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Partial molar volumes of proteins: amino acid side-chain contributions derived from the partial molar volumes of some tripeptides over the temperature range 10–90°C

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Abstract

The partial molar volumes of tripeptides of sequence glycyl-*X*-glycine, where *X* is one of the amino acids alanine, leucine, threonine, glutamine, phenylalanine, histidine, cysteine, proline, glutamic acid, and arginine, have been determined in aqueous solution over the temperature range 10–90°C using differential scanning densimetry. These data, together with those reported previously, have been used to derive the partial molar volumes of the side-chains of all 20 amino acids. The side-chain volumes are critically compared with literature values derived using partial molar volumes for alternative model compounds. The new amino acid side-chain volumes, along with that for the backbone glycyl group, were used to calculate the partial specific volumes of several proteins in aqueous solution. The results obtained are compared with those observed experimentally. The new side-chain volumes have also been used to redetermine residue volume changes upon protein folding.

Keywords: Partial molar volume; Amino acid side-chains; Group additivity; Protein partial specific volumes; Aqueous solution; Tripeptides

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1. Introduction

The interactions between water and protein constituent groups, such as the amino acid side-chains, play an important role in determining the stability of a protein in aqueous solution [1–3]. Because of the variety and complexity of these interactions in a macromolecule, there have been many attempts to characterise the thermodynamic and hydration behaviour of particular constituent groups using low molecular mass model compounds such as the amino acids [4–7], some neutral amino acid derivatives [8–10] or cyclic dipeptides [11].

In recent work to determine the heat capacities of the amino acid side-chains of proteins over a wide temperature range [12–14], we have used as an alternative set of model compounds tripeptides of sequence glycyl–*X*–glycine (gly–*X*–gly), where *X* is one of the naturally occurring amino acids. In these peptides the side-chain of amino acid *X* is adjacent to two peptide groups, which mimics the situation in a polypeptide or protein. In order to evaluate quantitatively the volume or heat capacity of a polypeptide chain using a group additivity scheme, it is preferable to choose model compounds that reflect as closely as possible the size, surface area, charge and hydrophobicity of the target moieties whose thermodynamic properties are to be evaluated. In this regard the tripeptides gly–*X*–gly are good models for the amino acid side-chains of proteins.

In recent papers on the heat capacities of the gly–*X*–gly peptides [12,14], we reported polynomial functions that describe the temperature dependencies of the standard state partial molar volume, V_2^0, of several tripeptides over the temperature range 10–90°C. However, no attempt was made to evaluate the partial molar volumes of the amino acid side-chains using these V_2^0 data. In this paper we report new V_2^0 data for tripeptides of sequence gly–*X*–gly, where *X* is one of the amino acids alanine, leucine, threonine, glutamine, phenylalanine, histidine, cysteine, proline, glutamic acid and arginine, in aqueous solution over the temperature range 10–90°C. V_2^0 data have also been determined for the tetrapeptides glycylglycylglycylglycine [$(gly)_4$] and glycyltryptophanylglycylglycine (glytrpglygly) because the tripeptide glytrpgly is very insoluble in water. The new V_2^0 data for the tripeptides and those reported earlier [12,14], along with the V_2^0 data for the tetrapeptides $(gly)_4$ and glytrpglygly, have been used to calculate the partial molar volumes of the side-chains for all 20 amino acids. These results were incorporated into a group additivity scheme to calculate the partial specific volumes of the unfolded forms of several proteins in aqueous solution.

2. Materials and methods

The tripeptides gly–*X*–gly where *X* is one of the amino acids ala, leu, phe, and his, and the peptide hydrate glythrgly.H_2O, were samples remaining from earlier studies. The purification and analyses of these peptides have been described in detail elsewhere [15–17]. The sample of glyglugly, which was obtained from Bachem Feinchemikalien, was recrystallised from water + ethanol mixture and dried under an oil pump vacuum at room temperature. The product was chromatographically pure as determined by TLC. Analysis by alkalimetric titration [18,19] gave a relative molar mass of 261.1 ± 2.0, which is in excellent agreement with that for the anhydrous compound (261.23). Elemental analyses gave: C, 41.5%; H, 5.9%; N, 16.0%; cf. calculated for $C_9H_{14}N_3O_6$: C, 41.4%; H, 5.8%; N, 16.1%. The tripeptide glyglngly used was the solid recovered after previous studies [20]. The material was recrystallised from water + ethanol to give a crystalline monohydrate. Alkalimetric titrimetry gave a relative molar mass of 281.5 ± 2.8, which is slightly higher than that expected for the monohydrate ($M_r = 278.27$). Elemental analyses gave: C, 38.8%; H, 6.6%; N, 20.1%; cf. calculated for $C_9H_{18}O_6N_4$: C, 38.9%; H, 6.7%; N, 20.0%. The sample of glycysgly used was that recovered by freeze-drying aqueous solutions used in previous work [20]. The purity of the tripeptide was confirmed chromatographically using TLC, by akalimetric titration and by spectrophotometric analysis of the

sulfhydryl group using dithionitrobenzoic acid (DTNB) [21]. Elemental analysis gave: C, 35.5%; H, 5.6%; N, 17.6%; cf. calculated for $C_7H_{13}O_4N_3S$: C, 35.7%; 5.6%; 17.9%. The tripeptide glyprogly was recovered from solutions used in previous work [20]. The material was twice recrystallised from water + ethanol and dried to constant weight under vacuum. The product obtained was a hydrate. Alkalimetric titration gave a relative molar mass of 246.7 ± 2.0 which is in good agreement with that expected for a monohydrate ($M_r = 247.25$).

The peptide glyarggly was obtained from Bachem Feinchemikalien as the acetate salt. A pH titration on a small amount of material indicated that excess acetic acid was present. The product was recrystallised from water + ethanol to give a white product that was chromatographically pure as determined by both TLC and ion exchange chromatography. Although the acidic contaminant was no longer present, we were unable to get satisfactory elemental analyses on the vacuum dried sample. Consequently, there is some doubt about the purity of this compound.

The tetrapeptide glytrpglygly, purchased from Bachem Feinchemikalien, was recrystallised twice from water + methanol and dried under vacuum. TLC indicated that the compound was chromatographically pure. Elemental analyses gave: C, 52.9%; H, 5.4%; N, 18.0%; cf. calculated for $C_{17}H_{21}N_5O_5$: C, 54.4%; H, 5.6%; N, 18.7%. The purification and analyses of tetraglycine have been described elsewhere [22].

Hydrochloric acid (0.1 M standard solution, Fluka) and sodium acetate (anhydrous GR, Merck) were used without further purification. The water used both to prepare solutions and as the reference solvent was deionized, twice glass-distilled and thoroughly degassed immediately prior to use. All solutions of the peptides were prepared by mass.

