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The partial molar heat capacities and volumes of some *N*-acetyl amino acid amides in aqueous solution over the temperature range 288.15 to 328.15 K

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The partial molar heat capacities, $C^0_{p,2}$, and partial molar volumes, V^0_2, at infinite dilution have been determined for the compounds *N*-acetylvalinamide and *N*-acetylleucinamide in aqueous solution at the temperatures 288.15, 298.15, 313.15 and 328.15 K and for *N*-acetylglycinamide and *N*-acetylalaninamide at the temperatures 288.15, 313.15 and 328.15 K. Partial molar volumes at infinite dilution have also been determined for the sparingly soluble *N*-acetylisoleucinamide in aqueous solution over the same temperature range. The $C^0_{p,2}$ and V^0_2 results have been used to calculate amino acid side-chain contributions to the thermodynamic properties. These side-chain contributions are critically compared with those obtained using other model compounds.

Introduction

The interactions between the solvent and the various constituent groups of a protein, such as the amino acid side-chains and the backbone peptide group, play a crucial role in the structure and function of proteins in aqueous solution.[1–4] Because of the complexity of these interactions in the intact macromolecule, one approach to characterize the thermodynamic and hydration behaviour of the various constituent groups of proteins is to study low molar mass compounds chosen to model specific structural features of a protein. These compounds are referred to as model compounds. This approach has received a lot of attention in recent years.[5–15]

In some of our recent work to determine the partial molar heat capacities,[16] volumes[14] and compressibilities[17] of the amino acid side-chains of proteins, we have used tripeptides of sequence glycyl-X-glycine (gly-X-gly), where X is one of the naturally occurring amino acids, as model compounds. The single side-chain in these peptides is flanked by two peptide groups which mimics the situation in a polypeptide or protein. The retention of the characteristic peptide backbone structure makes these small peptides good model compounds for investigating side-chain effects in proteins.

In a previous comprehensive study to determine the partial molar heat capacities of all protein constituent groups over a wide temperature range, Makhatadze and Privalov used small organic solutes as compounds to model many of the amino acid side-chains.[13] As outlined in detail elsewhere,[16] the results obtained differ significantly from those based on the gly-X-gly peptides as model compounds. In view of the importance of these quantities in group additivity schemes to derive the partial molar heat capacities of unfolded proteins,[16] it is desirable to validate the side-chain heat capacities obtained using the tripeptides. This can be achieved by choosing an alternative set of compounds that realistically model the amino acid side-chains of proteins. The *N*-acetyl amino acid amides are such compounds. These neutral amino acid derivatives have one secondary and one primary amide functional group adjacent to the side-chain which, although not identical, is structurally similar to that in a polypeptide. These amino acid derivatives have been used successfully as model compounds in previous work. The partial molar volumes and compressibilities of several amino acid side-chains over the temperature range 278.15 to 318.15 K have been derived using thermodynamic data for *N*-acetyl amino acid amides.[9,18] Also the partial molar heat capacities and volumes of the glycyl group over a wide temperature range obtained using some *N*-acetyl amino acid and peptide amides were shown to be in excellent agreement with those derived from thermodynamic data for the series of peptides alanyl(glycyl)$_x$glycine, $x = 1$–3.[15]

In this paper we report the partial molar heat capacities, $C^0_{p,2}$, and partial molar volumes, V^0_2, at infinite dilution of aqueous solutions of *N*-acetylglycinamide, *N*acetylalaninamide, *N*-acetylvalinamide and *N*-acetylleucinamide at the temperatures 288.15, 298.15, 313.15 and 328.15 K. V^0_2 data are also reported for *N*-acetylisoleucinamide at the same temperatures. These results have been used to derive the partial molar heat capacities and volumes for the various amino acid side-chains.

Experimental

Materials

The *N*-acetyl amino acid amides, *N*-acetylalaninamide ($AcalaNH_2$), *N*-acetylvalinamide ($AcvalNH_2$), *N*-acetylleucinamide ($AcleuNH_2$) and *N*-acetylisoleucinamide ($AcileNH_2$) were purchased from Bachem Feinchemikalien AG. *N*-acetylglycinamide ($AcglyNH_2$) was purchased from Aldrich. The $AcalaNH_2$ was recrystallized from ethanol + diethylether, m.p. 164–165 °C (lit.[19] 162 °C). Found

C, 46.04; H, 7.82; N, 21.47%; calc. for $C_5H_{10}O_2N_2$ C, 46.14; H, 7.75; N, 21.52%. The compound $AcvalNH_2$ was recrystallized from methanol + diethylether, m.p. 235–238 °C (lit.[19] 234–236 °C). Found C, 53.19; H, 9.18; N, 17.63%; calc. for $C_7H_{14}O_2N_2$ C, 53.15; H, 8.92; N, 17.71%. The $AcleuNH_2$ was recrystallized from chloroform + petroleum ether (b.p. 60–80 °C), m.p. 133–135 °C (lit.[19] 133–134 °C). Found C, 55.67; H, 9.62; N, 16.36%; calc. for $C_8H_{16}O_2N$ C, 55.79; H, 9.36; N, 16.27%. The $AcileNH_2$ was recrystallized from hot ethanol, m.p. 255–257 °C (lit.[20] 256.5 °C). Found C, 55.55; H, 9.56; N, 16.15%; calc. as for $AcleuNH_2$. The sample of $AcglyNH_2$ was recrystallized from hot ethanol, m.p. 140–141 °C (lit.[21] 139–140 °C). Found C, 41.33; H, 7.11; N, 24.18%; calc. for $C_4H_8O_2N_2$ C, 41.37; H, 6.95; N, 24.13%.

All the compounds were dried exhaustively under vacuum at room temperature before use. The water used to prepare solutions was deionized, glass distilled and thoroughly degassed immediately prior to use. Solutions were prepared by mass.

Apparatus and methods

Densities of solutions were measured using an Anton Paar digital density meter (model DMA 60/602) as outlined previously.[22,23] The reproducibility of an individual density measurement was to better than 3×10^{-6} g cm^{-3}. Heat capacity measurements were carried out using a Picker differential flow microcalorimeter.[24] The experimental procedures used were the same as those described in previous work[22,23] with the exception that the output from the calorimeter was connected to a Keithley model 181 nanovoltmeter that was interfaced to a PC.

