equation [22]

$$C_{\rho,\phi}(T) = c_\rho^0(T)V_\phi(T)/v^0(T) + \Delta(T)/n_2 \quad (7)$$

Table 4
The coefficients of Eq. (6)

Solute	p_1/cm^3 mol^{-1}	p_2/cm^3 mol^{-1} K^{-1}	10^4 p_3/cm^3 mol^{-1} K^{-2}	m[a]/mol kg^{-1}
AcglyNH$_2$	89.0	0.091	−1.06	0.067–0.130
AcglyglyNH$_2$	124.1	0.121	−1.58	0.040–0.081
ala(gly)$_2$	126.1	0.156	−6.98	0.031–0.057
ala(gly)$_3$	161.4	0.221	−10.76	0.017–0.040
ala(gly)$_4$	197.0	0.232	−10.30	0.019–0.038

[a] Molality range used.

where $c_p^0(T)$ and $v^0(T)$ are, respectively, the specific heat capacity and volume of the solvent at a temperature T, $V_\phi(T)$ is the apparent molar volume of the solute and n_2 is the number of moles of the solute in the calorimeter cell. Values of the specific volume of water over the temperature range 10–100°C were calculated using an equation which expresses the density as a power series in temperature [20]. The specific heat capacity of water at a given temperature was calculated using an equation derived by fitting literature heat capacity data [21] to a fourth order polynomial in temperature. Values of $V_\phi(T)$ were calculated using Eq. (6) and the coefficients given in Table 4, with the assumption that these coefficients are applicable over the temperature range 10–100°C.

Some experimental $C_{p,\phi}(T)$ data for the peptide ala(gly)$_2$ and ala(gly)$_4$ are shown in Fig. 1. The lower curve for ala(gly)$_2$ comprises four successive DSC scans for a single solution (0.0572 mol kg^{-1}, 11.51 mg cm^{-3}). The figure insert using an expanded scale illustrates the excellent reproducibility of successive scans. Despite the reproducibility of successive scans, thin – layer chromatograms indicated that a small degree of hydrolysis had occurred during the DSC measurements. As there was no evidence of hydrolysis following the DSD scans from 10 to 90°C, the calorimetric data used in the analysis were taken over this temperature range. For the peptide ala(gly)$_4$ a single scan is shown for each of four solutions over the molality range 0.019–0.038 mol kg^{-1}. The figure insert shows that a concentration dependence of $C_{p,\phi}$ is not discernable.

For each solute, DSC measurements were made on the same solutions that were used for the V_ϕ measurements. As no concentration dependence of $C_{p,\phi}$ could be detected, the $C_{p,\phi}(T)$ data for all solutions were combined and fitted to a polynomial of the form

$$C_{p,\phi} = \mathrm{a} + \mathrm{b}(T - 273.15) + \mathrm{c}(T - 273.15)^2 + \mathrm{d}(T - 273.15)^3 \quad (8)$$

where a, b, c and d are the fitted coefficients. This cubic function is the lowest order polynomial that gives a good representation of the apparent molar heat capacities over the temperature range of interest. The polynomial coefficients for the various solutes are given in Table 5. The uncertainties in the apparent molar heat capacities were estimated by the application of propagation of errors to Eq. (7) but with the assumption that the uncertainties in the specific volume and heat capacity of the solvent make a negligible contribution to the total error in $C_{p,\phi}$. Values of $C_{p,\phi}$ at 25°C along with their estimated uncertainties are given in Table 3. For the ala(gly)$_n$ peptides the agreement between the $C_{p,\phi}$ results obtained by DSC and those determined by flow microcalorimetry is excellent. For the acetyl amino acid and peptide amides, the $C_{p,\phi}$ values determined by DSC are approx. 3% higher than those determined using the Picker calorimeter.

