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Partial Molar Heat Capacities and Volumes of Aqueous Solutions of Some Peptides that Model Side-chains of Proteins†

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The partial molar volumes, V_2^∞, and partial molar heat capacities, $C_{p,2}^\infty$, at infinite dilution have been determined for the tripeptides glycyl-L-isoleucylglycine, glycyl-DL-threonylglycine, glycyl-L-asparagylglycine, glycylhistidylglycine, glycylmethionylglycine and glycylphenylalanylglycine in aqueous solution at 25 °C. These results, in conjunction with those for glycylglycylglycine, were used to estimate the amino acid side-chain contributions to V_2^∞ and $C_{p,2}^\infty$. The side-chain contributions are compared with those determined using other model compounds. For some side-chains the heat capacities were found to correlate well with the side-chain non-polar surface areas.

A knowledge of the origins of the stability of proteins in aqueous solution is essential to the understanding of their structure and function. The stability of a globular protein in aqueous solution can be determined by studying the disruption of its native structure, *i.e.* the process of denaturation.[1] In this process the native folded protein structure is converted into a form that is predominantly unfolded but can still possess some residual folded structure.[2] Consequently, the fully unfolded or random-coil state of a protein, which is the ideal reference state in discussions of the thermodynamic stability of proteins, is not always experimentally accessible.[2] Through model systems consisting of peptides or shortened proteins it is possible to gain a greater understanding of the properties of the important completely unfolded state of a protein.[3]

In earlier papers of this series[4,5] we reported the partial molar volumes V_2^∞ and the partial molar heat capacities $C_{p,2}^\infty$ at infinite dilution in aqueous solution at 25 °C of the tripeptides of sequence glycyl-Xaa-glycine (Gly-Xaa-Gly), where Xaa is one of the amino acids alanine, valine, leucine and serine. These peptides of sequence Gly-Xaa-Gly, where the side-chain of the amino acid Xaa is in the central position within the molecule, are reasonable models for investigating side-chain effects in proteins.[5] In this paper we report V_2^∞ and $C_{p,2}^\infty$ values at 25 °C for aqueous solutions of the tripeptides glycyl-L-isoleucylglycine (Gly-Ile-Gly), glycyl-DL-threonylglycine (Gly-Thr-Gly), glycyl-L-asparagylglycine (Gly-Asn-Gly), glycylhistidylglycine (Gly-His-Gly), glycylmethionylglycine (Gly-Met-Gly) and glycylphenylalanylglycine (Gly-Phe-Gly). Using these results the contributions of the side-chains to the thermodynamic properties of the peptides can be estimated.

Recently Privalov and co-workers have also used tripeptides of sequence Gly-Xaa-Gly as model compounds in studies to determine the heat capacities of amino acid side-chains over a wide temperature range.[6,7] The measurements were carried out using 0.5 mol dm^{-3} sodium acetate–acetic acid buffer at pH 4 as the solvent rather than using pure water. Their results obtained at 25 °C are compared with those reported herein.

Experimental

Preparation and Purification of Peptides

The tripeptides Gly-Ile-Gly, Gly-Thr-Gly and Gly-Asn-Gly were synthesized using the carbodiimide method.[8] The general procedure adopted was to couple the *N*-protected dipeptide Gly-Xaa with the benzyl ester of glycine using dicyclohexylcarbodiimide to effect peptide bond formation and *N*-hydroxysuccinimide as a trapping agent.[9] The protecting groups on the tripeptide were then removed by catalytic hydrogenation. The preparative procedures used are described as follows.

N-*Benzyloxycarbonylglycyl-L-isoleucylglycine Benzyl Ester*

N-Benzyloxcarbonylglycyl-L-isoleucine (10.7 g, 0.0331 mol, Sigma) was dissolved in a mixture of dichloromethane (200 cm^3) and dimethylformamide (10 cm^3). To this solution was added a mixture containing glycine benzyl ester *p*-toluenesulfonate (11.2 g, 0.0331 mol),[5] triethylamine (3.4 g), *N*,*N*′-dicyclohexylcarbodiimide (7.2 g, 0.035 mol) and *N*-hydroxysuccinimide (4.0 g, 0.035 mol) dissolved in dichloromethane (160 cm^3). The mixture was stirred overnight at room temperature. The precipitated dicyclohexylurea was removed by filtration and the filtrate washed successively with water, 2 mol dm^{-3} HCl, water, half-saturated $NaHCO_3$ solution and water. The solution was evaporated to dryness to obtain a creamy white solid. The product was recrystallised from 5 : 1 ethanol : dichloromethane–petroleum ether to give a white solid. The yield was 13.1 g, 85%; m.p. 164–165 °C; $[\alpha]_D^{22}$ − 9.4° (c = 0.5, acetone); the composition was found to be: C, 64.2%; H, 6.8%; N, 9.1%, *cf.* calculated 6.7%; N, 9.0%.

Glycyl-L-isoleucylglycine

N-Benzyloxycarbonylglycyl-L-isoleucylglycine benzyl ester (2 g) was dissolved in a mixture of ethanol (100 cm^3), dimethylformamide (6 cm^3) and water (50 cm^3) and hydrogenated using a Parr low-pressure shaker-type hydrogenator with 5% Pd/C as a catalyst. As the product formed is not soluble in the solvent mixture, water was added during the hydrogenation. The catalyst was removed by filtration and the solution was evaporated to dryness to yield a white solid. The products from several batch hydrogenations were combined and recrystallised from water–methanol. Overall yield 79%; m.p. 244–246 °C dec.; $[\alpha]_D^{22}$ − 34.7° (c = 0.4, H_2O). The molar mass determined by alkalimetric titration[10,11] was 246.7 ± 1.7 g mol^{-1}, in agreement with that expected for an anhydrous compound. Elemental analyses gave: C, 48.4%; H, 8.1%; N, 16.9%; *cf.* calculated composition for $C_{10}H_{19}O_4N_3$: C, 49.0%; H, 7.8%; N, 17.1%.

