ELSEVIER

Biophysical Chemistry 55 (1995) 279–288

Biophysical Chemistry

Partial molar heat capacities of the peptides glycylglycylglycine, glycyl-L-alanylglycine and glycyl-DL-threonylglycine in aqueous solution over the temperature range 50 to 125°C [1]

Colin J. Downes [a], Gavin R. Hedwig [b,*]

[a] *The New Zealand Institute for Industrial Research and Development, Gracefield Research Centre, PO Box 31-310, Lower Hutt, New Zealand*

[b] *Department of Chemistry and Biochemistry, Massey University, Palmerston North, New Zealand*

Received 14 November 1994; accepted in revised form 4 January 1995

Abstract

Partial molar heat capacities of the three tripeptides glycylglycylglycine, glycyl-L-alanylglycine and glycyl-DL-threonylglycine in aqueous solution at the temperatures 50, 75, 100 and 125°C have been determined using differential flow calorimetry. The results have been used to estimate the contributions to the partial molar heat capacities of peptides of the alanyl and threonyl side-chains. These side-chain contributions are compared with those reported in the literature.

Keywords: Heat capacity; Temperature dependence; Tripeptide; Aqueous solution; Solvation

1. Introduction

A knowledge of the interactions responsible for stabilizing the native state of a globular protein in aqueous solution is essential to the understanding of its structure and function. The stability of a protein in aqueous solution can be determined by studying its denaturation [1]. In this process the native folded structure is converted into a form that is predominantly unfolded but may still possess some residual folded structure [2]. The fully unfolded or random-coil state of a globular protein in aqueous solution is of some importance as it is generally used as the ideal reference state in discussions of the thermodynamic stability of proteins. As the random-coil state of a protein is not always experimentally accessible, thermodynamic parameters, such as the heat capacity, have been estimated for unfolded proteins using simple summations of the heat capacities of their constituent groups [3,4]. These group contributions are obtained using thermodynamic data for small solutes chosen to model the various functional groups of proteins.

Tripeptides of sequence glycyl-X-glycine(gly-X-gly), where X is one of the amino acids, have been used to model the amino acid side-chains in proteins.

[1] This paper is Part 13 of a series entitled 'Thermodynamic properties of peptide solutions'. Part 12: J.F. Reading and G.R. Hedwig, Thermochim. Acta, 242 (1994) 41.

* Corresponding author.

SSDI 0301-4622(95)00005-4

In earlier work [5] we determined the partial molar heat capacities of several of these peptides in pure water as the solvent at 25°C. In a comprehensive study to determine the heat capacities by DSC of protein constituent groups over a wide temperature range, some peptides of sequence gly-X-gly, as well as other small solutes, were used as compounds to model protein side-chains [3,6]. For the tripeptides, the partial molar heat capacities were determined using 0.5 M acetic acid-sodium acetate buffer at pH 4 as the solvent. A comparison of these results with those in pure water at 25°C revealed several inconsistencies. In order to investigate these further, we determined recently [7], using high sensitivity scanning densimetry and microcalorimetry, the partial molar volumes and heat capacities of the tripeptides gly-X-gly (X = met, asn, ile and gly) in both water and 0.5 M acetate buffer at pH 4. In a parallel study using differential flow calorimetry we report herein the partial molar heat capacities of the tripeptides glycylglycylglycine (glyglygly), glycyl-L-alanylglycine (glyalagly) and glycyl-DL-threonylglycine (glythrgly) in water as the solvent at the temperatures 50, 75, 100 and 125°C.

2. Materials and methods

Samples of the tripeptides glyglygly (Sigma Chemical Co.) and glyalagly(Bachem Feinchemikalien) were recrystallised from water + ethanol and dried under vacuum at room temperature. For glyglygly, analysis by alkalimetric titration [8,9] gave a relative molar mass of 189.5 ± 1.3 which is in agreement with that of the anhydrous compound (M_r = 189.17). Elemental analyses gave: C, 38.0%; H, 5.6%; N, 22.1%; cf. calculated composition for $C_6H_{11}N_3O_4$: C, 38.1%; H, 5.9%; N, 22.2%. Alkalimetric titrimetry for glyalagly gave a relative molar mass of 202.1 ± 1.3 which is in agreement with that for the anhydrous compound. Elemental analyses gave: C, 41.4%; H, 6.3%; N, 20.8%; cf. calculated composition for $C_7H_{13}N_3O_4$: C 41.4%; H, 6.5%; N, 20.7%. The sample of glythrgly was from a batch of material synthesized in earlier work [5]. Analyses for the crystalline monohydrate gave: M_r = 252.4 ± 1.6; elemental composition: C, 38.2%; H, 6.9%; N, 16.7%; cf. calculated composition for $C_8H_{17}O_6N_3$: C, 38.2%; H, 6.8%; N, 16.7%.