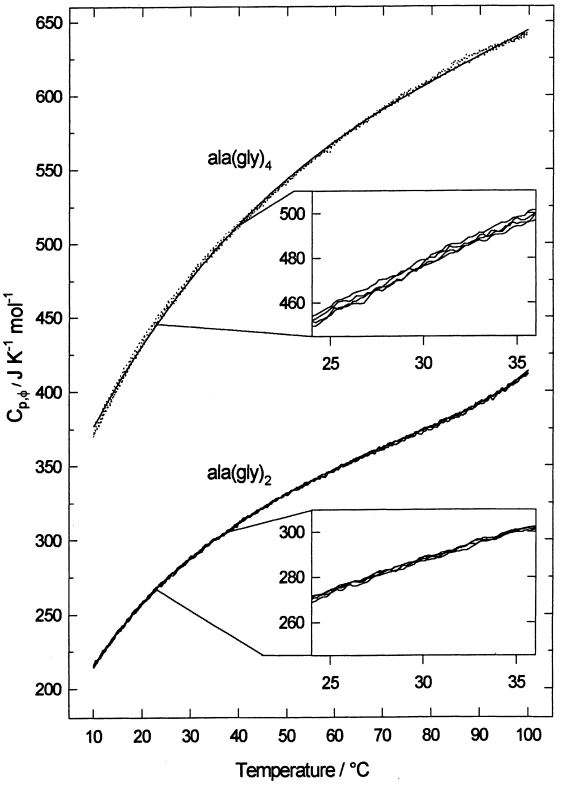

Fig. 1. Temperature dependence of the apparent molar heat capacity of two peptides in aqueous solution. For ala(gly)$_4$ one scan is shown for each of four solutions over the molality range $m = 0.019$–0.038 mol kg^{-1}. For ala(gly)$_2$ successive scans are shown for one solution of $m = 0.0572$ mol kg^{-1}.

Table 5
Coefficients from the fitting of experimental heat capacities to Eq. (8)

Solute	a/J K^{-1} mol^{-1}	b/J K^{-2} mol^{-1}	c/J K^{-3} mol^{-1}	10^4 d/J K^{-4} mol^{-1}
AcglyNH$_2$	199.3	2.44	−0.0204	0.74
AcglyglyNH$_2$	256.0	3.71	−0.0315	1.16
ala(gly)$_2$	176.4	5.40	−0.0561	2.61
ala(gly)$_3$	244.9	6.40	−0.0605	2.48
ala(gly)$_4$	312.5	7.01	−0.0585	2.16

The partial molar volumes of AcglyNH$_2$, tetra − and pentaglycine at 25°C and the partial molar heat capacity of tetraglycine at 25°C are compared with literature data in Table 6. The V_2^0 value for AcglyNH$_2$ determined in this study is slightly higher than the results from two previous determinations [23,24]. The V_2^0 result for pentaglycine is in reasonable agreement with two of the three literature results. On the other hand, the V_2^0 value for tetraglycine determined in this study is significantly lower than previous determinations. This result is somewhat surprising as in previous work [28] to determine the partial molar compressibilities of some oligoglycines, the densities of a few tetraglycine solutions were measured and found to be in reasonable agreement with those determined by Jolicoeur and Boileau [6]. Using a sample of tetraglycine, purchased recently from Sigma, gave results identical to those obtained using the Bachem material. The variation of the V_2^0 values determined for tetraglycine at 25°C perhaps illustrates the difficulty in working with a peptide that is both slow to dissolve and of low solubility.

There are fewer determinations of the $C_{p,2}^0$ value for tetraglycine at 25°C with which to com-

Table 6
A comparison with literature data at 25°C of the V_2^0 and $C_{p,2}^0$ results for tetraglycine and of the V_2^0 results for pentaglycine and acetylglycinamide in aqueous solution

Solute	V_2^0/ cm^3 mol^{-1}		$C_{p,2}^0$/ J K^{-1} mol^{-1}	
AcglyNH$_2$	91.02 (0.02)[a,b]	90.56 (0.05)[c] 90.84 (0.01)[d]		
Tetraglycine	149.0 (0.2)[a]	149.6 (0.5)[e] 149.6 (0.1)[f] 149.7 (0.1)[g] 150.0 (0.4)[h] 151.1 (1.0)[i]	271 (5)[a]	283 (2)[g] 265 (6)[j]
Pentaglycine	186.7 (0.5)[a]	186.9 (0.8)[e] 187.1 (0.2)[g] 189.5 (1.4)[i]		

[a] This work.
[b] Standard deviations are in parentheses.
[c] From Ref. [23].
[d] From Ref. [24].
[e] From Ref. [10].
[f] From Ref. [25].
[g] From Ref. [6].
[h] From Ref. [26].
[i] From Ref. [27].
[j] From Ref. [4].

pare our result. As can be seen from Table 6, the value of our result is between the two literature results.