N-*Benzyloxycarbonylglycyl-DL-threonine*

Glycyl-DL-threonine (9.6 g, 0.045 mol, Sigma) was dissolved in a mixture containing $NaHCO_3$ (8.0 g, 0.095 mol), water (80

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cm^3) and 1,4-dioxane (150 cm^3). A solution of *N*-(benzyloxycarbonyloxy)succinimide (12.6 g, 0.050 mol) in 1,4-dioxane (100 cm^3) was added to the mixture over a period of several hours. The reaction was monitored by TLC. On completion of the reaction, water (100 cm^3) was added, the mixture was filtered and the filtrate was extracted twice with diethyl ether. The volume of the water–1,4-dioxane solution was reduced by evaporation (to *ca.* 100 cm^3) and following acidification to pH 2 using 6 mol dm^{-3} HCl, the solution was extracted twice using ethyl acetate (80 cm^3). The combined ethyl acetate fractions were dried over anhydrous $MgSO_4$ and reduced in volume (to *ca.* 40 cm^3). The addition of petroleum ether gave a white oil-like product which solidified on stirring for several hours in an ice–salt bath. The product was recrystallised from hot ethyl acetate–petroleum ether. Overall yield 9.3 g, 67%; m.p. 108–110 °C; the composition was found to be: C, 54.1%; H, 5.9%; N, 9.0%; *cf.* calculated composition for $C_{14}H_{18}O_6N_2$: C, 54.2%; H, 5.9%; N, 9.0%.

N-*Benzyloxycarbonylglycyl-DL-threonylglycine Benzyl Ester*

N-Benzyloxycarbonylglycyl-DL-threonine (10.5 g, 0.0338 mol) was dissolved in a mixture of dichloromethane (400 cm^3) and dimethylformamide (12 cm^3). A solution containing glycine benzyl ester *p*-toluenesulfonate (11.4 g, 0.0338 mol), *N*,*N*′-dicyclohexylcarbodiimide (7.4 g, 0.0358 mol), triethylamine (3.4 g) and *N*-hydroxysuccinimide (4.1 g) in dichloromethane (400 cm^3) was added and the reaction mixture stirred overnight at room temperature. The precipitated dicyclohexylurea was removed by filtration and the filtrate was washed using the procedure outlined above for the preparation of the isoleucyl derivative. Evaporation of the solution to dryness gave a pale yellow oil which was solidified using ethanol–petroleum ether. The product was further purified by recrystallisations from ethanol–petroleum ether and from hot methanol. Overall yield 11.3 g, 73%; m.p. 137–138 °C; the composition was found to be: C, 60.4%; H, 6.0%; N, 9.2%; *cf.* calculated composition for $C_{23}H_{27}O_7N_3$: C, 60.1%; H, 6.0%; N, 9.1%.

Glycyl-DL-threonylglycine

N-Benzyloxycarbonylglycyl-DL-threonylglycine benzyl ester (2 g) was dissolved in a mixture of ethanol (150 cm^3) and water (10 cm^3) and hydrogenated using the procedure outlined above for Gly-Ile-Gly. The products obtained from several batch hydrogenations were combined and recrystallised from water–ethanol; yield 76%; m.p. 237–239 °C dec. Infrared spectrophotometry established that the product was a crystalline hydrate. The molar mass determined by alkalimetric titration was 252.3 ± 1.7 g mol^{-1} which is in good agreement with 251.2 g mol^{-1} expected for Gly-Thr-Gly monohydrate. Elemental analyses gave: C, 38.3%; H, 6.7%; N, 16.7%; *cf.* calculated composition for $C_8H_{17}O_6N_3$: C, 38.2%; H, 6.8%; N, 16.7%.

N-*Benzyloxycarbonylglycyl-L-asparagine*

Glycyl-L-asparagine (5.8 g, 0.0307 mol, Sigma) was dissolved in a mixture containing $NaHCO_3$ (5.2 g, 0.061 mol), water (70 cm^3) and 1,4-dioxane (60 cm^3). A solution of *N*-(benzyloxycarbonyloxy)succinimide (8.4 g, 0.0337 mol) in 1,4-dioxane (25 cm^3) was added in small portions over a 2 h period. When the reaction was complete, as indicated by TLC, the solution was evaporated to reduce the volume, then extracted twice with diethyl ether. The aqueous phase was acidified to pH 2 using 6 mol dm^{-3} HCl then reduced in volume to give an oil. After the product had been stored at 4 °C for 2 days a creamy white solid was obtained. The product was recrystallised from isopropyl alcohol–diethyl ether. Overall yield 6.0 g, 60%; m.p. 129–132 °C.

N-*Benzyloxycarbonylglycyl-L-asparagylglycine Benzyl Ester*

N-Benzyloxycarbonylglycyl-L-asparagine (7.7 g, 0.0238 mol) was dissolved in a mixture of dichloromethane (170 cm^3) and dimethylformamide (40 cm^3). To this solution was added a solution of dichloromethane (130 cm^3) containing glycine benzyl ester *p*-toluenesulfonate (8.0 g, 0.0238 mol), triethylamine (2.4 g), *N*,*N*′-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide (2.9 g, 0.0252 mol). The mixture was stirred at room temperature overnight. In this preparation the protected tripeptide precipitated out of the reaction mixture along with the dicyclohexylurea. The product was separated from the dicyclohexylurea by recrystallisation from hot methanol. Overall yield 61%; m.p. 192–195 °C; the composition was found to be: C, 58.7%; 5.5%; N, 12.2%; *cf.* calculated composition for $C_{23}H_{26}O_7N_4$: C, 58.7%; H, 5.6%; N, 11.9%.

Glycyl-L-asparagylglycine

N-Benzyloxycarbonylglycyl-L-asparagylglycine benzyl ester (1 g) was dissolved in warm ethanol (300 cm^3) and water (20 cm^3) was added. The solution was hydrogenated using the procedure described above for the Gly-Ile-Gly derivative. The products from several batch hydrogenations were combined and recrystallised from water–methanol. Overall yield 75%; m.p. 253–257 °C dec.; $[\alpha]_D^{22}$ − 31.4° (c = 0.5, H_2O); the composition was found to be: C, 38.9%; H, 5.6%; N, 22.6%; *cf.* calculated composition for $C_8H_{14}O_5N_4$: C, 39.0%; H, 5.7%; N, 22.7%. Alkalimetric titration gave a value for the molar mass that was in agreement, within the experimental error, with that of the anhydrous compound.

