

Thermodynamics of protein model compounds: An investigation of the apparent and partial molar heat capacities and volumes of aqueous solutions of alanyl and seryl side-chain containing cyclic dipeptides†

Andrew W. Hakin,* Benjamin Cavilla, Jin L. Liu and Brianne Zorzetti

Department of Chemistry and Biochemistry, The University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4. E-mail: hakin@uleth.ca

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This study reports relative densities and heat capacity ratios for the cyclic dipeptides cyclo-glycylserine, cyclo-alanyl-glycine, cyclo-alanylserine and cyclo-serylserine in water over the temperature range 288.15 to 328.15 K. These values are used to calculate apparent molar volumes, $V_{2,\phi}$, and heat capacities, $C_{p2,\phi}$, which have been modelled to give partial molar volumes and heat capacities at infinite dilution. The partial molar properties at infinite dilution have been used to calculate the thermodynamic parameters for the amino acid side-chains of alanine and serine, which have been compared to values previously reported in the literature.

Introduction

Several authors have reported group additivity schemes which may be utilized to estimate the thermodynamic properties of unfolded proteins in aqueous solution.^{1–7} Of particular note in this regard are the tripeptide model proposed by Hedwig and co-workers,^{3,4} the organic analogue approach proposed by Privalov and Makhatadze,^{1,2} and the semi-empirical model proposed by Amend and Helgeson.⁵ All of these models have been utilized, with some success, to estimate the partial molar heat capacities of denatured proteins and peptides in aqueous solution, however there are significant differences between the various values of amino acid side-chain contributions that have been generated from these models. The role of this investigation is therefore to further probe the use of cyclic dipeptides as potential protein model compounds (*i.e.*, compounds from which thermodynamic data for amino acid side-chains can be extracted) in order to verify previously reported values of the thermodynamic parameters for amino acid side-chains.

This study reports relative densities, $(\rho - \rho_1^0)$, and heat capacity ratios, $(c_p\rho/c_{p1}\rho_1^0) - 1$, for the cyclic dipeptides (diketopiperazines) cyclo-glycylserine (c(GlySer)), cyclo-alanyl-glycine (c(AlaGly)), cyclo-alanylserine (c(AlaSer)) and cyclo-serylserine (c(SerSer)) at temperatures of 288.15, 298.15, 313.15 and 328.15 K. These data were used to calculate apparent molar volumes, $V_{2,\phi}$, and heat capacities, $C_{p2,\phi}$, which were, in turn, utilized in the calculation of partial molar volumes and heat capacities at infinite dilution. Work presented in this paper is therefore a continuation of a recently published study⁸ in which volumetric and thermochemical data for cyclo(glycylglycine), cyclo(alanylalanine) and cyclo(sarcosylsarcosine) were reported and used to obtain partial molar volumes and heat capacities at infinite dilution of the glycyl group (CH₂CONH) and the alanyl side chain. In this paper, values for partial molar properties at infinite dilu-

tion have been used to calculate thermodynamic parameters for the seryl side-chain and also to re-evaluate thermodynamic parameters for the alanyl side-chain.

Although the cyclic dipeptides have been utilized in the modelling of interactions found in the solid core of globular proteins⁹ there have been surprisingly few studies^{8,9} of the thermodynamic properties of these systems in aqueous solution. Indeed, a review of the literature indicates that no previous volumetric or thermochemical measurements have been reported for the compounds selected in this study.

Experimental

Cyclo-glycylserine, cyclo-alanyl-glycine, cyclo-alanylserine and cyclo-serylserine were obtained from Bachem (product numbers G-1725, G-1660, G-1670 and G-1785 respectively) and were used, without further purification, to obtain volumetric and thermochemical data at 288.15 and 298.15 K. The solutes c(SerSer), c(AlaSer) and c(GlySer) were recovered by recrystallization from ethanol + water mixtures and their purities confirmed by IR and C, H and N elemental analysis before being used to obtain volumetric and thermochemical data at 313.15 and 328.15 K. Elemental analysis was performed by Glabraith Laboratories (Knoxville, TN, USA) and produced the following results. c(GlySer), found: C, 41.55%; H, 5.68%; N, 19.35%, *cf.* calculated composition: C, 41.67%; H, 5.59%; N, 19.44%. c(AlaSer), found: C, 45.52%; H, 6.49%; N, 17.56%, *cf.* calculated composition: C, 45.57%; H, 6.37%; N, 17.71%. c(SerSer), found: C, 41.67%; H, 5.91%; N, 16.02%, *cf.* calculated composition: C, 41.38%; H, 5.79%; N, 16.08%. The solute c(AlaGly) was recovered by recrystallization from methanol and although the IR spectrum of the recovered material was in good agreement with that of the original sample the results of C, H and N were not as satisfactory. c(AlaGly), (supplied from Bachem): C, 46.91%; H, 6.22%; N, 22.10%, recovered material: C, 45.70%; H, 6.40%; N, 22.79%, *cf.* calculated composition: C, 46.87%; H, 6.29%; N, 21.86%. There is therefore some uncertainty concerning the purity of

