Thermodynamic Properties of Peptide Solutions. 16. Partial Molar Heat Capacities and Volumes of Some Tripeptides of Sequence Gly-X-Gly in Aqueous Solution at 25 °C

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The partial molar volumes, V_2° and partial molar heat capacities, $C_{p,2}^\circ$, at infinite dilution have been determined for the tripeptides of sequence glycyl-X-glycine, where X is one of the amino acids glycine, tyrosine, glutamine, proline, and cysteine, in aqueous solution at 25 °C. Using these results, the partial molar heat capacities and volumes of the amino acid side chains were derived. The side chain heat capacities and volumes were compared with those obtained using other model compounds.

Introduction

There is currently considerable interest in the determination of various thermodynamic properties for amino acids, small peptides, and their derivatives in aqueous solution (Chalikian et al., 1994b; Hakin et al., 1995, 1997; Vogl et al., 1995; Kikuchi et al., 1995, 1996; Hedwig et al., 1996). As these small solutes incorporate some of the structural features found in proteins, they can be used to model specific features such as the amino acid side chains and the backbone glycyl unit, $-CH_2CONH-$, of proteins. The hydration of the various functional groups in proteins plays an important role in the conformational stability of proteins in aqueous solution (Makhatadze and Privalov, 1994; Chalikian et al., 1994a; Rupley et al., 1991; Némethy et al., 1981). The study of solute−solvent interactions in aqueous solutions of these model compounds can assist in the understanding of the conformational stability and unfolding behavior of proteins.

In previous work (Hedwig, 1993; Hedwig and Høiland, 1994; Vogl et al., 1995; Häckel et al., 1998), we used tripeptides of sequence glycyl-X-glycine (gly-X-gly), where X is one of the naturally occurring amino acids, as compounds to model the amino acid side chains of proteins. The side chain on the central amino acid in these tripeptides lies between two peptide groups, which is structurally analogous to that found in proteins. Consequently, these peptides should be reasonable models for investigating side chain effects in proteins.

In earlier papers of this series (Reading and Hedwig, 1990; Hedwig, 1993), we reported the partial molar volumes, V_2°, and the partial molar heat capacities, $C_{p,2}^\circ$, at infinite dilution in aqueous solution at 25 °C for 11 of the possible 16 gly-X-gly peptides with the neutral side chains that are found in proteins. This paper reports V_2° and $C_{p,2}^\circ$, results at 25 °C for aqueous solutions of triglycine and the four tripeptides glycyltyrosylglycine (glytyrgly), glycylglutaminylglycine (glyglngly), glycylprolylglycine (glyprogly), and glycylcysteinylglycine (glycysgly). The remaining tripeptide with a neutral side chain, glycyltryptophanylglycine, could not be studied as it is insoluble in pure water. The V_2° and $C_{p,2}^\circ$, results for the peptides have been used to estimate group contributions for the four amino acid side-chains.