Density measurements over the temperature range 10–90°C were carried out using a differential scanning densimetric system (DSD) comprising two matched Anton Paar DMA 602 HT cells coupled to a DMA 60 measuring unit. Details of the apparatus and operational procedures used have been described previously [12,23].

3. Results

The apparent molar volumes of the peptides, V_ϕ were obtained from the solution densities 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 the solvent. For each peptide, solution densities as a function of temperature were measured on a minimum of four solutions over the molality range shown in Table 1. For dilute solutions of peptides, 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. The precision of the DSD measurements was such that the molality dependence of V_ϕ could not be detected over the range used. The $V_\phi(T)$ data for all solutions were combined and fitted to a power series in temperature of the form

$$V_\phi = a + b(T - 273.15) + c(T - 273.15)^2 \quad (3)$$

where a, b and c are the fitted coefficients and T is the absolute temperature. The values of the coefficients along with the estimated uncertainties obtained from the data analysis are given in Table 1. Some typical $V_\phi(T)$ data are shown in Fig. 1. The $V_\phi(T)$ curves are for each of four solutions of the peptides glytyrgly and glyglugly, over the molality ranges 0.017–0.032 mol kg^{-1} and 0.031–0.061 mol kg^{-1}, respectively. Although the analysis using Eq. (3) gives, at any temperature, an average apparent molar volume over the molality range used, we have assumed that this can be approximated to the partial molar volume at infinite dilution, V_2^0. The concentration dependences of V_2^0 for many of the tripeptides have been determined from precise density measure-

Table 1
Coefficients from the fitting of the partial molar volumes of the peptides gly–*X*–gly to Eq. (3)

X	a/cm^3 mol^{-1}	b/cm^3 mol^{-1} K^{-1}	$10^4\,c$/cm^3 mol^{-1} K^{-2}	m/mol kg^{-1}
gly[a]	108.4	0.167	−8.68	
ala	126.4	0.180	−9.64	0.036–0.049
leu	172.6	0.163	−2.11	0.025–0.050
thr	142.0	0.183	−7.98	0.027–0.042
gln	157.7	0.210	−9.18	0.021–0.069
phe	187.2	0.251	−8.60	0.020–0.025
his	166.5	0.227	−10.0	0.007–0.012
cys	139.3	0.189	−7.95	0.032–0.055
pro	140.8	0.193	−8.07	0.022–0.059
glu	155.0	0.228	−10.9	0.031–0.061
lys$^+$Ac$^-$[b,c]	211.9	0.231	−10.8	
arg$^+$Ac$^-$[b]	218.2	0.298	−17.9	0.030–0.048
glygly	143.5	0.259	−13.8	0.011–0.020
trpgly	241.0	0.310	−11.4	0.020–0.028

[a] From Häckel et al. [14].
[b] Ac^- is the acetate ion.
[c] Data reported previously [14] were reprocessed. See text.

ments at a single temperature, 25°C [15,16,20]. Assuming that each concentration dependence is the same over the temperature range 10–90°C, then the calculated differences between V_ϕ and V_2^0 are typically in the range 0.05–0.4 cm^3 mol^{-1}. These differences are similar to the uncertainties in the average $V_\phi(T)$ values determined in this work, so, within the limits of experimental uncertainty, the average $V_\phi(T)$ can be taken as $V_2^0(T)$.

The side-chain carboxylic acid groups of the peptides glyglugly and glyaspgly ionise to a small extent in aqueous solution. The effect of ionisation can be considered in the analysis of the V_ϕ data by using a modified form of Eq. (2) [24,25].

$$V_\phi - \alpha\,\Delta V_i^0 = V_2^0 + S_v m \qquad (4)$$

where α is the degree of dissociation of the acidic side-chain and ΔV_i^0 is the standard state volume change upon ionisation. As neither the ΔV_i^0 values nor the acid dissociation constants required to evaluate α are known for these compounds over a wide temperature range, we are unable to correct the V_ϕ data to obtain the partial molar volumes of peptides with completely unionised side-chains. The magnitude of the $\alpha\,\Delta V_i^0$ term in Eq. (4) can be estimated for the temperature of 25°C. Using values of the side-chain dissociation constants for 0.1 M NaCl as the background electrolyte ($K = 9.12 \times 10^{-5}$ for glyaspgly and $K = 4.79 \times 10^{-5}$ for glyglugly) [26], along with a ΔV_i^0 value of −11.5 cm^3 mol^{-1}, which is the volume change for the ionisation of the carboxylic

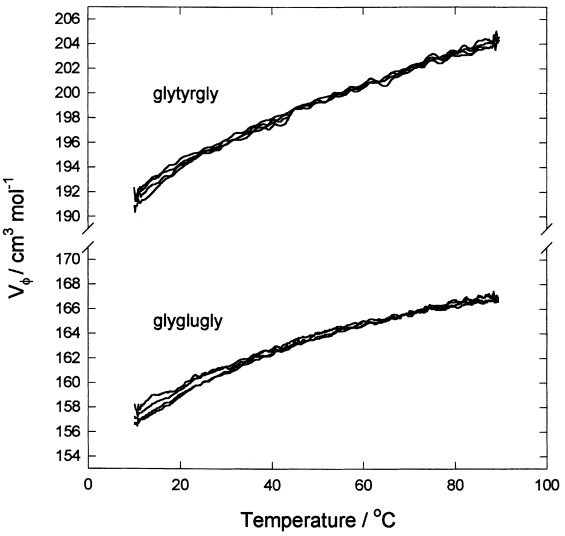

Fig. 1. Temperature dependence of the apparent molar volume for each of the peptides glytyrgly and glyglugly. One DSD scan is shown for each of four solutions over the molality ranges m = 0.017–0.032 mol kg^{-1} (glytyrgly) and m = 0.031–0.061 mol kg^{-1} (glyglugly).

acid group in acetic acid [27] and several substituted amino acids [25], the $\alpha\Delta V_i^0$ values range from -0.8 to -0.9 cm^3 mol^{-1} for glyaspgly and -0.3 to -0.5 cm^3 mol^{-1} for glyglugly over the concentration ranges used in our studies. Consequently, the partial molar volumes of glyaspgly and glyglugly with fully unionised side-chains will be higher by approximately 0.9 and 0.5 cm^3 mol^{-1}, respectively, than those calculated using the results represented in Table 1.