4. Discussion

4.1. Partial molar volumes

The partial molar volumes and heat capacities for homologous series of many solutes are linear functions of the number of repeating units in the series. For example, the partial molar volume of the series of *n*-alcohols from propanol to hexanol is an excellent linear function of the number of methylene groups in the alcohol [29]. End group effects prevail for the first few members of a homologous series so, in general, the thermodynamic properties for these solutes differ from those calculated using the parameters of the linear function for higher members of the series. It was shown some years ago [6] and again in a more recent study [10], that the homologous series of oligoglycines can be treated similarly. For triglycine and beyond the partial molar volume is an approximate linear function of the number of glycyl groups in the peptide [10]. This suggests that the $-CH_2CONH-$ group in these peptides is hydrated independently and that the contribution made by this group to the partial molar volume or heat capacity of a peptide can be evaluated from the slope of the plot of the partial molar property against the number of glycyl units in the molecule. The slope of such a plot of the partial molar volumes at 25°C for the ala(gly)$_n$ peptides, $n = 2–4$, is given in Table 7. Included in Table 7 are the results obtained from the analysis of V_2^0 data for the corresponding homoglycines at 25°C. The uncertainties in the slopes calculated by the least-squares computer program used in the data analysis were unrealistically small irrespective of whether weighted data or unit weights were used. The uncertainties given in Table 7 were obtained by using propagation of errors, or alternatively the standard deviation, on the results obtained when the V_2^0 data are taken in pairs. In each case the method chosen was that which gave the higher uncertainty. It is clear from the results in Table 7 that the value for the partial molar volume of the glycyl group obtained using ala(gly)$_n$ peptides as the model compounds is significantly smaller than that obtained using the homologous series gly(gly)$_n$. The difference is perhaps greater than might be expected from the limitations of volume additivity alone. It is instructive, therefore, to compare the results obtained from the oligoglycines with those derived using alternative model compounds. The difference between the V_2^0 values for the two neutral compounds AcglyglyNH$_2$ and AcglyNH$_2$ is simply the glycyl group contribution to the partial molar volume. As shown in Table 7, the result at 25°C is in excellent agreement with that obtained using the ala(gly)$_n$ series of peptides.

An alternative approach to obtain a value for $V(CH_2CONH)$ is to carry out a group contribution analysis on a series of compounds that contain the glycyl group. In this approach, the partial molar volume of the solute is represented by the expression

$$V_2^0 = \sum_i n_i V(i) \tag{9}$$

where $V(i)$ is the contribution to the partial molar volume of a solute of a group of type i and n_i is the number of these i groups. When using such a group contribution analysis to model the glycyl group of polypeptides, it is important to choose compounds that resemble the peptide groups as closely as possible. The compounds used are listed in Table 8. The groups used in the analysis were $-H$, $-CH_2-$, $-CONH-$ and $-CONH_2$. The experimental data given in Table 8 were fitted by least-squares methods using the following form of Eq. (9).

$$V_2^0 = n_H V(H) + n_{CH_2} V(CH_2) + n_{CONH} V(CONH) + n_{CONH_2} V(CONH_2) \tag{10}$$

The group volumes obtained were as follows: $V(H) = 11.26 \pm 0.27$ cm^3 mol^{-1}, $V(CH_2) = 15.83 \pm 0.12$ cm^3 mol^{-1}, $V(CONH) = 19.76 \pm 0.23$ cm^3 mol^{-1} and $V(CONH_2) = 28.68 \pm 0.24$ cm^3 mol^{-1}. The V_2^0 values calculated using these parameters are shown in Table 8. The value of $V(CH_2CONH)$

Table 7
A comparison of the partial molar volume of the $-CH_2CONH-$ group at 25°C obtained various model compounds

Compounds used	V (CH_2CONH) / cm^3 mol^{-1}
Peptides ala(gly)$_n$, $n = 2-4$	36.2 (0.3)[a,b], 36.3 (0.4)[c]
Peptides gly(gly)$_n$, $n = 2-4$	37.4 (0.3)[a], 37.6 (0.3)[d], 37.4 (0.4)[e], 38.3 (0.7)[f]
AcglyglyNH$_2$ and AcglyNH$_2$	36.35 (0.06)[a], 35.8 (0.7)[c], 36.81 (0.07)[g]
Selected simple amides, AcglyNH$_2$	
AcglyglyNH$_2$ and AcglyNHMe[j]	35.6 (0.3)[h]
N-acetyl amino acid and peptide amides	36.8 (2.2)[i]

[a] This work. Solution densities determined at MU.
[b] Estimated uncertainties are in parentheses.
[c] This work. Solution densities determined by DSD at WWU.
[d] From an analysis of V_2^0 data given in [6].
[e] V_2^0 data from [10].
[f] V_2^0 data from [27].
[g] V_2^0 data from [8].
[h] See text.
[i] [23].
[j] The compound AcglyNHMe is *N*-acetyl-*N′*-methylglycinamide.

at 25°C, which is just the sum of the V(CONH) and $V(CH_2)$ values, is given in Table 7. The result obtained is in reasonable agreement with that derived using V_2^0 data for the ala(gly)$_n$ peptides.