† Electronic Supplementary Information available. See <http://www.rsc.org/suppdata/cp/b1/b103629g/>

the c(AlaGly) samples used to obtain volumetric and thermochemical data at 313.15 and 328.15 K. All of the recrystallized compounds were dried and stored in a vacuum oven, over silica gel, at 323.15 K prior to use.

Water used in these investigations was obtained from an Omsonics model Aries High-purity D. I. Loop that can polish water to a resistance of 18.3 M Ω . All solutions were made by weight, using appropriate buoyancy corrections, on the molality concentration scale and were stored in sealed glass flasks.

Relative densities, $(\rho - \rho_1^0)$, were calculated from time-period data which were measured using a Sodev O2D vibrating tube densimeter.¹⁰ This densimeter was calibrated before and after each set of density measurements using the density of air (corrected for ambient humidity) and that of pure water. Values for the density of pure water, ρ_1^0 , used in these calibrations were taken from the compilations of Kell¹¹ ($\rho_1^0 = 0.999\,101, 0.997\,047, 0.992\,219$ and $0.985\,696$ g cm⁻³ at 288.15, 298.15, 313.15 and 328.15 K respectively). The overall uncertainty in a measured density is 5×10^{-6} g cm⁻³.

Heat capacity ratios, $(c_p \rho / c_{p1}^0 \rho_1^0) - 1$, were measured using a Picker dynamic microcalorimeter.¹² The calorimeter was calibrated using aqueous solutions of sodium chloride and its heat loss correction factor¹³ was calculated to be $F = 1.0106 \pm 0.0076$. This calibration factor was found to be independent of temperature. The uncertainty in a measured specific heat capacity, δc_p , is estimated to be 7×10^{-5} J K⁻¹ g⁻¹.

Results

Relative densities were calculated from measured time-period values using the equation:

$$(\rho - \rho_1^0) = K(\tau^2 - \tau_1^2) \quad (1)$$

where ρ is the density of the solution, τ and τ_1 are the oscillation periods of the tube containing sample and pure water respectively and K defines a temperature dependent calibration constant for the vibrating tube densimeter.

Apparent molar volumes were calculated from relative densities using the equation:

$$V_{2,\phi} = \frac{M}{\rho} = \frac{(\rho - \rho_1^0)}{m\rho\rho_1^0} \quad (2)$$

where m is the molality of the investigated solution and M is the molar mass of the solute. Details of how the uncertainties in apparent molar volumes, $\delta V_{2,\phi}$, were calculated have been

discussed previously.¹⁴ Relative densities, apparent molar volumes and their uncertainties are reported for c(GlySer), c(AlaGly), c(AlaSer) and c(SerSer) in Tables S1–S4.†

The concentration dependences of the calculated apparent molar volumes were found to be well modelled at each investigated temperature by a linear equation of the form:

$$V_{2,\phi} = V_2^0 + S_v m \quad (3)$$

In this equation V_2^0 defines a partial molar volume at infinite dilution and S_v is a calculated slope. Values of V_2^0 and S_v were obtained by fitting eqn. (3) to the relevant sets of apparent molar volume data using a weighted least squares analysis procedure. Weights used in these fits were calculated as $1/(\delta V_{2,\phi})^2$. Values of V_2^0 and S_v , and their estimated uncertainties, are contained in Table 1. In cases where values of apparent molar volume were found to be independent of concentration the reported V_2^0 values were calculated as the mean of the $V_{2,\phi}$ values. Uncertainties in these infinite dilution values were calculated as the root mean squares of the uncertainties in the apparent molar values.