Results

Partial molar volumes

Densities at the temperatures 288.15, 298.15, 313.15 and 328.15 K for solutions of $AcglyNH_2$, $AcalaNH_2$, $AcvalNH_2$, $AcleuNH_2$ and $AcileNH_2$ are given in Tables S1–S5 of the Supplementary Data, respectively.† These data were used to calculate the apparent molar volumes of the solutes, V_ϕ, using the equation

$$V_\phi = (M_2/\rho) - (\rho - \rho_1^0)/(m\rho\rho_1^0), \quad (1)$$

where M_2 is the solute molar mass, ρ and ρ_1^0 are, respectively, the densities of the solution and solvent, and m is the solution molality. The ρ_1^0 values for water at the various temperatures used were those reported by Kell[25] (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 V_ϕ values, together with their uncertainties estimated using the procedures outlined earlier,[22] are given in the electronic supplementary data.† For the dilute solutions used in this study, the molality dependence of V_ϕ can be represented by the linear equation

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

where V_2^0 is the partial molar volume of the solute at infinite dilution and S_V is the experimental slope. Values of V_2^0, S_V and their standard errors obtained from weighted least-squares analyses of the apparent molar volume data using eqn. (2), are given in Table 1. As the solute $AcileNH_2$ is sparingly soluble in water, values of S_V could not be determined. The V_2^0 results given in Table 1 are actually the mean values of the V_ϕ data. For some other systems, the values of S_V obtained from the least-squares analyses were statistically not different from zero. In these cases the V_2^0 results are also mean values of the V_ϕ data over the molality range studied.

The temperatures 288.15 and 298.15 K chosen in this work are identical to those used in the earlier study by Kikuchi *et al.*[9] Table 2 gives a comparison of the results obtained for the systems common in the two studies. For $AcvalNH_2$ the V_2^0 results obtained in this study and those reported by Kikuchi *et al.* are concordant. For the other solutes our V_2^0 values are 0.15–0.30 cm^3 mol^{-1} higher than those reported earlier. With one exception, the agreement between the S_V values determined herein and those reported by Kikuchi *et al.*[9] is reason-

† Available as electronic supplementary information. See http://www.rsc.org/suppdata/cp/a9/a910296p

Table 1 Partial molar volumes and heat capacities at infinite dilution and the S_V and S_C values for some *N*-acetyl amino acid amides in aqueous solution at 288.15, 298.15, 313.15, and 328.15 K

T/K	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}
$AcglyNH_2$				
288.15	90.03(0.02)[a]	−0.55(0.1)[a]	220.3(1.5)[a]	[b]
298.15	91.02(0.02)[c]	−0.14(0.09)[c]	240.6(0.6)[c]	2.8(2.6)[a,c]
313.15	92.36(0.01)	−0.24(0.07)	265.2(0.7)	[b]
328.15	93.53(0.02)	[b]	278.2(1.6)	13(11)
$AcalaNH_2$				
288.15	107.18(0.01)	-0.4_3(0.1)	329.6(0.8)	[b]
298.15	108.06(0.01)[d]	−0.12(0.01)[d]	346.4(0.6)[e]	5(4)[e]
313.15	109.31(0.03)	$+0.4_4$(0.2)	362.6(0.8)	10(5)
328.15	110.75(0.04)	-0.4_2(0.3)	371.5(2.1)	[b]
$AcvalNH_2$				
288.15	137.80(0.02)	-1.4_4(0.2)	492.4(0.8)	−20(7)
298.15	138.92(0.03)	-0.8_4(0.2)	502.4(1.6)	[b]
313.15	140.71(0.04)	-0.7_7(0.3)	520.2(2.0)	−33(16)
328.15	142.50(0.02)	[b]	526.8(1.8)	[b]
$AcleuNH_2$				
288.15	154.77(0.02)	-1.5_3(0.2)	585.7(1.6)	[b]
298.15	156.09(0.02)	-1.2_5(0.1)	599.1(1.4)	−16(12)
313.15	158.15(0.03)	[b]	608.4(1.7)	[b]
328.15	160.31(0.03)	[b]	614.8(2.0)	[b]
$AcileNH_2$				
288.15	152.9(0.1)	[b]		
298.15	153.9(0.3)	[b]		
313.15	156.0(0.2)	[b]		
328.15	158.3(0.1)	[b]		

[a] Standard errors are in parentheses. [b] No concentration dependence was detected. See text. [c] Ref. 15. [d] Ref. 26. [e] Ref. 27.

Table 2 A comparison of V_2^0 and S_V values with literature results

Solute	T/K	V_2^0/cm^3 mol^{-1}		S_V/cm^3 kg mol^{-2}	
		This work	Literature	This work	Literature
AcglyNH$_2$	288.15	90.03(0.02)[a]	89.76(0.02)[a,b]	-0.5_5(0.1)[a]	-1.07(0.23)[a,b]
AcalaNH$_2$	288.15	107.18(0.01)	107.03(0.02)[b]	-0.4_3(0.1)	1.31(0.27)[b]
AcvalNH$_2$	288.15	137.80(0.02)	137.74(0.01)[b]	-1.4_4(0.2)	-1.92(0.12)[b]
	298.15	138.92(0.03)	138.96(0.01),[a] 139.00(0.14)[c]	-0.8_4(0.2)	-1.52(0.10),[b] -1.15(0.38)[c]
AcleuNH$_2$	288.15	154.77(0.02)	154.47(0.01)[b]	-1.5_3(0.2)	-1.55(0.17)[b]
	298.15	156.09(0.02)	155.88(0.01)[b]	-1.2_5(0.1)	-1.13(0.12)[b]

[a] Standard errors are in parentheses. [b] Ref. 9. [c] Ref. 26.

able, given that S_V values reported in the literature for a particular system often vary considerably. For AcalaNH$_2$ at 288.15 K the S_V value determined in this work is negative whereas that reported by Kikuchi *et al.* is positive. We have no satisfactory explanation for this difference.

As the temperature range used in this work overlaps that used in the previous study by Kikuchi *et al.*[9] it is of interest to compare the partial molar volumes for the *N*-acetyl amino acid amides over a wide temperature range. The $V_2^0(T)$ curves are displayed in Figs. 1 and 2. For both AcvalNH$_2$ and AcalaNH$_2$, the agreement between the two $V_2^0(T)$ curves is satisfactory while for AcglyNH$_2$ and to a lesser extent for AcleuNH$_2$, the differences alluded to above for the lower temperatures diminish as the temperature increases.