A group contribution analysis has been reported [23] of a limited set of *N*-acetyl amino acid and peptide amides. The value obtained for the $-NHCH_2CO-$ group at 25°C was 36.8 ± 2.2 cm^3

Table 8
Experimental and calculated partial molar volumes and heat capacities at 25°C of the amides used in the group contribution analysis

Solute	V_2^0/cm^3 mol^{-1}		$C_{p,2}^0$/J K^{-1} mol^{-1}	
	Experimental	Calculated	Experimental	Calculated
CH_3CONH_2	55.82[a]	55.76	162.4 (1)[d]	163
$CH_3CH_2CONH_2$	71.54[a]	71.58	253.6 (1)[d]	251
$CH_3(CH_2)_2CONH_2$	87.10[a]	87.40	–	–
$CH_3(CH_2)_4CONH_2$	119.20[a]	119.05	–	–
$CH_3CONHCH_3$	74.04[a]	73.92	258 (3)[d]	251
$CH_3CONHCH_2CH_3$	90.72[a]	89.74	343 (5)[d]	340
$CH_3CH_2CONHCH_3$	89.75[a]	89.74	334 (4)[d]	340
$CH_3CONH(CH_2)_2CH_3$	105.09[a]	105.57	437 (5)[d]	429
$CH_3CH_2CONHCH_2CH_3$	105.39[a]	105.57	–	–
$CH_3CH_2CONH(CH_2)_2CH_3$	121.53[a]	121.39	–	–
$CH_3CONHCH_2CONH_2$	91.02[b]	91.34	240.6 (0.6)[e]	242
$CH_3CONHCH_2CONHCH_2CONH_2$	127.37[c]	126.92	322.1 (0.8)[d]	321
$CH_3CONHCH_2CONHCH_3$	108.93[c]	109.50	–	–
$CH_3(CH_2)_2CONHCH_3$	–	–	434 (5)[d]	429
$CH_3(CH_2)_3CONHCH_3$	–	–	524 (4)[d]	517
$CH_3CONH(CH_2)_3CH_3$	–	–	516 (1)[d]	517

[a] From [30].
[b] This work.
[c] From [23].
[d] From [8].
[e] From Table 3.

mol^{-1} which, within the admittedly large uncertainty, is in agreement with the result derived using V_2^0 data for the ala(gly)$_n$ peptides.

It has been suggested [7,31] that the compound 2,5-diketopiperazine [cyclo(glygly)] could be used to model the glycyl group. Half the value of the partial molar volume of cyclo(glygly) would correspond to $V(CH_2CONH)$. The value of V_2^0 for cyclo(glygly) at 25°C is 76.73 ± 0.07 cm^3 mol^{-1} [25] so the estimate of $V(CH_2CONH)$ is 38.4 cm^3 mol^{-1}. This is considerably higher than most of the other results given in Table 7 and is probably a manifestation of the restricted exposure to the solvent of each glycyl group in cyclo(glygly) compared with that in a linear peptide.

Perusal of the results presented in Table 7 indicates that the $V(CH_2CONH)$ value derived using V_2^0 data for the ala(gly)$_n$ series of peptides is indeed in reasonable agreement with the results obtained using alternative model compounds. It remains then to account for the higher values that have been obtained using the gly(gly)$_n$ peptides. Given the structural similarities between the two series, it is difficult to imagine how the hydration of the $-CH_2CONH-$ group could differ significantly in the two sets of peptides. Perhaps the difference arises because of the experimental difficulties associated with tetra- and pentaglycine. It is noteworthy that the V_2^0 value for tetraglycine determined in this study is significantly lower than other determinations in the literature and as mentioned in the experimental section, the sample of pentaglycine used in this study did not give satisfactory elemental analyses. As the solubilities of ala(gly)$_3$ and ala(gly)$_4$ are much higher than the respective glycyl compounds and as such more reliable V_2^0 values can be determined, the $V(CH_2CONH)$ value of 36.2 cm^3 mol^{-1} at 25°C is likely to a better estimate of the partial molar volume of the glycyl group in an unfolded polyglycine at 25°C than the result obtained from the short-chain oligoglycines.