The temperature dependences of the calculated partial molar volumes at infinite dilution of the cyclic dipeptides were modelled by equations of the form:

$$V_2^0 = a_0 + a_1(T - \theta) + a_2(T - \theta)^2 \quad (4)$$

where a_i ($i = 0$ to 2) are fitting coefficients, T is the temperature in K and the temperature θ is assigned a value of 308.15 K which is the mid-point of the investigated temperature range. Values of the fitting coefficients for each investigated system are reported in Table 2 together with their estimated uncertainties. These coefficients were obtained through weighted regression analyses in which weights were calculated as the inverse squares of the uncertainties in the reported V_2^0 values.

Apparent molar heat capacities, $C_{p2,\phi}$, were determined from heat capacity ratios using the equation:

$$C_{p2,\phi} = M c_p + \frac{(c_p - c_{p1}^0)}{m} \quad (5)$$

where c_p defines the specific heat capacity of the sample solution and c_{p1}^0 defines the specific heat capacity of pure water¹⁵ ($c_{p1}^0 = 4.1855, 4.1793, 4.1783$ and 4.1821 J K⁻¹ g⁻¹ at 288.15, 298.15, 313.15 and 328.15 K respectively). The $C_{p2,\phi}$ values reported in Tables S1–S4 are mean values obtained from the analyses of the recorded calorimetric signals at four separate points during the course of a run. These values have been cor-

Table 1 Calculated values of V_2^0 and C_{p2} and the slopes S_v and S_c for eqn. (3) and (6)

T/K	$V_2^0/\text{cm}^3 \text{ mol}^{-1}$	$S_v/\text{cm}^3 \text{ kg mol}^{-2}$	$C_{p2}^0/\text{J K}^{-1} \text{ mol}^{-1}$	$S_c/\text{J kg K}^{-1} \text{ mol}^{-2}$
c(GlySer)				
288.15	$93.3_1 \pm 0.1_2$	5.5 ± 1.7	177.8 ± 6.3	
298.15	$94.8_2 \pm 0.1_3$	2.2 ± 1.8	201.6 ± 2.7	
313.15	$96.3_2 \pm 0.1_2$	—	236.5 ± 2.5	-59 ± 37
328.15	$97.7_9 \pm 0.1_3$	-2.7 ± 1.9	253.4 ± 4.3	
c(AlaGly)				
288.15	$93.3_8 \pm 0.1_3$	2.7 ± 1.8	199.4 ± 3.2	
298.15	$94.4_6 \pm 0.1_2$	4.5 ± 1.6	217.5 ± 2.7	
313.15	$96.2_7 \pm 0.1_3$	1.9 ± 1.8	243.8 ± 2.6	—
328.15	$97.8_4 \pm 0.1_2$	—	257.2 ± 4.1	—
c(AlaSer)				
288.15	$110.6_0 \pm 0.1_2$	—	288.3 ± 7.3	—
298.15	$111.4_8 \pm 0.1_2$	3.2 ± 1.8	306.0 ± 1.4	77 ± 27
313.15	$113.0_6 \pm 0.1_2$	4.2 ± 1.8	336.2 ± 3.9	—
328.15	$114.3_8 \pm 0.1_2$	—	351.0 ± 3.3	87 ± 43
c(SerSer)				
288.15	$110.3_g \pm 0.1_3$	—	245.9 ± 4.8	—
298.15	$112.1_g \pm 0.1_3$	-3.1 ± 1.9	273.0 ± 3.1	—
313.15	$113.3_6 \pm 0.2_3$	12.2 ± 5.5	304.5 ± 4.3	—
328.15	$114.5_4 \pm 0.1_4$	3.9 ± 1.9	330.4 ± 6.0	—

Table 2 Calculated coefficients for eqn. (4)^a

Compound	$a_0/\text{cm}^3 \text{ mol}^{-1}$	$a_1/\text{cm}^3 \text{ mol}^{-1} \text{ K}^{-1}$	$a_2/\text{cm}^3 \text{ mol}^{-1} \text{ K}^{-2}$
c(GlySer)	95.8 ₇ (0.1 ₀)	0.110 ₀ (0.004 ₁)	-0.0007 ₉ (0.0003 ₆)
c(AlaGly)	95.62 ₃ (0.06 ₂)	0.112 ₅ (0.004 ₁)	—
c(AlaSer)	112.50 ₀ (0.03 ₅)	0.095 ₈ (0.002 ₆)	—
c(SerSer)	113.2 ₃ (0.1 ₃)	0.101 ₄ (0.004 ₆)	-0.0019 ₅ (0.0004 ₆)

^a Estimated uncertainties are shown in parentheses.

rected for the effects of heat leaks¹³ using the reported correction factor. The reported values for heat capacity ratios have not been corrected in this manner. Uncertainties in apparent molar heat capacities, $\delta C_{p2,\phi}$, have been calculated as the standard deviations of each set of four $C_{p2,\phi}$ values.