Other Materials

Samples of the tripeptides glycylhistidylglycine, glycylmethionylglycine and glycylphenylalanylglycine, which were obtained as a customer accommodation through Sigma, were recrystallised from water–methanol. Each peptide was chromatographically pure as determined by TLC. For Gly-Met-Gly, analysis by alkalimetric titration gave a molar mass of 263.5 ± 1.7 g mol^{-1}, which is in excellent agreement with that expected for the anhydrous compound. Elemental analyses gave: C, 40.9%; H, 6.7%; N, 15.9%; *cf.* calculated composition for $C_9H_{17}O_4N_3S$: C, 41.0%; H, 6.5%; N, 16.0%. Alkalimetric titrimetry for Gly-His-Gly gave a molar mass of 271.7 ± 1.9 g mol^{-1}, which is 0.9% higher than that expected for the anhydrous compound. Elemental analyses gave C, 44.4%; H, 6.1%; N, 26.3%; *cf.* calculated composition for $C_{10}H_{15}O_4N_5$: C, 44.6%; H, 5.6%; N, 26.0%. Satisfactory microanalytical data were not obtained for the peptide Gly-Phe-Gly. In view of this, the sample was further analysed by HPLC, mass spectrometry and chromatography using a Pharmacia LKB 4151 Alpha plus amino acid analyser. No significant amount of any impurity could be detected. Alkalimetric titrimetry gave a molar mass that was in satisfactory agreement with that for the anhydrous compound.

All water used, both to prepare solutions and as the reference solvent, was deionized glass-distilled and degassed immediately prior to use. Solutions were prepared by mass and corrections were made for air buoyancy. All the peptides, except Gly-Thr-Gly monohydrate were dried under vacuum at room temperature before use.

Apparatus and Methods

Solution densities were determined using an Anton Paar digital density meter (model DMA 60/602) as outlined pre-

Table 1 Densities and apparent molar heat capacities of aqueous solutions of tripeptides at 25 °C

m/mol kg^{-1}	d/g cm^{-3}	$C_{p,\phi}{}^{a}$/J K^{-1} mol^{-1}	m/mol kg^{-1}	d/g cm^{-3}	$C_{p,\phi}{}^{a}$/J K^{-1} mol^{-1}
		glycyl-L-isoleucylglycine			
0.022 23	0.998 560	—	0.075 16	1.002 106	534.4 (2.8)
0.033 02	0.999 290	—	0.081 72	—	534.2 (3.4)
0.038 98	0.999 690	—	0.085 71	1.002 797	536.2 (5.2)
0.042 04	—	531.2 (4.6)	0.089 62	—	535.4 (2.5)
0.046 01	1.000 162	533.9 (4.2)	0.094 90	1.003 406	537.2 (2.4)
0.052 88	1.000 619	534.0 (3.8)	0.106 27	1.004 142	537.4 (2.2)
0.062 02	1.001 231	534.0 (3.3)	0.115 18	1.004 732	536.2 (2.4)
0.069 90	1.001 756	535.3 (3.1)	0.126 37	1.005 462	538.0 (2.4)
			0.135 14	1.006 020	538.6 (1.8)
		glycyl-DL-threonylglycine			
0.030 01	0.999 654	—	0.089 65	1.004 753	344.9 (3.2)
0.039 96	1.000 510	340.3 (4.8)	0.099 28	1.005 564	345.2 (2.5)
0.050 03	1.001 373	342.4 (3.6)	0.110 45	1.006 509	346.2 (2.7)
0.059 20	1.002 163	342.3 (4.6)	0.119 93	1.007 293	347.0 (3.2)
0.069 91	1.003 073	344.0 (3.9)	0.130 20	1.008 157	346.0 (2.1)
0.080 31	1.003 953	344.3 (3.4)	0.141 61	1.009 111	346.4 (4.7)
			0.149 55	1.009 765	348.3 (2.1)
		glycyl-L-asparagylglycine			
0.020 04	0.999 020	—	0.039 99	1.000 966	—
0.025 08	0.999 515	—	0.041 81	1.001 150	287.4 (4.8)
0.029 94	0.999 995	—	0.044 63	1.001 418	285.8 (4.5)
0.030 00	0.999 998	—	0.046 72	1.001 624	288.4 (4.5)
0.031 95	1.000 189	—	0.049 94	1.001 937	284.7 (4.3)
0.031 98	1.000 193	—	0.052 04	—	289.5 (4.3)
0.032 09	—	285.3 (6.3)	0.054 60	1.002 389	287.1 (3.9)
0.034 00	1.000 387	—	0.057 92	1.002 710	288.5 (3.7)
0.034 89	—	280.9 (5.8)	0.060 55	1.002 956	286.8 (3.5)
0.037 02	1.000 684	—	0.063 97	1.003 290	288.6 (3.7)
0.037 73	—	281.6 (5.4)	0.068 64	1.003 738	288.7 (3.3)
0.038 52	1.000 826	—			
		glycylmethionylglycine			
0.020 01	0.998 808	—	0.070 31	1.003 167	430.8 (4.8)
0.025 01	0.999 250	—	0.074 92	—	433.2 (3.5)
0.030 87	0.999 759	432.0 (6.5)	0.079 67	1.003 962	435.5 (3.5)
0.034 95	—	432.9 (5.8)	0.084 04	1.004 337	435.3 (3.5)
0.040 06	1.000 558	429.5 (5.0)	0.089 90	1.004 833	432.7 (3.3)
0.044 98	—	434.2 (4.7)	0.095 04	—	433.5 (3.2)
0.050 50	1.001 461	431.7 (5.4)	0.100 18	1.005 701	434.2 (3.0)
0.054 99	—	428.6 (4.8)	0.105 00	—	434.8 (2.9)
0.060 38	1.002 310	—	0.110 08	1.006 537	434.7 (2.6)
			0.119 67	—	434.7 (2.6)
		glycylphenylalanylglycine			
0.004 01	0.997 392	—	0.019 06	—	506.9 (8.5)
0.006 01	0.997 564	—	0.019 99	0.998 761	—
0.008 01	0.997 734	—	0.021 09	—	511.9 (7.9)
0.010 03	0.997 912	—	0.022 00	—	510.1 (7.6)
0.012 01	0.998 080	—	0.022 14	0.998 946	—
0.013 02	—	501 (12)	0.022 73	—	507.6 (7.3)
0.014 03	0.998 250	503 (12)	0.023 05	—	503.2 (7.2)
0.015 00	—	503 (11)	0.023 85	—	510.1 (7.0)
0.015 97	0.998 421	—	0.024 21	0.999 119	—
0.016 99	—	506.1 (9.5)	0.024 96	—	505.7 (6.9)
0.018 01	0.998 593	510.3 (9.0)			
		glycylhistidylglycine			
0.003 98	0.997 443	—	0.009 80	—	364.2 (9.2)
0.005 00	0.997 544	—	0.009 98	0.998 035	—
0.006 00	0.997 644	—	0.010 00	—	353.3 (9.0)
0.006 44	0.997 684	—	0.010 29	—	357.4 (8.8)
0.007 01	0.997 743	—	0.010 91	0.998 125	—
			0.011 00	0.998 138	—
0.007 97	—	360 (10)	0.011 02	—	347.2 (8.2)
0.008 00	0.997 839	—	0.011 30	—	357.5 (8.9)
0.008 51	—	359 (11)	0.011 39	0.998 174	—
0.008 70	0.997 907	350 (10)	0.011 57	0.998 190	352.9 (7.8)
0.008 98	0.997 939	—	0.011 58	—	366.5 (9.5)
0.009 20	—	349.5 (8.7)	0.011 93	0.998 228	—
0.009 51	—	370.5 (9.5)	0.011 99	—	366.4 (9.2)