All solutions were prepared by mass using water that was deionised and glass-distilled. Prior to loading into the sample loop of the high temperature calorimeter, solutions were filtered using Sartorius Minisart filters (0.2 μm) and an all glass syringe. As the densities of filtered and unfiltered solutions determined using a digital density meter were the same within the combined experimental uncertainties, it can be assumed that the filtering process did not significantly alter the solution molality.

Solutions of glyglygly were monitored for possible decomposition products at 125°C. The densities of solutions before and after storage in sealed glass vials at 125°C for 1 h were the same within the combined experimental uncertainties. This suggests that peptide decomposition is negligible under the conditions used in this study.

Heat capacity measurement at 50°C were carried out using a Picker flow microcalorimeter. Details of the instrument and procedures of operation have been described in previous work [10,11]. In order to check the accuracy of the calorimeter at 50°C, specific heat capacities were determined for numerous aqueous solutions of sodium chloride in the molality range 0.30 to 1.0 mol kg^{-1}. The apparent molar heat capacities obtained were in excellent agreement with those given by Clarke and Glew [12]. Densities of aqueous solutions at 50°C, which were required to convert the volumetric heat capacities to heat capacities per unit mass of solution, were determined using an Anton Paar digital density meter (model DMA 60/602). The experimental procedures used have been reported previously [10,11].

Heat capacity measurements at the nominal temperatures 75, 100 and 125°C were carried out using a differential flow calorimeter designed for studies at high temperatures and pressures [13]. The instrument, which is similar in design to that described by Smith-Magowan and Wood [14], has a maximum operating temperature in excess of 450°C. At the relatively low temperatures used in this study, the instrument is less convenient to operate because only a low power dissipation in the heater is required to maintain the temperature of the copper block thermostat which makes it more susceptible to minor changes in room temperature. Sample solutions are

injected into the flowline on the working side of the calorimeter using a Rheodyne 7125 HPLC valve to switch in a 13 cm^3 sample loop thermostatted at 25°C. To evaluate the specific heat capacity of a solution, the ratio of the solution density to that of the pure solvent at 25°C and the operating pressure of 1 MPa is required. As densities at 1 MPa are not available for these peptide solutions, we have used instead the density ratios at 0.1 MPa. Calculations using isentropic compressibility data for the peptide solutions and for the solvent indicate that the use of density ratios at 0.1 MPa introduces negligible error. Heat loss corrections were made using the methods described previously [13].

3. Results

Densities of aqueous solutions of the tripeptides at 50°C are given in Table 1. Although the purpose of these densities was to enable specific heat capacities to be derived, the results were used to calculate apparent molar volumes of the solutes, V_ϕ, using the equation

$$V_\phi = M_2/d - (d - d_0)/mdd_0 \quad (1)$$

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 pure solvent (0.988038 g cm^{-3} at 50°C [15]). The partial molar volumes of the peptides

Table 1
Densities and apparent molar heat capacities of aqueous solutions of the tripeptides at 50°C and 0.1 MPa

m (mol kg^{-1})	d (g cm^{-3})	$C_{p,\phi}$ [a] (J K^{-1} mol^{-1})	m (mol kg^{-1})	d (g cm^{-3})	$C_{p,\phi}$ [a] (J K^{-1} mol^{-1})
glycylglycylglycine					
0.17422	1.000783	260.8 (1.5)	0.10047	0.995472	255.3 (2.1)
0.16001	0.999769	259.2 (1.6)	0.08471	0.994323	252.9 (2.5)
0.15054	0.999087	258.5 (1.7)	0.07009	0.993248	253.3 (3.0)
0.13992	0.998328	258.5 (1.8)	0.06104	0.992584	253.1 (3.5)
0.13033	0.997628	258.1 (1.9)	0.05005	0.991772	250.7 (4.0)
0.12520	0.997262	259.2 (2.0)	0.04204	0.991175	251.1 (4.8)
0.12003	0.996884	257.9 (2.0)	0.03512	0.990667	–
0.10988	0.996155	256.2 (2.0)	0.03000	–	248.8 (6.4)
			0.02993	0.990280	251.5 (6.4)
glycyl-L-alanylglycine					
0.15246	–	352.8 (2.0)	0.07999	0.993695	349.5 (3.4)
0.15193	0.998648	–	0.07513	–	348.7 (2.8)
0.13966	0.997807	351.8 (2.1)	0.06949	0.992961	348.2 (2.9)
0.12994	0.997150	351.7 (2.4)	0.05999	0.992293	349.0 (3.4)
0.11974	0.996447	351.7 (2.3)	0.05489	–	346.2 (3.9)
0.10862	0.995681	349.7 (2.2)	0.05024	0.991612	346.8 (5.2)
0.09987	0.995071	–	0.04688	–	344.5 (3.2)
0.09793	–	351.0 (2.9)	0.03913	0.990827	346.9 (4.6)
0.09012	0.994396	349.8 (2.4)	0.03209	0.990327	–
			0.02501	0.989824	–
glycyl-DL-threonylglycine					
0.12172	0.998132	412.1 (2.5)	0.07069	0.993954	407.4 (2.9)
0.10963	–	411.2 (2.1)	0.06209	0.993247	405.1 (3.4)
0.10889	0.997089	–	0.04980	0.992229	–
0.10179	0.996516	408.6 (2.3)	0.05410	–	407.9 (3.7)
0.09582	0.996025	409.0 (2.2)	0.04560	–	406.3 (4.4)
0.09130	0.995654	407.2 (3.0)	0.04118	0.991507	406.0 (4.9)
0.08306	0.994984	407.8 (2.5)	0.03495	0.990986	403.9 (5.5)
0.08014	0.994740	–	0.02999	0.990570	–
			0.02190	0.989889	–

