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Group additivity schemes for the calculation of the partial molar heat capacities and volumes of unfolded proteins in aqueous solution

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Abstract

A critical review is given of the present state of group additivity schemes for the calculation of partial molar volumes and heat capacities of unfolded proteins. The comparison between the experimental values and the predictions based on the different models shows clearly that only the peptide-based additivity scheme represents properly both the absolute values and the temperature dependence of these thermodynamic quantities.

Keywords: Additivity schemes; Unfolded proteins; Volume; Heat capacity; Oligopeptides; Tripeptides; Model compounds

1. Introduction

Empirical additivity schemes to evaluate thermodynamic properties of compounds from the knowledge of their molecular structure have received widespread attention over many years [1–9], and are indeed still of current interest [10–12]. For the thermodynamic property of heat capacity, for example, various additivity schemes based on parameters for atom, bond and group contributions can successfully predict the heat capacity of gaseous molecules [9] and of liquid hydrocarbons [13]. The heat capacity changes accompanying the dissolution of a wide range of organic molecules into water have been successfully analysed using a group additivity approach [14], as has the partial molar heat capacity for a wide range of organic solutes in water [3,6,7].

Although the main purpose of any additivity scheme is its predictive utility, significant differences between experimental results and those predicted using group additivity can also provide additional insight into the intramolecular interactions amongst the various functional groups on molecules [10].

The application of group additivity methods to specifically evaluate thermodynamic properties of proteins also has a long history. In the context of this special issue of *Biophysical Chemistry*, it is significant to note that John T. Edsall, in collaboration with his co-worker Edwin J. Cohn, were first to report, in their seminal monograph [15],

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the use of group additivity methods to evaluate the partial specific volume of proteins. Since this 1943 publication, a modified Cohn–Edsall procedure [16,17] and several alternative group additivity schemes [11,18–21] have been proposed to calculate the partial molar (or specific) volume of polypeptides and proteins in aqueous solution. Furthermore, additivity schemes to calculate the partial molar compressibility [11,18] and partial molar heat capacity [21–23] of aqueous polypeptide and protein systems have also been reported.

Much of the current interest in additivity models to evaluate protein thermodynamic properties, in particular the heat capacity, stems from the contribution they can make in discussions of protein stability [24]. Since the stability of a protein is defined as the difference between the Gibbs energy of the native and denatured states, both states are equal in importance when considering the stability of proteins. Within the conformational ensemble that constitutes the denatured state of proteins, there may be, for a particular protein, a subset of conformations that retains a significant degree of structure. In an analysis of the stability of such a protein, it is useful to consider the unfolded or random-coil form of the protein as an ideal reference state [24]. The conformations that characterise this state are essentially without structure and are well exposed to the solvent [25,26]. Since the random-coil ideal reference state is experimentally inaccessible for many proteins, its thermodynamic properties, such as the partial molar volume and heat capacity, can be estimated using group additivity methods.

As all proteins are comprised of just 20 different amino acids, it is not surprising that most of the additivity schemes developed to derive thermodynamic properties of proteins are based on the group contributions for the various amino acid residues. The constituent groups of a protein are usually chosen as the various amino-acid side chains, the repeating unit of the backbone chain, and the chain terminal groups, which at neutral pH are the ionic amino and carboxyl functional groups. Based on the principle of group additivity, which assumes that each constituent group of a molecule interacts with the surrounding solvent independently of adjacent groups, the partial molar thermodynamic property for a protein at infinite dilution, Y_2^o, is given by the expression:

$$Y_2^o = Y_2^o(NH_3^+ + CHCOO^-) + (N-1)Y_2^o(CHCONH) + \sum Y_2^o(R_i) \quad (1)$$

where N is the number of amino acid residues in the protein, R_i is the side chain of the ith amino acid, CHCONH is the repeating unit of the backbone peptide chain, and NH_3^+ and $CHCOO^-$ are the ionic end-groups of the peptide chain. The contributions to the thermodynamic property of these constituent groups are obtained using thermodynamic data for small solutes that are chosen to model the various components of a protein molecule. While it is well recognised that group additivity schemes can only ever be good approximations at best, it is, however, clear that the ultimate success of any scheme depends on how reliably the various group contributions can be estimated.

In several of the reported [11,17,18] additivity schemes to calculate volumetric properties of proteins, the amino-acid side chains, R_i, were modelled using the simple zwitterionic amino acids. In these molecules the charged NH_3^+ and COO^- groups are adjacent to the side chain and, as such, there will be considerable overlap among the respective hydration co-spheres of the side chain and charged functional groups. Consequently, side-chain contributions derived using thermodynamic data for the amino acids will not necessarily be appropriate to use for a polypeptide or protein that has neutral peptide groups adjacent to the side chains. A further limitation of these schemes is that they are valid at only a single temperature of 25 °C. In an attempt to address this temperature limitation, a scheme was reported [19] in which the partial molar volume of polypeptides could be calculated over a wide temperature range. However, this scheme has several deficiencies, some of which are discussed below (see Section 5.2).

With regard to additivity schemes for the calculation of the partial molar heat capacity of unfolded proteins, Makhatadze and Privalov [22] proposed the use of the partial molar heat capacity values of a disparate set of small organic molecules to derive the contributions of the amino-acid side

chains. For example, the side chains of the amino acids leucine and glutamine were modelled using *n*-butane and propionamide, respectively. Although such model compounds appear, at first sight, to be a better choice than, say, the amino acids, they also have their limitations [23,27], as is shown herein.

To ensure the best quantitative success of 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 for which the thermodynamic properties are to be evaluated. With this in mind, we proposed several years ago [28] that small peptides of sequence glycyl–X–glycine (Gly–X–Gly), where X represents one of the 20 amino acids, would be ideal compounds to model the amino-acid side chains of polypeptides and proteins. In these tripeptides, the single side chain, R, of amino acid X is flanked by two peptide groups, which is structurally identical to that found in a protein. In this regard, the tripeptides Gly–X–Gly are the best compounds used hitherto for the derivation of side-chain thermodynamic properties.

In several papers over the past few years [20,23,27–29] we have carried out thermodynamic studies of these tripeptides in aqueous solution, and also of compounds chosen to model the other constituent groups given in Eq. (1). The objective of this short review is to summarise the salient features of our work that has been directed towards establishing a definitive additivity scheme to evaluate the partial molar heat capacity and volume of unfolded proteins in aqueous solution over a wide temperature range. This peptide-based group additivity model is outlined and some distinct advantages it has over previously proposed models are discussed. We also show that this peptide-based model has considerable promise as a means to give reliable estimates of the partial molar heat capacity and volume of unfolded proteins.

2. Peptide-based group additivity model

A feature that distinguishes our additivity model from others reported in the literature is that the compounds chosen as models are themselves portions of proteins, i.e. peptide molecules. The method used to derive the amino-acid side chain contributions is illustrated here for the thermodynamic property, heat capacity. The partial molar heat capacity of any side chain R is obtained using the equation:

$$C_p^o(R) = C_{p,2}^o(\text{Gly–X–Gly}) - C_{p,2}^o(\text{GlyGlyGly}) + C_p^o(H) \quad (2)$$

where $C_{p,2}^o(\text{Gly–X–Gly})$ and $C_{p,2}^o(\text{GlyGlyGly})$ are the partial molar heat capacity at infinite dilution for the peptides Gly–X–Gly and GlyGlyGly, respectively, and $C_p^o(H)$ is the heat capacity of the hydrogen atom of the methylene moiety of triglycine. Values for $C_p^o(H)$ were taken from the literature [22], although, as outlined below, the values chosen are of little consequence in group additivity calculations.

The assumption inherent in the derivation of $C_p^o(R)$ using Eq. (2) is that any interactions between the side-chain R and the charged amino and carboxyl groups in the tripeptide Gly–X–Gly make negligible contributions to the side-chain heat capacity. This assumption seems reasonable based on results from volumetric studies of two homologous series of compounds, the α,ω-aminocarboxylic acids, $^+NH_3(CH_2)_nCO_2^-$, $n=1$–7, and the oligoglycines, $(Gly)_n$, $n=1$–5. For the α,ω-amino acids, the charged end-groups have no effect on the central position in the molecule when separated by five or more carbon atoms [30], and similarly for the oligoglycines when there are two or more peptide groups present [31]. The tripeptides Gly–X–Gly are in this category. Furthermore, we have shown [32] that the side-chain heat capacity values derived using $C_{p,2}^o$ data for the tetrapeptides GlyPheGlyGly and GlyGlyAlaGly, and for the pentapeptides GlyGlySerGlyGly and GlyGlyLeuGlyGly are identical, within the combined experimental uncertainties, to those derived using tripeptides as model compounds. If any interactions between the ionic end-groups and the central side-chain were indeed significant in the Gly–X–Gly peptides, then different values for $C_p^o(R)$ would have been obtained when $C_{p,2}^o$ data for tetra- and pentpeptides are used, since in these peptides each side chain is at a greater distance from the ionic end-groups than in the tripeptides.