For the peptides glyarggly acetate and glylysgly acetate, which are strong electrolytes, the apparent molar volume data were analysed using an equation of the form [28,29]:

$$V_\phi = V_2^0 + A_v(d_0 m)^{1/2} + B_v m \quad (5)$$

where, A_v is the limiting slope derived from the Debye–Hückel theory and B_v is an adjustable parameter. Values for the term $A_v(d_0)^{1/2}$ at five degree intervals over the range 0–100°C given by Archer and Wang [30] were fitted to a polynomial in temperature to give the expression:

$$A_v(d_0)^{1/2} = 1.514 + 7.886\times10^{-3}\,(T-273.15) + 1.789\times10^{-4}(T-273.15)^2 \quad (6)$$

with a correlation coefficient of $R^2 = 0.9999$. For both glylysgly acetate and glyarggly acetate no concentration dependence of the quantity $V_\phi - A_v(d_0 m)^{1/2}$ could be detected so the value of B_v was taken as zero. The data for the various solutions were combined and fitted to a polynomial expression analogous to Eq. (3) but with V_ϕ replaced by $V_\phi - A_v(d_0 m)^{1/2}$. The polynomial coefficients obtained for glylysgly acetate differ slightly from those reported previously [14] because in the earlier analysis the contribution of the Debye–Hückel term was not included.

For the peptides triglycine and tetraglycine, partial molar volumes at various temperatures have been reported previously by several authors [31–34]. A comparison of these literature results with those determined in this work is shown in Fig. 2a,b. Fig. 2a indicates that the $V_2^0(T)$ polynomial for triglycine obtained from the analysis of the solution densities determined by DSD is in excellent agreement, within the combined experimental uncertainties, with most of the results determined isothermally. The results at 5, 25 and 75°C reported by Makhatadze et al. [32] are somewhat higher than those determined in this work and by others [22,31,33]. For tetraglycine, the results displayed in Fig. 2b show that there is more variation among the literature results than for triglycine. This is probably an illustration of the difficulty in working with material that is both slow to dissolve and of low solubility. In general, the results in this work are in good agreement with other determinations. The V_2^0 values at 5 and 25°C reported by Makhatadze et al. [32] are

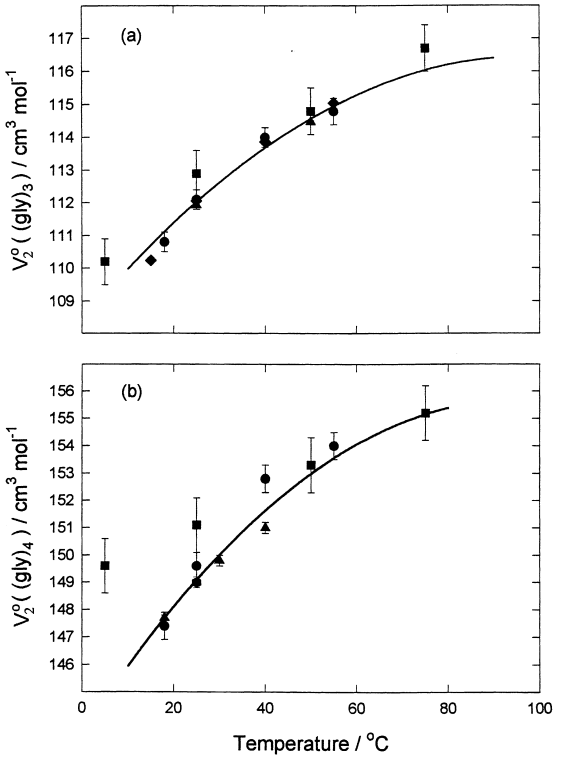

Fig. 2. Temperature dependence of the partial molar volume of oligoglycines: (a) triglycine — this work, ● V_2^0 data from Chalikian et al., [31], ■ V_2^0 data from Makhatadze et al., [32], ▲ V_2^0 data from Downes et al., [13], ◆ V_2^0 data from Hakin, [33]; and (b) tetraglycine — this work, ● V_2^0 data from Chalikian et al., [31], ■ V_2^0 data from Makhatadze et al., [32], ▲ V_2^0 data from Hakin et al., [34], ⬮ V_2^0 value from Häckel et al., [22].

significantly higher than the other determinations shown in Fig. 2b.

For several of the tripeptides used in this study, the partial molar volumes at 25°C have been determined in previous work [15,16,20]. Table 2 shows a comparison of literature results with the results at 25°C calculated using the polynomial coefficients in Table 1. For all peptides there is excellent agreement, within the combined uncertainties, between the results obtained using DSD and those determined isothermally. In earlier work [13], a V_2^0 value of 149.05 ± 0.04 cm^3 mol^{-1} was reported for the peptide glythrgly in aqueous solution at 50°C. The value calculated using the polynomial coefficients given in Table 1 is 149.2 ± 0.3 cm^3 mol^{-1} which is in excellent agreement with the previous determination. Although the new polynomial coefficients given in Table 1 for the peptide glyalagly differ somewhat from those reported in our previous paper [14], the average difference between V_2^0 values calculated using the two sets of coefficients and Eq. (3) is only approximately 0.5 cm^3 mol^{-1} which is within the combined uncertainties.

4. Discussion

4.1. Side-chain partial molar volumes

The partial molar volume of an unfolded protein in aqueous solution can be obtained using the method of group additivity which assumes that the partial molar volumes of the various constituent groups of proteins, such as the amino acid side-chains and the peptide backbone unit, can be added to give the total volume. The partial molar volumes of the tripeptides can be used to derive the side-chain partial molar volumes. The difference between the partial molar volumes of the peptides gly–*X*–gly and glyglygly gives the contribution to the partial molar volume on replacing a methylene hydrogen atom of the glycyl unit, CH_2CONH, by the side-chain *R* of amino acid *X*:

$$V^0(R) = V_2^0(R) - V_2^0(\mathrm{H}) = V_2^0(\text{gly-}X\text{-gly}) - V_2^0(\text{glyglygly}) \quad (7)$$

In Eq. (7) $V_2^0(R)$ and $V_2^0(\mathrm{H})$ represent the absolute values of the partial molar volumes of the side-chain *R* and the hydrogen atom, respectively. Reliable values of $V_2^0(\mathrm{H})$ are not available over a wide temperature range so it is not possible to derive the absolute values of the partial molar volumes of the side-chains. This is not a limitation, however, because for the group additivity approach used in this work (see Section 4.2) it is sufficient to know the values of the quantity $V^0(R)$ for the various amino acid side-chains.