[a] The estimated uncertainty of each $C_{p,\phi}$ is given in parentheses.

Table 2
Standard state partial molar volumes of the tripeptides in aqueous solution at 50°C and 0.1 MPa

Tripeptide	V_2^0 ($cm^3\ mol^{-1}$)	S_v ($cm^3\ kg\ mol^{-2}$)
glyglygly	114.48 (0.04)	3.4 (0.3)
glyalagly	132.20 (0.03)	3.4 (0.3)
glythrgly	149.05 (0.04)	4.3 (0.4)

at infinite dilution, V_2^0, were obtained from the V_ϕ results by least squares analysis using the equation

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

where S_v is the experimental slope.

Values of V_2^0 and S_v together with their standard deviations are given in Table 2. In a recent study, V_2^0 values were reported for glyglygly in aqueous solution at the temperatures 18, 25, 40 and 55°C [16]. Based on a polynomial fit of these data, the value of V_2^0 at 50°C is $114.7 \pm 0.3\ cm^3\ mol^{-1}$ which is in agreement, within the combined uncertainties, with that determined in this work. The V_2^0 value for glyglygly at 50°C determined by Makhatadze et al. [17] ($V_2^0 = 114.8 \pm 0.7\ cm^3\ mol^{-1}$) is also in agreement with the result given in Table 2.

The apparent molar heat capacities, $C_{p,\phi}$, of the tripeptides at 50°C were calculated from the experimental specific heat capacities, c_p, 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 pure water and the other symbols are as defined for Eq. (1). The $C_{p,\phi}$ data along with their estimated uncertainties, determined as described in previous work [10], are given in Table 1. In some cases specific heat capacity measurements were made on solutions for which densities were not determined. For these solutions the densities were calculated using a power series in the solution molality of the form

$$d = d_0 + p_1 m + p_2 m^2 \quad (4)$$

where p_1 and p_2 are parameters determined by least squares fitting to the density data given in Table 1. Over the molality range studied, $C_{p,\phi}$ was found to vary linearly with the solution molality, as is shown

Table 3
Apparent molar heat capacities of aqueous solutions of the tripeptides at the temperatures 75.5, 100.4 and 125.4°C and at 1.0 MPa

Temperature (°C)	m ($mol\ kg^{-1}$)	$C_{p,\phi}$ ($J\ K^{-1}\ mol^{-1}$)	m ($mol\ kg^{-1}$)	$C_{p,\phi}$ ($J\ K^{-1}\ mol^{-1}$)	m ($mol\ kg^{-1}$)	$C_{p,\phi}$ ($J\ K^{-1}\ mol^{-1}$)
glycylglycylglycine						
75.5	0.10026	294.1	0.08797	293.1	0.07615	291.6
75.5	0.09334	294.7	0.08188	293.2	0.06944	293.5
100.4	0.10656	335.9	0.09166	330.5	0.07439	335.1
100.4	0.09875	337.2	0.08371	335.0	0.06824	334.9
125.4	0.10751	364.1	0.09184	364.5	0.07590	367.5
125.4	0.09941	366.4	0.08397	365.5	0.06790	370.7
glycyl-L-alanylglycine						
75.5	0.09879	392.4	0.08205	387.5	0.06456	387.3
75.5	0.09063	390.7	0.07284	392.3		
100.4	0.09558	430.3	0.08603	428.8	0.07080	431.1
100.4	0.09125	429.5	0.08091	430.9	0.06606	428.9
125.4	0.10100	463.7	0.08510	463.2	0.07177	460.6
125.4	0.09182	467.0	0.07901	460.5	0.06594	457.5
glycyl-DL-threonylglycine						
75.5	0.07979	453.3	0.06989	456.6	0.06503	454.4
75.5	0.07506	454.3				
100.4	0.07945	488.4	0.06926	485.8	0.06482	492.4
100.4	0.07500	486.6				
125.4	0.07979	513.4	0.06977	514.2	0.06484	513.7
125.4	0.07554	512.5				