The partial molar heat capacity of the backbone peptide group, CHCONH, required for group additivity calculations using Eq. (1) is derived from the partial molar heat capacity of the glycyl group, CH_2CONH, using the equation:

$$C_p^o(\mathrm{CHCONH}) = C_p^o(\mathrm{CH_2CONH}) - C_p^o(\mathrm{H}) \quad (3)$$

where $C_p^o(\mathrm{H})$ is as previously defined for Eq. (2). It is evident from a comparison of Eqs. (2) and (3) that the sum $\{C_p^o(\mathrm{CHCONH}) + C_p^o(\mathrm{R})\}$ is independent of the value chosen for $C_p^o(\mathrm{H})$, and hence is unaffected by any debate there may be over the correct $C_p^o(\mathrm{H})$ values to use for tripeptide model compounds [27]. The partial molar heat capacity of the glycyl group was derived using $C_{p,2}^o$ data over the temperature range 10–100 °C for a series of peptides of sequence $\mathrm{Ala(Gly)}_n$, $n = 2$–4, using methods described in Section 3.2.

The contribution to the heat capacity of a polypeptide of the ionic end-groups NH_3^+ and CH_2COO^- was derived using the equation:

$$C_p^o(\mathrm{NH_3^+ + CH_2COO^-}) = C_{p,2}^o(\mathrm{GlyGlyGly}) - 2C_p^o(\mathrm{CH_2CONH}) \quad (4)$$

where $C_{p,2}^o(\mathrm{GlyGlyGly})$ is the partial molar heat capacity of triglycine at infinite dilution. The partial molar heat capacity of the end groups $(\mathrm{NH_3^+ + CHCOO^-})$ is obtained from the quantity $C_p^o(\mathrm{NH_3^+ + CH_2COO^-})$ by subtraction of the heat capacity of the H atom.

These estimates of the heat capacity of the side-chains, the backbone peptide group and the ionic end-groups are then combined using Eq. (1) to obtain the partial molar heat capacity of an unfolded protein of known amino acid sequence.

The partial molar volume of an unfolded protein can be derived using essentially the same procedure as outlined for heat capacity. However, rather than using absolute values of side-chain volumes, the quantities used are defined by the equation:

$$V^o(\mathrm{R}) = V_2^o(\mathrm{Gly{-}X{-}Gly}) - V_2^o(\mathrm{GlyGlyGly}) \quad (5)$$

The quantity $V^o(\mathrm{R})$, which gives the contribution to the partial molar volume on replacing the methylene hydrogen atom of the glycyl unit with the side-chain R, is then coupled with the partial molar volume for the glycyl group, $V^o(\mathrm{CH_2CONH})$, in an additivity relationship analogous to Eq. (1). This approach was used for side-chain volume because there were fewer comparisons that could be made with literature results obtained using other model systems, in contrast to the case for heat capacity.

3. Group partial molar heat capacity

3.1. Amino-acid side chains

The partial molar heat capacity at infinite dilution, $C_{p,2}^o$, of the various Gly–X–Gly peptides were determined over the temperature range 10–100 °C using sensitive differential scanning calorimetry (DSC) [23]. The partial molar heat capacity of all 20 amino acid side-chains, derived from these $C_{p,2}^o$ data using Eq. (2), was analysed using a polynomial in temperature of the form:

$$C_p^o(\mathrm{R}) = a + b(T - 273.15) + c(T - 273.15)^2 + d(T - 273.15)^3 \quad (6)$$

where a, b, c and d are the fitted coefficients. This third-order polynomial was chosen because it was the lowest-order expression that gave a good representation of the experimental heat capacity data over the temperature range used. The polynomial coefficients obtained for each amino side chain are given in Table 1. The coefficients for glycine, which has no side chain, describe the temperature dependence of $C_p^o(\mathrm{H})$ [27]. For each of the cationic side chains of lysine and arginine, the contribution of the acetate anion of the peptide salt used was eliminated using literature data for the appropriate strong electrolytes [23]. The coefficients given in Table 1 are those that represent the temperature dependence of the quantity $\{C_p^o(\mathrm{R^+}) - C_{p,2}^o(\mathrm{H^+})\}$, where $C_{p,2}^o(\mathrm{H^+})$ is the partial molar heat capacity at infinite dilution of the proton, and $\mathrm{R^+}$ represents the charged side chain. Until estimates of the heat capacity of the proton over a wide temperature range become available, it is not possible to obtain the absolute values of the heat capacity of the protonated side chains of lysine and arginine. In our study of the peptide [GlyArgGly]acetate, there was some doubt about the analytical purity of the compound [23]. To verify whether the heat capacity data obtained for this compound are reasonable,

Table 1
Coefficients of Eq. (6) that represent the temperature dependence of the heat capacity for the constituent groups[a]

Group	a (J K^{-1} mol^{-1})	b (J K^{-2} mol^{-1})	c (J K^{-3} mol^{-1})	$10^4 d$ (J K^{-4} mol^{-1})
Gly[b]	83.4	−0.22	−0.0003	0.01
Ala	204.3	−0.99	0.0078	−0.24
Val	359.9	−1.64	0.0186	−1.16
Leu	444.8	−1.47	0.0107	−0.60
Ile	451.3	−1.38	0.0067	−0.47
Ser	151.6	−0.58	0.0106	−0.87
Thr	247.7	−0.64	0.0094	−0.70
Asn	160.8	1.28	−0.0253	1.49
Gln	206.2	0.16	0.0050	−0.67
Phe	427.3	−1.63	0.0155	−0.83
Tyr	336.7	0.26	−0.0044	0.08
Trp	440.4	−0.21	−0.0128	0.92
His	235.4	0.67	0.0428	−3.52
Cys	256.9	−1.73	0.0207	−1.22
Met	342.0	−1.23	0.0177	−1.47
Pro	206.2	−1.40	0.0256	−1.25
Asp	157.8	0.54	−0.0112	0.18
Glu	219.3	−0.17	0.0058	−0.77
(Lys^+–H^+)	360.6	−0.26	0.0148	−0.27
(Arg^+–H^+)	241.0	0.63	−0.0095	0.27
CHCONH	−15.3	1.02	−0.0009	−0.24
$NH_3^+ + CHCO_2^-$	−148.4	4.77	−0.0644	3.68

[a] From Häckel et al. [23].
[b] Values of C_p^o(H).

we recently carried out a study of the hydrochloride salts of the amino acids L-lysine and L-arginine [33]. It was clear, from the side-chain heat capacity values derived using the amino acid data, that further work is indeed required to establish the reliability of the results for the Arg side chain given in Table 1. This work is currently in progress [34].

Comparisons between the side-chain heat capacity obtained using peptides as model compounds and the values derived using small organic solutes [22] have been described in considerable detail elsewhere [23,27]. In general, there are very significant differences between the C_p^o(R) results, in particular the temperature dependence, obtained using the two sets of model compounds. This is illustrated in Fig. 1 for the leucyl and glutamyl side chains. The results presented in Fig. 1a show that the temperature dependence of the heat capacity of the leucyl side chain derived using heat capacity data for tripeptides is larger than that obtained when butane is used as a model compound [22]. In more recent work [35,36], we have derived some side-chain heat capacity values using as model compounds the neutral *N*-acetyl amino acid amides, $CH_3CONHCH(R)CONHY$ (Ac-XNHY), where R is the side-chain of amino acid X (Gly, Ala, Val or Leu), and Y is either the H or CH_3 functional group. The side chains in these compounds are flanked by two peptide groups, which is identical to that found in the Gly–X–Gly peptides. The results obtained for the leucyl side chain, which are shown in Fig. 1a, are approximately 20 J K^{-1} mol^{-1} (ca. 5%) larger than those derived using tripeptide heat capacity data. This difference is larger than might be expected, given the structural similarities between the two sets of model compounds. However, as shown in Fig. 1a, there is excellent agreement between the temperature dependence of C_p^o(Leu) for these two sets of model compounds. Similar results are also found for the side chains of alanine and valine [35,36].

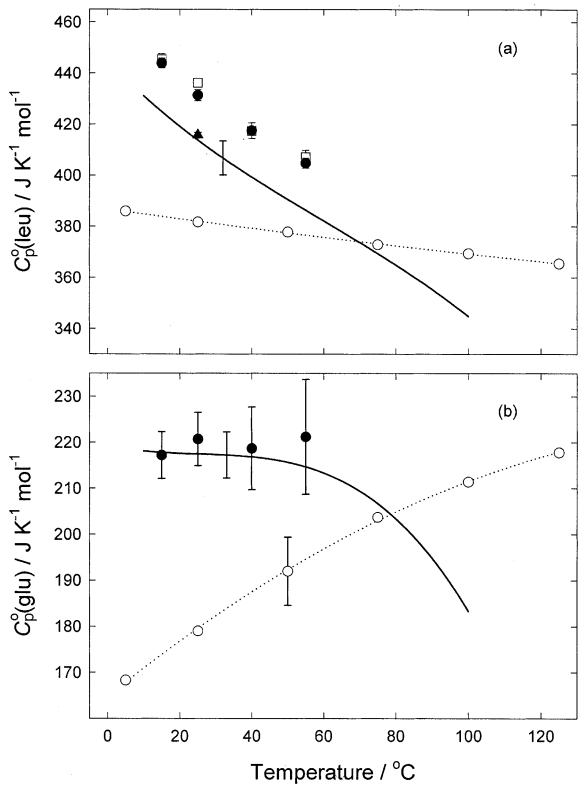

Fig. 1. Partial molar heat capacity of the leucyl and glutamyl side-chains as a function of temperature. (a) ● from [36], AcXNHMe compounds; □ from [35], $AcXNH_2$ compounds; ▲ from [28], Gly–X–Gly tripeptides; — from [23], Gly–X–Gly tripeptides, DSC; ○ from [22]. (b) ● calculated using amino acid $C_{p,2}^o$ data from [37,38]; — from [23], Gly–X–Gly tripeptides, DSC; ○ from [22].