Experimental Section

The sample of glygylgly used was that prepared for previous studies. The purification and analyses of glyglygly have been reported elsewhere (Downes and Hedwig, 1995). The peptide glytyrgly, which was obtained as a customer accommodation through Sigma, was recrystallized from water + methanol. The product was chromatographically pure as determined by TLC and HPCE. Analysis by alkalimetric titration (Kumaran et al., 1983; Kolthoff and Stenger, 1947) gave a relative molar mass of (298.5 ± 2.1), which is slightly higher than that for the anhydrous compound (M_r = 295.30). Elemental analyses gave C, 52.7%; H, 5.7%; N, 14.1%; cf. calculated composition for $C_{13}H_{17}O_5N_3$: C, 52.9%; H, 5.8%; N, 14.2%. Glycysgly (Bachem Feinchemikalien) was used as supplied in order to avoid possible oxidation of the side chain −SH group during a recrystallization. The sample was shown to be chromatographically pure by TLC. The relative molar mass determined by titration was (237.9 ± 1.9), which differs from that expected (235.26) by 1.1%. Elemental analyses gave C, 36.0%; H, 5.8%; N, 17.9%; cf. calculated composition for $C_7H_{13}O_4N_3S$: C, 35.7%; H, 5.6%; N, 17.9%. The peptides glyprogly and glyglngly (Bachem Feinchemikalien) were recrystallized from water + ethanol. Analyses by TLC and HPCE confirmed that each sample was chromatographically pure. Infrared spectrophotometry indicated that both products were crystalline hydrates. For glyprogly the relative molar mass determined by alkalimetric titration was (247.1 ± 1.6), which is in excellent agreement with that for a monohydrate (M_r = 247.25). Elemental analyses gave C, 43.4%; H, 7.1%; N, 17.1%; cf. calculated composition for $C_9H_{17}O_5N_3$: C, 43.7%; H, 6.9%; N, 17.0%. The hydrated peptide glyglngly was found to be hygroscopic. Regular vacuum-drying was carried out when working with this compound. Alkalimetric titration gave a relative molar mass of (277.0 ± 2.2), which is in agreement with that calculated for glyglngly monohydrate (M_r = 278.27). Elemental analyses gave C, 38.8%; H, 6.4%; N, 20.2%; cf. calculated composition for $C_9H_{18}O_6N_4$: C, 38.9%; H, 6.5%; N, 20.1%. The anhydrous tripeptides were dried under vacuum at room temperature prior to being used for preparing solutions. All solutions were prepared by mass using deionized glass-distilled water that had been freshly degassed. The uncertainties in the solution mola-

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lities were in the range 0.02−0.06%. For the peptide glycysgly, solution density measurements and thin layer chromatograms as a function of time were carried out to check for possible decomposition of the peptide in solution. The results showed that over a period of about 1 h, which was the maximum time needed to prepare a solution and carry out the thermodynamic measurements, there was no detectable decomposition.

Densities of solutions were measured using an Anton Paar digital density meter (model DMA 60/602) as outlined previously (Reading and Hedwig, 1990; Hedwig, 1988). The reproducibility of an individual density measurement was to better than (3×10^{-6}) g·cm^{-3}. Heat capacity measurements were carried out using a Picker flow microcalorimeter. Details of the instrument and procedures of operation have been described in previous work (Reading and Hedwig, 1990; Hedwig, 1988).

Results and Discussion

Densities of aqueous solutions of the various tripeptides at 25 °C are given in Table 1. These data were used to calculate the apparent molar volumes of the solutes, $V_ø$, using the equation

$$V_ø = M_2/\rho - (\rho - \rho_1)/(m\rho\rho_1) \quad (1)$$

where M_2 is the solute molar mass, ρ and ρ_1 are, respectively, the densities of the solution and solvent, and m is the solution molality. The ρ_1 value used was that reported by Kell (1967). For the dilute solutions used in this study, the molality dependence of $V_ø$ can be represented by the linear equation

$$V_ø = V_2^\circ + S_v m \quad (2)$$

where V_2° is the partial molar volume of the solute at infinite dilution and S_v is the experimental slope. Values of V_2° and S_v were obtained from a weighted least-squares analysis of the apparent molar volume data using eq 2. The weighting factors for the $V_ø$ results were calculated using the procedures described previously (Hedwig, 1988). The V_2° and S_v results together with their standard deviations are given in Table 2. The value of S_v for the peptide glytyrgly is significantly larger than those for the other peptides. The low solubility of glytyrgly in water restricts the molality range available for study, resulting in a value for S_v that is less reliable than one determined using $V_ø$ data over a wider molality range. A similar effect was noted previously for the sparingly soluble peptide glyhisgly (Hedwig, 1993). A least-squares analysis of the $V_ø$ data for the peptide glyglngly gave a value for S_v that was not statistically significant, despite the wide molality range used. This is a manifestation of the hygroscopic nature of this compound. The value of V_2° given in Table 2 is actually the mean value of all the $V_ø$ data.