Partial molar heat capacities at infinite dilution, C_{p2}° , were calculated using weighted linear regression analyses. The linear expression shown as eqn. (6) was used to model the concentration dependences of apparent molar heat capacities at each investigated temperature.

$$C_{p2,\phi} = C_{p2}^{\circ} + S_c m \quad (6)$$

In this equation S_c defines a calculated slope. The weights utilized in these analyses were calculated as the inverse squares of the reported $\delta C_{p2,\phi}$ values. For those analyses in which values of apparent molar heat capacity were found to be independent of solution concentration, partial molar heat capacities at infinite dilution were calculated as mean values of the reported $C_{p2,\phi}$ data. Uncertainties in these infinite dilution values were calculated as the root mean squares of the $\delta C_{p2,\phi}$ values. Calculated values of C_{p2}° , S_c , and their uncertainties are contained in Table 1.

Discussion

The functionality of a globular protein in aqueous solution is dictated by its unique three-dimensional, folded structure. For example, folding is the final step in the overall process of gene expression. The native state of a protein is often experimentally more accessible than the fully unfolded, denatured, state and has been investigated for many systems using such techniques as NMR spectroscopy and X-ray crystallography. However, investigations of the fully unfolded states are important because they may be utilized as ideal reference states in discussions of thermodynamic stability in aqueous solution; the stability of a globular protein in aqueous solution being dictated by the magnitude of the equilibrium constant associated with the unfolding process. Direct experimental investigation of the ideal reference state is possible, but has been criticized on the grounds that some residual structure could remain in the system.¹⁶ To overcome such criticisms some researchers have turned to the use of additivity models to predict the thermodynamic properties of proteins in the denatured state.^{1,5} This approach utilizes thermodynamic data obtained from small organic compounds, known as protein model compounds, which can be manipulated to yield thermodynamic data for amino acid side-chains. In probing the structures of proteins in aqueous solution using the model compound approach it has become apparent that there are significant differences between various sets of thermodynamic parameters for amino acid side-chains. It is clear therefore, that some critical evaluation of thermodynamic parameters for amino acid side-chains is in order and in this regard we have turned to the thermodynamic properties of the cyclic dipeptides. Several authors have suggested the use of these small molecules as protein model compounds because they contain many of the structural characteristics that one looks for in such systems. For example, they contain no charged end groups in aqueous solution and, as indicated earlier, they have been shown to possess many of the interactions required to

quantitatively characterize the interactions of the solid-like core of globular proteins.⁹

The analysis presented in this paper compares thermodynamic parameters for the alanyl and seryl side-chains which can be calculated from partial molar properties at infinite dilution for the cyclic dipeptides with those previously reported in the literature. The partial molar properties at infinite dilution reported in Table 1 for c(AlaGly) and c(AlaSer) may be combined with previously reported values⁸ for the cyclic dipeptide cyclo-glycylglycine, c(GlyGly), to yield alanyl and seryl side-chain contributions to partial molar volumes and heat capacities at infinite dilution. Using the principles of group additivity the following equations may be used to obtain the required side-chain contributions:

$$Y_2^{\circ}(\text{CH}_3) = Y_2^{\circ}(\text{c(AlaGly)}) - Y_2^{\circ}(\text{c(GlyGly)}) + Y_2^{\circ}(\text{H}) \quad (7)$$

$$Y_2^{\circ}(\text{CH}_2\text{OH}) = Y_2^{\circ}(\text{c(GlySer)}) - Y_2^{\circ}(\text{c(GlyGly)}) + Y_2^{\circ}(\text{H}) \quad (8)$$

where $Y_2^{\circ}(\text{CH}_3)$, $Y_2^{\circ}(\text{CH}_2\text{OH})$, $Y_2^{\circ}(\text{H})$ define contributions of the alanyl side-chain, the seryl side-chain and of a hydrogen atom, respectively, to the thermodynamic property of interest, Y , at infinite dilution. Polynomial equations which define the temperature dependence of $V_2^{\circ}(\text{H})$ and $C_{p2}^{\circ}(\text{H})$ have been previously reported⁸ and have been used in the current study without modification. Values for $V_2^{\circ}(\text{CH}_3)$, $C_{p2}^{\circ}(\text{CH}_3)$, $V_2^{\circ}(\text{CH}_2\text{OH})$ and $C_{p2}^{\circ}(\text{CH}_2\text{OH})$, that were calculated using eqn. (7) and (8), are reported in Table 3 with their estimated uncertainties.