The results for the hydrophilic glutamyl side-chain given in Fig. 1b indicate markedly different temperature dependence for the heat capacity data derived using the two types of model compounds. When propanoic acid is used to model the side chain [22], C_p^o(Glu) increases with an increase in temperature, whereas when tripeptides are used, a more complex temperature dependence is observed. Over the temperature range 10–40 °C, C_p^o(Glu) is approximately constant, but it decreases significantly with a further increase in temperature. The values of C_p^o(Glu) calculated using $C_{p,2}^o$ data for the amino acids [37,38] are also shown in Fig. 1b. Although, because of charged end-group effects [20], these results are not expected to be identical to those based on the tripeptides, it is interesting to note that the values are also approximately constant over the temperature range 15–55 °C. The results presented in Fig. 1b suggest that when the functional group $(CH_2)_2COOH$ is adjacent to either polar peptide groups (as in Gly–X–Gly peptides) or charged groups (as in the amino acids), the group heat capacities and their temperature dependence are quite different from those for the analogous simple organic acid.

3.2. Backbone peptide group

The contribution of the glycyl group, CH_2CONH, from which the heat capacity of the backbone peptide group is obtained using Eq. (3), was derived using two methods [29]. Prior to our study, heat capacity data for the glycyl group were available at only five temperatures in the range 5–100 °C [22]. The $C_p^o(CH_2CONH)$ values were derived from plots of $C_{p,2}^o$ against the number of glycyl groups for the oligoglycines, $(Gly)_n$, $n=3$–5. Although this method is sound, there are experimental limitations due to the very low solubility in pure water of the peptides tetra- and pentaglycine. To surmount this difficulty, we used an alternative series of peptides, $Ala(Gly)_n$, $n=2$–4, the higher members of which are more soluble in water than the corresponding oligoglycines [29]. In a second approach, values for $C_p^o(CH_2CONH)$ were obtained from the differences between the $C_{p,2}^o$ data for the two neutral *N*-acetyl peptide and amino acid amides, $CH_3CONHCH_2CONHCH_2CONH_2$ ($AcGlyGlyNH_2$), and $CH_3CONHCH_2CONH_2$ ($AcGlyNH_2$) [29].

The temperature dependence of the partial molar heat capacity of the glycyl group is shown in Fig. 2. The agreement between the heat capacity–temperature curves derived using the two sets of model compounds is within the combined estimated uncertainties. Also displayed in Fig. 2 are the results obtained by Makhatadze and Privalov [22] using the oligoglycines. Clearly, these results are in poor agreement with those derived using the $Ala(Gly)_n$ series of peptides. Recently, Amend and Helgeson [21] proposed a new method, based on group additivity algorithms, to predict various thermodynamic properties of unfolded proteins

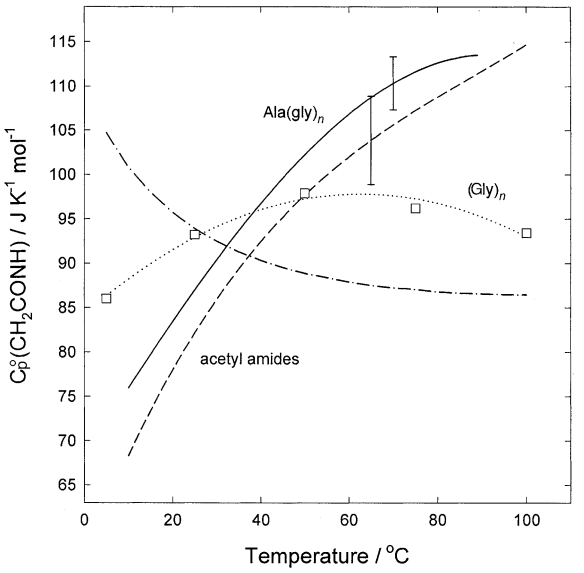

Fig. 2. Temperature dependence of the partial molar heat capacity of the glycyl group: — derived from $C^o_{p,2}$ data for the Ala(Gly)$_n$ peptides [29]; – – – derived from $C^o_{p,2}$ data for $AcGlyNH_2$ and $AcGlyGlyNH_2$ [29]; □ from [22]; – • – • – calculated using data from [21].

over extended ranges of temperature and pressure. A critique of the Amend–Helgeson scheme has been presented elsewhere [39] but, for the purposes of comparison, their prediction for the heat capacity of the glycyl group is included in Fig. 2. The temperature dependence of C^o_p (CH_2CONH) differs very significantly from that we obtained.

The glycyl group heat capacities derived from the $C^o_{p,2}$ data for the Ala(Gly)$_n$ peptides were used to calculate the heat capacity of the backbone peptide group, CHCONH [23]. The relevant polynomial coefficients that describe its temperature dependence are given in Table 1.

3.3. Additivity of side-chain heat capacities

The side-chain group heat capacities derived using the Gly–X–Gly peptides give results that are appropriate for isolated side chains in a polypeptide. Are they indeed valid for real proteins, which contain, of course, adjacent side chains? To answer this question, the concept of group additivity, as it applies to the partial molar heat capacity of polypeptides, needs to be experimentally verified. As a first step to achieving this objective, we recently carried out [40] syntheses of several tetrapeptides of sequence Gly–X–Y–Gly and pentapeptides of sequence Gly–X–Y–Z–Gly, where X, Y and Z are amino acids with neutral side chains. As these peptides have at least two adjacent side-chains, they can be used to verify the concept of group additivity for small peptides. A comparison is given in Table 2 of the partial molar heat capacity at 25 °C obtained experimentally by DSC with the values derived using the constituent group parameters given in Table 1. For all peptides, the partial molar heat capacities calculated using group additivity are in excellent quantitative agreement with those obtained by experiment.

For three selected peptides, a comparison is given in Fig. 3 of the experimental partial molar heat capacity over the temperature range 10–100 °C with the values calculated using group additivity. Each peptide chosen has a combination of side chains with different characteristics. The peptide GlyLeuLeuGly has two adjacent hydrophobic side chains, GlyGlySerAlaGly has a hydrophilic side chain adjacent to a hydrophobic side chain, and there are two adjacent hydrophilic side chains in the peptide GlySerThrGly. As shown in Fig. 3, in each case there is excellent agreement over a wide temperature range between the experimental heat capacities and those calculated using group additivity. Similar results were also obtained for the other peptides studied [32]. These results unequiv-

Table 2
A comparison of the partial molar heat capacity at 25 °C obtained experimentally and using group additivity

Peptide	$C^o_{p,2}$ (J K^{-1} mol^{-1})	
	Experimental[a]	Group additivity
GlyPheAlaGly	710 (9)	698 (12)
GlyLeuLeuGly	955 (10)	947 (11)
GlyPheSerGly	654 (7)	656 (12)
GlySerThrGly	499 (5)	498 (9)
GlyAsnAlaGly	472 (10)	482 (9)
GlySerSerGly	410 (6)	405 (10)
GlyPheValGlyGly	933 (6)	930 (13)
GlyGlySerAlaGly	527 (5)	533 (8)

Estimated uncertainty in parentheses.
[a] From Häckel et al. [31].

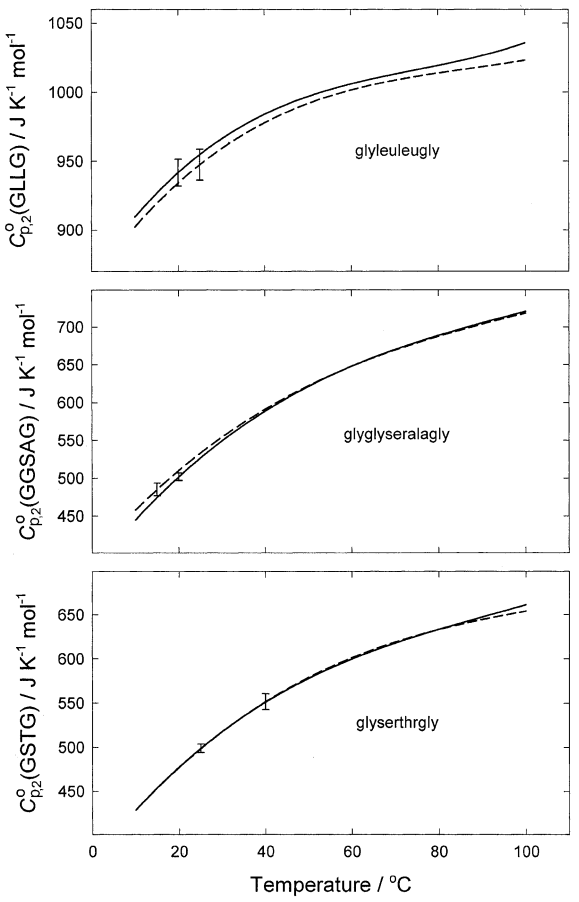

Fig. 3. Temperature dependence of the partial molar heat capacity of some peptides: — experimental DSC data from [32]; – – – calculated using the constituent group heat capacities in Table 1.

ocally show that group additivity is indeed valid over a wide temperature range for the partial molar heat capacities of small peptides with neutral side chains, irrespective of the side-chain sequence. By inference, group additivity should also be valid for fully unfolded proteins, but for completeness, further work is required to verify that additivity indeed extends to ionic side chains.