The V_2° value for triglycine at 25 °C obtained in this study is in good agreement with an earlier result from this laboratory (111.92 ± 0.03) cm^3·mol^{-1} (Hedwig, 1998), and also with the value of (112.06 ± 0.01) cm^3·mol^{-1} reported recently by Hakin et al. (1997). Volumetric data for aqueous solutions of the peptide glytyrgly over the temperature range (10 to 90) °C were obtained recently using a differential scanning densimetric method (Häckel et al., 1998). The value of V_2° derived at 25 °C, (195.0 ± 0.5) cm^3·mol^{-1}, is in good agreement with the result obtained in this work.

The apparent molar heat capacities, $C_{p,ø}$, of the peptides in aqueous solution were calculated from the experimental specific heat capacities, c_p, using the equation

$$C_{p,ø} = M_2 c_p + (c_p - c_{p,1})/m \quad (3)$$

where $c_{p,1}$ is the specific heat capacity of water (4.1793 J·K^{-1}·g^{-1} at 25 °C (Stimson, 1955)) and the remaining symbols are as defined for eq 1. The $C_{p,ø}$ results along with their estimated uncertainties, determined as described in previous work (Hedwig, 1988), are given in Table 1. For

Table 1. Densities and Apparent Molar Heat Capacities of Aqueous Solutions of Tripeptides at (25.00 ± 0.01) °C