Partial molar heat capacities

The apparent molar heat capacities, $C_{p,\phi}$, of the *N*-acetyl amino acid amides in aqueous solution were calculated from the experimental specific heat capacities, c_p, using the equation

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

where c_p^0 is the specific heat capacity of pure water and the remaining symbols are defined as for eqn. (1). The c_p^0 values used are those given by Stimson[28] (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 $C_{p,\phi}$ results and their estimated uncertainties, determined as described in previous work,[22] are given in Tables S1–S4 of the electronic supplementary data.† The solubility of AcileNH$_2$ was insufficient to enable specific heat capacities to be obtained. In a few cases, specific heat capacity measurements were made on solutions for which the density was not measured. For these solutions, the densities, which are needed to convert the heat capacities per unit volume into specific heat capacities, were calculated using a power series in molality of the form

$$\rho = \rho_1^0 + p_1 m + p_2 m^2, \quad (4)$$

where p_1 and p_2 are parameters obtained by least-squares fitting to the density data for all the other solutions used.

For each *N*-acetyl amino acid amide, the $C_{p,\phi}$ data were analysed by a weighted least-squares method 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

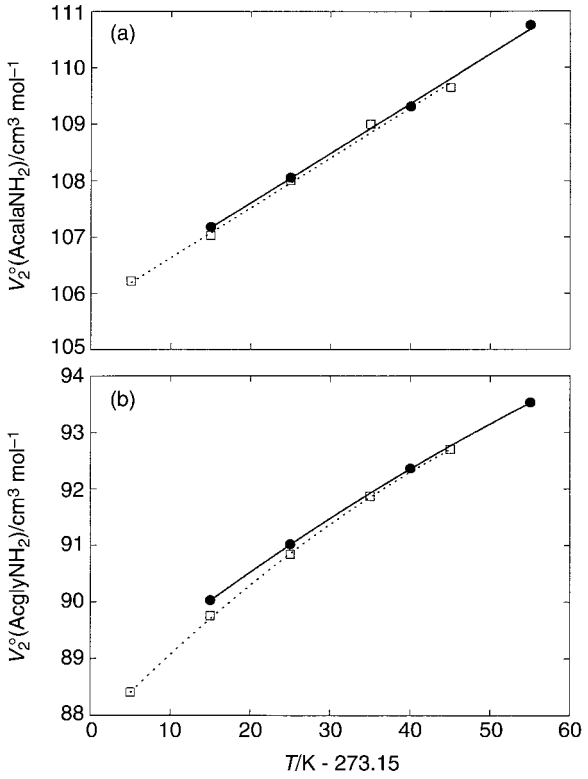

Fig. 1 Temperature dependence of the partial molar volumes of (a) AcalaNH$_2$ and (b) AcglyNH$_2$. ● This work; □ from ref. 9.

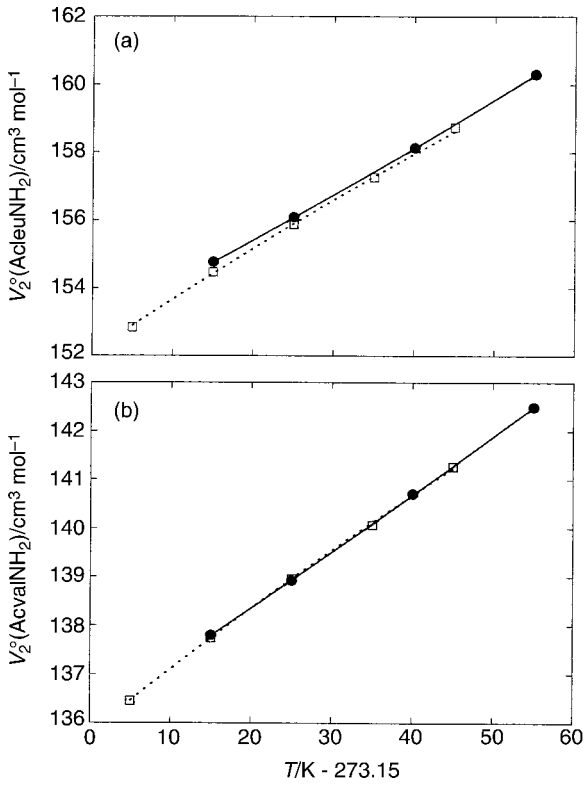

Fig. 2 Temperature dependence of the partial molar volumes of (a) AcleuNH$_2$ and (b) AcvalNH$_2$. ● This work; □ from ref. 9.

$C^0_{p,2}$ and S_C, together with their standard deviations, are given in Table 1. The analyses for several systems studied gave S_C values that were not statistically different from zero. In these cases the $C^0_{p,2}$ values given in Table 1 are the means of all the $C_{p,\phi}$ data.

In an earlier study by one of us,[15] the partial molar heat capacity of $AcglyNH_2$ was determined over the temperature range 283.15 to 373.15 K using high sensitivity differential scanning calorimetry (DSC). A comparison of these results with those obtained in this study is shown in Fig. 3. The $C^0_{p,2}$ values determined using DSC are, on average, 3% higher than those determined herein.

Partial molar expansibilities

The equation

$$V^0_2 = a + b(T - 308.15) + c(T - 308.15)^2 \quad (6)$$

was fitted to the V^0_2 data for each solute using a weighted least-squares procedure. The quantity $(T - 308.15)$ was chosen as the independent variable because the temperature 308.15 K is the mid-point of the range used in this study. The weighting factor for V^0_2 each value was taken as $1/(\delta V^0_2)^2$, where δV^0_2 is the standard error of V^0_2. The polynomial coefficients of eqn. (6) along with their standard deviations obtained from the least-squares analysis are given in Table 3. For $AcglyNH_2$ and $AcleuNH_2$ the low standard deviations arise because, somewhat fortuitously, the V^0_2 values obtained happen to give an excellent fit to a quadratic function. However, these low standard deviations underestimate the true uncertainty. For example, if the V^0_2 values for $AcglyNH_2$ at 288.15 and 298.15 K are changed by their standard errors to give 90.05 and 91.00 $cm^3\ mol^{-1}$ respectively, then the errors for the parameters a, b and c obtained in the least-squares procedure become 0.01, 0.0008 and 0.5, respectively. For $AcalaNH_2$ a fit to eqn. (6) gave a value for the coefficient c that was statistically insignificant. The values of the coefficients a and b for this solute given in Table 3 are those obtained from a fit of a linear equation to the V^0_2 data.