4. Group partial molar volumes

4.1. Amino-acid side chains

The partial molar volumes at infinite dilution, V^o_2, of the various Gly–X–Gly tripeptides were determined over the temperature range 10–90 °C using a differential scanning densimetric (DSD) method [41]. Although the results obtained using DSD are not as precise as those determined isothermally, the advantage of this method is that in a single scan, partial molar volume data are obtained across a wide temperature range. Precise density measurements at 25 °C have also been made for aqueous solutions of most of the tripeptides [28,42,43]. There is excellent agreement, within the combined uncertainties, between the partial molar volumes at 25 °C derived from these density data and those obtained using DSD [20].

The quantities $V^o(R)$, which give the volume changes on replacing hydrogen atoms of the backbone glycyl group by the various side chains, were calculated from the partial molar volume data for the tripeptides using Eq. (5). These $V^o(R)$ results for each side chain were analysed using the polynomial in temperature:

$$V^o(R) = a_v + b_v(T - 273.15) + c_v(T - 273.15)^2 \quad (7)$$

where a_v, b_v and c_v are the coefficients fitted using least-squares methods. The values of these coefficients for 19 amino-acid side chains are given in Table 3. For the side chains of lysine and arginine, the coefficients given represent the temperature dependence of the quantity $\{V^o(R) - V^o_2(H^+)\}$, where $V^o_2(H^+)$ is the partial molar volume of the proton in aqueous solution at infinite dilution. Using the recommended value [44,45] of $V^o_2(H^+) = -5.5$ cm^3 mol^{-1} at 25 °C, coupled with information about the partial molar expansibility of the proton [46], estimates can be made of the partial molar volume of the proton over a wide temperature range [20]. Further studies are required to confirm the reliability of these estimations.

The volume–temperature profiles of just two side-chains are shown in Fig. 4. These examples were selected to illustrate the temperature dependence of partial molar volumes that are representative of hydrophobic and hydrophilic side chains. For the leucyl side chain, the partial molar volume increases with an increase in temperature, which is consistent with that found for hydrophobic solutes in aqueous solution [47,48]. For the pur-

Table 3
Coefficients of Eq. (7) for the temperature dependence of the partial molar volumes of amino-acid side chains[a]

Side chain	a_v ($cm^3\ mol^{-1}$)	b_v ($cm^3\ mol^{-1}\ K^{-1}$)	$10^4 c_v$ ($cm^3\ mol^{-1}\ K^{-2}$)
Ala	18.0	0.013	−0.96
Val	48.1	−0.002	3.32
Leu	64.2	−0.004	6.57
Ile	64.3	0.017	6.81
Ser	17.7	0.015	−1.19
Thr	33.6	0.016	0.70
Asn[b]	33.4	0.082	−4.46
Gln	49.3	0.043	−0.50
Phe	78.8	0.084	0.08
Tyr	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	63.6	0.051	4.66
Pro	32.4	0.026	0.61
Asp	30.4	0.058	−2.22
Glu	46.6	0.061	−2.22
$(Lys^+–H^+)$	63.9	0.016	3.53
$(Arg^+–H^+)$	70.2	0.083	−3.56
CH_2CONH[c]	35.5	0.038	−1.67
$NH_3^+ + CH_2CO_2^-$[d]	37.5	0.090	−5.34

[a] From Häckel et al. [20].
[b] Derived using new V_2^o data for GlyAsnGly from Häckel et al. [40].
[c] From Häckel et al. [29].
[d] See text.

poses of comparison, side-chain partial molar volumes derived from V_2^o data for the neutral *N*-acetyl amino acid amides (see Section 3.1) are also shown in Fig. 4a. The temperature profile for V^o(Leu) obtained using the amide model compounds [35,36] parallels that based on tripeptides. Furthermore, the two sets of V^o(Leu) results are in good agreement within the combined estimated uncertainties.

In contrast to that for the hydrophobic leucyl side-chain, the polynomial coefficient c_v for the glutamyl side chain has a negative value, and consequently the volume–temperature profile is convex. This pattern is similar to those observed for electrolytes [49,50] and for polar solutes [51,52] in aqueous solution. Included in Fig. 4b are the V^o(Glu) values derived using partial molar volume data determined for the zwitterionic amino acids over the temperature range 15–55 °C [37,38]. The V^o(Glu) values based on the amino acids are smaller than those derived using tripeptide data, which is to be expected because of the ionic end-group effects that are present in the amino acids [42]. However, what is more significant in the present context is that the temperature dependence of V^o(Glu) for the amino acids is in good qualitative agreement with that derived using partial molar data for the tripeptide model compounds.

The model proposed by Makhatadze et al. [19] to calculate the partial molar volume of proteins uses a disparate set of solutes as models for the amino-acid side chains. For the glutamyl side chain, the absolute value of the side-chain partial molar volume was obtained by subtracting from the V_2^o value for propanoic acid an estimated value for the partial molar volume of the hydrogen atom [19]. The values of V^o(Glu) derived from the results reported by Makhatadze et al. [19] are also shown in Fig. 4b. It is clear that these data are in very poor agreement with those derived using the tripeptide GlyGluGly as a realistic model of the

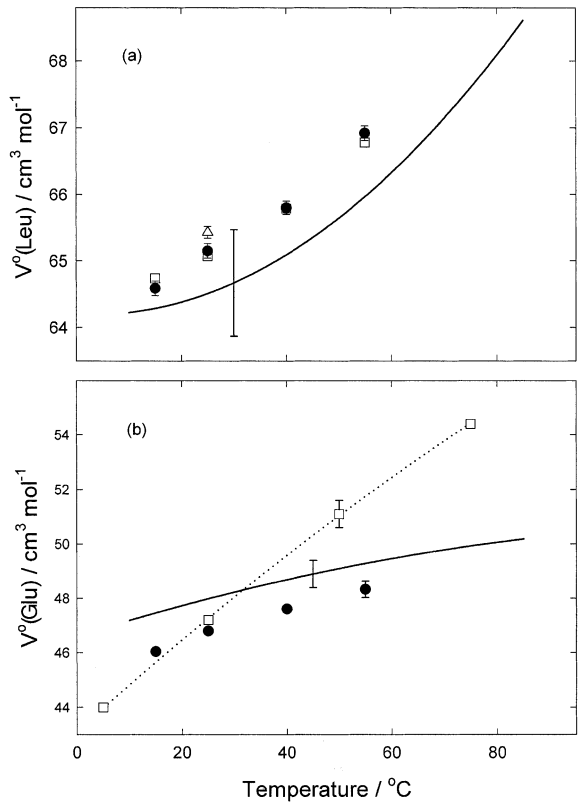

Fig. 4. Temperature dependence of the partial molar volume of the leucyl and glutamyl side chains. (a) ● from [36], AcXNHMe compounds; □ from[35], $AcXNH_2$ compounds; △ from [28], Gly–X–Gly tripeptides; — from [20], Gly–X–Gly tripeptides, DSD. (b) — from [20], Gly–X–Gly tripeptides, DSD; ● calculated using amino acid V_2^o data from [37,38]; □ from [19].

glutamyl side chain. The origin of this large discrepancy may be due to the method that was used to estimate the group contribution of the hydrogen atom, as outlined below (see Section 5.2).

4.2. Glycyl group

Since the side-chain partial molar volumes expressed as V^o(R) are volume changes on replacing H groups of the peptide backbone by R groups, the backbone contribution for group additivity calculations is simply based on the partial molar volume of the repeating glycyl unit. The partial molar volume of the glycyl group, $V^o(CH_2CONH)$, over the range 10–80 °C was derived [29] from V_2^o data for both the Ala(Gly)$_n$ peptides and the acetyl amino acid and peptide amides, using the same methods as outlined for the heat capacity in Section 3.2. The results displayed in Fig. 5 show that there is good agreement, over the complete temperature range, between the $V^o(CH_2CONH)$ values derived using the peptides and acetyl amides as model compounds. In a more recent study [53], partial molar volumes at infinite dilution of the peptides Ala(Gly)$_n$, $n=2$–4, were determined at the four temperatures 18, 25, 30 and 40 °C. The values of $V^o(CH_2CONH)$ derived from these results are in excellent agreement with those obtained using DSD methods, as shown in Fig. 5.