m/(mol·kg^{-1})	ρ/(g·cm^{-3})	$C_{p,\phi}^a$/(J·K^{-1}·mol^{-1})	m/(mol·kg^{-1})	ρ/(g·cm^{-3})	$C_{p,\phi}$/(J·K^{-1}·mol^{-1})
Glycylglycylglycine					
0.042 02		191.6 (4.1)	0.114 33		191.4 (1.9)
0.044 86	1.000 494		0.124 26	1.006 465	
0.048 43		188.0 (4.0)	0.128 12		190.8 (1.9)
0.054 57	1.001 230		0.136 73		192.2 (1.7)
0.073 84	1.002 697		0.139 73	1.007 614	
0.074 26		190.9 (2.3)	0.146 96		192.0 (1.7)
0.074 68		190.8 (3.0)	0.149 89	1.008 364	
0.078 69	1.003 060		0.155 30		194.1 (1.4)
0.089 58	1.003 872		0.159 99		192.1 (1.6)
0.091 65		190.3 (2.8)	0.170 09	1.009 868	
0.098 67		191.0 (2.2)	0.173 89		196.1 (1.4)
0.100 14	1.004 676		0.190 83		196.6 (1.3)
0.110 69	1.005 457		0.191 83	1.011 442	
0.111 10		192.6 (1.7)			
Glycylcysteinylglycine					
0.021 98	0.999 066	334.8 (8.2)	0.044 11		332.0 (4.1)
0.023 70	0.999 226	329.8 (7.6)	0.045 20	1.001 177	335.7 (5.1)
0.026 46	0.999 477	335.2 (6.8)	0.046 84		332.0 (3.9)
0.028 88	0.999 695	333.4 (6.6)	0.049 01	1.001 524	332.3 (3.9)
0.031 82	0.999 964	335.7 (5.7)	0.050 02	1.001 614	332.7 (4.0)
0.034 01	1.000 166	330.9 (5.6)	0.051 96	1.001 784	335.7 (3.7)
0.036 05	1.000 346		0.053 83	1.001 958	334.0 (3.8)
0.037 70	1.000 500	335.9 (5.1)	0.055 19	1.002 079	334.6 (3.5)
0.040 07	1.000 713	336.6 (4.8)	0.058 14	1.002 345	336.6 (3.3)
0.042 06	1.000 892	335.2 (4.6)			
Glycyltyrosylglycine					
0.005 99	0.997 651		0.017 93	0.998 844	448.6 (10.7)
0.007 50	0.997 805		0.019 12	0.998 965	447.8 (9.5)
0.008 98	0.997 952		0.020 97	0.999 147	448.2 (9.1)
0.010 02	0.998 059		0.022 01	0.999 249	448.1 (8.7)
0.012 04	0.998 258		0.024 08	0.999 455	450.0 (7.9)
0.013 03	0.998 353		0.025 15		448.2 (7.6)
0.014 01	0.998 451	449.4 (13.6)	0.025 79	0.999 625	447.3 (7.0)
0.014 99	0.998 554	453.6 (12.1)	0.026 21	0.999 668	452.5 (7.7)
0.016 00	0.998 656	448.6 (11.9)	0.027 98	0.999 837	452.6 (7.6)
0.016 47	0.998 697	448.3 (11.6)	0.028 86	0.999 931	444.7 (7.0)
0.016 99		446.8 (10.6)	0.030 79	1.000 119	448.9 (6.6)
Glycylprolylglycine					
0.016 55	0.998 448	290.3 (11.5)	0.035 06	1.000 007	287.0 (5.8)
0.017 61	0.998 534	291.0 (10.8)	0.037 55	1.000 204	291.7 (5.4)
0.020 87	0.998 810		0.040 14	1.004 260	289.0 (5.0)
0.023 43	0.999 028	287.8 (8.2)	0.042 04	1.000 588	
0.025 91	0.999 233	291.1 (7.4)	0.044 89	1.000 830	289.4 (4.7)
0.029 88		287.5 (6.7)	0.046 27	1.000 943	291.0 (4.6)
0.030 98	0.999 665	285.8 (6.5)	0.050 41	1.001 284	288.2 (4.2)
0.031 96	0.999 745	289.9 (6.3)	0.052 65	1.001 470	288.7 (4.0)
0.032 96	0.999 830	287.4 (6.1)	0.055 60	1.001 718	290.0 (3.8)
0.034 08	0.999 921	290.0 (5.9)	0.057 67	1.001 896	291.0 (3.7)
Glycylglutaminylglycine					
0.023 85	0.999408		0.070 00	1.003 948	311.5 (3.9)
0.028 02		315.5 (6.5)	0.074 86	1.004 387	312.8 (3.9)
0.030 11	1.000 027		0.080 17	1.004 892	315.4 (3.4)
0.039 44	1.000 943		0.086 20	1.005 514	311.2 (4.0)
0.044 76	1.001 454	316.7 (3.6)	0.092 65	1.006 127	313.8 (3.2)
0.049 93	1.001 977	311.2 (3.9)	0.100 05	1.006 825	313.9 (2.9)
0.055 81	1.002 529	313.8 (3.8)	0.129 32	1.009 635	316.7 (2.7)
0.065 83	1.003 488	318.7 (3.9)			

[a] Estimated uncertainties are in parentheses.

Table 2. Partial Molar Volumes and Heat Capacities of Tripeptides in Aqueous Solution at (25.00 ± 0.01) °C[a]

peptide	V_2°/ ($cm^3 \cdot mol^{-1}$)	S_v/($cm^3 \cdot kg \cdot mol^{-2}$)	$C_{p,2}^\circ$/($J \cdot K^{-1} \cdot mol^{-1}$)	S_c/$J \cdot kg \cdot K^{-1} \cdot mol^{-2}$)
glyglygly	111.96 (0.06)	3.4 (0.4)	186.1 (1.3)	50 (9)
glycysgly	143.04 (0.05)	8.7 (1.1)	334.2 (1.9)	
glytyrgly	194.5 (0.1)	23 (5)	449.0 (2.3)	
glyprogly	144.58 (0.09)	2.3 (1.9)	287.7 (1.5)	39 (33)
glyglngly	161.1 (0.2)		314.3 (2.4)	

[a] Standard deviations are in parentheses.