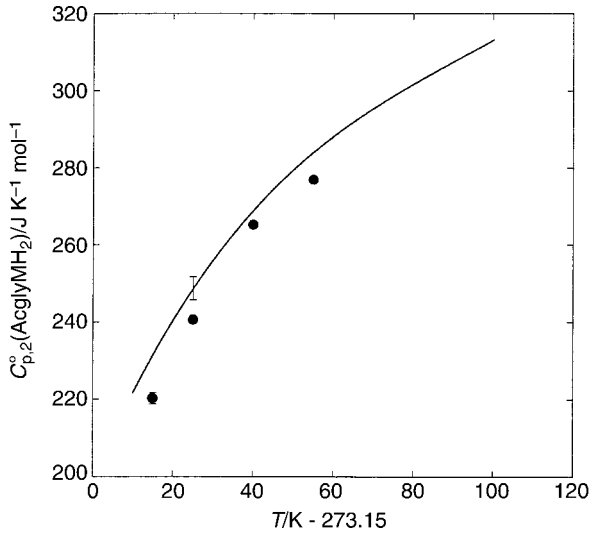

Fig. 3 Temperature dependence of the partial molar heat capacity of $AcglyNH_2$. ● This work; —— from ref. 15.

The partial molar expansibility of a solute at infinite solution, $E^0_2 = (\partial V^0_2/\partial T)_p$ can be derived using the coefficients given in Table 3. Differentiation of eqn. (6) with respect to temperature at constant pressure leads to

$$(\partial V^0_2/\partial T)_p = b + 2c(T - 308.15). \quad (7)$$

It follows from eqn. (7) that the quantity $(b - 20c)$ is equivalent to E^0_2 at a temperature of 298.15 K. Values of E^0_2, along with those derived using V^0_2 data taken from the literature, are given in Table 4. For $AcalaNH_2$ and $AcvalNH_2$ the E^0_2 values obtained in this work are in good agreement with those derived using the V^0_2 data reported by Kikuchi *et al.*[9] whereas for $AcglyNH_2$ and $AcleuNH_2$ the differences between the E^0_2 values are more significant (13% and 7% respectively).

Discussion

Partial molar volumes

As the *N*-acetyl amino acid amides are simple derivatives of their parent amino acids, it is of interest to compare the V^0_2 results obtained in this work with those reported for the amino acids.[29,30] At each temperature the linear equation

$$V^0_2(AcXNH_2) = mV^0_2(aa) + c, \quad (8)$$

was fitted to the V^0_2 data given in Table 1. In this equation $V^0_2(aa)$ is the partial molar volume of the parent amino acid at infinite dilution and m and c are adjustable parameters. Values for the slope m and intercept c obtained using unit weighting, along with the regression coefficients, r^2, are given in Table 5. The values obtained for r^2 indicate that at each temperature there is an excellent linear relationship between $V^0_2(AcXNH_2)$ and $V^0_2(aa)$. This relationship can be rationalized using the following scheme:

(A) $$CH_3CONHCH(R)CONH_2 = NH_2CH(R)CO_2H + CH_3CONH_2 - H_2O$$

(B) $$NH_2CH(R)CO_2H \rightleftharpoons {}^{+}NH_3CH(R)CO_2{}^{-}.$$

Using the principle of group additivity on process (A), it follows that the difference between the partial molar volume of an acetyl amino acid amide and the neutral form of its parent amino acid is equal to the difference between the partial molar volumes of acetamide and water. At 298.15 K this difference is 37.54 $cm^3\ mol^{-1}$.[26] Based upon process (A) alone, the values for m and c in eqn. (8) would be 1.000 and 37.54 $cm^3\ mol^{-1}$ at 298.15 K which is not the case, as shown in Table 5. However, amino acids in aqueous solution exist in the zwitterionic form (see process (B) above). As is well known,[31–33] ionic groups induce a considerable contraction in volume because of electrostrictive effects on the surrounding solvent molecules. This electrostrictive effect is principally responsible for the c values given in Table 5 being approximately 10 $cm^3\ mol^{-1}$ larger than those predicted from process (A). It is worth noting that the values obtained for the parameter m in eqn. (8) are not exactly unity. Values of $m = 1$ are what would be expected on the basis of perfect additivity

Table 3 Coefficients of eqn. (6)

Solute	a/$cm^3\ mol^{-1}$	b/$cm^3\ mol^{-1}\ K^{-1}$	10^4c/$cm^3\ mol^{-1}\ K^{-2}$
$AcglyNH_2$	91.9322(0.0004)[a]	0.087 49(0.000 03)[a]	−3.80(0.02)[a]
$AcalaNH_2$	108.93(0.02)	0.0878(0.0015)	—
$AcvalNH_2$	140.10(0.02)	0.1176(0.0005)	1.3(0.6)
$AcleuNH_2$	157.4527(0.0006)	0.138 49(0.000 03)	2.18(0.02)
$AcileNH_2$	155.27(0.07)	0.1352(0.0013)	8.3(1.9)

[a] Standard errors are in parentheses.

Table 4 Partial molar expansibilities of *N*-acetyl amino amides in aqueous solution at 298.15 K

Solute	E_2^0/cm^3 mol^{-1} K^{-1}	
	This work	Literature[a]
$AcglyNH_2$	0.095 09(0.000 05)[b]	0.1073(0.0008)[b]
$AcalaNH_2$	0.088(0.002)	0.089(0.003)
$AcvalNH_2$	0.115(0.001)	0.119(0.001)
$AcleuNH_2$	0.134 13(0.000 05)	0.143(0.003)
$AcileNH_2$	0.119(0.004)	

[a] Derived from an analysis of the $V_2^0(T)$ data given in ref. 9 using the equation $V_2^0 = a + b(T - 298.15) + c(T - 298.15)^2$. [b] Standard errors are in parentheses.

Table 5 Coefficients of eqn. (8)

T/K	m	c/cm^3 mol^{-1}	r^2
288.15	1.007_4(0.002)[a]	47.1_5(0.2)[a]	0.999991
298.15	1.009_7(0.003)	47.2_0(0.3)	0.999974
313.15	1.013_9(0.004)	47.5_9(0.4)	0.999959
328.15	1.018_6(0.001)	48.1_5(0.1)	0.999995

[a] Standard errors are in parentheses.

of group partial molar volumes. The most significant contribution to these deviations from unity presumably arises from the mutual interaction between the charged $^+NH_3$ and CO_2^- groups and the adjacent side-chain, **R**, in the zwitterionic amino acids.