The polynomial functions (Eq. (6)) for the ala(gly)$_n$ peptides and those for the acetyl amides can be used to generate polynomials that describe the temperature dependence of the quantity $V(CH_2CONH)$. For the acetyl amides this polynomial is simply the difference between those for

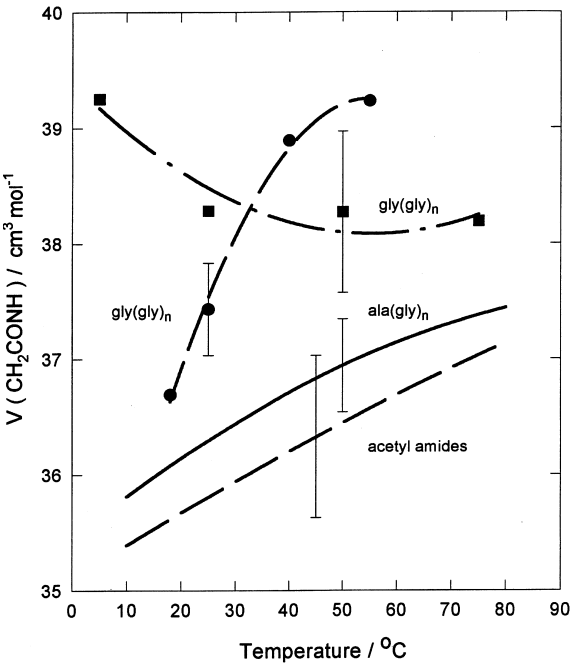

Fig. 2. Temperature dependence of the partial molar volume of the glycyl group. ——— this work; - - - this work; - - ● - - V_2^0 data from [10]; -·- ■ -·- V_2^0 data from [27].

the two compounds $AcglyglyNH_2$ and $AcglyNH_2$. For the ala(gly)$_n$ peptides, V_2^0 values calculated at specific temperatures using the polynomials given in Table 4, were analysed by linear least-squares to obtain values of $V(CH_2CONH)$ at those temperatures. These $V(CH_2CONH)$ results were then fitted to a second order polynomial in temperature to give the expression

$$V(CH_2CONH) = 35.45 + 0.0383(T - 273.15) - 1.672 \cdot 10^{-4}(T - 273.15)^2 \quad (11)$$

The results obtained are displayed in Fig. 2. For both sets of model compounds, $V(CH_2CONH)$ increases with an increase in temperature over the range 10–80°C. The increase is approx. 1 cm^3 for a temperature change of around 40°C. Within the combined uncertainties, the temperature dependence of $V(CH_2CONH)$ obtained using the ala(gly)$_n$ compounds is the same as that derived using the acetyl amide model compounds.

Also shown in Fig. 2 are values of $V(CH_2CONH)$ derived by least-squares fitting of V_2^0 data for the gly(gly)$_n$ peptides at specific temperatures taken from two literature sources [10,27]. The disparity amongst the temperature dependences of $V(CH_2CONH)$ derived using the various model compounds is immediately obvious. In contrast to the results of this study, the $V(CH_2CONH)$ values derived using the V_2^0 data for the gly(gly)$_n$ peptides reported by Makhatadze et al. [27] give a negative temperature coefficient for $V(CH_2CONH)$. Although the temperature coefficient of the $V(CH_2CONH)$ results derived using V_2^0 data reported by Chalikian et al. [10] is positive, its magnitude is significantly different from that obtained in this work. The marked contrast between the $V(CH_2CONH)$-temperature curves derived from the two sets of literature data for the gly(gly)$_n$ peptides is, perhaps, a further illustration of the difficulty in determining reliable V_2^0 data for compounds of very low solubility. As the V_2^0 data for the ala(gly)$_n$ series of peptides are more reliable than those obtained using the oligoglycines, the temperature dependence of $V(CH_2CONH)$ derived using these model compounds should better represent that for the glycyl group in an oligoglycine than results determined hitherto.