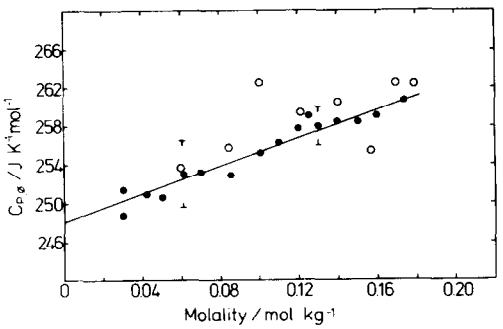

Fig. 1. The molality dependence of the apparent molar heat capacity of glyglygly in aqueous solution at 50°C and 0.1 MPa. The open circles were determined using the high temperature instrument. See text.

for glyglygly in Fig. 1. The $C_{p,\phi}$ data at 50°C were analysed by weighted least squares using the equation

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

where $C^0_{p,2}$ is the partial molar heat capacity of the solute at infinite dilution and S_c is the experimental slope. Values of $C^0_{p,2}$ and S_c along with their standard deviations are given in Table 4.

At the higher temperatures, the apparent molar heat capacities of aqueous solutions of the tripeptides were derived from the experimental specific heat capacities using Eq. (3). The $C_{p,\phi}$ values obtained at a pressure of 1 MPa are given in Table 3. Unfortunately, the instrument was not sensitive enough to enable the molality dependence of $C_{p,\phi}$ to be determined. For each peptide an average $C_{p,\phi}$ value was calculated from the $C_{p,\phi}$ results obtained over a narrow molality range. These average values along with their uncertainties are given in Table 4. The uncertainty in each average $C_{p,\phi}$ value was estimated from the standard errors in the $C_{p,\phi}$ values obtained from the least squares fitting procedure [13].

In order to check that there were no systematic differences between the two calorimeters used in this study, the apparent molar heat capacities for a few solutions of glyglygly were determined at 50°C and 1 MPa using the high temperature instrument. For the purposes of comparison, the $C_{p,\phi}$ values obtained were corrected to a pressure of 0.1 MPa using the method outlined below. As these $C_{p,\phi}$ results shown in Fig. 1 are consistent with those obtained using the Picker calorimeter, any systematic difference between the two instruments is negligible.

Table 4
Standard state partial molar heat capacities of the tripeptides in aqueous solution at various temperatures [a]

Peptide	Temperature (°C)	$C^0_{p,2}$ (J K^{-1} mol^{-1})	S_c (J kg K^{-1} mol^{-2})
glyglygly	25.0 [b]	188.3 (0.7) [c]	52 (6)
	50.0	248.2 (0.8)	72 (6)
	75.5	293.4 (2.0)	
	100.4	334.8 (4.6)	
	125.4	366.4 (7.3)	
glyalagly	25.0 [d]	289.8 (0.3)	38 (3)
	50.0	344.1 (0.7)	59 (7)
	75.5	390.0 (4.0)	
	100.4	429.9 (2.6)	
	125.4	462.1 (6.7)	
glythrgly	25.0 [d]	339.4 (0.7)	58 (6)
	50.0	401.5 (1.2)	80 (14)
	75.5	454.6 (3.3)	
	100.4	488.3 (3.6)	
	125.4	513.5 (4.9)	

[a] The $C^0_{p,2}$ at 25 °C and 50 °C were determined at $p = 0.1$ MPa; those at other temperatures were determined at $p = 1.0$ MPa.
[b] Ref. [10].
[c] Uncertainties are in parentheses. See text.
[d] Ref. [5].

For each peptide the $C^0_{p,2}$ results given in Table 4 are plotted as a function of temperature in Fig. 2.

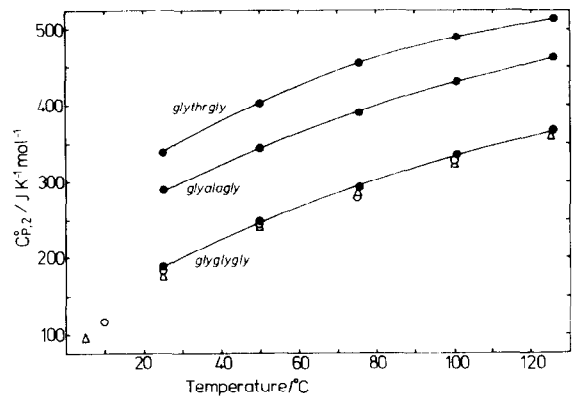

Fig. 2. The temperature dependence of $C^0_{p,2}$ for the tripeptides. The curves have been drawn using Eq. (6) and the parameters in Table 5. (●) This work; (△) from Ref. [3]; (○) from Ref. [7].

Table 5
Coefficients of Eq. (6)

Peptide	a (J K^{-2} mol^{-1})	b (J K^{-3} mol^{-1})
glyglygly	2.59 (0.06) [a]	−0.0087 (0.0011)
glyglygly	2.59 (0.07) [b]	−0.0090 (0.0013) [b]
glyalagly	2.32 (0.01)	−0.0062 (0.0002)
glythrgly	2.75 (0.03)	−0.0101 (0.0004)

[a] Standard deviations are in parentheses.
[b] Derived using $C^0_{p,2}$ values at 1 MPa. See text.