Using the V_2^0 data for the tripeptides presented

Table 2
A comparison with literature data of the partial molar volumes of the peptides in aqueous solution at 25°C

Peptide	V_2^0/cm^3 mol^{-1}	
	This work	Literature
glyalagly	130.3 (0.4)	129.67 (0.01)[a], 130.7 (0.5)[b]
glyleugly	176.5 (0.7)	177.35 (0.06)[a]
glythrgly	146.1 (0.3)	146.15 (0.04)[c]
glyglngly	162.4 (0.4)	161.1 (0.2)[d]
glyphegly	193.0 (0.3)	193.3 (0.1)[c]
glyhisgly	171.6 (0.5)	169.9 (0.2)[c]
glycysgly	143.5 (0.4)	143.04 (0.05)[d]
glyprogly	145.1 (0.3)	144.58 (0.09)[d]

[a] From Reading et al., [15].
[b] Calculated using the polynomial in *T* given in [14].
[c] From Hedwig, [16].
[d] From Schwitzer et al., [20].

as polynomials in temperature given in Table 1, along with the results reported earlier [12,14], the quantity $V^0(R)$ has been derived as a function of temperature for 19 amino acid side-chains. The results given in Table 3 are the coefficients of the equation

$$V^0(R) = a_r + b_r(T - 273.15) + c_r(T - 273.15)^2 \quad (8)$$

For the lys and arg side-chains, the $V^0(R)$ values derived using Eq. (7) include contributions from the acetate anion. Without an acceptable scale of single-ion partial molar volumes over a broad range of temperature, volumes for the protonated lysine and arginine side-chains cannot be calculated. The contribution of the acetate ion can be eliminated by combining V_2^0 data for the electrolytes NaCl, HCl and NaAc using the two equations

$$V_2^0\left(\text{gly-}\overset{+}{X}\text{-gly}\right) - V_2^0(\text{H}^+) = V_2^0(\text{gly-}X\text{-glyAc}) - V_2^0(\text{NaAc}) + V_2^0(\text{NaCl}) - V_2^0(\text{HCl}) \quad (9)$$

$$V^0\left(\overset{+}{R}\right) - V_2^0(\text{H}^+) = V_2^0\left(\text{gly-}\overset{+}{X}\text{-gly}\right) - V_2^0(\text{glyglygly}) - V_2^0(\text{H}^+) \quad (10)$$

The quantity obtained is the difference between the side-chain volume $V^0(\overset{+}{R})$ and the partial molar volume of the proton in aqueous solution, $V_2^0(\text{H}^+)$.

The partial molar volume of NaCl at infinite dilution over the temperature range 10–90°C was obtained using the polynomial in temperature

Table 3
Coefficients of Eq. (8) for the temperature dependence of the partial molar volumes of amino acid side-chains, $V^0(R)$

Side-chain	a_r cm^{-3} mol^{-1}	b_r cm^{-3} mol^{-1} K^{-1}	$10^4\, c_r$ cm^{-3} mol^{-1} K^{-2}
ala	18.0	+0.013	−0.96
val[a]	48.1	−0.002	3.32
leu	64.2	−0.004	6.57
ile[b]	64.3	0.017	6.81
ser[a]	17.7	0.015	−1.19
thr	33.6	0.016	0.70
asn[b]	36.6	0.058	0.13
gln	49.3	0.043	−0.50
phe	78.8	0.084	0.08
tyr[a]	81.2	0.068	0.49
trp	97.5	0.051	2.40
his	58.1	0.060	−1.32
cys	30.9	0.022	0.73
met[b]	63.6	0.051	4.66
pro	32.4	0.026	0.61
asp[a]	30.4	0.058	−2.22
glu	46.6	0.061	−2.22
$\overset{+}{\text{lys}}\ \text{Ac}^-$	103.5	0.064	−2.12
$\overset{+}{\text{arg}}\ \text{Ac}^-$	109.8	0.131	−9.22
$\overset{+}{\text{lys}} - \text{H}^+$[c]	63.9	0.016	3.53
$\overset{+}{\text{arg}} - \text{H}^+$[c]	70.2	0.083	−3.56

[a] V_2^0 data from Häckel et al., [14].
[b] V_2^0 data from Vogl et al., [12].
[c] See text.

$$V_2^0(\mathrm{NaCl}) = a_0 + a_1/T + a_2 \ln T + a_3 T + a_4 T^2 \quad (11)$$

and the coefficients reported by Connaughton et al. [35]. Over the temperature range 10–55°C, reliable V_2^0 data for HCl selected from the literature [36–39] were fitted to a fourth order polynomial in temperature to give

$$\begin{aligned} V_2^0(\mathrm{HCl}) = {} & 16.63 + 0.045(T - 273.15) \\ & + 1.27 \times 10^{-3}(T - 273.15)^2 \\ & - 5.80 \times 10^{-5}(T - 273.15)^3 \\ & + 4.84 \times 10^{-7}(T - 273.15)^4 \end{aligned} \quad (12)$$

As literature data for V_2^0(HCl) in the range 55–85°C are unavailable, we carried out density measurements on solutions of HCl (0.1 M) using DSD. The V_ϕ data, which can be taken as V_2^0 within the limits of the experimental uncertainty, are represented by the equation

$$\begin{aligned} V_\phi(\mathrm{HCl}) = {} & 17.2 + 0.0395(T - 273.15) \\ & - 5.67 \times 10^{-4}(T - 273.15)^2 \end{aligned} \quad (13)$$

For the electrolyte NaAc literature V_2^0 data at atmospheric pressure are available only over the narrow temperature range 10–40°C [40]. Apparent molar volumes of aqueous solutions of NaAc at molalities in the range 0.035–0.109 mol kg^{-1} were determined in this work using DSD over the temperature range 10–90°C. The results can be represented by the polynomial

$$\begin{aligned} V_\phi(\mathrm{NaAc}) = {} & 36.9 + 0.117(T - 273.15) \\ & - 8.89 \times 10^{-4}(T - 273.15)^2 \end{aligned} \quad (14)$$

For the temperatures 10, 25 and 40°C the results obtained using Eq. (14) are in good agreement, within the combined experimental uncertainties, with the V_2^0 data reported by Allred and Woolley [40].

As the quantity $V^0(R)$ represents the difference between the partial molar volumes of the side-chains R and H, its interpretation in terms of the subtleties of solute–water interactions is rather complex. However, it is apparent from a perusal of the coefficients in Table 3 that the side-chains can be divided into two groups based upon the sign of the c_r coefficient. Side-chains which are typically hydrophobic in character have relatively large positive c_r coefficients while those that are polar or ionic have negative values for c_r. Side-chains that possess both hydrophobic and hydrophilic moieties have values of c_r that lie between these two extremes. These general observations are consistent with a proposal outlined some years ago by Hepler [41]. On the basis of the thermodynamic relation,

$$\left(\partial C_{p,2}^0/\partial p\right)_T = -T(\partial^2 V_2^0/\partial T^2)_p \quad (15)$$

it was argued that solutes that are 'structure-making' in terms of their effect on the solvent should have positive $(\partial^2 V_2^0/\partial T^2)_p$ values while those that are 'structure-breaking' should have negative values [41]. From the differentiation of Eq. (8), the value of $(\partial^2 V^0(R)/\partial T^2)_p$ for any side-chain R is simply $2c_r$. Although such a proposal has its limitations [41,42] it is, nevertheless, useful because a distinction can be made between ionic and very polar solutes and those for which hydrophobic hydration is dominant [41,43].