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

$$V_2^o = \sum n_i V(i) \quad (8)$$

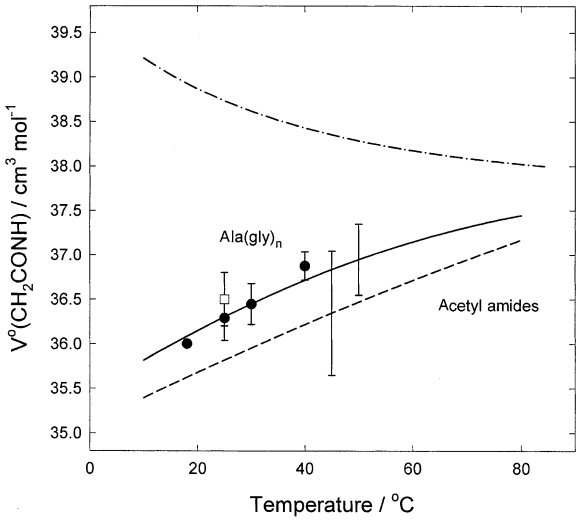

Fig. 5. Temperature dependence of the partial molar volume of the glycyl group. — derived from V_2^o data for the Ala(Gly)$_n$ peptides [29]; ● from V_2^o data for the Ala(Gly)$_n$ peptides [53]; – – – derived from V_2^o data for $AcGlyNH_2$ and $AcGlyGlyNH_2$ [29]; – · – · – calculated using data from [21]; □ from [36], group additivity using Eq. (8).

where $V(i)$ is the contribution to the partial molar volume of each solute of a group of type i and n_i is the number of these i groups. New partial molar volume data at 25 °C for two series of acetyl amino acid amides, $AcXNH_2$ and $AcXNHCH_3$, where X is one of Gly, Ala, Val or Leu, were recently determined [35,36]. These results were combined with data at 25 °C for other acetyl amides and some simple amides, to update a previous group-contribution analysis [29]. The groups chosen in the analysis using Eq. (8) were the peptide group, CONH, the primary amide group, $CONH_2$, the methylene group, CH_2, and a terminal H group [36]. The sum of the two group parameters $V(CONH)$ and $V(CH_2)$ is an estimate of the partial molar volume for the glycyl group. The result obtained is 36.5 ± 0.3 cm^3 mol^{-1}, which, as shown in Fig. 5, is in excellent agreement with that derived using the $Ala(Gly)_n$ peptides.

For comparison, the temperature dependence of the glycyl group reported recently by Amend and Helgeson [21] is also included in Fig. 5. This was derived from regression analyses using partial molar volume data for the oligoglycines selected from the literature [19]. It is worth noting that if an alternative set of oligoglycine data had been chosen [31], the temperature dependence of $V^o(CH_2CONH)$ would have the opposite sign to that shown in Fig. 5. This is an illustration of how difficult it is to obtain reliable thermodynamic data for compounds such as tetra- and pentaglycine that are both sparingly soluble and slow to dissolve.

The polynomial parameters given in Table 3 that describe the temperature dependence of $V^o(CH_2CONH)$ were derived from an analysis of the results based on the $Ala(Gly)_n$ peptides. The partial molar volume of the ionic end-groups was obtained using the volumetric equivalent of Eq. (4). The parameters that describe the temperature dependence of $V^o(NH_3^+ + CH_2COO^-)$ are also given in Table 3.

4.3. Additivity of side-chain volumes

To confirm that group additivity methods are applicable to the partial molar volumes of polypeptides, V_2^o data were determined for some tetra- and pentapeptides with amino acid sequences of Gly–X–Y–Gly and Gly–X–Y–Z–Gly, respectively, where X, Y and Z are amino acids with neutral side chains. Solution densities were measured at 25 °C and for several peptides over the temperature range 10–90 °C using DSD [40]. For each peptide, the V_2^o results at 25 °C obtained using the two methods were concordant within the combined experimental uncertainties [40]. In Table 4, a comparison is given of the partial molar volumes at infinite dilution obtained from the experimental solution densities at 25 °C with values calculated using the group additivity parameters given in Table 3. There is excellent agreement between the V_2^o results calculated using group additivity and those obtained by experiment. With the exception of the peptide GlyProAlaAlaGly, the differences between the calculated and experimental values are in the range 0.2–0.6% of V_2^o. Even for GlyProAlaAlaGly the difference is only 1.2%, which is still small considering the quantitative limitations of the group additivity principle [10,54].

Table 4
A comparison of the partial molar volumes at 25 °C obtained experimentally and using group additivity

Peptide	V_2^o (cm^3 mol^{-1})	
	Experimental[a]	Group additivity
GlyPheAlaGly	247.8 (0.2)	247.3 (0.4)
GlyPheSerGly	247.0 (0.2)	247.5 (0.7)
GlySerThrGly	201.7 (0.1)	200.4 (0.7)
GlyAsnAlaGly	202.08 (0.08)	201.3 (0.3)
GlyGlySerAlaGly	220.9 (0.2)	220.1 (0.7)
GlyPheValGlyGly	314.98 (0.07)	314.2 (0.5)
GlyProAlaAlaGly	255.4 (0.2)	252.4 (0.4)

Estimated uncertainty in parentheses.
[a] From Häckel et al. [40].

The results presented in Table 4 categorically establish that group additivity for small peptides with neutral amino-acid side chains is valid at 25 °C. To verify that it also holds over a wide temperature range, V_2^o data were determined from 10 to 90 °C for five of the peptides. The results obtained for the peptides GlyPheSerGly and GlyGlySerAlaGly are shown in Fig. 6. For both peptides, there is excellent agreement over the entire temperature range between the experimental

volume–temperature curves and those calculated using the group partial molar volumes given in Table 3. Similar agreement was also observed for the other peptides studied [40].

The general conclusion that can be drawn from the results presented in Table 4 and Fig. 6 is that group additivity for partial molar volumes of small peptides is indeed valid over a wide temperature range. Since fully unfolded polypeptides and proteins have the same structural characteristics as these small peptides, the implication that follows from our work is that group additivity should also be applicable to these larger molecules.

5. Comparisons with other models

5.1. Heat capacity

Alternative additivity schemes to calculate the partial molar heat capacity of unfolded polypeptides in aqueous solution have been reported by both Makhatadze and Privalov [22] and Amend and Helgeson [21]. It is worthwhile, therefore, to compare these models with our peptide-based scheme and to explore how well they can predict the partial molar heat capacity of some polypeptides.

The scheme proposed by Makhatadze and Privalov [22] uses mainly small organic solutes, RH, as models for the amino-acid side chains. The partial molar heat capacity of each side chain R was obtained from the experimental partial molar heat capacity of the organic solute in water by subtraction of an estimated value for the heat capacity of a hydrogen atom:

$$C_p^o(R) = C_{p,2}^o(RH) - C_p^o(H) \quad (9)$$

As mentioned in Section 3 and discussed in some detail elsewhere [23], there are very significant differences between the side-chain heat capacities derived using our tripeptide model compounds and those based on these organic analogues. We have suggested [23,27] that, at least for some side chains, the origin of these differences may lie in the choice of values for $C_p^o(H)$ used in the calculations. In this context, it is worth stressing one very important difference between our peptide-based model and that proposed by Makhatadze and Privalov [22]. Whereas the partial molar heat capacity of a polypeptide calculated using the peptide-based model is independent of the value chosen for the absolute heat capacity of the H group (see Section 2), this is not the case for the model proposed by Makhatadze and Privalov [22]. In their scheme, the partial molar heat capacity of the backbone peptide group, C_p^o (CHCONH), was also derived from the glycyl group using Eq. (3). From a comparison of Eqs. (3) and (9), it is clear that the sum $\{C_p^o(CHCONH) + C_p^o(R)\}$ is not independent of $C_p^o(H)$. In other words, in the Makhatadze and Privalov scheme, the accuracy of the heat capacity calculated for any polypeptide depends on the reliability of the $C_p^o(H)$ values used in their calculations.

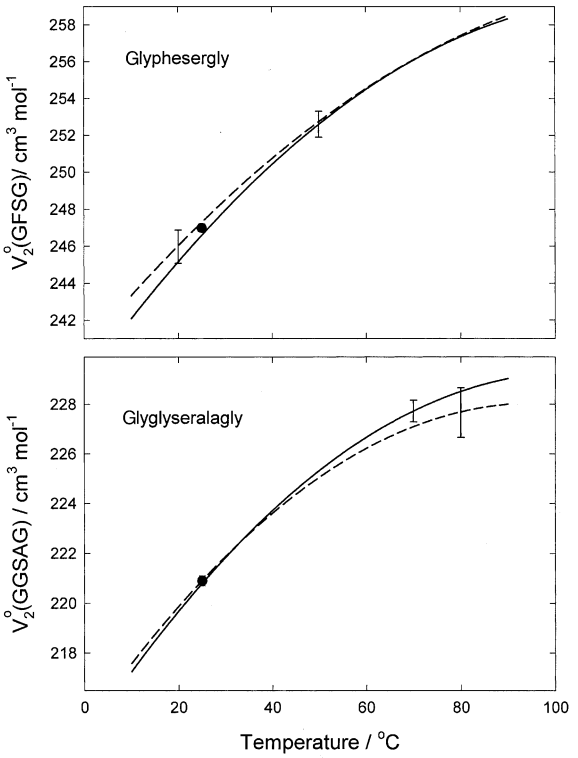

Fig. 6. Partial molar volume of the peptides GlyPheSerGly and GlyGlySerAlaGly as a function of temperature. — experimental DSD data from [40]; – – – calculated using the constituent group volumes in Table 3; ● experimental V_2^o results at 25 °C from Table 4.