For the isomeric compounds $AcleuNH_2$ and $AcileNH_2$, the difference between the V_2^0 values is about 2 cm^3 mol^{-1} in the temperature range 288.15–328.15 K. Although this difference is similar to that observed for the parent zwitterionic amino acids over the same temperature range,[30] it is much larger than might be expected based on V_2^0 data for the tripeptides gly-leu-gly and gly-ile-gly.[14,34] For these peptides each side-chain is in the central position within the molecule and is adjacent to two peptide (–CONH–) functional groups, which is structurally similar to the acetylated amino acid amide derivatives. Despite these structural similarities, the difference between the V_2^0 values for these two tripeptides at 298.15 K is only 0.11 cm^3 mol^{-1}.[34] Perhaps the similar partial molar volumes for the isomeric tripeptides are a reflection of a particular folding within the molecules that does not occur for the acetylated amino acid amides.

For $AcXNH_2$, X = gly, ala, val and leu, there is at each temperature a good linear relationship between V_2^0 and the number of carbon atoms in the side-chain. Similar linear relationships exist for the zwitterionic amino acids,[35] dipeptides of sequence glyX[23] and tripeptides of sequence gly-X-gly.[23] The slopes of these linear relationships give the contributions of the side-chain methylene groups, $V^0(CH_2)$, to the partial molar volumes of the solutes at infinite dilution. The $V^0(CH_2)$ values obtained from linear least-squares analyses with unit weights of the V_2^0 data for the $AcXNH_2$ compounds, with the correlation coefficients in parentheses, are as follows: 288.15 K, $16.0_1 \pm 0.3$ cm^3 mol^{-1} (0.9995); 298.15 K, $16.1_0 \pm 0.2$ cm^3 mol^{-1} (0.9994); 313.15 K, $16.3_0 \pm 0.2$ (0.9996); 328.15 K, $16.5_3 \pm 0.2$ (0.9996). The temperature dependence of $V^0(CH_2)$ is displayed in Fig. 4. For the purposes of comparison, $V^0(CH_2)$ values derived using V_2^0 data for the amino acids (gly,[29] ala,[29] val,[30] leu,[30] and α-aminobutyric acid[36]), for the tripeptides of sequence gly-X-gly,[14,23] and for the *n*-alcohols[37] are also given in Fig. 4. In the interests of clarity the error bars have been omitted for some of the results shown in Fig. 4. Within the combined estimated uncertainties, the methylene group contribution obtained from an analysis of the V_2^0 data for the neutral acetyl amino acid amides has a

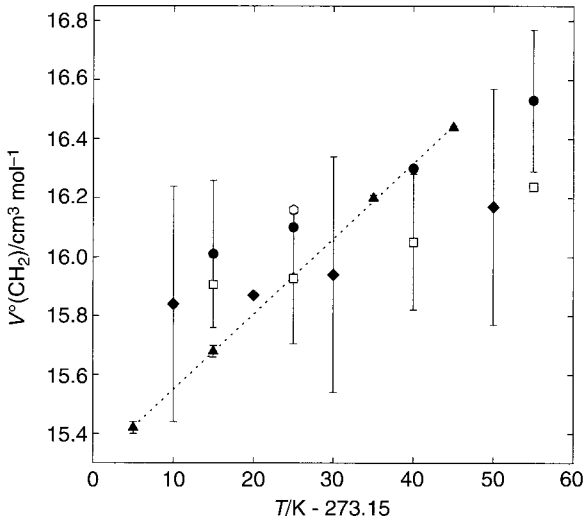

Fig. 4 Temperature dependences of the methylene group contribution to V_2^0 derived using different solutes. ● This work; □ amino acids, V_2^0 data from ref. 29, 30, 36; ○ tripeptides of sequence gly-X-gly, V_2^0 data from ref. 23; ◆ tripeptides of sequence gly-X-gly, V_2^0 data from ref. 14; ▲ *n*-alcohols, V_2^0 data from ref. 37.

temperature dependence that is the same as that obtained for both the zwitterionic amino acids and tripeptides. This temperature dependence is, however, smaller than that obtained from an analysis of V_2^0 data for the *n*-alcohols, $H(CH_2)_mOH$, $m = 3$–6. Presumably, this is a manifestation of differences between the hydrophobic hydration around the alkyl chain of the linear alcohols and that around the side-chain of amino acids, peptides and their derivatives for which the alkyl groups are adjacent to polar functional groups.

The partial molar volumes of the side-chains of the amino acids ala, val, leu and ileu can be estimated using the partial molar volume data for the $AcXNH_2$ compounds given in Table 1. The side-chain partial molar volume, $V^0(R)$, is given by

$$V^0(R) = V_2^0(AcXNH_2) - V_2^0(AcglyNH_2), \quad (9)$$

where $V_2^0(AcXNH_2)$ and $V_2^0(AcglyNH_2)$ are, respectively, the partial molar volumes at infinite dilution of the compounds $AcXNH_2$ and $AcglyNH_2$. The quantity $V^0(R)$ is not the absolute value of the partial molar volume of side-chain **R**, but gives the contribution to the volume on replacing a C–H group by a C–**R** group. Values of $V^0(R)$ for the various side-chains are given in Table 6. Figs. 5 and 6 show comparisons of these $V^0(R)$ values with those obtained using the V_2^0 data reported by Kikuchi *et al.*[9] and also with those obtained using V_2^0 data for tripeptides of sequence gly-X-gly.[14,23,38] For the valyl and leucyl side-chains, the differences between the $V^0(R)$ values at 25 °C obtained using the $AcXNH_2$ and tripeptides as model compounds are small (*ca.* 1.2% and 0.6% for the valyl and leucyl side-chains respectively). Furthermore, the temperature dependences of $V^0(R)$ obtained in this study are in good agreement, within the combined experimental uncertainties, with those obtained by differential scanning densimetry using the gly-X-gly peptides.[14] For the side-chain of isoleucine, the large differences between the V^0(ile) values obtained for the two sets of model compounds are a manifestation of the differences in the V_2^0 values, as mentioned above. It is noteworthy, however, that the temperature dependence of V^0(ile) obtained using the acetylated amino acid amides parallels that based on the tripeptides. For the smaller alanyl side-chain, the differences between the V^0(ala) values derived using V_2^0 data for the $AcXNH_2$ compounds and those based on tripeptide model compounds are slightly larger (*ca.* 4%) than those for the valyl and leucyl side-chain. This is probably due to a subtle difference in hydration of primary and secondary amide groups. In the $AcXNH_2$ compounds the side-chain is