The temperature dependences of $V^0(R)$ for selected side-chains are displayed in Figs. 3 and 4. We have chosen to show the volume–temperature profiles that are typical for side-chains of a particular type, rather than present the results for all 19 amino acid side-chains. For the hydrophobic side-chains phe (Fig. 3a) and val (Fig. 3b), $V^0(R)$ increases with increasing temperature and, as mentioned above, the second derivative of volume with respect to T is positive. These volume temperature profiles are typical of those for hydrophobic solutes in aqueous solution [44–46]. On the other hand, for polar side-chains such as ser (Fig. 4a) and glu (Fig. 4b) which have negative values for c_r, $V^0(R)$ increases with increasing T to reach a maximum after which $V^0(R)$ decreases with a further increase in temperature. For the seryl side-chain the maximum value for $V^0(R)$ happens to lie within the temperature range used in this study. These volume–temperature profiles

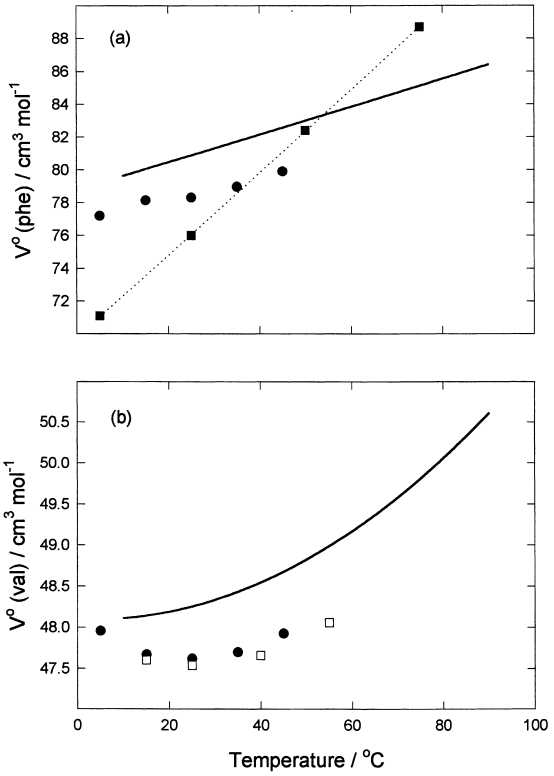

Fig. 3. Partial molar volumes of the phenylalanyl and valyl side-chains as a function of temperature: (a) phe side-chain — this work, ● amino acid V_2^0 data from Kikuchi et al., [4], ■ from Makhatadze et al., [32]; and (b) val side-chain — this work, ● amino acid V_2^0 data from Kikuchi et al., [4], □ amino acid V_2^0 data from Duke et al., [5].

are similar to those observed for electrolytes [35,47,48] and for polar solutes [49–51] in aqueous solution.

As this is the first study to determine the partial molar volumes of the gly–*X*–gly peptides over a wide temperature range, there are no literature $V^0(R)$-temperature profiles available for a direct comparison with the results presented herein. One comparison that can be made is with $V^0(R)$ data derived from the partial molar volumes of the amino acids. The partial molar volumes at infinite dilution for several amino acids over the temperature ranges 5–45°C and 15–55°C have been determined in careful studies by two research groups over the past few years [4,5,52–54]. The $V^0(R)$ results for the side-chains phe, val, ser and glu derived from the V_2^0 data for the amino acids are shown in Figs. 3 and 4. At any given temperature each $V^0(R)$ value obtained using amino acid V_2^0 data is less than that based on volumetric data for the tripeptides. As discussed elsewhere [16,55], this difference arises because of ionic end-group effects in the amino acids. More significant in the present context is the observation that the temperature dependences of the $V^0(R)$ results obtained from the amino acid V_2^0 data are in good qualitative agreement with the $V^0(R)$ results obtained in this work, as shown in Figs. 3 and 4.

Also shown in Figs. 3 and 4 are $V^0(R)$ values for the side-chains of phe, glu and ser that were derived by Makhatadze et al. [32] using as model compounds either small organic solutes or pep-

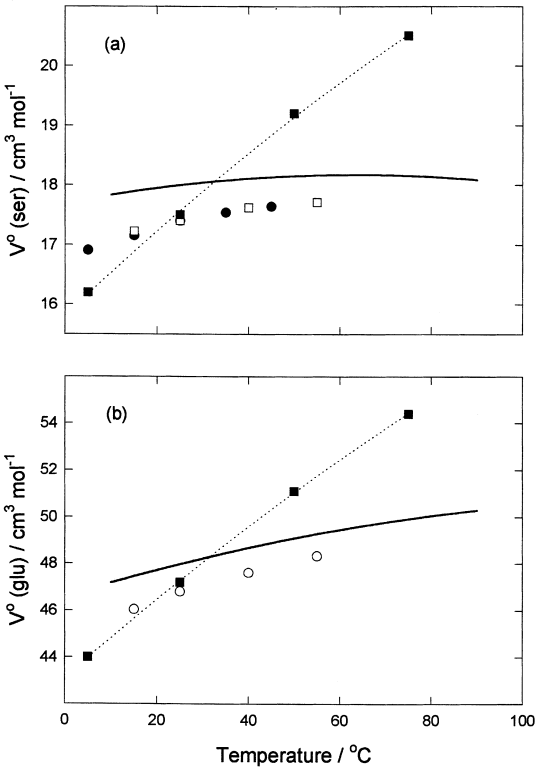

Fig. 4. Partial molar volumes of the seryl and glutamyl side-chains as a function of temperature: (a) ser side-chain — this work, ● amino acid V_2^0 data from Mizuguchi et al., [52], □ amino acid V_2^0 data from Hakin et al., [53], ■ from Makhatadze et al., [32]; and (b) glu side-chain — this work, ○ amino acid V_2^0 data from Hakin et al., [54], ■ from Makhatadze et al., [32].