The model proposed by Amend and Helgeson combines the revised Helgeson–Kirkham–Flowers (HKF) equations of state with group additivity algorithms to calculate various thermodynamic properties of unfolded proteins at elevated temperatures and pressures [21]. As this general approach has been discussed several times [21,55,56] in some detail, only a brief outline as it applies to proteins is given here. Any partial molar thermodynamic property is considered to be represented as the sum of a solvation (or electrostatic) and a non-solvation (or structural) contribution [57]. The electrostatic contribution is modelled using the Born equation and empirical equations are used to represent the structural components. The various equation-of-state parameters required are obtained from regression analyses using thermodynamic data taken from the literature. For the glycyl group, the parameters were generated from thermodynamic data for the $(Gly)_n$, $n=3$–5, peptides [55]. Parameters for all the amino-acid side chains, with the exception of those for glycine, were generated from literature data for the zwitterionic amino acids [56]. To obtain the equation-of-state parameters of the peptide backbone group of proteins, CHCONH, Amend and Helgeson combined group additivity methods with regression analyses using thermodynamic data from the literature [19,58] for four denatured proteins, along with their own computed group coefficients for the amino-acid side chains and the glycyl group. The parameters for the side chain of glycine were then derived from the difference between those for the glycyl group and the peptide backbone. The ionic end-groups of a protein were modelled using a moiety referred to as the amino acid backbone, which has the structure $CHNH_2COOH$. The parameters for this unit were derived from those for the amino acid serine by subtraction of the CH_2OH group contribution [56].

The experimental partial molar heat capacities of the peptides GlySerThrGly and GlyGlySerAlaGly are compared in Fig. 7 with those calculated by group additivity methods using the constituent group contributions given in Table 1, those reported by Makhatadze and Privalov [22], and those given by Amend and Helgeson [21]. The calculated heat capacities were obtained using

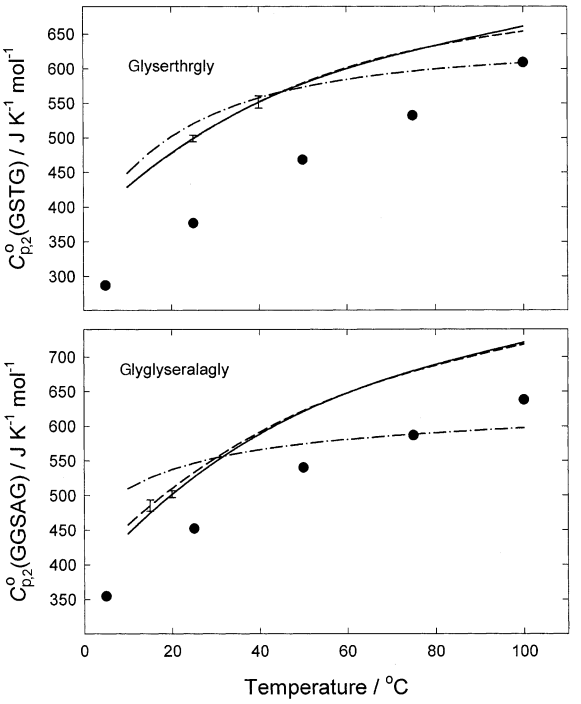

Fig. 7. Partial molar heat capacity of GlySerThrGly and GlyGlySerAlaGly as a function of temperature. — experimental DSC data from [32]; – – – calculated using the constituent group heat capacities in Table 1; ● calculated using the group heat capacities from [22]; – · – · – calculated using the group coefficients from [21].

Eq. (1), which for the peptide GlySerThrGly, for example, takes the form:

$$C^o_{p,2}(\text{calc}) = C^o_p(\text{end-groups}) + 3C^o_p(\text{CHCONH}) + C^o_p(\text{Ser}) + C^o_p(\text{Thr}) + 2C^o_p(\text{H}) \quad (10)$$

For each peptide, the agreement between the experimental heat capacity–temperature curve and that calculated using our peptide-based group additivity model is excellent over the complete temperature range. The Amend and Helgeson [21] additivity scheme gives a poor representation of the experimental heat capacity data for GlyGlySerAlaGly. For GlySerThrGly the quantitative agreement with the experimental results is somewhat better, at least in the temperature range from 10 to 60 °C; however, their model has not pre-

dicted the correct shape of the heat capacity–temperature profile.

The Makhatadze and Privalov [22] model predicts, to a reasonable approximation, the correct temperature profiles of the heat capacities of the two peptides GlySerThrGly and GlyGlySerAlaGly. This result is rather surprising, as for most side chains the heat capacity–temperature curves obtained when using peptides as model compounds are very different from those derived using small organic solutes to model the side chains [23]. For example, C_p^o(Ser) decreases with an increase in temperature when the side chain is modelled using tripeptides, whereas when using methanol as a model compound the side-chain heat capacity increases with an increase in temperature [22]. Moreover, the temperature dependence of C_p^o(CHCONH) calculated by Makhatadze and Privalov differs from that we obtained [23], as is evident from the heat capacity data for the glycyl group given in Fig. 2. It would appear, therefore, that there are some fortuitous compensatory effects in the Makhatadze and Privalov scheme. When group contributions having very different temperature profiles from those in our peptide-based model are combined, a heat capacity–temperature curve that qualitatively matches that obtained from the peptide-based model would not be the expected outcome.

Heat capacity data for the pentapeptide GlyGlyLeuGlyGly are given in Fig. 8. The partial molar heat capacity calculated using the peptide-based additivity model is in excellent agreement with that obtained experimentally over the temperature range 10–100 °C. The Makhatadze and Privalov scheme happens to give qualitatively the correct temperature profile, but again, in view of the comparison of heat capacity data for the leucyl side chain given in Fig. 1, this maybe somewhat fortuitous. In contrast, $C_{p,2}^o$ calculated using the Amend and Helgeson additivity scheme gives a temperature dependence that is the inverse of that found experimentally.

From the results presented in Figs. 7 and 8 and in other work [32], it is clear that the peptide-based additivity model is able to predict quantitatively the heat capacities of peptides over a wide temperature range. By inference, it is expected that the model will also be able to predict the partial molar heat capacities of longer polypeptides and unfolded proteins.

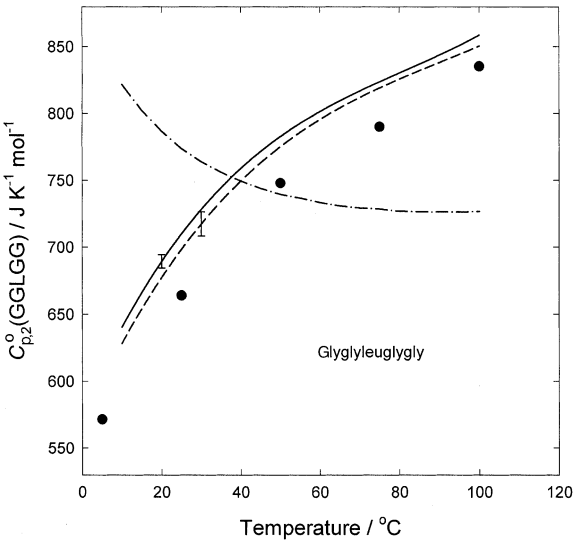

Fig. 8. Partial molar heat capacity of GlyGlyLeuGlyGly as a function of temperature. — experimental DSC data from [32]; – – – calculated using the constituent group heat capacities in Table 1; ● calculated using the group heat capacities from [22]; – · – · – calculated using the group coefficients from [21].

5.2. Volumes

Many of the additivity methods that have been proposed [11,15–18] to calculate the partial molar or specific volumes of polypeptides and proteins are based on volumetric data for the zwitterionic amino acids. As mentioned in Section 1, there are limitations in using the amino acids as model compounds because of the influence of the charged amino and carboxyl groups. However, in the most recent of these schemes, Kharakoz [11] has attempted to take better account of the contribution from ionised functional groups in evaluating the group contributions of the various amino acid residues of a polypeptide or protein. Although the method proposed by Kharakoz is limited to a temperature of 25 °C, it is, nevertheless, useful to compare the results obtained with those of our peptide-based model.

Two other schemes have been reported [19,21] that can predict the partial molar volumes of polypeptides over a wide temperature range. The approach used by Amend and Helgeson [21] to evaluate partial molar volumes is the same as outlined above for heat capacity. Makhatadze et al. [19] reported a method that uses a mixture of compounds to derive the volumetric contributions of the amino-acid side chains. For nine amino acids, the side-chain contributions were obtained from the partial molar volumes of small organic solutes in water by subtraction of an estimated value of the partial molar volume of a hydrogen atom, $V^{\circ}(H)$. Tripeptides of sequence Gly–X–Gly were used to model the side-chains of eight amino acids, but the partial molar volume data were determined using 0.5 M acetate buffer at pH 4.0 as the solvent rather than pure water. The side-chain contributions of the amino acids cysteine and tryptophan were based on literature data at 25 °C [4] for the zwitterionic amino acids, with the assumption that the partial molar volumes for these side chains are independent of temperature. The partial molar volume of the backbone peptide group was derived using experimental data for the oligoglycines, $(Gly)_n$, $n=3–5$. Makhatadze et al. [19] did not evaluate a group contribution for the ionic end-groups of a protein. In the comparison of additivity schemes that follows, we have used the end-group contributions derived from our peptide-based model along with the other contributions given by Makhatadze et al. [19].