Table 3. Coefficients of Eq 4

peptide	$10^{-3}p_1$/($kg^2 \cdot m^{-3} \cdot mol^{-1}$)	$10^{-3}p_2$/($kg^3 \cdot m^{-3} \cdot mol^{-2}$)
glyglygly	0.07730 (0.00006)[a]	−0.0117 (0.0004)
	0.07733 (0.00003)[b]	−0.0120 (0.0002)[b]
glycysgly	0.09237 (0.00005)	−0.0216 (0.0011)
glytyrgly	0.1011 (0.0001)	−0.043 (0.005)
glyprogly	0.08483 (0.00009)	−0.014 (0.002)
glyglngly	0.0993 (0.0002)	−0.015 (0.002)

[a] Standard deviations are in parentheses. [b] Hedwig (1988).

the peptide glyglygly and in a few cases for the other peptides, specific heat capacity measurements were made on solutions for which the density was not measured. For these solutions the densities, which are needed to convert the heat capacities per unit volume into specific heat capacities, were calculated using a power series in solution molality of the form

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

where p_1 and p_2 are parameters determined by least-squares fitting to the density data given in Table 1. The parameters p_1 and p_2 along with their standard deviations are given in Table 3. For the peptide glyglygly, the p_1 and p_2 values obtained in this work are in excellent agreement with those reported previously (Hedwig, 1988).

For dilute solutions of tripeptides, $C_{p,\phi}$ varies linearly with solution molality. The $C_{p,\phi}$ results were analyzed by weighted least-squares using the equation

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

where $C_{p,2}^\circ$ is the partial molar heat capacity of the solute at infinite dilution and S_c is the experimental slope. Values of $C_{p,2}^\circ$ and S_c, together with their standard deviations, are given in Table 2. For the peptides glycysgly, glytyrgly, and glyglngly, the values of S_c obtained from the least-squares analyses were (45 ± 47, −22 ± 123, and 15 ± 26) $J \cdot kg \cdot K^{-1} \cdot mol^{-2}$, respectively. As these results are not statistically different from zero, the $C_{p,2}^\circ$ values shown in Table 2 are the means of the $C_{p,\phi}$ values given in Table 1.

The partial molar heat capacity of the peptide glytyrgly in aqueous solution was determined recently over the temperature range (10−100) °C using differential scanning calorimetry (Häckel et al., 1998). The result at 25 °C was (451 ± 4) $J \cdot K^{-1} \cdot mol^{-1}$, which is in good agreement with that determined in this study. For triglycine the $C_{p,2}^\circ$ value given in Table 2 is in good agreement with an earlier result determined in this laboratory, (188.3 ± 0.7) $J \cdot K^{-1} \cdot mol^{-1}$ (Hedwig, 1988), and with the value of (186.3 ± 0.9) $J \cdot K^{-1} \cdot mol^{-1}$ reported recently by Hakin et al. (1997).

The partial molar volume of an amino acid side chain, R, can be estimated from the difference between the partial molar volume for the tripeptide gly-X-gly and that for the tripeptide triglycine, which has no side chain

$$V°(R) = V_2^\circ(\text{gly-X-gly}) - V_2^\circ(\text{glyglygly}) \quad (6)$$

It should be stressed that the quantity $V°(R)$ is not the absolute partial molar volume of the side-chain residue, but it gives the contribution to V_2° of the peptide on replacing a C−H group by a C−R group. These $V°(R)$ values for the various amino acid side chains found in proteins, along with the estimates of the partial molar volumes of the glycyl unit, $-CH_2CONH-$, and the ionic end groups of a polypeptide, are the quantities needed to estimate the partial molar volume of an unfolded protein in aqueous solution. Values of $V°(R)$ calculated using the V_2° results given in Table 2 are shown in Table 4. For the purposes of comparison, $V°(R)$ values calculated by the method outlined for the tripeptides but using V_2° data for the corresponding amino acids (Jolicoeur et al., 1986; Hakin et al., 1995) are also given in Table 4.