Alternatively, the principles of additivity may be used to extract thermodynamic parameters for the seryl side-chain from the partial molar properties of the cyclic dipeptides c(SerSer) and c(AlaSer) using the equations:

$$Y_2^{\circ}(\text{CH}_2\text{OH}) = \{Y_2^{\circ}(\text{c(SerSer)}) - Y_2^{\circ}(\text{c(GlyGly)}) + Y_2^{\circ}(\text{H})\}/2 \quad (9)$$

$$Y_2^{\circ}(\text{CH}_2\text{OH}) = (Y_2^{\circ}(\text{c(AlaSer)}) - Y_2^{\circ}(\text{c(AlaGly)}) + Y_2^{\circ}(\text{H})) \quad (10)$$

Seryl side-chain contributions that were calculated using eqn. (9) and (10) are also reported in Table 3.

Finally, a second route to the calculation of alanyl side-chain contributions is provided by combining partial molar thermodynamic properties at infinite dilution for c(AlaSer) with those for c(GlySer) using the equation:

$$Y_2^{\circ}(\text{CH}_3) = Y_2^{\circ}(\text{c(AlaSer)}) - Y_2^{\circ}(\text{c(AlaSer)}) - Y_2^{\circ}(\text{c(GlySer)}) + Y_2^{\circ}(\text{H}) \quad (11)$$

Values for $V_2^{\circ}(\text{CH}_3)$ and $C_{p2}^{\circ}(\text{CH}_3)$ which were calculated using eqn. (11) are contained in Table 3 together with their estimated uncertainties.

A comparison of the temperature dependences of values for the partial molar volume of the alanyl side-chain is presented in Fig. 1. This figure indicates that there is good agreement between the values reported by Hakin *et al.*,⁸ Häckel *et al.*³ and those which may be calculated from the semi-empirical equations of state reported by Amend and Helgeson,⁵ however, it is clear that the values of $V_2^{\circ}(\text{CH}_3)$ reported by Makhatazde *et al.*¹⁷ oppose this trend. Indeed, the temperature dependence of the latter data is reversed compared to

Table 3 Alanine and serine side-chain contributions to partial molar volumes and heat capacities at infinite dilution. Values have been calculated using eqn. (7), (8), (9), (10) and (11)^a

<i>T</i> /K	$V_2^0(\text{CH}_3)/\text{cm}^3 \text{ mol}^{-1}$	$C_{p2}^0(\text{CH}_3)/\text{J K}^{-1} \text{ mol}^{-1}$
Eqn. (7):		
288.15	28.2 ₁ (0.1 ₅)	157.6(3.4)
298.15	27.8 ₆ (0.1 ₄)	153.4(2.9)
313.15	27.8 ₄ (0.1 ₅)	151.2(2.8)
328.15	27.8 ₅ (0.1 ₄)	147.0(4.2)
Eqn. (11):		
288.15	27.8 ₅ (0.1 ₈)	190.5(9.7)
298.15	26.9 ₁ (0.1 ₉)	182.1(3.1)
313.15	26.6 ₀ (0.1 ₈)	173.9(4.6)
328.15	26.1 ₃ (0.1 ₉)	168.2(5.4)
<hr/>		
	$V_2^0(\text{CH}_2\text{OH})$	$C_{p2}^0(\text{CH}_2\text{OH})$
Eqn. (8):		
288.15	28.1 ₄ (0.1 ₄)	135.6(6.4)
298.15	28.2 ₂ (0.1 ₅)	137.5(2.9)
313.15	27.8 ₅ (0.1 ₄)	143.9(2.7)
328.15	27.8 ₀ (0.1 ₅)	143.2(4.4)
Eqn. (9):		
288.15	27.8 ₉ (0.1 ₆)	141.9(4.9)
298.15	27.9 ₂ (0.1 ₆)	143.3(3.3)
313.15	27.4 ₀ (0.2 ₅)	143.0(4.5)
328.15	27.0 ₅ (0.1 ₇)	145.3(6.1)
Eqn. (10):		
288.15	27.7 ₈ (0.1 ₉)	168.9(8.0)
298.15	27.2 ₇ (0.1 ₈)	166.2(3.1)
313.15	26.6 ₂ (0.1 ₉)	166.6(4.7)
328.15	26.0 ₈ (0.1 ₈)	164.4(5.3)