The partial molar volumes obtained experimentally [40] over the temperature range 10–90 °C for the peptides GlyGlyLeuGlyGly, GlyPheAlaGly and GlyAsnAlaGly are given in Fig. 9, along with data calculated using four different group-additivity models. For the pentapeptide and for GlyAsnAlaGly, there is excellent agreement over the complete temperature range studied between the experimental results and those calculated using our peptide-based group additivity scheme [20]. Although for the peptide GlyPheAlaGly there is some deviation between the experimental and calculated curves at high temperature, there is good agreement within the combined estimated uncertainties over the range from 10 to approximately 80 °C.

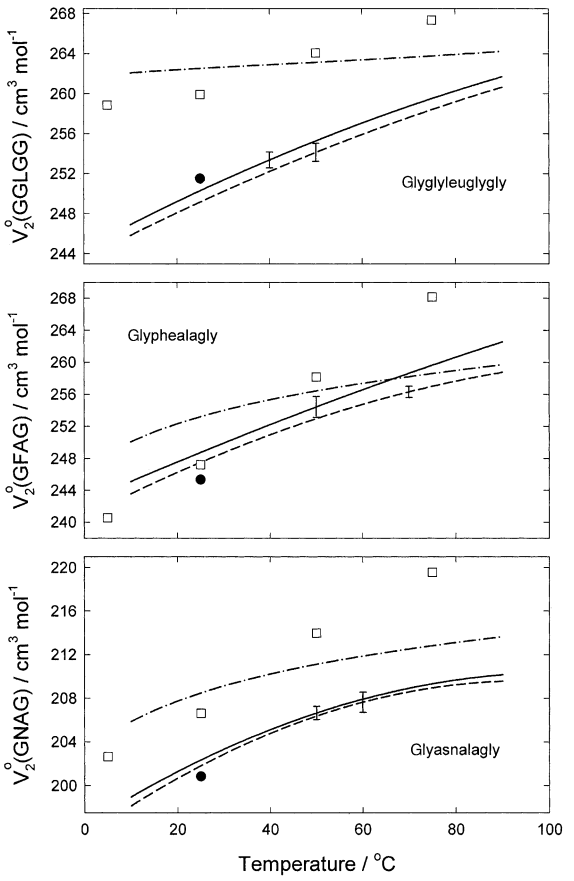

Fig. 9. Partial molar volume of the peptides GlyGlyLeuGlyGly, GlyPheAlaGly and GlyAsnAlaGly as a function of temperature. — experimental V_2° results from [40]; – – – calculated using the constituent group volumes in Fig. 3; ● calculated using the group partial molar volumes from [11]; – · – · – calculated using the group coefficients from [21]; □ calculated using the group partial molar volumes from [19].

The partial molar volumes calculated using the additivity scheme reported by Kharakoz [11] are in fair agreement with the experimental values, considering the general limitations of amino acids as side-chain model compounds. The differences between the calculated and experimental partial molar volumes are 0.4, 1.2 and 2.4 cm^3 mol^{-1} for the peptides GlyGlyLeuGlyGly, GlyAsnAlaGly and GlyPheAlaGly, respectively.

It is clear from Fig. 9 that both the Amend and Helgeson method [21] and the additivity scheme proposed by Makhatadze et al. [19] give poor

representations of the partial molar volumes of these three peptides. Moreover, in previous work [39] we used the equation-of-state parameters given by Amend and Helgeson to derive the partial molar volumes at 25 °C of nine different tetra- and pentapeptides. The differences between these calculated values and those observed experimentally were in the range 5–14 $cm^3\ mol^{-1}$, i.e. approximately 10-fold greater than those obtained using the peptide-based group additivity model. As previously suggested [39], one factor that could be responsible for the poor predictive ability of the Amend and Helgeson scheme is the reliability of the thermodynamic data used in the generation of the various equation-of-state parameters. A model of this type that uses data from the literature for the generation of its parameters can only ever be as reliable as the input data sets used in its construction.

There may be several reasons why the mixed side-chain model of Makhatadze et al. [19] gives a poor representation of the partial molar volume for these peptides. Since the side-chain volumes for alanine and leucine were derived using volumetric data for the tripeptides in acetate buffer, some systematic differences between experimental heat capacities for the peptides in water and those calculated using their group additivity scheme would not be unexpected.

The method used by Makhatadze et al. to derive values for V^o(H) assumes negligible differences between acetate buffer and pure water as solvent systems. From a consideration of volumes of transfer data of solutes from water to aqueous salt solutions [59], this seems unlikely. Furthermore, the V^o(H) results obtained by Makhatadze et al. [19] are inconsistent with those derived using reliable literature data for some alcohols and polyols [20]. As accurate values of V^o(H) are crucial when using organic solutes as models to estimate side-chain volumes and their temperature dependence, this may be one factor contributing to the poor group additivity shown in Fig. 9. Certainly, the side-chain volumes derived using these organic analogues in water are very different from those derived using tripeptides in water [20], as is illustrated for the glutamyl side chain in Fig. 4.

From a historical viewpoint, it is of interest to see how well the original additivity scheme of Cohn and Edsall [15] can predict the partial molar volumes of the three peptides given in Fig. 9. Using the terminal ionisation corrections given by Kharakoz [11], along with the amino acid residue contributions given by Cohn and Edsall [15], the partial molar volumes of the peptides GlyGlyLeuGlyGly, GlyAsnAlaGly and GlyPheAlaGly at 25 °C are smaller than the experimental values by 4.8, 6.7 and 9.1 $cm^3\ mol^{-1}$, respectively. It is worth noting that these differences arise not from incorrect experimental values of the apparent molar volumes of the amino acids, but rather from the estimations of the various group contributions that were, of course, based on information available at that time.

6. Application of group additivity to proteins

It is clear from the results presented in Sections 4 and 5 that our peptide-based group additivity model successfully predicts the partial molar volumes and heat capacities of small peptides over a wide temperature range. These small peptides have the same structural characteristics as those found in fully hydrated and completely unfolded polypeptides and proteins, so the peptide-based additivity model should, in principle, be able to predict the partial molar volumes and heat capacities for these larger molecules in aqueous solution. Since the group contributions derived for the protonated side chains of the amino acids lysine and arginine include contributions from the free proton (see Tables 1 and 3), a rigorous test of how well the model predicts the partial molar heat capacity and volume of unfolded proteins must await new data on the partial molar properties of the proton over a wide temperature range. Nevertheless, it is instructive to apply our model in its current form, and to compare the results obtained with those using alternative additivity schemes, in particular those reported by Amend and Helgeson [21] and by Makhatadze and co-workers [19,22].

An ideal polypeptide or protein to use in any test of group additivity would be one that exists in the fully unfolded state over a wide temperature range, i.e. it is devoid of the secondary and tertiary

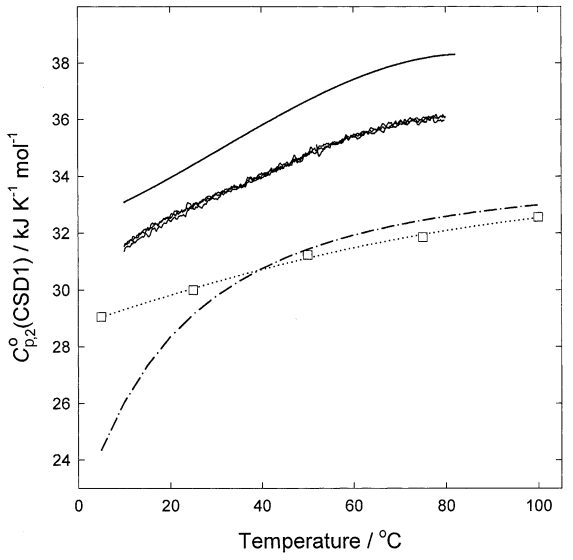

Fig. 10. Temperature dependence of the partial molar heat capacity of human calpastatin domain I. ∼ three experimental DSC scans, 5.47 mg ml^{-1}, in water, pH 6.2, from [61]; — calculated using the group heat capacities in Table 1; □ calculated using the group heat capacities from [22]; – · – · – calculated using the group coefficients from [21].

structural elements normally associated with the native states of proteins. One protein that has these requirements is calpastatin domain 1 (CSD1). This small protein has been characterised to be unfolded, even at 20 °C, based on experimental evidence from techniques such as NMR, CD and small-angle X-ray scattering [60]. In recent work [61], heat capacity measurements have been made on aqueous solutions of both the porcine (148 amino acids) and human (142 amino acids) variants of CSD1. The experimental results obtained for the human protein, and the calculated heat capacities obtained using three different group-additivity models, are shown in Fig. 10. Since there is no unfolding transition for this protein, the experimental partial molar heat capacity is simply a smooth function of temperature over the range studied. The heat capacity–temperature curve calculated using the group contributions given in Table 1 lies above that of the experimental curve. In contrast, the heat capacity–temperature curves calculated using the group additivity models of Makhatadze and Privalov [22] and Amend and Helgeson [21] lie below the experimental curve. The Amend and Helgeson scheme gives a particularly poor representation of the shape of the heat capacity–temperature profile at low temperatures.