[a] The estimated uncertainty of each $C_{p,\phi}$ is given in parentheses.

viously.[4,5] The reproducibility of an individual density measurement was to better than 3×10^{-6} g cm^{-3}. Specific heat capacities per unit volume of the solutions were measured relative to pure water using a Picker differential flow microcalorimeter. The experimental procedures used have been described in previous work.[4,5]

Results

Partial Molar Volumes

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

$$V_\phi = M_2/d - (d - d_0)/mdd_0 \quad (1)$$

where M_2 is the solute molar mass, m is the solution molality and d and d_0 are, respectively, the densities of the solution and pure solvent (0.997 047 g cm^{-3} at 25 °C[12]). Since, for each solute, the apparent molar volume was found to be a linear function of molality over the range studied, the partial molar volume of the solute at infinite dilution, V_2^∞, was obtained using the equation

$$V_\phi = V_2^\infty + S_v m \quad (2)$$

where S_v is the experimental slope. In the weighted least-squares analyses, weighting factors for the V_ϕ results were calculated using the procedures outlined earlier.[4] Values of V_2^∞ and S_v together with their standard deviations are given in Table 2. For the peptides Gly-Phe-Gly and Gly-His-Gly the standard deviations are large for V_2^∞ and in particular, for S_v. This is because of the very low solubility of these compounds in water. The accessible concentration range is far too narrow to enable reliable values of S_v to be determined. If the concentration dependence of V_ϕ is ignored, a value of V_2^∞ can be estimated by averaging the V_ϕ results. The means and standard deviations of the V_ϕ data for Gly-Phe-Gly and Gly-His-Gly are 193.5 ± 0.1 and 170.2 ± 0.2 cm^3 mol^{-1}, respectively. These values are the same, within experimental error, as the values of V_2^∞ obtained using eqn. (2).

In the recent study by Makhatadze *et al.*[13] the reported values of V_2^∞ for the peptides Gly-Ile-Gly, Gly-Met-Gly and Gly-His-Gly in the solvent 0.5 mol dm^{-3} sodium acetate–acetic acid buffer at pH 4.0 were 171.3 ± 1.1, 177.3 ± 1.1 and 169.2 ± 1.1 cm^3 mol^{-1}, respectively. Although the solvent system used negates any direct comparison with the results in this study, it is worth noting that for Gly-Ile-Gly and Gly-His-Gly the V_2^∞ results in water are larger than those in the buffer system. This is contrary to the results for other tripeptides of sequence Gly-Xaa-Gly which have V_2^∞ values in water that are smaller than those determined in the acetate buffer system.[14]

Partial Molar Heat Capacities

The apparent molar heat capacities, $C_{p,\phi}$, of the tripeptides were calculated from the specific heat capacities, c_p, using the equation

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

where c_p^0 is the specific heat capacity of pure water (4.1793 J K^{-1} g^{-1} at 25 °C[15]) and the other symbols are as defined for eqn. (1). The uncertainty in each $C_{p,\phi}$ was calculated from the estimated error in the specific heat capacity as described in earlier work.[4] The $C_{p,\phi}$ data along with their uncertainties are given in Table 1. In some cases specific heat capacity measurements were made on solutions for which the densities were not determined. For these solutions the densities required to convert volumetric heat capacities into specific heat capacities were calculated using a power series in the solution molality of the form

$$d = d_0 + p_1 m + p_2 m^2 \quad (4)$$

where p_1 and p_2 are parameters determined by least-squares fitting to the density data given in Table 1. For each tripeptide the $C_{p,\phi}$ data were analysed by a weighted least-squares method using an equation of the form

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

where $C_{p,2}^\infty$ is the partial molar heat capacity of the solute at infinite dilution and S_c is the experimental slope. Values of $C_{p,2}^\infty$ and S_c, along with their standard deviations, are given in Table 2. For the peptides Gly-Phe-Gly and Gly-His-Gly values of S_c have not been determined because of the low solubility of these compounds in water. The tabulated values of $C_{p,2}^\infty$ are the means for the $C_{p,\phi}$ data given in Table 1. The value of S_c for Gly-Asn-Gly is high compared with the results for other tripeptides in this study and in previous work.[5] The maximum solubility of this compound in aqueous solution is about $m = 0.07$ mol kg^{-1} near room temperature so the $C_{p,\phi}$ values have been determined over only a narrow molality range.