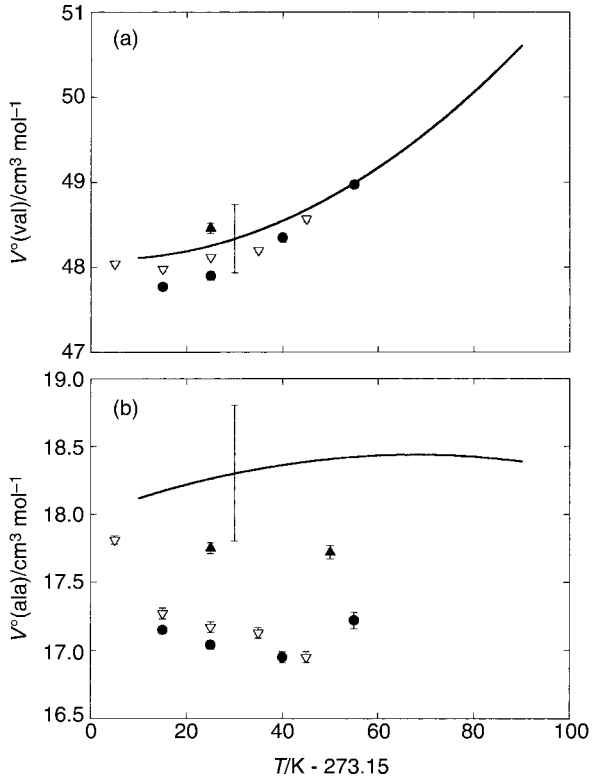

Fig. 5 Partial molar volumes of the alanyl and valyl side-chains as a function of temperature. (a) Valyl side-chain, ● this work; ▽ V_2^0 data for $AcXNH_2$ from ref. 7; ▲ V_2^0 data for gly-X-gly from ref. 23.; —— V_2^0 data for gly-X-gly from ref. 14. (b) Alanyl side-chain, ● this work; ▽ V_2^0 data for $AcXNH_2$ from ref. 9; ▲ V_2^0 data for gly-X-gly from ref. 38; —— V_2^0 data for gly-X-gly from ref. 14.

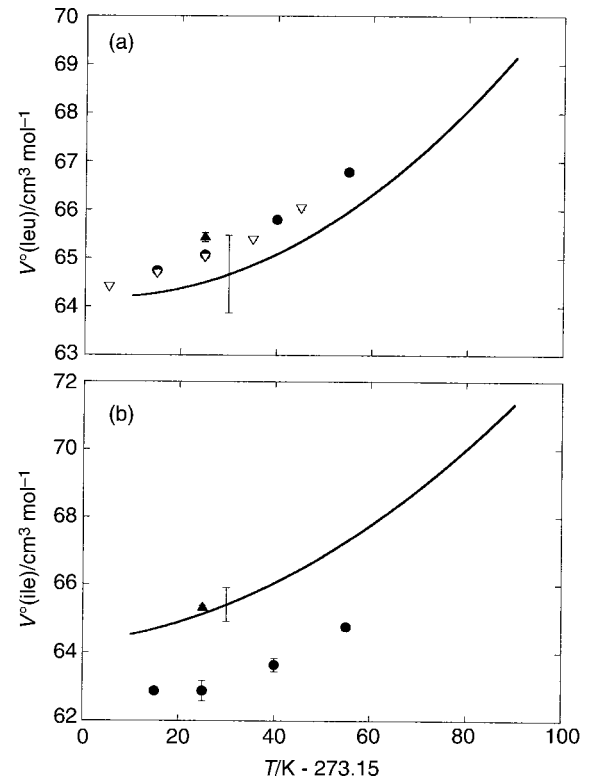

Fig. 6 Partial molar volumes of the leucyl and isoleucyl side-chains as a function of temperature. (a) Leucyl side-chain, ● this work; ▽ V_2^0 data for $AcXNH_2$ from ref. 9; ▲ V_2^0 data for gly-X-gly from ref. 23; —— V_2^0 data for gly-X-gly from ref. 14. (b) Isoleucyl side-chain; ● this work; ▲ V_2^0 data for gly-X-gly from ref. 23; —— V_2^0 data for gly-X-gly from ref. 39.

Table 6 Amino acid side-chain contributions to V_2^0 and $C_{p,2}^0$ derived using *N*-acetyl amino acid amides as model compounds

Side-chain (R)	T/K	V^0(R)[a]/cm^3 mol^{-1}	C_p^0(R)[b]/J K^{-1} mol^{-1}
ala	288.15	17.15(0.03)[c]	189.3(1.7)[c]
	298.15	17.04(0.03)	183.5(0.9)
	313.15	16.95(0.04)	171.6(1.1)
	328.15	17.22(0.06)	163.9(2.6)
val	288.15	47.77(0.04)	352.1(1.7)
	298.15	47.90(0.05)	339.5(1.7)
	313.15	48.35(0.05)	329.2(2.1)
	328.15	48.97(0.04)	319.2(2.4)
leu	288.15	64.74(0.04)	445.4(2.2)
	298.15	65.07(0.04)	436.2(1.5)
	313.15	65.79(0.04)	417.4(1.8)
	328.15	66.78(0.05)	407.2(2.6)
ile	288.15	62.9(0.1)	
	298.15	62.9(0.3)	
	313.15	63.6(0.2)	
	328.15	64.8(0.1)	

[a] Derived using eqn. (9). [b] Derived using eqn. (10). [c] Estimated uncertainties are in parentheses.

flanked by one primary and one secondary amide whereas the gly-X-gly peptides contain only secondary amides. The effect of amide type in the mutual interaction between a side-chain and its adjacent amides becomes more significant as the size of the hydrophobic side-chain decreases. The influence of primary and secondary amides is also evident in the V^0(ala) values derived using V_2^0 data for the isomeric peptide derivatives *N*-acetylglycyl-L-alaninamide ($AcglyalaNH_2$) and *N*-acetyl-L-alanylglycinamide ($AcalaglyNH_2$).[27] In $AcglyalaNH_2$, the side-chain is flanked by one primary and one secondary amide while for $AcalaglyNH_2$ the side-chain is adjacent to two secondary amide functional groups. The V^0(ala) value at 25 °C based on $AcalaglyNH_2$ is 18.06 cm^3 mol^{-1} which is in good agreement with that obtained using the gly-X-gly peptides while that obtained using $AcglyalaNH_2$ is 17.04 cm^3 mol^{-1} which is in excellent agreement with that obtained using the *N*-acetyl amino acid amides as model compounds.