For the side chains of cys and tyr, the $\Delta V°(R)$ values ($\Delta V°(R)$ is the difference between the $V°(R)$ values derived using tripeptide and amino acid V_2° data) are positive. This is consistent with the results observed earlier for a range of neutral side chains (Hedwig, 1993). The positive differences arise because of ionic end-group effects in the amino acids. In a zwitterionic amino acid, the charged $-NH_3^+$ and $-CO_2^-$ functional groups are adjacent to the side chain, which results in a significant mutual interaction between the hydrated side chain and the charged groups with their associated cospheres (Hedwig, 1993). Such interactions are not significant in a tripeptide of sequence gly-X-gly because the side chain is well separated from the ionic end-groups.

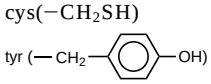
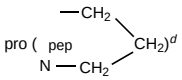
For the side chain of gln, the value of $\Delta V°(R)$ is negative, which is not what would be expected on the basis of the results for other neutral side chains. At present we are unable to provide a satisfactory explanation for this unexpected result. Although the $\Delta V°(R)$ value for the side-chain of proline is large and negative, the comparison of $V°(R)$ values given in Table 4 is not strictly valid for this side chain. The unique structural feature of the imino acid proline means that the quantity $V°(R)$ includes a contribution from the difference in hydration between the imino groups of proline and the amino group of glycine. Similarly, the $V°(R)$ value derived using V_2° data for the tripeptides glyprogly and triglycine will include a contribution from the difference in hydration of the peptide groups adjacent to the prolyl side chain of the tripeptide and that in triglycine. Given the structural similarities between the prolyl side chain in the tripeptide and in a protein, the $V°(R)$ value derived using V_2° data for the tripeptides ought to be a better estimation of the volumetric contribution of the side chain in a protein molecule than that based on V_2° data for the amino acids.

The contribution of a side chain to the heat capacity of a peptide can be estimated from the differences between $C_{p,2}^\circ$ for the peptide gly-X-gly and that for triglycine

$$C_p^\circ[(R) - (H)] = C_{p,2}^\circ(\text{gly-X-gly}) - C_{p,2}^\circ(\text{glyglygly}) \quad (7)$$

The quantity $C_p^\circ[(R) - (H)]$ gives the contribution to the heat capacity on replacing a C−H group by a C−R group, where R is the side chain of amino acid X. Values of $C_p^\circ[(R) - (H)]$ calculated using the heat capacity data determined in this work are given in Table 5, along with the values calculated using $C_{p,2}^\circ$ data for the corresponding amino acids taken from the literature (Jolicoeur et al., 1986; Hakin et al., 1994, 1995). For the side chains of the amino acids cys, pro, and gln, the $C_p^\circ[(R) - (H)]$ values derived using the tripeptides are less than those obtained

Table 4. Comparison of Side Chain Contributions to Partial Molar Volumes of Peptides in Aqueous Solution at 25 °C

side chain (R)	$V°$(R)/(cm^3·mol^{-1}) tripeptide	$V°$(R)/(cm^3·mol^{-1}) amino acid	$\Delta V°$(R)[a]/(cm^3·mol^{-1})
cys(−CH_2SH)	31.08 (0.08)	30.37 (0.06)[b]	0.7
tyr (— CH_2—  —OH)	82.5 (0.1)	80 (1)[b]	2.5
pro ()	32.6 (0.1)	39.40 (0.03)[b]	−6.8
gln(−$CH_2CH_2CONH_2$)	49.1 (0.2)	50.65 (0.05)[c]	−1.6

[a] $\Delta V°$(R) = $V°$(R)(tripeptide) − $V°$(R)(amino acid). [b] Based on V_2° data taken from Jolicoeur et al. (1986). [c] V_2° for glutamine from Hakin et al. (1995). [d] N^{pep} is the nitrogen atom of the peptide group adjacent to the prolyl side chain.