A comparison of the $C_{p,2}^\infty$ results obtained for some of the tripeptides determined in this study and in earlier work[4,5] with those determined in 0.5 mol dm^{-3} sodium acetate–acetic acid buffer at pH 4.0[6,7] is shown in Table 3. With the exception of Gly-Gly-Gly, the $C_{p,2}^\infty$ values in water are all larger than those determined in acetate buffer. For the peptide Gly-Met-Gly the difference between the $C_{p,2}^\infty$ values is considerable. Although the solvent systems used are different, it is of interest to see whether the differences between the two sets of results in Table 3 are what might be expected. The heat capacities of transfer from water to aqueous sodium chloride solutions of a selection of amino acids and dipeptides with hydrophobic side-chains are all positive.[17] The heat capacity of transfer of the tripeptide Gly-Gly-Gly from water to aqueous sodium chloride is also positive.[17] Assuming that a change from sodium chloride to sodium acetate does not alter the sign of the heat capacity of transfer of peptide, then the $C_{p,2}^\infty$ values of tripeptides in acetate buffer would be expected to be larger than those in water. This is contrary to what is observed. The solution pH is another factor to consider in the comparison of the $C_{p,2}^\infty$ values in

Table 2 Partial molar volumes and heat capacities of tripeptides in aqueous solution at 25 °C[a]

peptide	V_2^∞/cm^3 mol^{-1}	S_v/cm^3 kg mol^{-2}	$C_{p,2}^\infty$/J K^{-1} mol^{-1}	S_c/J kg K^{-1} mol^{-2}
Gly-Ile-Gly	177.24 (0.04)	2.7 (0.4)	530.4 (0.8)	60 (7)
Gly-Thr-Gly	146.15 (0.04)	2.3 (0.3)	339.4 (0.7)	58 (6)
Gly-Asn-Gly	147.35 (0.05)	8.0 (1.0)	279.2 (2.6)	146 (47)
Gly-Met-Gly	175.10 (0.03)	5.6 (0.4)	429.9 (1.3)	43 (14)
Gly-Phe-Gly	193.3 (0.1)	9 (7)	506.6 (3.5)	[b]
Gly-His-Gly	169.9 (0.2)	35 (24)	358 (7)	[b]

[a] Standard deviations are given in parentheses. [b] See text.

Table 3 Comparison of the partial molar heat capacities of some tripeptides in aqueous solution and in acetate buffer at 25 °C

peptide	$C^{\infty}_{p,2}$/J K^{-1} mol^{-1}	
	H_2O	acetate buffer[a]
Gly-Gly-Gly	188.3 (0.7),[b] 185.9 (0.9),[c] 175.3 (5.0)[d]	195 (11)[d]
Gly-Ala-Gly	289.8 (0.3)[b]	282 (13)[e]
Gly-Val-Gly	443.6 (0.7)[f]	413 (15)[e]
Gly-Leu-Gly	526.1 (0.7)[f]	503 (16)[e]
Gly-Ile-Gly	530.4 (0.8)	488 (16)[e]
Gly-Ser-Gly	263 (1)	198 (14)[e]
Gly-Met-Gly	429.9 (1.3)	293 (9)[d]
Gly-His-Gly	358 (7)	296 (14)[d]

[a] 0.5 mol dm^{-3} sodium acetate–acetic acid buffer, pH 4.0. [b] Ref. 4. [c] Ref. 16. [d] Ref. 6. [e] Ref. 7. [f] Ref. 5.

Table 3. The structure that a peptide without ionizing side-chains adopts when dissolved in pure water is predominantly the zwitterionic form of the molecule. At pH 4 the zwitterion will still be the major species, but there will be an increase in the concentration of the species that has a protonated carboxyl group. The $C^{\infty}_{p,2}$ values for un-ionized carboxylic acids[18] are greater than those for the carboxylate ions.[19] It is expected therefore, that the $C^{\infty}_{p,2}$ values for the peptides in a buffer at pH 4 would be greater than those in pure water. We are unable to provide a satisfactory explanation as to why the $C^{\infty}_{p,2}$ values of the peptides in acetate buffer are lower than the corresponding values in water.

Discussion

Partial Molar Volumes

Some years ago[20] it was shown that the partial molar volume of a solute at infinite dilution can be related to the potential of mean force between one molecule of solute and one of solvent in the pure solvent, w^{11}, by the equation

$$V^{\infty}_2 = 4\pi N_A \int_0^{\infty} [1 - \exp(-w^{11}/kT)]r^2\,dr + RT\kappa \quad (6)$$

where r is the distance between the molecules, κ is the isothermal compressibility of the solvent and the remaining symbols have their usual meanings. Although eqn. (6) provides a direct link between the thermodynamic property V^{∞}_2 and solute–solvent interactions, it has received little attention because of the difficulty in evaluating w^{11} for molecules of any complexity.[21] Semiempirical models provide an alternative approach that has been widely used to rationalise V^{∞}_2 data for organic solutes in water.[22] One such model is based on the equation

$$V^{\infty}_2 = V_{int} + V_v + V_s \quad (7)$$

where V_{int} is the intrinsic volume occupied by the solute, V_v is the void or empty volume that arises because of thermal motion and packing effects[23–25] and V_s is the contribution that arises from interactions of the solute with the solvent. The V_s term will also incorporate contributions associated with changes in solvent–solvent interactions that are a consequence of the interaction of the solvent with the solute.[26] In order to interpret V^{∞}_2 data in terms of solute–solvent interactions estimates are needed for the V_{int} and V_v terms. One approach that can be used to estimate V_{int} is to equate this volume with the van der Waals volume of the solute, V_w. This can be obtained by the addition of the van der Waals increments for the individual atoms that make up the molecule.[27,28] The van der Waals volumes for the tripeptides were calculated using

$$V_w(\text{Gly-Xaa-Gly}) = V_w(\text{Gly-Gly-Gly}) - V_w(H) + V_w(R) \quad (8)$$

where V_w(Gly-Gly-Gly) is the van der Waals volume of Gly-Gly-Gly and V_w(H) and V_w(R) are, respectively, the van der Waals volumes of the hydrogen atom and the side-chain R of the amino acid Xaa. The van der Waals increments used in the calculations are those given by Edward.[28] A plot of V^{∞}_2 *vs.* V_w for the various tripeptides of sequence Gly-Xaa-Gly is shown in Fig. 1. For Gly-Gly-Gly and the peptides with hydrophobic side chains (Xaa = Ala, Val, Leu, Ile and Phe) there is a reasonable linear relationship

$$V^{\infty}_2 = aV_w + b \quad (9)$$

with $a = 1.56 \pm 0.02$, $b = -33.8 \pm 2.3$ cm^3 mol^{-1} and a correlation coefficient of 0.999. As the value of a is not unity it follows from eqn. (7) and (9) that for these peptides the sum $(V_v + V_s)$ is also a function of V_w. The value obtained for the constant a is identical, within experimental error, to that obtained in the analogous plot for a series of *n*-alcohols[24] and is similar to those for other neutral organic solutes in water.[24]