4.2. Partial molar heat capacities

A plot of the partial molar heat capacities for the ala(gly)$_n$ peptides at 25°C as a function of the number of glycyl groups is shown in Fig. 3. The slope of the linear function, which is the glycyl group contribution to the partial molar heat capacity of an oligoglycine, $C_p(CH_2CONH)$, was obtained by a linear least-squares analysis using unit weights. The value of $C_p(CH_2CONH)$ and its estimated uncertainty, which was calculated as described for the volumes, is given in Table 9. Also given in the table is the $C_p(CH_2CONH)$ result at 25°C derived using the partial molar heat capacities of the ala(gly)$_n$ peptides calculated using Eq. (8) and the polynomial coefficients given in Table 5. There is excellent agreement between this result derived using DSC data and that based on data from the isothermal calorimetric measurements. Fig. 3 also shows plots for two sets of literature data [4,6] for the gly(gly)$_n$ peptides. The $C_{p,2}^0$ data reported by Jolicoeur and Boileau [6] were analysed as outlined for the ala(gly)$_n$ peptides to obtain the $C_p(CH_2CONH)$ result given in Table 9. For the second plot, the $C_p(CH_2CONH)$ result given in Table 9 is that reported by Makhatadze and Privalov [4].

Table 9
A comparison of the partial molar heat capacity of the $-CH_2CONH-$ group at 25°C obtained using various model compounds

Compounds used	$C_p(CH_2CONH)$/J K^{-1} mol^{-1}
Peptides ala(gly)$_n$, $n = 2-4$	84.8 (1.4)[a,b], 87 (3)[c]
Peptides gly(gly)$_n$, $n = 2-4$	93.6 (3.6)[d], 93.2 (6.2)[e]
AcglyglyNH$_2$ and AcglyNH$_2$	81.5 (1.0)[g], 82 (5)[c]
Selected simple amides, AcglyNH$_2$ and AcglyglyNH$_2$	79.1 (1.0)[f], 77[h]

[a] This work. $C_{p,2}^0$ data determined using the Picker calorimeter.
[b] Estimated uncertainties are in parentheses.
[c] This work. $C_{p,2}^0$ data determined by DSC.
[d] From an analysis of $C_{p,2}^0$ data given in [6].
[e] From [4].
[f] See text.
[g] $C_{p,2}^0$ data from Table 3.
[h] From [8].

A comparison between the $C_p(CH_2CONH)$ values obtained using the two peptide series shows that the result obtained using the new ala(gly)$_n$ peptides is approx. 9 J K^{-1} mol^{-1} smaller than that obtained using the gly(gly)$_n$ series. In order to decide if the new $C_p(CH_2CONH)$ result obtained in this study is a reasonable estimate of the heat capacity of a glycyl group in an oligoglycine, it is useful to look at the results obtained using alternative model compounds.

The difference between the partial molar heat capacities of the two neutral compounds AcglyglyNH$_2$ and AcglyNH$_2$ gives the contribution of a $-CH_2CONH-$ group. The results obtained are given in Table 9. The value obtained using heat capacity data obtained from DSC measurements is in excellent agreement with that based on measurements using the Picker instrument. The $C_p(CH_2CONH)$ value obtained using these neu-

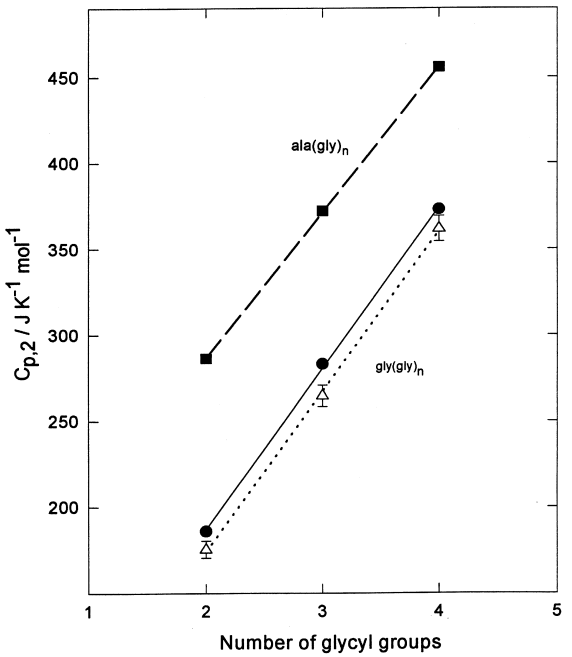

Fig. 3. Partial molar heat capacities of oligopeptides at 25°C as a function of the number of glycyl groups in the peptide. - - ■ - - this work; - ● - $C^0_{p,2}$ data from [6]; · Δ · $C^0_{p,2}$ data from [4].

tral acetyl amides is in reasonable agreement with that derived using the ala(gly)$_n$ series of peptides.