^a Estimated uncertainties are shown in parentheses.

those obtained in this and previous investigations. In attempting to explain this discrepancy it is noted that although the $V_2^0(\text{CH}_3)$ values reported by Makhatadze *et al.*¹⁷ are obtained by subtracting partial molar volumes at infinite dilution for the tripeptide GlyGlyGly from those for GlyAlaGly, in the same manner as those reported by Häckel *et al.*,³ the solvent environment is not the same in the two studies. The values of partial molar volume at infinite dilution for GlyGlyGly and GlyAlaGly reported by Makhatadze *et al.*¹⁷ are obtained in a pH 4.0, 0.5 M sodium acetate buffer. Solute–solvent interactions in such buffer systems, which are reflected in values of

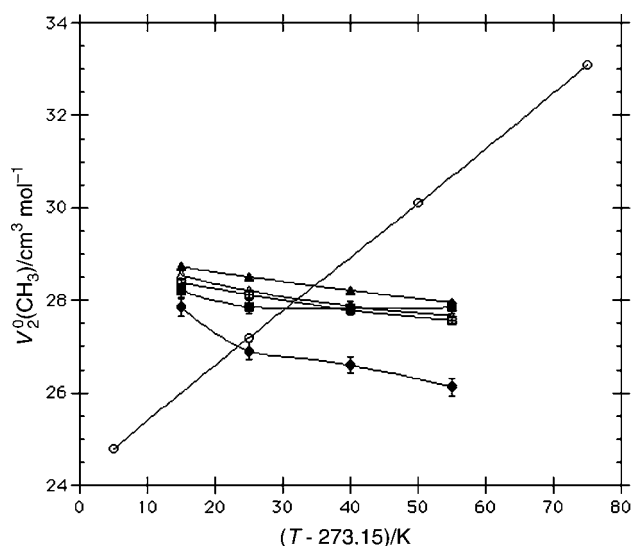


Fig. 1 The temperature dependence of the partial molar volume at infinite dilution of the alanyl side-chain. (Δ) Amend and Helgeson,⁵ (\blacktriangle) Häckel *et al.*,³ (\circ) Makhatadze *et al.*,¹⁷ (\bullet) eqn. (11), (\square) Hakin *et al.*,⁸ (\blacksquare) eqn. (7).

partial molar volumes at infinite dilution, are necessarily different from those in which pure water is utilized as the solvent.

Fig. 2 shows a comparison of values of $V_2^0(\text{CH}_2\text{OH})$ obtained in this study with those previously reported in the literature. Although the $V_2^0(\text{CH}_2\text{OH})$ values calculated from eqn. (10) show the same general temperature dependence as those calculated using eqn. (8) and (9) and those reported by Amend and Helgeson⁵ and Häckel *et al.*,³ the difference between the former values and the latter appear to be larger than the sum of the combined uncertainties. Although there are clearly many structural similarities between the cyclic dipeptides used in this study to calculate values for $V_2^0(\text{CH}_2\text{OH})$ via eqn. (8), (9) and (10) we are forced to conclude that the differences between the various values stem from small differences in the hydration of the seryl side-chain within the individual cyclic dipeptides. As noted in a previous study,³ the values of $V_2^0(\text{CH}_2\text{OH})$ reported by Makhatadze *et al.*¹⁷ are in very poor agreement with other serine side-chain values. An explanation for this discrepancy follows the same reasoning offered above with respect to the $V_2^0(\text{CH}_3)$ values reported by Makhatadze *et al.*¹⁷

Turning to side-chain partial molar heat capacities, Fig. 3 compares the temperature dependences of values for $C_{p2}^0(\text{CH}_3)$ obtained in this study with those previously reported in the literature. There is good agreement between the tripeptide derived values reported by Häckel *et al.*⁴ and the values obtained in this study using eqn. (11). There is also good agreement between the values of $C_{p2}^0(\text{CH}_3)$ obtained by Hakin *et al.*⁸ from the cyclic dipeptide c(AlaAla) and the organic analogue (methane) derived values reported by Makhatadze and Privalov.¹ Values of $C_{p2}^0(\text{CH}_3)$ calculated using eqn. (7) are somewhat lower in value than those reported by Hakin *et al.*⁸ and Makhatadze and Privalov¹ but nevertheless remain in reasonable agreement. The agreement between these two groups of data is however, larger than the sum of the combined uncertainties. A possible explanation for this discrepancy has been previously discussed^{8,18} and identifies the values of $C_{p2}^0(\text{H})$ used within the calculation of the amino acid side-chain contribution as being a probable source of error. In brief, it has been shown that the values of $C_{p2}^0(\text{H})$ are dependent upon the class of molecule used within the additivity analysis. For example, a value for $C_{p2}^0(\text{H})$ at $T = 298.15$ K obtained from a series of simple hydrocarbons was found to be considerably different from a value that was derived from a group additivity analysis of *N*-acetyl amino acid amides, peptide amides, and simple amides.¹⁸ Clearly, although this