Partial molar heat capacities

The partial molar heat capacities of the side-chains of the amino acids ala, val and leu can be estimated using the partial molar heat capacities of the $AcXNH_2$ compounds given in Table 1. The side-chain partial molar heat capacity, C_p^0(R), is given by the equation

$$C_p^0(\mathrm{R}) = C_{p,2}^0(\mathrm{AcXNH_2}) - C_{p,2}^0(\mathrm{AcglyNH_2}) + C_p^0(\mathrm{H}), \quad (10)$$

where $C_{p,2}^0(AcXNH_2)$ and $C_{p,2}^0(AcglyNH_2)$ are, respectively, the partial molar heat capacities at infinite dilution for the acetyl amino acids $AcXNH_2$ and $AcglyNH_2$, and C_p^0(H) is the heat capacity of the hydrogen atom of the methylene moiety in $AcglyNH_2$. For the purposes of comparison with previous work,[16,39,40] we have chosen to derive the absolute value of the partial molar heat capacity of the side-chain R rather than calculate the difference between the C–H and C–R groups as outlined above for the side-chain partial molar volumes. As in earlier work,[16,40] the values of C_p^0(H) used in the calculations are those based on the values reported by Makhatadze and Privalov.[13] Although these C_p^0(H) values may not be appropriate for H atoms that are adjacent to polar functional groups,[16,40] it should be noted that any uncertainties in the C_p^0(R) results that arise from the choice of C_p^0(H) values are not manifested in the partial molar heat capacities of unfolded proteins using a group additivity scheme. This is because the heat capacity of the backbone peptide group, –CHCONH–, is derived from the partial molar heat capacity of the glycyl

group, $C_p^0(CH_2CONH)$, by subtracting the same estimated heat capacity of the hydrogen atom, $C_p^0(H)$.[15]

The $C_p^0(R)$ values for the amino acid side-chains ala, val and leu at the temperatures 288.15, 298.15, 313.15 and 328.15 K are given in Table 6. The uncertainty given for each $C_p^0(R)$ does not include any contribution from the $C_p^0(H)$ term in eqn. (10). A comparison is given in Fig. 7 of the results for the alanyl side-chain with those derived previously using alternative model compounds. In our earlier work, tripeptides of sequence gly-X-gly were used to derive the partial molar heat capacities of amino acid side-chains. The results for the alanyl side-chain obtained over a wide temperature range using both flow calorimetry[23,38] and DSC[16,40] are shown in Fig. 7. Within the combined uncertainties there is good agreement between the C_p^0(ala) values derived using the neutral acetyl amino acid amides and those based on the zwitterionic tripeptides as model compounds.

Also included in Fig. 7 are the C_p^0(ala) results reported by Makhatadze and Privalov.[13] These C_p^0(ala) values were derived by subtracting from the standard state partial molar heat capacity of methane in water an estimated value of the heat capacity of a hydrogen atom. The $C_{p,2}^0$ values for methane were derived by combining heat capacity of solution data determined over the range 273.15–323.15 K[41] with C_p^0(g) data. The $C_{p,2}^0$ values for methane over the range 323.15–398.15 K were evaluated by assuming that the temperature dependence of $C_{p,2}^0$ in this temperature range is the same as that over the range 273.15–323.15 K. Although the uncertainties in the C_p^0(ala) values cannot be assessed because the source of the C_p^0(g) data used was not given by Makhatadze and Privalov,[13] it is clear from Fig. 7 that the C_p^0(ala) values based on methane as a side-chain model compound are significantly smaller than those derived using either the *N*-acetyl amino acid amides or the tripeptides as model compounds.

In Fig. 8(a) the partial molar heat capacities of the valyl side-chain derived using heat capacity data for the acetylated amino acid amides are compared with those based on tripeptides as model compounds[23,40] and with those based on $C_{p,2}^0$ data for the organic analogue propane.[13] Over the temperature range 288.15–328.15 K, the results based on the $AcXNH_2$ compounds are about 10 to 14 J K^{-1} mol^{-1} (*ca.* 5%) larger than those obtained using $C_{p,2}^0$ data for the tripeptides. However, the temperature dependence of C_p^0(val) is in good agreement with that obtained using tripeptides. As

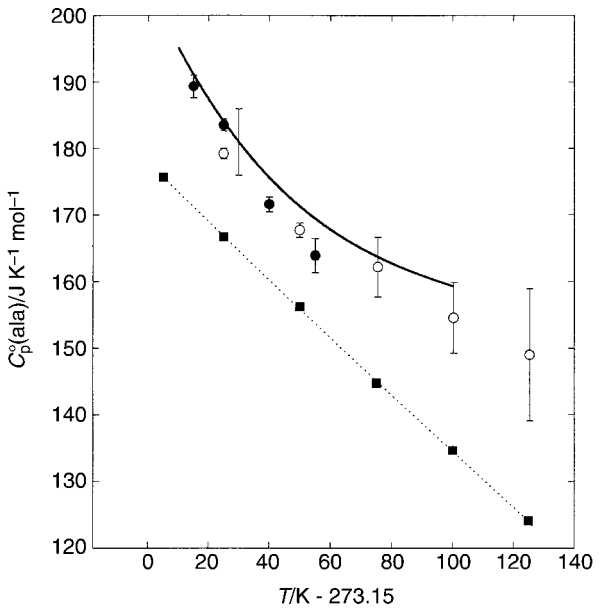

Fig. 7 Partial molar heat capacities of the alanyl side-chain as a function of temperature. ● This work; ○ $C_{p,2}^0$ data for gly-X-gly peptides from ref. 38; —— $C_{p,2}^0$ data for gly-X-gly peptides from ref. 40; ■ $C_{p,2}^0$ data for methane from ref. 13.