tides of sequence gly–*X*–gly, but with 0.5 M sodium acetate/acetic acid buffer at pH 4.0 as the solvent rather than pure water as in this study. For example, the absolute value of the partial molar volume of the glu side-chain was obtained by subtracting from the V_2^0 value of propanoic acid, an estimated value of the partial molar volume of the hydrogen atom $V^0(H)$. The results displayed in Figs. 3 and 4 indicate that the $V^0(R)$ values, and in particular the temperature dependence of $V^0(R)$, derived using V_2^0 data for small organic side-chain analogues [32] are in very poor agreement with the results determined in this study. The origin of this discrepancy may be the method used by Makhatadze et al. [32] to evaluate $V^0(H)$ values. In their method, V_2^0 data for methanol in pure water were combined with V_2^0 data for glysergly and glyglygly in acetate buffer at pH 4.0 to obtain $V^0(H)$ values at various temperatures. This approach assumes that there are negligible differences between water and acetate buffer as solvent systems, which indeed is not the case [12]. Furthermore, the following analysis of the partial molar volume data of some alcohols and diols taken from the literature [45,56,57] also suggests that there are some inconsistencies in the group evaluation method used by Makhatadze et al. [32]. The V_2^0 data for the *n*-alcohols $H(CH_2)_mCH_2OH$, m = 2–5, are good linear functions of the number of methylene groups in the alcohol. The slopes $V^0(CH_2)$ and intercepts $[V^0(H) + V^0(CH_2OH)]$ obtained from linear least squares analyses of the V_2^0 data are given in Table 4. Similarly, for the series of diols $HOCH_2(CH_2)_mCH_2OH$, m = 2–4, the intercept obtained from least squares analysis is simply the quantity $2V^0(CH_2OH)$. Combining these results enables $V^0(H)$ and $V^0(CH_2OH)$ to be evaluated for the three temperatures 5, 25 and 45°C. Values of the quantity $V^0(ser)$, which is just $V^0(CH_2OH) - V^0(H)$, are 16.90, 17.66 and 17.55 $cm^3\ mol^{-1}$ at the temperatures 5, 25 and 45°C, respectively. These results are in reasonable agreement with the $V^0(ser)$ values derived using V_2^0 data for the amino acids (see Fig. 4a) but are in poor agreement with those based on side-chain data reported by Makhatadze et al. [32].

4.2. Partial molar volumes of unfolded proteins

Using the principle of group additivity, the partial molar volume of any oligopeptide or unfolded protein of known amino acid composition can be calculated using the equation

$$V_2^0 = V^0(NH_3^+ + CH_2CO_2^-) + (N-1)V^0(CH_2CONH) + \sum_{i=1}^{N} V^0(R) \quad (16)$$

where N is the number of amino acids in the peptide, $V^0(R)$ is the contribution from the amino acid side-chain R, as defined by Eq. (8), $V^0(CH_2CONH)$ is the contribution of the backbone glycyl group, and $V^0(NH_3^+ + CH_2CO_2^-)$ rep-

Table 4
Partial molar volumes of CH_2, CH_2OH and H groups obtained from an analysis of V_2^0 data for alcohols and diols

Solutes	*T*°C	$V(CH_2)$/ $cm^3\ mol^{-1}$	$[V(CH_2OH) + V(H)]$/ $cm^3\ mol^{-1}$	$V(CH_2OH)$/ $cm^3\ mol^{-1}$
$H(CH_2)_mCH_2OH$	5[a]	15.42	39.58	
m = 2–5	25[b]	15.99	38.68	
	45[a]	16.44	38.77	
	5	15.68		28.24
$HOCH_2(CH_2)_mCH_2OH$[c]	25	16.02		28.17
m = 2–4	45	16.44		28.16

[a] V_2^0 data from Sakurai et al. [45].
[b] V_2^0 data from Høiland [57].
[c] V_2^0 data from Nakajima et al. [56].

resents the contributions from the ionic end-groups of the polypeptide chain. Values of $V^0(CH_2CONH)$ over the temperature range 10–90°C were derived using the equation reported in earlier work [22]

$$V^0(CH_2CONH) = 35.45 + 0.0383(T - 273.15) - 1.67 \times 10^{-4}(T - 273.15)^2 \quad (17)$$

The sum of the partial molar volumes of the ionic end-groups $-NH_3^+$ and $-CH_2CO_2^-$ was obtained using the equation:

$$V^0(NH_3^+ + CH_2CO_2^-) = V_2^0[(gly)_3] - 2V^0(CH_2CONH) \quad (18)$$

where $V_2^0[(gly)_3]$ is the partial molar volume of the peptide triglycine. Volumetric properties of proteins are usually expressed as specific rather than molar quantities. The partial specific volume of a protein, v^0, is just V_2^0 divided by the molar mass of the protein. The v^0 values for several

Table 5
Partial specific volumes of some proteins in aqueous solution at 25°C

Protein	Solvent and conditions	v^0/cm^3 g^{-1}		
		Expt.[a]	Calc.[b]	Calc.(l)[c]
Ribonuclease A (bovine pancreas)	H_2O, isoionic protein	0.704 (0.001)[d]	0.705	0.705
	10 mM cacodylic acid buffer/10 mM NaCl, pH = 6.0	0.704 (0.003)[e]		
Lysozyme (chicken egg white)	H_2O, isoionic protein	0.712 (0.001)[d]	0.710	0.713
	H_2O, dialysed protein	0.702 (0.003)[e]		
	0.1 M phosphate buffer, pH = 6.5–7.0	0.725[h,i]		
α-Chymotrpsinogen A (bovine pancreas)	H_2O, isoionic protein	0.733 (0.001)[d]	0.726	0.729
	H_2O, dialysed protein	0.730 (0.003)[e]		
α-Lactalbumin (bovine milk)	Phosphate buffer, pH = 7, I = 0.1 M	0.736 (0.002)[d]	0.715	0.718
	10 mM cacodylic acid buffer, pH = 6, 2 mM $CaCl_2$	0.713 (0.003)[e]		
	H_2O, adjusted to pH = 7.0	0.731 (0.005)[f]		
Cytochrome C (horse heart)	H_2O, dialysed protein	0.725 (0.002)[g]	0.724[j]	0.726[j]
	H_2O, dialysed protein	0.738 (0.003)[e]		
	0.1 M phosphate buffer, pH = 7.0	0.708 (0.005)[f]		
Myoglobin				
(Whale skeletal muscle)	H_2O, isoionic protein	0.742 (0.001)[d]	0.736[j]	0.738[j]
(Horse heart)	H_2O, dialysed protein	0.745 (0.003)[e]	0.734[j]	
(Sperm whale)	H_2O, adjusted to pH 6.7	0.730[h]		
(Apo form)	50 mM phosphate buffer, pH = 7.2	0.730[h,i]		