The results were analysed using a polynomial in temperature, t, of the form

$$C^0_{p,2} = C^0_{p,2}(25) + a(t-25) + b(t-25)^2 \qquad (6)$$

where $C^0_{p,2}(25)$ is the partial molar heat capacity of each peptide at 25°C and a and b are the fitted coefficients. These polynomial coefficients are given in Table 5. As shown in Fig. 2, a second order polynomial gives a good fit to the experimental $C^0_{p,2}$ data for each peptide over the temperature range used in this study. Although the $C^0_{p,2}(T)$ curves appear satisfactory, it should be stressed that the $C^0_{p,2}$ values have not all been determined at the same pressure. At 25 and 50°C the $C^0_{p,2}$ values were determined at 0.1 MPa while at the higher temperatures the pressure was 1.0 MPa. It is possible to adjust the $C^0_{p,2}$ values to the same pressure by making use of the thermodynamic equation [18]

$$\left(\frac{\partial C^0_{p,2}}{\partial p}\right)_T = -T\left(\frac{\partial^2 V^0_2}{\partial T^2}\right)_p \qquad (7)$$

For glyglygly, analysis using a second order polynomial in temperature of the V^0_2 data determined by Chalikian et al. [16], and those determined at 50°C in this study and at 25°C in earlier work [10], gave $(\partial^2 V^0_2/\partial T^2)_p = -0.0044$ cm^3 mol^{-1} K^{-2}. Using this result, the changes in $C^0_{p,2}$ on correction of the data at 25°C and 50°C to a pressure of 1.0 MPa are 1.2 J K^{-1} mol^{-1} and 1.3 J K^{-1} mol^{-1}, respectively, which are smaller than the uncertainties in the $C^0_{p,2}$ values determined using the high temperature calorimeter. The coefficients of Eq. (6) that are obtained from an analysis of the $C^0_{p,2}$ data for glyglygly all at a pressure of 1.0 MPa are given in Table 5. These coefficients are the same, within the combined uncertainties, as those obtained using the $C^0_{p,2}$ data at the two pressures. In view of this finding we have chosen to use the experimental $C^0_{p,2}$ values in further analyses of the data.

Partial molar heat capacities of glyglygly over wide temperature ranges have been determined in two previous studies using high sensitivity DSC methods [3,7]. A comparison of these results with those determined in this work is shown in Fig. 2. In general, there is reasonable agreement among the $C^0_{p,2}$ values for glyglygly in water as a solvent.

4. Discussion

4.1. Relaxation effects

In dilute aqueous solutions, amino acids and linear peptides exist in two forms which are in equilibrium. These forms are the neutral molecule and the dipolar ion (or zwitterion) in which the terminal amino and carboxyl groups are charged. For the tripeptide glyglygly the equilibrium can be represented by

$$NH_2(CH_2CONH)_2CH_2CO_2H \rightleftharpoons \overset{+}{N}H_3(CH_2CONH)_2CH_2CO_2^- \qquad (8)$$

The thermodynamic equilibrium constant for this reaction is given by

$$K_z = \frac{\left[\overset{+}{N}H_3(CH_2CONH)_2CH_2CO_2^-\right]\gamma^i}{\left[NH_2(CH_2CONH)_2CH_2CO_2H\right]\gamma^u} \qquad (9)$$

where γ^i and γ^u are the activity coefficients of the dipolar ion and unionized form, respectively. The presence of such a temperature-dependent equilibrium in aqueous solutions complicates the interpretation of solution heat capacities. During the measurement of the heat capacity, the temperature is increased which leads to a shift in the equilibrium state and a consequent contribution to the heat capacity. This contribution, which in some cases can be a significant portion of the total heat capacity, is known as the 'relaxation' contribution [19]. It is worthwhile to explore the significance of this relaxation contri-

bution in aqueous solutions of the tripeptides over the temperature range of interest.

The relaxation heat capacity, $C_{p,rel}$, can be derived from a consideration of the temperature dependence of the equilibrium constant. General methods have been proposed for the evaluation of the relaxation heat capacity contributions for different types of chemical equilibria in solution [19,20]. For the equilibrium represented by Eq. (8), $C_{p,rel}$ is given by [19–21]