Fig. 1 also shows that the points corresponding to the peptides with hydrophilic side-chains lie, to varying degrees, below the straight line for the peptides with hydrophobic side-chains. In other words, the V^{∞}_2 value for each of these peptides is smaller than that of a hypothetical peptide having a non-polar side-chain and with a V_w value the same as that of the peptide itself. These smaller V^{∞}_2 values can be interpreted in terms of a volume decrease that arises because of polar group–water interactions, presumably hydrogen bonding. For the seryl and threonyl side-chains which contain the hydroxy group, the volume decreases are 4.5 and 5.4 cm^3 mol^{-1}, respectively. These are similar to estimates of the shrinkage due to hydrogen bonding between hydroxy functional groups and water reported for other solute

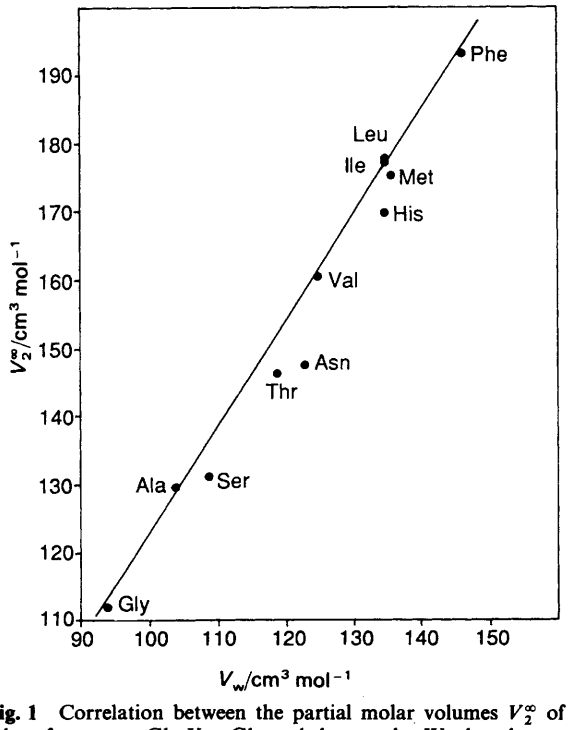


Fig. 1 Correlation between the partial molar volumes V^{∞}_2 of peptides of sequence Gly-Xaa-Gly and the van der Waals volume of the peptides

systems.[22,24,29,30] The largest difference between the experimental value of V_2^∞ and that calculated using eqn. (9) is for the peptide with an asparagyl side chain (the difference is 10.4 $cm^3\ mol^{-1}$). This is consistent with the fact that the amide functional group is able to form hydrogen bonds with water both through the carbonyl and the amine moieties.[31] Similarly, V_2^∞ for Gly-His-Gly is lower than that calculated using eqn. (9) because of the multiple hydrogen-bonding sites on the imidazole group of the histidyl side-chain. Although the thioether functional group is a poor hydrogen-bond acceptor,[32] the slight deviation of V_2^∞ for Gly-Met-Gly from the straight line in Fig. 1 suggests that there is some interaction of sulfur atom with water that is not typically hydrophobic.

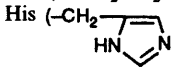
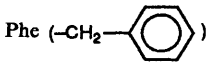
The partial molar volumes of the various amino acid side-chain residues can be estimated from the difference between V_2^∞ for each peptide and that for the corresponding peptide without a side chain

$$V^\infty(\mathrm{R}) = V_2^\infty(\text{Gly-Xaa-Gly}) - V_2^\infty(\text{Gly-Gly-Gly}) \quad (10)$$

It should be stressed that $V^\infty(\mathrm{R})$ is not the absolute partial molar volume of the side-chain R, but it gives the contribution to V_2^∞ on replacing a C—H group by a C—R group. The actual partial molar volume of each side-chain R can be obtained by adding the partial molar volume of the H atom to each $V^\infty(\mathrm{R})$ value. Values of $V^\infty(\mathrm{R})$ calculated using the V_2^∞ results in this work and the V_2^∞ value for Gly-Gly-Gly determined previously[4] are given in Table 4, along with some data determined in earlier studies.[4,5] In a comprehensive study of the amino acids in aqueous solution, Jolicoeur *et al.*[33] reported $V^\infty(\mathrm{R})$ values for 20 amino acid side-chains. For the purposes of comparison some of these values are also given in Table 4. The results in Table 4 show that there are small but significant differences between the side-chain $V^\infty(\mathrm{R})$ values determined using amino acid and peptide V_2^∞ data. The differences range from *ca.* 0.5 $cm^3\ mol^{-1}$ for the alanyl and threonyl side-chains to 3.1 $cm^3\ mol^{-1}$ for the isoleucyl side-chain. These differences arise because of ionic end-group effects in the amino acids. As the charged NH_3^+ and CO_2^- groups are adjacent to the side-chain in a zwitterionc amino acid, there will be a significant mutual interaction between the solvated side-chain and the charged groups with their associated cospheres. Consequently, $V^\infty(\mathrm{R})$ values based on V_2^∞ data for the amino acids will not give accurate estimates of the contributions of isolated side-chains. In the tripeptides of sequence Gly-Xaa-Gly the side-chains are separated from both charged end groups so $V^\infty(\mathrm{R})$ values determined using V_2^∞ data for these tripeptides ought to be better estimations of the volumetric contributions from solvent-accessible side-chains in proteins.

For the isomeric leucyl and isoleucyl side-chains, the $V^\infty(\mathrm{R})$ values determined using V_2^∞ data for the tripeptides are identical within the combined experimental uncertainties whereas a difference of 2.1 $cm^3\ mol^{-1}$ is observed using amino acid data. The similar $V^\infty(\mathrm{R})$ values observed for these isomeric side-chains are consistent with results for structurally related compounds. For example, the V_2^∞ values for isobutyl alcohol and butan-2-ol,[34] which model the leucyl and isoleucyl side-chains respectively, differ by only 0.22 $cm^3\ mol^{-1}$.