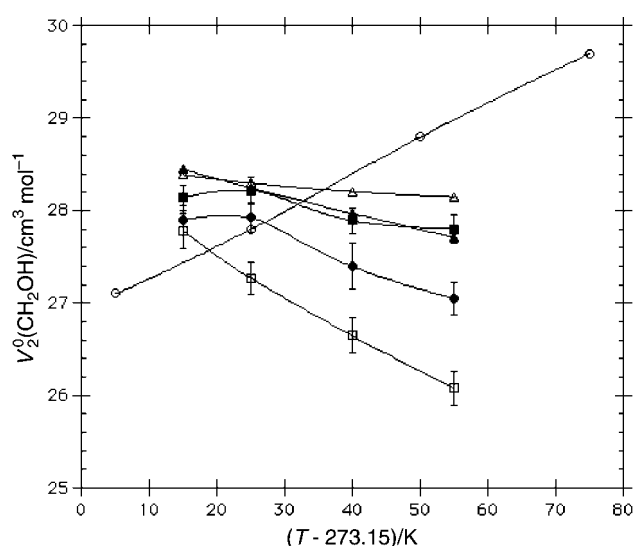


Fig. 2 The temperature dependence of the partial molar volume at infinite dilution of the seryl side-chain. (Δ) Amend and Helgeson,⁵ (\blacktriangle) Häckel *et al.*,³ (\circ) Makhatadze *et al.*,¹⁷ (\bullet) eqn. (9), (\square) eqn. (10), (\blacksquare) eqn. (8).

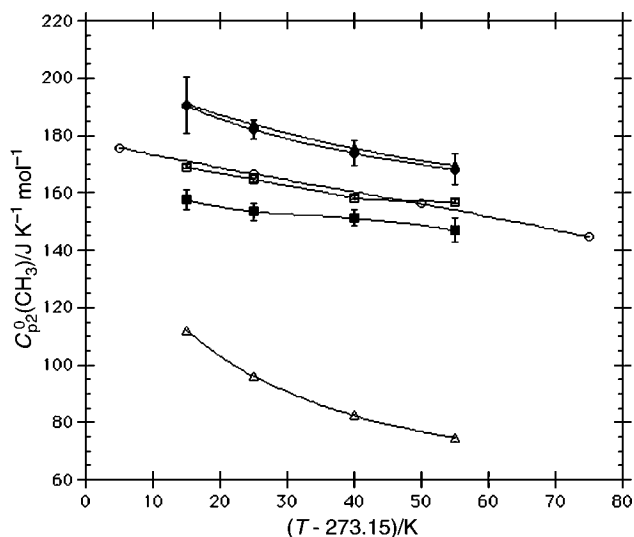


Fig. 3 The temperature dependence of the partial molar heat capacity at infinite dilution of the alanyl side-chain. (Δ) Amend and Helgeson,⁵ (\blacktriangle) Häckel *et al.*,⁴ (\circ) Makhatadze and Privalov,¹ (\bullet) eqn. (11), (\square) Hakin *et al.*,⁸ (\blacksquare) eqn. (7).

line of argumentation appears logical with respect to the differences between the alanyl side-chains reported by Häckel *et al.*¹⁸ and Makhatadze and Privalov¹ it would not appear to apply to the values of side-chain heat capacities obtained from the cyclic-dipeptides.