[a] Experimental values for proteins in their native states.
[b] This work calculated for the unfolded form of the protein under isoionic conditions.
[c] From Kharakoz, [60].
[d] From Gekko et al., [61].
[e] From Chalikian et al., [62].
[f] From Hinz et al., [23].
[g] From Gekko et al., [63].
[h] From Gavish et al., [64].
[i] At a temperature of 22°C.
[j] For the protein chain only.

proteins in aqueous solution at 25°C were calculated using Eqs. (16)–(18) and the side-chain coefficients given in Table 3. As outlined in Section 4.1, the partial molar volumes for the side-chains of lysine and arginine given in Table 3 include a contribution from the proton. For the temperature of 25°C, the contribution from the proton can be eliminated using the recommended value of $V_2^0[H^+(aq)] = -5.5$ cm^3 mol^{-1} [58,59]. Similarly, the side-chain contributions for asp and glu can be corrected for the effect of ionisation using a value for the volume change on ionisation of $\Delta V_i^0 = -11.5$ cm^3 mol^{-1}. The calculated v^0 values for various proteins are given in Table 5. Included in Table 5 are the v^0 values determined experimentally for the proteins in their native states. In general, the agreement between the experimental and calculated values is surprisingly good considering that the calculated v^0 values correspond to the completely unfolded random coil form of the protein. This is a manifestation of the fact that the volume changes observed experimentally for the complete unfolding of many small globular proteins are close to zero [23,65,66].

For the purposes of comparison with the calculated v^0 values obtained in this work, we have included in Table 5 the results reported recently by Kharakoz [60] who used the simple zwitterionic amino acids as model compounds. Although several other additivity schemes based on amino acids as model compounds have been reported previously [67–69], we chose to use the most recent scheme because side-chain ionisation effects were evaluated. With the exception of ribonuclease A, the v^0 values obtained in this study are slightly smaller than those derived by Kharakoz. The agreement is perhaps better than expected given that the mutual interaction between the ionic end-groups and the side-chain in an amino acid gives rise to a side-chain group contribution that is significantly smaller than that derived using partial molar volume data for tripeptides [15,16,20]. The group contribution of the backbone glycyl unit used by Kharakoz [60], which is an average of several literature values based on the oligoglycines as model compounds, is, however, 1.2 cm^3 mol^{-1} greater than the value we determined in earlier work [22], and which has been used herein. Consequently, there is a compensation of these differences to such an extent that v^0 values calculated using amino acids as model compounds are in closer agreement to those based on peptide model compounds than might have been expected from a consideration of ionic end-group effects in amino acids.

The v^0 values for proteins calculated over a wide temperature range are, at present, less reliable than those at a temperature of 25°C. This is because of the uncertainties associated with the corrections for the temperature dependence of the partial molar volume of the proton and also for the extent of ionisation of asp and glu side-chains. Despite these limitations, we have estimated the partial specific volumes for the two proteins ribonuclease A and lysozyme over the temperature range 10–90°C. Some years ago [70] Millero reviewed the various methods that had been used to derive the ionic partial molar volume of the proton. Although the $V_2^0(H^+)$ values obtained by the various methods differed significantly, it was noted that the partial molar expansibility of the proton, $E_2^0(H^+) = (\partial V_2^0(H^+)/\partial T)_p$, of all the methods appeared to be a smooth function of temperature. The 'best-fit' straight line through the data was found to be [70]:

$$E_2^0(H^+) = -0.008 - 3.40 \times 10^{-4}\ (T - 273.15) \quad (19)$$

On integrating this equation, using the value $V_2^0(H^+) = -5.5$ cm^3 mol^{-1} at 25°C [58,59], we were able to estimate the partial molar volume of the proton over the temperature range of interest. With regard to the correction for ionisation of the acidic side-chains of asp and glu, we have assumed that, to a first approximation, the volume change upon ionisation is independent of temperature. The $v^0(T)$ curves for the two proteins are shown in Fig. 5. There is a paucity of experimental v^0 data for unfolded proteins in water with which to compare our calculated $v^0(T)$ curves. Makhatadze et al. [32] reported v^0 values at selected temperatures over the range 5–65°C for ribonuclease A and lysozyme with carboxymethylated cysteine residues, but the measurements

were carried out in 10 mM glycine buffer under acidic conditions [32,71]. In earlier work from this laboratory [23], v^0 data were obtained for several proteins over the temperature range 10–90°C using DSD. However, for ribonuclease A and lysozyme the solvent conditions were 0.1 M phosphate buffer, at pH = 2.5 and 2.0, respectively, which negate direct comparisons with the curves derived in this work. The only partial specific volume data with which comparisons can be made are those for the native states in pure water [62,72]. These results are shown in Fig. 5. Although the agreement between the two sets of literature data for lysozyme is not good, it is apparent from Fig. 5 that the quantity dv^0/dT for the native state of these two proteins is smaller than that predicted for the unfolded state. This observation is consistent with previous findings that the temperature coefficients of the partial specific volumes of the native states of proteins are smaller than those for the denatured states [23,66,73]. Also included in Fig. 5 are the calculated partial specific volumes of the unfolded proteins given by Makhatadze et al. [32]. In their additivity scheme, some amino acid side-chains were modelled using organic analogues in water while others were modelled using tripeptides of sequence gly–X–gly in buffer solution at pH = 4. Clearly, the results obtained are significantly larger than those predicted using the additivity scheme used in this work and are in poor agreement with experimental data.

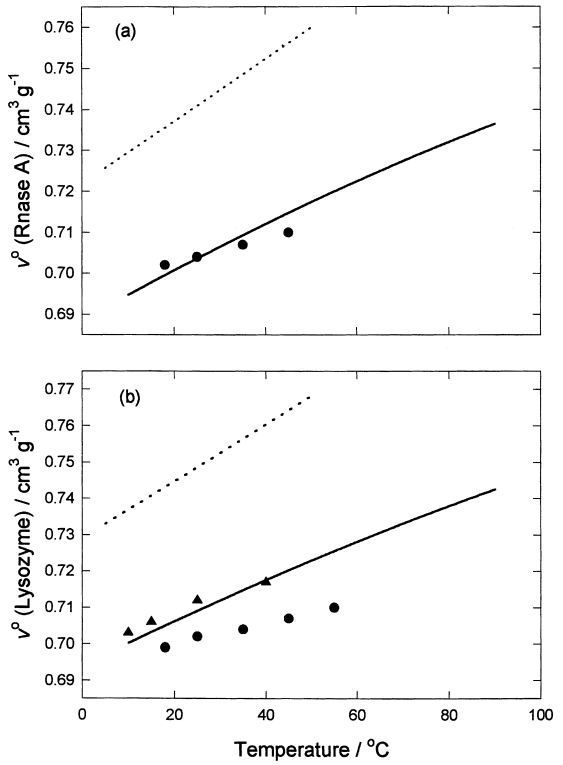

Fig. 5. Temperature dependence of the partial specific volume of ribonuclease A and lysozyme: (a) ribonuclease A — this work, ● native state protein, 10 mM cacodylic acid buffer/10 mM NaCl, pH = 6 [62] ····· calculated values from Makhatadze et al., [32]; and (b) lysozyme — this work, ● native state protein, H_2O [62], ▲ native state protein, H_2O [72] ····· calculated values from Makhatadze et al., [32].