Table 4 Contributions of amino acid side-chains to V_2^∞ of peptides in aqueous solution at 25 °C

side-chain (R)	$V^\infty(\mathrm{R})^a/cm^3\ mol^{-1}$ tripeptide	amino acid[b]
Ala ($-CH_3$)	17.75 (0.04)[c]	17.20 (0.02)
Val ($-CH(CH_3)_2$)	48.46 (0.06)[d]	47.54 (0.02)
Leu ($-CH_2CH(CH_3)_2$)	65.43 (0.09)[d]	64.32 (0.06)
Ile ($-CH(CH_3)CH_2CH_3$)	65.32 (0.07)	62.20 (0.06)
Ser ($-CH_2OH$)	19.21 (0.08)[d]	17.37 (0.02)
Thr ($-CH(OH)CH_3$)	34.23 (0.07)	33.61 (0.04)
Asn ($-CH_2CONH_2$)	35.43 (0.08)	33.9 (0.02)
Met ($-CH_2CH_2SCH_3$)	63.18 (0.06)	62.1 (0.2)
His ()	58.0 (0.2)	55.89 (0.05)
Phe ()	81.4 (0.2)	78.67 (0.03)

[a] Estimated uncertainties are in parentheses. [b] Ref. 33. [c] Ref. 4. [d] Ref. 5.

Partial Molar Heat Capacities

The contribution of the side-chain to the $C_{p,2}^\infty$ of a peptide can be derived from the difference between $C_{p,2}^\infty$ for the peptide of sequence Gly-Xaa-Gly and that for the analogous compound with no side-chain,

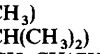
$$C_p^\infty(\mathrm{R}) = C_{p,2}^\infty(\text{Gly-Xaa-Gly}) - C_{p,2}^\infty(\text{Gly-Gly-Gly}) \quad (11)$$

The quantity $C_p^\infty(\mathrm{R})$ 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 Xaa. Values of $C_p^\infty(\mathrm{R})$ derived from the results in this work and those determined in earlier studies[4,5] are given in Table 5, along with results based on $C_{p,2}^\infty$ data for the amino acids.[33] A set of $C_p^\infty(\mathrm{R})$ values based on the $C_{p,2}^\infty$ data for tripeptides reported by Privalov and co-workers[6,7] (see Table 3) have not been included in Table 5 because the solvent system used in their study was not pure water. However, Makhatadze and Privalov[6] determined partial molar heat capacities in water of a variety of small solutes that were used to model some of the amino acid side-chains in proteins. For example, methanol was chosen as a model for the side-chain of serine, acetamide was the analogue for the side-chain of asparagine and methane was used for the side-chain of alanine.[6] The absolute value of each side-chain heat capacity was derived from $C_{p,2}^\infty$ value for the solute by subtracting an estimated value for the heat capacity of the H atom (78 $J\ K^{-1}\ mol^{-1}$).[6] These side-chain heat capacities have been converted into $C_p^\infty(\mathrm{R})$ values by subtracting the absolute value of the side-chain heat capacity of glycine (the H atom). The results obtained, along with the estimated uncertainties, are given in column 4 of Table 5. The value of the heat capacity of the H atom, which was taken as the mean of four estimates from the literature,[6] has a standard deviation of 10 $J\ K^{-1}\ mol^{-1}$. This is the reason for the high uncertainty associated with these $C_p^\infty(\mathrm{R})$ values.

The results in Table 5 show that, in general, the $C_p^\infty(\mathrm{R})$ values based on the $C_{p,2}^\infty$ data for tripeptides are lower than those determined using amino acid data. However, in some cases the differences are quite small. For the asparagyl side-chain the $C_p^\infty(\mathrm{R})$ derived from tripeptide $C_{p,2}^\infty$ data is in fact slightly larger than that obtained using the amino acids. Given the mutual interactions that exist between the ionic end groups and the adjacent side-chain in the amino acids,[5,16] the small differences between the results in columns 2 and 3 of Table 5 are somewhat surprising. As the side-chain in a tripeptide of sequence Gly-Xaa-Gly is adjacent to two peptide functional groups, which is structurally analogous to that found in proteins, the $C_p^\infty(\mathrm{R})$ data obtained using tripeptide model compounds should be a better set of values to use in additivity schemes for proteins than those derived using amino acid data.

For the hydrophobic side-chains, except that for leucine, the values of $C_p^\infty(\mathrm{R})$ obtained using the small solute side-

Table 5 Contributions of the amino acid side-chains to $C_{p,2}^{\infty}$ of peptides in aqueous solution at 25 °C

side-chain (R)	C_p^{∞}(R)[a]/J K^{-1} mol^{-1} tripeptide	amino acid[b]	analogue[c]
Ala (—CH_3)	102 (1)[d]	102.2 (0.6)	89 (23)[e]
Val (—$CH(CH_3)_2$)	255 (1)[f]	263 (1)	236 (23)[e]
Leu (—$CH_2CH(CH_3)_2$)	338 (1)[f]	359 (1)	304 (29)[e]
Ile (—$CH(CH_3)CH_2CH_3$)	343 (2)[f]	344.1 (0.8)	324 (43)[e]
Ser (—CH_2OH)	75 (2)[f]	78.2 (0.5)	2 (23)
Thr (—$CH(OH)CH_3$)	151 (1)	171 (2)	107 (24)
Asn (—CH_2CONH_2)	91 (3)	86 (1)	11 (26)
Met (—$CH_2CH_2SCH_3$)	242 (2)	254 (29)	—
His (—CH_2— )	170 (8)	202 (1)	—
Phe (—CH_2— )	318 (4)	345 (2)	305 (50)

[a] Estimated uncertainties are in parentheses. [b] Ref. 33. [c] Ref. 6. [d] Ref. 4. [e] The estimated uncertainty does not include a contribution from the C_p^0(g) term. [f] Ref. 5.

chain analogues are the same, within the admittedly large uncertainties, as those derived using the $C_{p,2}^{\infty}$ data for the tripeptides. This result is consistent with the observation that the partial molar heat capacity of a hydrocarbon chain HC in a compound HC—Z is not too dependent on the nature of the group Z.[35] For the hydrophilic side-chains of serine, threonine and asparagine the differences between the C_p^{∞}(R) values derived using tripeptide model compounds and the side-chain analogues are quite large. This suggests that for these side-chains the hydration of R in each of the analogue compounds R—H is quite different from that when R is part of a peptide chain.