The temperature dependence of the partial molar heat capacity of the alanyl side-chain that can be calculated from the semi-empirical equations reported by Amend and Helgeson⁵ is out of line with those reported in this and other investigations. This lack of agreement with other alanyl side-chain values invites some doubt as to the validity of the approach utilized by Amend and Helgeson to obtain their equation for the partial molar heat capacity of the alanyl side-chain

Fig. 4 compares $C_{p2}^o(\text{CH}_2\text{OH})$ values obtained in this study with those previously reported in the literature. There is very good agreement between the values calculated using eqn. (8) and (9) and those obtained from the tripeptide model of Häckel *et al.*⁴ There is also good agreement between the values of $C_{p2}^o(\text{CH}_2\text{OH})$ derived from the organic analogue methanol¹ and the values that can be calculated from the semi-empirical equations reported by Amend and Helgeson.⁵

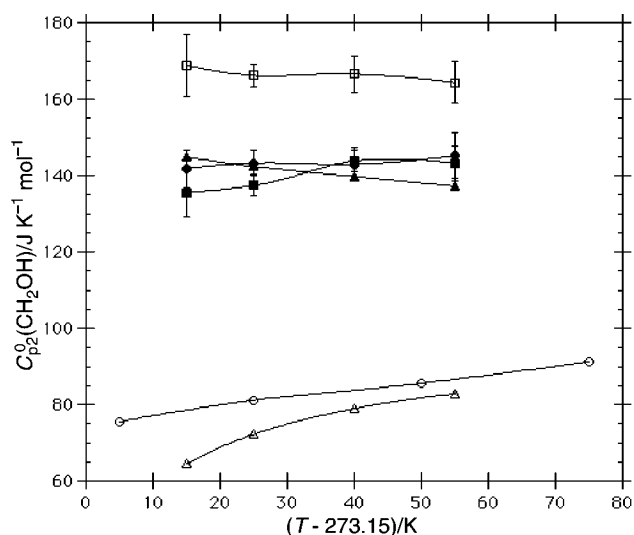


Fig. 4 The temperature dependence of the partial molar heat capacity at infinite dilution of the seryl side-chain. (Δ) Amend and Helgeson,⁵ (\blacktriangle) Häckel *et al.*,⁴ (\circ) Makhatadze and Privalov,¹ (\bullet) eqn. (9), (\square) eqn. (10), (\blacksquare) eqn. (8).

The latter agreement is not surprising as the parameters utilized in the Amend and Helgeson equation for the partial molar heat capacity of the seryl side-chain were derived from a group additivity analysis of organic analogue data and not from a direct regression of heat capacity data for the parent amino acid. Paralleling the behaviour that is observed for values for $V_2^o(\text{CH}_2\text{OH})$ which were calculated from eqn. (10), the values for $C_{p2}^o(\text{CH}_2\text{OH})$ obtained from eqn. (10) are also observed to be larger than other reported values.

The observation that the equations of Amend and Helgeson appear to yield values which agree with peptide derived model compound results with respect to the calculation of partial molar volumes of the alanyl and seryl side-chains and yet are apparently in poor agreement with the peptide derived side-chain values for the partial molar heat capacities merits further discussion. With respect to volumes, the Amend and Helgeson equations for the alanyl and seryl side-chain contributions were derived from regressions of volumetric data for the parent amino acids.¹⁹ Side-chain values obtained from amino acids have previously been shown to be in good agreement with peptide derived side-chain values.³ However, with respect to heat capacities the Amend and Helgeson equations for the alanyl and seryl side-chain contributions could not be obtained from the regression of parent amino acid data because of the paucity of heat capacity data available in the literature at temperatures removed from 298.15 K. Rather, the parameters used in equations for seryl and alanyl side-chains heat capacities were obtained from a group additivity analysis of organic analogue data.²⁰ Although the principles of group additivity are obviously being challenged it would appear that the values for side-chain parameters are dependent upon the class of compound from which they are derived. This observation is also apparent with the organic analogue derived heat capacities of the alanyl and seryl side-chains reported by Makhatadze and Privalov.¹

Although, seryl and alanyl side-chain parameters obtained from the cyclic parameters appear, in general, to support the tripeptide derived side-chain values reported by Häckel *et al.*^{3,4} there remain several discrepancies between the sets of data. Given the many structural similarities between the tripeptides and the cyclic dipeptides, solid explanations for these discrepancies are not immediately obvious. Indeed, the larger question must surely be why do different, cyclic dipeptide based, approaches to the calculation of amino acid side-chain parameters yield different values. One possible explanation for these discrepancies may stem from subtle differences in hydration of the side-chains within the cyclic dipeptides. In this regard an investigation of the water accessible surface-areas of the side-chains with the cyclic dipeptides may be warranted. Water accessible surface areas have been previously found to correlate to the partial molar heat capacities of amino acid side-chains.¹

In summary, it is becoming clearer that values for side-chain parameters, intended for use in the modelling of thermodynamic parameters for unfolded proteins, should be derived from compounds which resemble, as closely as possible, the environments of the side-chains within the proteins.

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