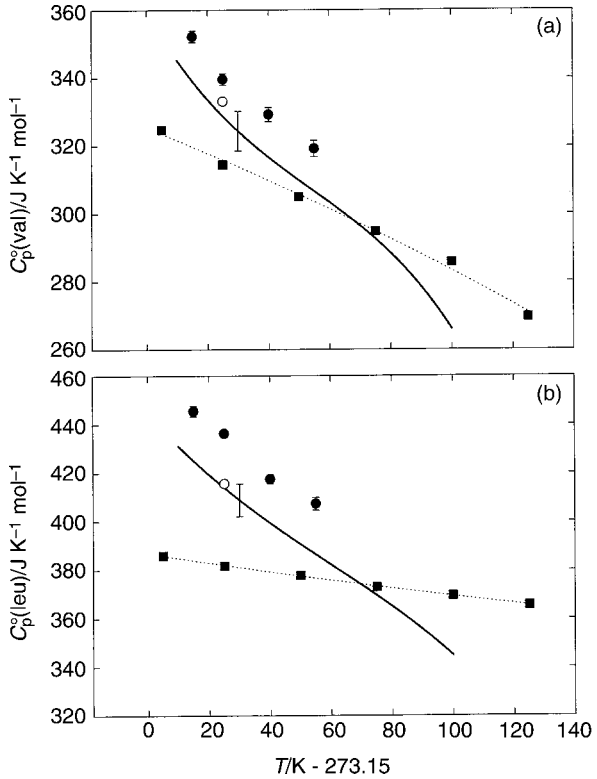

Fig. 8 Partial molar heat capacities of the valyl and leucyl side-chains as a function of temperature. (a) Valyl side chain, ● this work; ○ $C_{p,2}^0$ data for gly-X-gly peptides from ref. 23; —— $C_{p,2}^0$ data for gly-X-gly peptides from ref. 40; ■ $C_{p,2}^0$ data for propane from ref. 13. (b) Leucyl side-chain, ● this work; ○ $C_{p,2}^0$ data for gly-X-gly peptides from ref. 23; —— $C_{p,2}^0$ data for gly-X-gly peptides from ref. 16; ■ $C_{p,2}^0$ data for butane from ref. 13.

noted previously,[40] there is a significant difference between the temperature dependence of C_p^0(val) obtained using tripeptide or $AcXNH_2$ heat capacity data and that derived using heat capacity data for propane.

As shown in Fig. 8(b), a similar pattern is observed for the leucyl side-chain. The C_p^0(leu) values obtained in this study are about 18 to 21 J K^{-1} mol^{-1} (*ca.* 5%) larger than those reported previously using tripeptides as model compounds.[16,23] Given the structural similarities between the acetyl amino acid amides and the tripeptides, the differences between the $C_p^0(R)$ values for val and leu obtained using the two sets of model compounds are larger than might be expected. One possible reason for the differences may be because the side-chain in an acetyl amino acid amide is flanked by one secondary and one primary amide whereas in the tripeptides the side-chain is flanked by two secondary amides. On the other hand, as shown in Fig. 7 such a difference does not appear to be significant for the smaller alanyl side-chain. Additional experimental work is required in order to clarify this further. The temperature dependence of C_p^0(leu) obtained in this work is concordant with that based on the tripeptides but, as shown in Fig. 8(b), the temperature dependence of C_p^0(leu) derived using *n*-butane as a model compound[13] is significantly different. The observation that the ($C_p^0(R)$, *T*) plots obtained in this work are very similar to those obtained using the tripeptides is further support for the conclusion reached in earlier work,[16] namely that the side-chain heat capacities derived using tripeptides as model compounds should give a very good representation of the side-chain heat capacities in the random coil form of an unfolded protein in aqueous solution.

Partial molar expansibilities

The partial molar volumes of small organic solutes in aqueous solution are often interpreted using semiempirical models.[11,42]

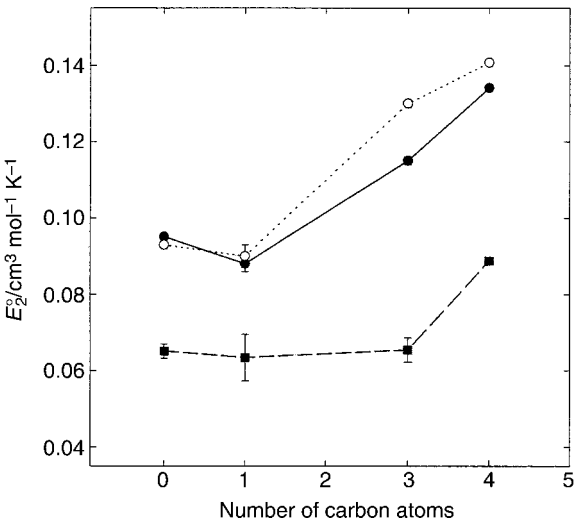

Fig. 9 Partial molar expansibilities at 298.15 K of some solutes as a function of the number of carbon atoms in the side-chain. ● $AcXNH_2$, this work; ○ glycyl dipeptides glyX, ref. 45; ■ amino acids. E_2^0 values derived using V_2^0 data from ref. 29, 30.

One such model is to regard V_2^0 as comprising three terms given by the equation

$$V_2^0 = V_{int} + V_v + V_s, \quad (11)$$

where V_{int} is the intrinsic volume occupied by the solute, V_v is the void volume that arises because of thermal motion and packing effects,[43,44] and V_s is the contribution from solute–solvent interactions. From the differentiation of eqn. (11) with respect to temperature, the partial molar expansibility at infinite dilution $E_2^0 = (\partial V_2^0/\partial T)_p$ can be expressed as

$$E_2^0 = (\partial V_{int}/\partial T)_p + (\partial V_v/\partial T)_p + (\partial V_s/\partial T)_p. \quad (12)$$

For solutes of low molar mass, the temperature dependence of V_{int} is governed mainly by the expansibility of covalent bonds, hence the term $(\partial V_{int}/\partial T)_p$ can be neglected which reduces eqn. (12) to

$$E_2^0 = (\partial V_v/\partial T)_p + (\partial V_s/\partial T)_p. \quad (13)$$

As reliable methods to estimate the temperature dependence of V_v are currently unavailable,[45] the rationalization of E_2^0 using eqn. (13) cannot be developed at present. However, eqn. (13) does suggest that the quantity E_2^0 ought to be a sensitive measure of solute–solvent interactions. This appears to be the case as illustrated in Fig. 9. Whereas the partial molar heat capacities and volumes of the $AcXNH_2$ compounds at infinite dilution are approximate linear functions of the number of carbon atoms in the side-chain, no such relationship is observed for E_2^0. Presumably this is a result of the subtle interplay between the side-chain and backbone moieties and their respective hydration cospheres. For comparison, E_2^0 results at 298.15 K for the parent amino acids[29,30] and for the zwitterionic depeptides[45] of sequence glyX are also included in Fig. 9. The trend of E_2^0 with the number of side-chain carbon atoms for the dipeptides is similar to that for the acetyl amino acid amides. The compounds $AcXNH_2$ and glyX are structurally rather similar in the side-chain region of the molecules, the difference being that the amide moiety in $AcXNH_2$ is replaced by a CO_2^- group in the dipeptide. The results depicted in Fig. 9 suggest that the two functional groups $CONH_2$ and CO_2^- behave in a similar manner as far as expansibility is concerned. The different trend for the amino acids of E_2^0 with the number of carbon atoms in the side-chain is perhaps not surprising given the close proximity to the side-chain of the two charged $^+NH_3$ and CO_2^- functional groups.

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