Differences between side-chain heat capacities determined using tripeptides in pure water and those determined using tripeptides in buffered solution at pH 4 and also using the side-chain analogues have been noted in a previous study.[7] It was suggested that for the tripeptides in pure water the central side-chain may be influenced to some extent by the ionic end groups. In Table 6 the side-chain heat capacity of the alanyl side-chain, $C_p^{\infty}(CH_3)$ determined using the model tripeptides is compared with those obtained from using some cyclodipeptides[7] and some neutral acetyl amino acid and peptide amides.[36] Given the variation in the $C_p^{\infty}(CH_3)$ values using the various combinations of neutral peptide derivatives, the result obtained using zwitterionic tripeptides does not look unreasonable. It would appear that the ionic end groups do not significantly affect the value of $C_p^{\infty}(CH_3)$.

For non-polar solutes in aqueous solution, the partial molar heat capacities generally correlate with the solvent-accessible surface area.[37] In a previous paper[5] we reported, for a small number of tripeptides, a linear correlation between the C_p^{∞}(R) value and the solvent-accessible surface area of the hydrophobic component of the side-chain R. In Fig. 2 this correlation is extended to include the peptide side-chains in this study. The hydrophobic solvent-accessible surface areas for the various side-chains, taken from the work of Jolicoeur *et al.*,[33] were obtained from calculations actually performed on tripeptides of sequence Gly-Xaa-Gly. For the hydrocarbon side-chains and for the side-chains of serine and threonine, there is a reasonably good linear correlation. These data were fitted to a straight line passing through the origin with a slope of 238.8 J K^{-1} mol^{-1} nm^{-2} and a correlation coefficient of 0.999. The linear relationship suggests

Table 6 Comparison of the $C_p^{\infty}(CH_3)$ value derived using various peptide model compounds in aqueous solution

solute[a]	$C_p^{\infty}(CH_3)$[b]/J K^{-1} mol^{-1}
Gly-Ala-Gly–Gly-Gly-Gly	102 (1)[c]
c(Gly-Ala)–c(Gly-Gly)	97 (11)[d]
[c(Ala-Ala)–c(Gly-Gly)]/2	92 (12)[d]
c(Ala-Ala)–c(Gly-Ala)	86 (13)[d]
Ac-Ala-NH_2–Ac-Gly-NH_2	108 (1)[e]
Ac-Gly-Ala-NH_2–Ac-Gly-Gly-NH_2	100 (2)[e]
Ac-Ala-Gly-NH_2–Ac-Gly-Gly-NH_2	109 (1)[e]
(Ac-Ala-Ala-NH_2–Ac-Gly-Gly-NH_2)/2	108 (2)[e]

[a] Abbreviations used: c(Gly-Gly) = 2,5-diketopiperazine; c(Gly-Ala) = 3-methyl-2,5-diketopiperazine; c(Ala-Ala) = 3,6-dimethyl-2,5-diketopiperazine; Ac-Gly-NH_2 = *N*-acetylglycinamide; Ac-Ala-NH_2 = *N*-acetyl-L-alaninamide; Ac-Gly-Ala-NH_2 = *N*-acetylglycyl-L-alaninamide; Ac-Gly-Gly-NH_2 = *N*-acetylglycylglycinamide; Ac-Ala-Gly-NH_2 = *N*-acetyl-L-alanylglycinamide; Ac-Ala-Ala-NH_2 = *N*-acetyl-L-alanyl-L-alaninamide. [b] Estimated uncertainties in parentheses. [c] Ref. 4. [d] Ref. 7. [e] Ref. 36.

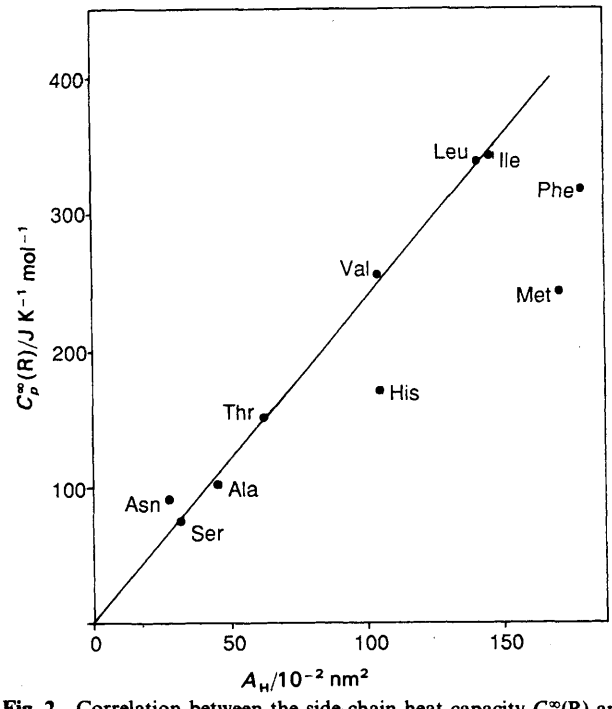


Fig. 2 Correlation between the side-chain heat capacity C_p^{∞}(R) and the hydrophobic solvent-accessible surface area, A_H

that at 25 °C, the OH group contributes little to the C_p^∞(R) value for the side-chains of serine and threonine. The deviation from the line for the side-chain of asparagine is presumably due to the hydration of the $CONH_2$ moiety. The significant deviations from the line for the side-chains with ring systems (Phe and His) and that containing a sulfur atom (Met) are consistent with the results reported earlier for the amino acids.[33] The side-chain of methionine is generally regarded as being hydrophobic.[6] The hydrophobic solvent-accessible surface area of the side-chain used in Fig. 2 includes the —S— group.[33] This may not be valid. The $C_{p,2}^\infty$ values for some alkyl sulfides in aqueous solution have been determined recently.[38] Although there are some irregularities in the $-CH_2-$ group contributions for the short series of compounds studied, it does appear that the contribution of the —S— group to the $C_{p,2}^\infty$ value is negative.[38] This finding is certainly consistent with the position of methionine given Fig. 2.

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