$$C_{p,rel} = \frac{\Delta H_z^2}{RT^2} \cdot \frac{K_z}{(1+K_z)^2} \quad (10)$$

where ΔH_z is enthalpy change for the reaction. At 25°C the value of ΔH_z (-42.4 kJ mol^{-1}) was obtained from the sum of the enthalpy of protonation of the amine group of glyglygly [22] and the enthalpy of ionization of the carboxylic acid group of the amino acid derivative N-acetylglycine [23]. A value for K_z was calculated using the method outlined by Cohn and Edsall [24]. This method requires a knowledge of the equilibrium constants for the protonation of the amine and carboxylate groups of glyglygly and also for the protonation of the amine group of a glyglygly derivative, such as $NH_2(CH_2CONH)_2CH_2CONH_2$, which does not form a zwitterion. These equilibrium constants have been determined at 25°C and at an ionic strength of 0.1 M ($NaClO_4$) [25,26]. Although the ionic strength conditions differ from those in this study, the estimated value of K_z obtained using these protonation constants ($K_z = 4.27 \times 10^4$) is of sufficient accuracy for the present purposes. Using these values of ΔH_z and K_z the relaxation heat capacity at 25°C calculated using Eq. (10) is negligibly small ($C_{p,rel} = 0.06$ J K^{-1} mol^{-1}). Negligible relaxation heat capacities have been assumed in recent studies of the zwitterionic amino acids in aqueous solution over the temperature range 15 to 55°C [27]. However, the contribution of relaxation effects is not necessarily neglible at higher temperatures. The change in heat capacity corresponding to the process of zwitterion formation for the ω-amino acids has been calculated to be -167 J K^{-1} mol^{-1} [28]. This value will be a reasonable estimate for the change in heat capacity, $\Delta C_{p,z}$, for the reaction represented by Eq. (8). Assuming that $\Delta C_{p,z}$ is independent of temperature, ΔH_z can be expressed by

$$\Delta H_z(T) = \Delta H_0 + \Delta C_{p,z} \cdot T \quad (11)$$

where ΔH_0 is the enthalpy change when the temperature is zero. It follows that the temperature dependence of K_z can be determined using the van't Hoff equation in the form

$$\frac{d \ln K_z}{dT} = \frac{\Delta H_0}{RT^2} + \frac{\Delta C_{p,z}}{RT} \quad (12)$$

Using Eqs. (11) and (12) the values of K_z and ΔH_z at 100°C are 858 and -54.9 kJ mol^{-1}, respectively. These lead to a value of $C_{p,rel}$ of 3.0 J K^{-1} mol^{-1}. This is of the same order as the uncertainty in the partial molar heat capacity of glyglygly at this temperature. The pressure dependence of K_z can be ignored because of the very small volume change on formation of the zwitterionic structure for glyglygly [29] and because of the small pressure range covered in this study. At 125°C the value of $C_{p,rel}$ calculated using Eq. (10) is 9.7 J K^{-1} mol^{-1}. Although this is greater than the uncertainty in the value of $C^0_{p,2}$ at this temperature, it is only about 3% of the total heat capacity. However, as the temperature is further increased, the contribution of the relaxation heat capacity will become an increasingly more significant fraction of the total heat capacity.

The appropriate thermodynamic quantities are not available to allow relaxation heat capacities to be derived for the other peptides. As the central side-chains in these tripeptides do not interact significantly with the ionic end groups [11], it is anticipated that the $C_{p,rel}$ values for glyalagly and glythrgly will be very similar to those for glyglygly.

4.2. Side-chain heat capacities

The partial molar heat capacities of the tripeptides can be used to obtain group heat capacities for the alanyl and threonyl side-chains. The heat capacity contribution of a side-chain, $C^0_p(-R)$, can be calculated using the equation

$$C^0_p(-R) = C^0_{p,2}(\text{gly-X-gly}) - C^0_{p,2}(\text{glyglygly}) + C^0_p(-H) \quad (13)$$

where $C^0_p(-H)$ is the partial molar heat capacity of

Table 6
Standard state partial molar heat capacities $C_p^0(-R)$ of the alanyl and threonyl side-chains at various temperatures [a]

Side-chain (R)	Temperature/°C				
	25	50	75	100	125
$-CH_3$	179.5 [b]	167.6	163.0	154.8	149.6
$-CH(OH)CH_3$	229.1 [c]	225.0	227.6	213.2	201.0

[a] Units of $C_p^0(-R)$ are J K^{-1} mol^{-1}.
[b] Calculated using $C_{p,2}^0$ data from Ref. [10].
[c] Calculated using $C_{p,2}^0$ data from Ref. [5].

the hydrogen atom. For the purposes of comparison we have chosen to use the same values of $C_p^0(-H)$ as used in the earlier study by Makhatadze and Privalov [3]. At 25°C the value of $C_p^0(-H)$ was derived by taking the mean of four estimates of the heat capacity of the H atom taken from the literature [3]. At other temperatures the value of $C_p^0(-H)$ was determined from the difference between the partial molar heat capacities of the $-CH_3$ and $-CH_2-$ groups [3]. The use of uncorrected $C_{p,2}^0$ data in deriving the group contributions using Eq. (13) assumes that the $C_{p,rel}$ terms largely cancel.