The group additivity approach, as used above for the analysis of the partial molar volumes of solutes, is also a useful method for the analysis of the partial molar heat capacities of small solutes in aqueous solution. The partial molar heat capacity of a solute is represented by the expression

$$C^0_{p,2} = \sum_i n_i C_p(i) \qquad (12)$$

where C_p(i) is the contribution to the partial molar heat capacity of the solute from a group of type i and n_i is the number of i groups in the solute. In a previous paper by one of us [8], the partial molar heat capacities in water at 25°C for several neutral *N*-acetyl amino acids and peptide amides, along with literature data for 11 simple amides were analysed, using Eq. (12). The analysis indicated that the values for several of the group heat capacities differed from those obtained previously [32] in an analysis of the $C^0_{p,2}$ data of the simple amides alone. In particular, the value we obtained for the −CONH− group (−13 J K^{-1} mol^{-1}) differed markedly from that obtained in the analysis of simple amides (−61 J K^{-1} mol^{-1}) [32]. Clearly, group contributions based on the simple amides alone are not useful for predicting the partial molar heat capacities of more complex molecules, such as peptides.

As the heat capacity of the glycyl group is the quantity of interest in this study, we have chosen to repeat our previous group contribution analysis but with a different set of chemical groups and using amides without a methyne group or a hydrogen bonded to a −CONH− moiety. The groups chosen were the glycyl group, −CH_2CONH−, the methylene group and the −H group. The experimental heat capacities given in Table 8 were fitted to Eq. (12) in the form

$$C^0_{p,2} = n_H C_p(H) + n_{CH_2} C_p(CH_2) + n_{CH_2CONH} C_p(CH_2CONH) \qquad (13)$$

The value obtained for $C_p(CH_2)$ is 88.7 ± 0.6 J K^{-1} mol^{-1}, which is in good agreement with estimates based on other model compounds [32–34]. The value obtained for C_p(H) is 41.9 ± 1.1 J K^{-1} mol^{-1}. As shown in Table 9, the $C_p(CH_2CONH)$ result derived using this group contribution analysis is similar to that obtained from the sum of the contributions of the peptide group and the methylene group obtained in our previous analysis [8] and is in better agreement with the result obtained using the ala(gly)$_n$ peptides than that based on $C^0_{p,2}$ data for the gly(gly)$_n$ peptides.

It is possible that the higher value for $C_p(CH_2CONH)$ obtained from the gly(gly)$_n$ series of peptides is due to the difficulty in obtaining reliable $C^0_{p,2}$ results for compounds of low solubility. Jolicour and Boileau [6] analysed their $C_{p,\phi}$ data for pentaglycine by least-squares methods using an equation analogous to Eq. (5) but with molar concentration instead of molality. The value obtained for the experimental slope was −3000 ± 2600 J L K^{-1} mol^{-2}. Not only is this value excessively large but the sign is opposite to that

usually observed for zwitterionic peptides in aqueous solution. An alternative and acceptable approach is to take the mean of the $C_{p,\phi}$ results as an estimate of $C^0_{p,2}$. The mean and standard deviation of the $C_{p,\phi}$ data deposited with CISTI [35] is 366 ± 13 J K^{-1} mol^{-1}. If this value is used along with the $C^0_{p,2}$ values for tri- and tetraglycine the $C_p(CH_2CONH)$ values becomes 90 ± 6 J K^{-1} mol^{-1}. Furthermore, if the value of 366 ± 13 J K^{-1} mol^{-1} for pentaglycine is used in conjunction with our own $C^0_{p,2}$ results for tetraglycine (Table 3) and triglycine [13] the value calculated for $C_p(CH_2CONH)$ is 89 ± 6 J K^{-1} mol^{-1}. Within the large uncertainties these results are in agreement with that obtained using the ala(gly)$_n$ series of peptides.