If the various constituent groups are similarly hydrated in both the unfolded protein and the compounds chosen to model these groups, then on the basis of group additivity the calculated and experimental heat capacity–temperature curves should be coincident. The fact that the heat capacity–temperature curve calculated using our peptide-based model, in its present form, lies above the experimental curve is eminently reasonable. Firstly, as mentioned already, the group heat capacities for the side chains of arginine and lysine include contributions from the free proton. Corrections for this effect, and also for any contributions from side-chain ionisation of glutamic and aspartic acids, are expected to lead to a decrease in the heat capacity calculated [23]. Secondly, if there is indeed any small degree of residual 'structure' remaining in the unfolded protein, then the partial molar heat capacity of the protein should be smaller than that based on group additivity.

On the other hand, it is difficult to find a rational explanation for why a protein heat capacity, calculated on the basis of contributions from model compounds in which the appropriate functional groups are all well hydrated, should be lower than that observed experimentally. It is worth noting that in using the Makhatadze and Privalov [22] group additivity scheme we chose, for the purposes of comparison with the peptide-based model, to adopt their side-chain contributions for arginine and lysine in the form $\{C^{o}_{p}(R^{+}) - C^{o}_{p,2}(H^{+})\}$. This means that the same corrections mentioned above also apply to the heat capacity–temperature curve calculated using their model. When these corrections are applied, the heat capacity–temperature curve calculated will shift even lower.

The general conclusion that can be drawn from the results presented in Fig. 10 is that the group additivity scheme that best represents the heat capacity of an unfolded protein is our scheme that uses small peptides as compounds to model the various constituent groups of unfolded proteins.

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

Protein	Solvent and conditions	v^o ($cm^3\ g^{-1}$)		
		Experimental[a]	Calculated[b]	Calculated[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]		
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]		
Cytochrome *c* (horse heart)	H_2O, dialysed protein	0.725 (0.002)[f]	0.724[g]	0.726[g]
	H_2O, dialysed protein	0.738 (0.003)[e]		

[a] Experimental values for proteins in their native states.
[b] Calculated for the unfolded form of the protein under isoionic conditions.
[c] From Kharakoz [11].
[d] From Gekko et al. [62].
[e] From Chalikian et al. [63].
[f] From Gekko et al. [64].
[g] For the protein chain only.

In a previous paper [20], we applied the peptide-based group additivity model to calculate the partial specific volume, v^o, of several proteins in aqueous solution at 25 °C. The contribution of the proton to the side-chain volumes for lysine and arginine was eliminated by using the recommended value of $V_2^o(H^+_{(aq)}) = -5.5\ cm^3\ mol^{-1}$ [44,45]. Corrections were also made for the effect of ionisation of the side chains of aspartic and glutamic acids [20]. Some of the results obtained in these group additivity calculations are shown in Table 5. Included in Table 5 are the partial specific volumes determined experimentally under conditions in which the proteins are in their native states. The agreement between the experimental and calculated values is surprisingly good, considering that the additivity model is applicable to only the completely unfolded, random-coil state of a protein. This agreement is, in fact, consistent with the experimental observation that the volume changes that occur on the complete unfolding of many small globular proteins are close to zero [41,65]. For comparison, the partial specific volumes calculated by Kharakoz [11] using the additivity scheme based on amino acids as model compounds are also given in Table 5. With the exception of ribonuclease A, the v^o values derived using the peptide-based additivity model are slightly smaller than those calculated by Kharakoz. Perhaps larger differences might have been expected, given the limitations of amino acids as models, but, as mentioned previously [20], there may be some compensating effects occurring in the Kharakoz model.

In principle, the group parameters given in Table 3 can be used to calculate the partial specific volume of an unfolded protein over a wide temperature range. Use of the peptide-based model in its current form requires some assumptions to be made about the proton contributions, as outlined above for heat capacity calculations. On the basis of some observations about the partial molar expansibility of the proton made several years ago by Millero [46], we were able to estimate the partial molar volume of the proton over a wide temperature range [20]. This approximation, along with the assumption that the volume changes on ionisation of aspartic and glutamic acids are, to a first approximation, independent of temperature, enables the calculation of v^o as a function of temperature for unfolded proteins. Another problem in our attempt to test the peptide-based additivity model is that there is a paucity of experimental v^o data over a wide temperature

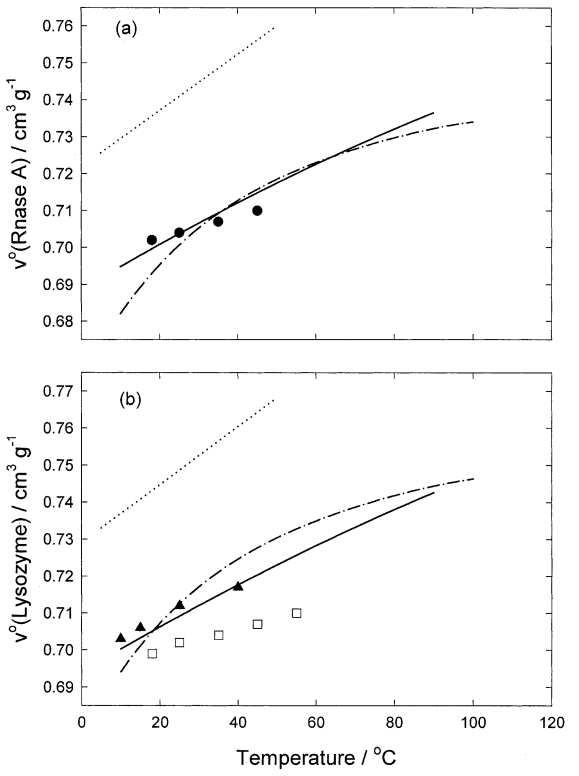

Fig. 11. Partial specific volume of ribonuclease A and lysozyme as a function of temperature. ● ribonuclease A, native state, 10 mM cacodylic acid buffer/10 mM NaCl, pH 6, from [63]; □ lysozyme, native state, H_2O, from [63]; ▲ lysozyme, native state, H_2O, from [66]; – · – · – calculated using the group coefficients from [21]; · · · · · calculated using the group volumes from [19].

range for unfolded proteins in pure water as a solvent. The only partial specific volume data for proteins in water with which comparisons can be made are for the native states. Nevertheless, such comparisons are useful, if only to enable various group-additivity models to be critically assessed.

The partial specific volumes determined at several temperatures for ribonuclease A and lysozyme are shown in Fig. 11. Also included in Fig. 11 are the partial specific volumes calculated using the peptide-based model [20], the group additivity scheme of Makhatadze et al. [19], and the equation-of-state parameters given by Amend and Helgeson [21]. For both proteins, the peptide-based model gives results that seem reasonable, given that the volume changes accompanying the unfolding of these proteins are small. It is clear that the v^{o} data obtained using the additivity scheme proposed by Makhatadze et al. [19] are in particularly poor agreement with the experimental values. For ribonuclease A, the results obtained using the Amend–Helgeson scheme are in good agreement with those derived using the peptide-based model over the temperature range 30–70 °C. However, as a consequence of the curvature of the v^{o}–temperature plot, the Amend–Helgeson additivity method gives a poor representation of the temperature dependence of v^{o} at low temperatures. The same effect is also apparent for lysozyme, as shown in Fig. 11b. The temperature dependence of v^{o} is poorly represented by the equation-of-state parameters given by Amend and Helgeson [21]. In summary, the results presented in Fig. 11 suggest that the group additivity scheme that best represents the partial molar, or specific, volumes of unfolded proteins in water is that based on small peptides as model compounds.

In protein chemistry, it is common practice to study proteins in aqueous buffer systems as the solvent rather than pure water. A valid question to ask is whether a peptide-based additivity scheme developed using pure water as a solvent is applicable to unfolded proteins in buffer solutions. If the buffer ionic strength is not too high, it would be expected, based on information from studies of the transfer thermodynamics of model compounds, that the additivity scheme could also be used in buffer solutions. Experimental work is in progress [67] to establish whether this is indeed the case. It is, however, worth pointing out that all but one of the group additivity schemes that have been developed are, in fact, based on thermodynamic data for model compounds in water. The one exception is the volume additivity model of Makhatadze et al. [19], which uses a mixture of model compounds in water and in acetate buffer solution at high ionic strength.

7. Concluding remarks

The success of any group additivity scheme to calculate the solution thermodynamic properties of an unfolded protein depends, ultimately, on how

well the chosen model compounds represent the various constituent groups of the protein. The distinct advantage of our peptide-based additivity scheme is that the model compounds are themselves fragments of protein molecules. Our studies on small peptides with adjacent side chains have confirmed that this group additivity method has excellent predictive utility. Since polypeptides and proteins are essentially just longer versions of such peptides, the peptide-based model should apply equally well to the larger molecules. Certainly, at this stage it shows considerable promise as a reliable method for calculations on unfolded proteins. The comparisons made herein amongst various group-additivity models that have been reported in the literature suggest that our peptide-based scheme is the most reliable method developed hitherto for the estimation of the partial molar heat capacity and volume of unfolded proteins in aqueous solution.

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