Values of $C_p^0(-R)$ derived using the results in this work, and $C_{p,2}^0$ data at 25°C determined previously [5,10], are given in Table 6. The group heat capacity for the alanyl side-chain decreases as the temperature increases. This trend is typical of hydrophobic substances in water [30,31]. As the temperature increases the hydrophobic hydration around the alkyl group gradually breaks down which is manifested as a decrease in heat capacity. The temperature dependence of the heat capacity of the threonyl side-chain is more complex. The results in Table 6 indicate that across the temperature range 25 to 75°C $C_p^0(-CH(OH)CH_3)$ is approximately independent of temperature while at higher temperatures it decreases with increasing temperature. This suggests that at the lower temperatures the contributions of the hydrophobic and hydrophilic (the –OH group) moieties to the temperature dependence of $C_p^0(-CH(OH)CH_3)$ are approximately balanced while at higher temperatures the hydrophobic moiety dominates.

In Fig. 3 the heat capacities of the threonyl side-chain determined in this work are compared with those reported by Makhatadze and Privalov [3]. In the latter study ethanol was used as a model for the threonyl side-chain. Values of the side-chain heat capacities at various temperatures were obtained by subtracting the $C_p^0(-H)$ values from the $C_{p,2}^0$ data for ethanol [32]. It is clear from Fig. 3 that the two sets of results differ considerably. As discussed previously [5], it would appear that the hydration of the structural unit $-CH(OH)CH_3$ in the compound CH_3CH_2OH is quite different from that when it is part of a peptide chain. As the side-chain in the peptide gly-X-gly is structurally the same as that found in proteins, the $C_p^0(-CH(OH)CH_3)$ values obtained in this work should better represent the side-chain values in proteins than results using ethanol as a model compound.

The heat capacities of the alanyl side-chain at various temperatures have been derived previously using $C_{p,2}^0$ data for some cyclodipeptides in aqueous solution [6] and also using $C_{p,2}^0$ data for methane [3]. A comparison of these results with those determined in this work given in Fig. 4. The $C_p^0(-CH_3)$ results derived in this work are larger than the previous estimations. The side-chain heat capacities based on the cyclodipeptides that are shown in Fig. 4 were calculated using $C_{p,2}^0$ data for c(glygly) and c(alagly) [6]. Using the standard deviations reported for these results, the uncertainties in the $C_p^0(-CH_3)$ data obtained by propagation of errors are ± 8 J K^{-1} mol^{-1}. For the present purpose, the error in the quantity $C_p^0(-H)$ has been neglected. The corresponding uncertainties in the $C_p^0(-CH_3)$ data determined in this study are typically ± 5 J K^{-1} mol^{-1} so, within the

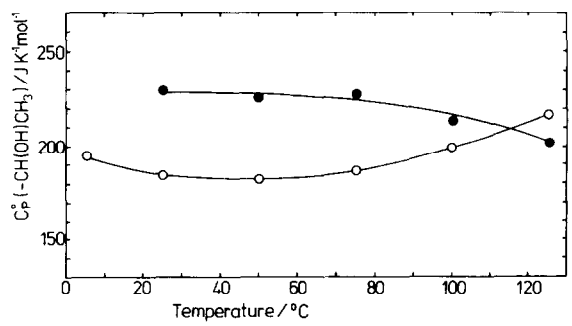

Fig. 3. A comparison of $C_p^0(-CH(OH)CH_3)$ values with literature results: (●) this work; (○) from Ref. [3].

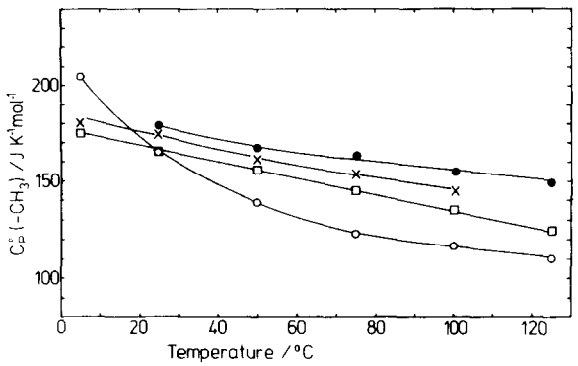

Fig. 4. A comparison of $C_p^0(-CH_3)$ values derived using heat capacity data for various model compounds (●) this work; (×) from data for cyclodipeptides, Ref. [6]; (□) from data for methane, Ref. [3]; (○) from data for tripeptides in acetate buffer, pH 4, Ref. [6].

combined uncertainties, our $C_p^0(-CH_3)$ results are in agreement with those obtained using the cyclodipeptides as model compounds.

The $C_{p,2}^0$ data for methane used by Makhatadze and Privalov were derived from the $C_p^0(g)$ data for methane and from heat capacity of solution data determined over the range 0 to 50°C [33]. The temperature dependence of the partial molar heat capacity of methane above 50°C was assumed to be the same as that below this temperature [3]. The $C_p^0(-CH_3)$ values were obtained by subtracting the values of $C_p^0(-H)$ from the $C_{p,2}^0$ data for methane. We are unable to comment on the uncertainties of these $C_p^0(-CH_3)$ results used in Fig. 4 because the source of the methane $C_p^0(g)$ data was not given in Ref. [3]. As the methyl side-chain in glyalagly is structurally identical to that found in proteins, the $C_p^0(-CH_3)$ results derived using $C_{p,2}^0$ data for tripeptides should better represent the side-chain value found in proteins than the results based on methane.