4.3. Changes in side-chain volumes on protein folding

The calculations described in Section 4.2 show that protein partial molar volumes at room temperature can be predicted moderately well using a group additivity scheme based on tripeptides as model compounds. This success is due primarily to the fact that the volume changes on protein folding observed experimentally are rather small, typically less than 0.5% of the partial specific volume of the protein. However, this does not imply that there are no changes in amino acid side-chain volumes upon protein folding, as was demonstrated recently by Chothia and coworkers [74]. In their study, the mean volumes occupied by residues completely buried within the interior of proteins were calculated using the Voronoi polyhedra procedure and a set of 108 proteins whose structures have been determined to high resolution. The differences between the volumes of each amino acid residue and the glycyl residue, V_p, are shown in Table 6. To calculate the volume of side-chains in solution, Chothia and coworkers [74] choose to use partial molar volume data for the amino acids as these were the most complete set of data available at that time. However, these side-chain volumes are not exactly comparable with those in proteins because the ionic amino and carboxyl groups in a zwitterionic amino acid interfere with the hydration of the adjacent side-

chain [16,55]. Volumes of hydrated protein side-chains are better represented by using as model compounds tripeptides of sequence gly–*X*–gly for which the side-chain is well separated from the ionic end-groups. Now that we have determined the partial molar volumes for the peptides gly–*X*–gly with all 20 amino acid side-chains, it is of interest to use these new results to calculate the change in side-chain volume on folding. The results obtained are represented in Table 6. Where possible, we have chosen to use side-chain volumes in solution that are based on peptide partial molar volumes determined isothermally rather than by DSD as they are of higher precision. For comparison, the changes in side-chain volume on folding reported previously [74] are also included in Table 6. As noted in previous work [16,20] and in Section 4.1, the partial molar volumes of side-chains, $V^0(R)$, derived using V_2^0 data for tripeptides are slightly larger than those based on V_2^0 data for amino acids. As a consequence of this, the changes in side-chain volume on folding derived in this work, $(V_p - V_{tri})$, are, for most side-chains, more negative than those reported by Harpaz et al. In some cases the differences are significant enough to warrant a change in the classification of the side-chains suggested previously [74]. For example, based on the $(V_p - V_{aa})$ values the side-chains of phe and tyr were grouped with those showing only small volume changes on transfer between the two environments [74] whereas, based on the $(V_p - V_{tri})$

Table 6
Volumes occupied by side-chains in protein interiors and in gly–*X*–gly tripeptides in aqueous solution at 25°C

X	$10^{24}\ V_p$[a]/ cm^3 residue^{-1}	$10^{24}\ V_{tri}$[b]/ cm^3 residue^{-1}	$10^{24}\ (V_p - V_{tri})$/ cm^3 residue^{-1}	$10^{24}\ (V_p - V_{aa})$[h,i]/ cm^3 residue^{-1}
gly	–	–	–	–
ala	26.3	29.5[c]	−3.2	−2.6
val	75.3	80.5[c]	−5.2	−3.6
leu	100.8	108.6[c]	−7.8	−6.2
ile	101.1	108.5[c]	−7.4	−2.6
ser	30.4	29.9[d]	+0.5	+1.4
thr	56.2	56.8[c]	−0.6	+0.3
asn	63.7	58.8[c]	+4.9	+7.0
gln	85.6	81.5[e]	+4.1	+1.3
phe	129.7	135.2[c]	−5.5	−0.9
tyr	133.3	137.0[e]	−3.7	−0.3
trp	167.9	164.0[d]	+3.9	+0.6
his	95.5	96.3[c]	−0.8	+3.3
cys	49.4	51.6[e]	−2.2	−1.0
met	103.9	104.9[c]	−1.0	+0.7
pro	59.3	54.1[e]	+5.2	−6.2
asp^-	53.3	44.2[d,f]	+9.1	+11.9
glu^-	77.0	70.6[d,f]	+6.4	+8.5
lys^+	106.2	98.0[d,g]	+8.2	+7.6
arg^+	129.0	110.5[d,g]	+18.5	+7.9

[a] From Harpaz et al., [74]. V_p represents the difference between the volume of a buried amino acid residue and that for the glycyl residue.
[b] $V_{tri} = [V_2^0(\text{gly-}X\text{-gly}) - V_2^0(\text{glyglygly})]/N_A$.
[c] V_2^0 data from Hedwig, [16].
[d] This work.
[e] V_2^0 data from Schwitzer et al., [20].
[f] Calculated for the fully ionised side-chain using $\Delta V_i = -11.5$ cm^3 mol^{-1} and $V_2^0(H^+) = -5.5$ cm^3 mol^{-1}.
[g] Calculated for the charged side-chain using $V_2^0(H^+) = -5.5$ cm^3 mol^{-1}.
[h] $V_{aa} = [V_2^0(X) - V_2^0(\text{gly})]/N_A$.
[i] From Harpaz et al., [74].

results in this work, these side-chains can now be grouped with the aliphatic side-chains. A perusal of Table 6 indicates that, in general, the $(V_p - V_{tri})$ values for typically hydrophobic side-chains are relatively large and negative, those for ionic and typically polar side-chains are relatively large and positive and those for side-chains which have both hydrocarbon and polar moieties are somewhat smaller and may be positive or negative. There are some anomalies however. The $(V_p - V_{tri})$ value for the trp side-chain $(+3.9 \times 10^{-24}$ cm^3 $residue^{-1})$ is larger than might be expected and the value for the arg^+ side-chain seems excessively large. We are unable to provide a satisfactory explanation for these results. Although the $(V_p - V_{tri})$ and $(V_p - V_{aa})$ values for pro differ significantly, such a comparison is not strictly valid for this side-chain [20]. This is because of the unique structural feature of the amino acid proline. The quantity V_{aa} includes a contribution from the difference in hydration between the imino group of proline and the amino group of glycine. Similarly, V_{tri} includes a contribution from the difference in hydration of the peptide group adjacent to the prolyl side-chain and that in triglycine.

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