The polynomials that describe the temperature dependences of the ala(gly)$_n$ peptides and the acetyl amides (Eq. (8) and the coefficients in Table 5) can be used to derive the temperature dependence of the quantity $C_p(CH_2CONH)$. For the acetyl amides the polynomial coefficients for $C_p(CH_2CONH)$ are obtained from the differences between those for the compounds AcglyglyNH$_2$ and AcglyNH$_2$. For the three ala(gly)$_n$ peptides, $C^0_{p,2}$ values calculated for specific temperatures using the polynomial coefficients in Table 5 were fitted by least squares methods to obtain $C_p(CH_2CONH)$ values at these temperatures. These $C_p(CH_2CONH)$ results were then fitted to a polynomial in temperature to give the expression

$$C_p(CH_2CONH) = 68.1 + 0.801(T - 273.15) - 1.20 \cdot 10^{-3}(T - 273.15)^2 - 2.32 \cdot 10^{-5}(T - 273.15)^3 \quad (14)$$

The results obtained are displayed in Fig. 4. There is excellent agreement between the temperature dependences obtained using the two sets of model compounds. Also displayed in Fig. 4 are two sets of $C_p(CH_2CONH)$ results derived using $C^0_{p,2}$ data for the model compounds cyclo(glygly) [31] and the gly(gly)$_n$ series of peptides [4]. As is clearly seen in Fig. 4, neither set of results is in agreement with those determined in this study. It is likely that the difference between the $C_p(CH_2CONH)$ values derived using $C^0_{p,2}$ data for cyclo(glygly) and those determined using the ala(gly)$_n$ peptides is due to the more limited exposure to the solvent of the glycyl groups in the cyclic compound. This explanation, which is eminently reasonable, was suggested earlier [31] to account for the difference between the $C_p(CH_2CONH)$ results obtained using the cyclo(glygly) and gly(gly)$_n$ peptides as model compounds. Given the structural similarities between the ala(gly)$_n$ and the gly(gly)$_n$ series of peptides, rather similar $C_p(CH_2CONH)$-temperature curves would be expected for the two series of compounds. We are unable to provide a satisfactory explanation for the discrepancy between the curves shown in Fig. 4 other than to reiterate that reliable partial molar properties at infinite dilution are difficult to determine when a solute is not very soluble.

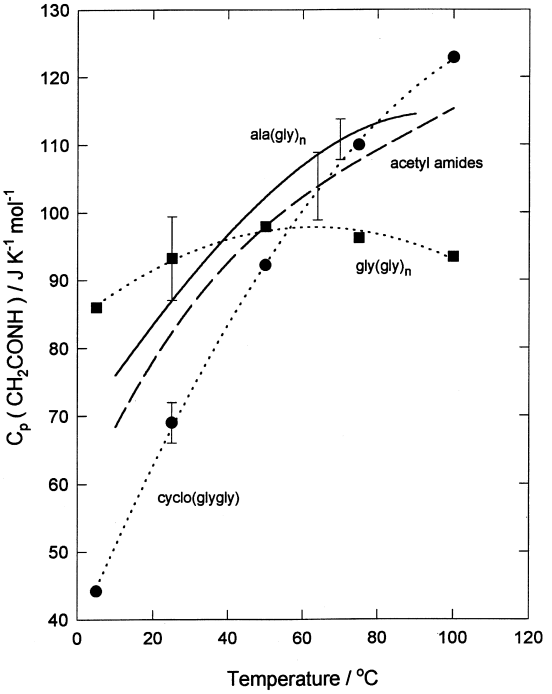

Fig. 4. Temperature dependence of the partial molar heat capacity of the glycyl group. ———— this work; - - - this work; ·· ■ ·· data from [4]; ·· ● ·· data from [31].

5. Conclusions

From this study it is clear that the $C_p(CH_2CONH)$ and $V(CH_2CONH)$ results derived using, respectively, $C^0_{p,2}$ and V^0_2 values for the ala$(gly)_n$ series of peptides differ significantly from those obtained using data for the oligoglycines. As the partial molar properties at infinite dilution can be determined more reliably for ala$(gly)_3$ and ala$(gly)_4$ than for the corresponding oligoglycines we believe that the results obtained in this study better represent the partial molar volume and heat capacity of a glycyl group in a polypeptide than those based on the gly$(gly)_n$ series reported by Makhatadze and Privalov [4]. We recommend that in calculations to derive the partial molar heat capacities of unfolded proteins in aqueous solution over a wide temperature range, the $C_p(CH_2CONH)$ values that should be used, along with reliable heat capacities of the amino acid side-chains, are those given by Eq. (14) viz.

$$C_p(CH_2CONH) = 68.1 + 0.801(T - 273.15) - 1.20 \cdot 10^{-3}(T - 273.15)^2 - 2.32 \cdot 10^{-5}(T - 273.15)^3$$

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