Fig. 4 also shows the $C_p^0(-CH_3)$ results derived using $C_{p,2}^0$ data for tripeptide model compounds but with 0.5 M sodium acetate-acetic acid buffer at pH 4 as the solvent. Although the solvent system used negates any direct comparison with the results in water, it is worth noting that the temperature dependence of $C_p^0(-CH_3)$ differs significantly from that determined in this study. This result is similar to that observed earlier for the peptides glyilegly and glymetgly [7].

Acknowledgement

This work was supported by the Foundation for Research Science and Technology.

References

[1] S. Lapanje, Physicochemical Aspects of Protein Denaturation, Wiley, New York, 1978.
[2] P.L. Privalov, Adv. Protein Chem., 33 (1979) 167.
[3] G.I. Makhatadze and P.L. Privalov, J. Mol. Biol., 213 (1990) 375.
[4] P.L. Privalov and G.I. Makhatadze, J. Mol. Biol., 213 (1990) 385.
[5] G.R. Hedwig, J. Chem. Soc. Faraday Trans., 89 (1993) 2761.
[6] G.I. Makhatadze, S.J. Gill and P.L. Privalov, Biophys. Chem., 38 (1990) 33.
[7] T. Vogl, H.-J. Hinz and G.R. Hedwig, Biophys. Chem., 54 (1995) 261.
[8] M.K. Kumaran, I.D. Watson and G.R. Hedwig, Aust. J. Chem., 36 (1983) 1813.
[9] I.M. Kolthoff and V.A. Stenger, Volumetric Analysis, Vol. 2, Wiley Interscience, New York, 1947, p. 158.
[10] G.R. Hedwig, J. Solution Chem., 17 (1988) 383.
[11] J.F. Reading and G.R. Hedwig, J. Chem. Soc. Faraday Trans., 86 (1990) 3117.
[12] E.C.W. Clarke and D.N. Glew, J. Phys. Chem. Ref. Data, 14 (1985) 489.
[13] D.R. White and C.J. Downes J. Solution Chem., 17 (1988) 733.
[14] D. Smith-Magowan and R.H. Wood, J. Chem. Thermodyn., 13 (1981) 1047.
[15] G.S. Kell, J. Chem. Eng. Data, 12 (1967) 66.
[16] T.V. Chalikian, A.P. Sarvazyan, T. Funck and K.J. Breslauer, Biopolymers, 34 (1994) 541.
[17] G.I. Makhatadze, V.N. Medvedkin and P.L. Privalov, Biopolymers, 30 (1990) 1001.
[18] L.G. Hepler, Can. J. Chem., 47 (1969) 4613.
[19] G.J. Mains, J.W. Larson and L.G. Hepler, J. Phys. Chem., 88 (1984) 1257.
[20] M.J. Blandamer, J. Burgess and J.M.W. Scott, J. Chem. Soc. Faraday 1, 80 (1984) 2881.
[21] C. Jolicoeur, in D. Glick (Editor), Methods of Biochemical Analysis, Vol. 27, Wiley and Sons, New York, 1981, p. 171.
[22] A.P. Brunetti, M.C. Lim and G.H. Nancollas, J. Am. Chem. Soc., 90 (1968) 5120.
[23] G.D. Fasman, Handbook of Biochemistry and Molecular Biology, 3rd edn, Physical and chemical data, Vol. I, CRC Press, Florida, 1976, p. 151.
[24] E.J. Cohn and J.T. Edsall, Proteins, Amino Acids and Peptides, Am. Chem. Soc. Monographs, Hafner Pub. Co., New York, 1965, p. 96.
[25] H. Hauer, E.J. Billo and D.W. Margerum, J. Am. Chem. Soc., 93 (1971) 4173.

[26] T.F. Dorigatti and E.J. Billo, J. Inorg. Nucl. Chem., 37 (1975) 1515.
[27] A.W. Hakin, M.M. Duke, J.L. Marty and K.E. Preuss, J. Chem. Soc. Faraday Trans., 90 (1994) 2027.
[28] S. Cabani, G. Conti, E. Matteoli and A. Tani, J. Chem. Soc. Faraday Trans. 1, 73 (1977) 476.
[29] S. Cabani, G. Conti, E. Matteoli and M.R. Tiné, J. Chem. Soc. Faraday Trans. 1, 77 (1981) 2377.
[30] G.I. Makhatadze and P.L. Privalov, J. Chem. Thermodyn., 20 (1988) 405.
[31] P.L. Privalov and S.J. Gill, Adv. Protein Chem., 39 (1988) 191.
[32] G.I. Makhatadze and P.L. Privalov, J. Solution Chem., 18 (1989) 927.
[33] H. Naghibi, S.F. Dec and S.J. Gill, J. Phys. Chem., 90 (1986) 4621.