Table 5. Comparison of Side Chain Heat Capacities Derived Using $C_{p,2}^\circ$ Data for Various Model Compounds

side chain (R)	C_p°[(R) − (H)]/(J·K^{-1}·mol^{-1}) tripeptide[a]	C_p°[(R) − (H)]/(J·K^{-1}·mol^{-1}) amino acids[b]	$C_p°$(R)/(J·K^{-1}·mol^{-1}) tripeptide A[c]	tripeptide B[d]	analogue[e]
cys	148 (2)	150.4 (0.7)			
tyr	263 (3)	261 (20)	341	308	302
pro	102 (2)	135 (1)			
gln	128 (3)	137 (1)	206	173	180

[a] This work. [b] $C_{p,2}^\circ$ for the amino acids cys, tyr, and pro from Jolicoeur et al. (1986). $C_{p,2}^\circ$ for gln from Hakin et al. (1995). $C_{p,2}^\circ$ for glycine from Hakin et al. (1994). [c] Using $C_p°$(H) = 78 J·K^{-1}·mol^{-1}. [d] Using $C_p°$(H) = 45 J·K^{-1}·mol^{-1}. [e] From Makhatadze and Privalov (1990).

using heat capacity data for the amino acids. The differences are consistent with those reported previously for other neutral side chains (Hedwig, 1993). The high uncertainty in the $C_{p,2}^\circ$ value for the amino acid tyrosine masks any difference between the $C_{p,2}^\circ$[(R) − (H)] values for the tyrosyl side-chain.

The absolute value of the heat capacity contribution of a side chain, $C_p°$(R) can be obtained from the $C_p°$[(R) − (H)] values using the equation

$$C_p°(\mathrm{R}) = C_p°[(\mathrm{R}) - (\mathrm{H})] + C_p°(\mathrm{H}) \qquad (8)$$

where $C_p°$(H) is an estimate of the absolute value of the heat capacity of the hydrogen atom. One advantage of deriving these $C_p°$(R) values is that it enables comparisons to be made with side chain heat capacities estimated using $C_{p,2}^\circ$ data for other model compounds.

In a study by Makhatadze and Privalov (1990), various small organic solutes were chosen as compounds to model the side chains of many of the amino acids. For the side chains of the amino acids tyr and gln, which are part of this study, 4-methylphenol and propionamide, respectively, were used as model compounds. The values of $C_p°$(R) at 25 °C were estimated by subtracting from the $C_{p,2}^\circ$ value for the compound an estimate of the heat capacity of the hydrogen atom (78 J·K^{-1}·mol^{-1}). The results obtained are given in column 6 of Table 5. The $C_p°$(R) values for the corresponding side chains obtained using eq 8 are given in column 4 of Table 5. The agreement between the results in these two columns is not good. Although a value of 78 J·K^{-1}·mol^{-1} seems reasonable for a hydrogen atom that is part of a hydrocarbon chain, it has been proposed (Häckel et al., 1998) that it is better to use a $C_p°$(H) value of 45 J·K^{-1}·mol^{-1} when calculating $C_p°$(R) values based on heat capacity data for peptides. The results obtained using this $C_p°$(H) value, which are given in column 5 of Table 5, are certainly in better agreement with those based on $C_{p,2}^\circ$(R) data for the organic analogues. However, propionamide was one compound in the collection of amides, *N*-acetyl amino acid, and peptide amides that were used in a group contribution analysis which gave the $C_p°$(H) value of 45 J·K^{-1}·mol^{-1} (Hedwig et al., 1991) used above. Perhaps it would be more appropriate to use this result rather than $C_p°$(H) = 78 J·K^{-1}·mol^{-1}, in deriving the heat capacity of the glutaminyl side chain from the $C_{p,2}^\circ$ value for glutamine. The $C_p°$(R) value obtained is 213 J·K^{-1}·mol^{-1}, which is in poor agreement with the value of 173 J·K^{-1}·mol^{-1} obtained using the tripeptide model compounds.

Given that the side chains in peptides of sequence gly-X-gly are structurally the same as those found in proteins, heat capacities and volumes derived using these peptide model compounds should give a good representation of the side-chain contributions for unfolded proteins in aqueous solution.

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