Volumetric Properties of Tripeptides with Polar Side-Chains: Partial Molar Volumes at (288.15 to 313.15) K and Partial Molar Expansions at 298.15 K of Some Peptides of Sequence Gly-X-Gly in Aqueous Solution†

Daniel C. Parr,‡ Gavin R. Hedwig,*,‡ and Andrew W. Hakin§

Institute of Fundamental Sciences—Chemistry, Massey University, Private Bag 11 222, Palmerston North, New Zealand, and Department of Chemistry and Biochemistry, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta T1K 3M4, Canada

Solution densities have been determined for aqueous solutions of the tripeptides of sequence glycyl-X-glycine, where X is one of the amino acids serine, threonine, asparagine, glutamine, cysteine, histidine, and tyrosine, at T = (288.15, 303.15, and 313.15) K. These solution densities were used to calculate the partial molar volumes at infinite dilution, V_2°, for the tripeptides. The V_2° results were combined with those determined in previous work for T = 298.15 K to obtain the partial molar isobaric expansions at infinite dilution, E_2° $\{E_2^\circ = (\partial V_2^\circ/\partial T)_p\}$, for the tripeptides at T = 298.15 K. The contribution of amino acid side-chain hydration to the E_2° results has been discussed.

Introduction

The volumetric properties of aqueous solutions of small solutes that model specific structural features of proteins are of particular interest because of their use in group additivity schemes to evaluate the volumetric properties of fully unfolded proteins.[1−4] To ensure the quantitative success of any group additivity scheme, it is desirable to choose model compounds that match as close as possible the size, surface area, charge distribution, and hydrophobicity of the target moieties whose properties are to be evaluated. With this in mind, we proposed several years ago[5] that small peptides of amino acid sequence glycyl-X-glycine (gly-X-gly), where X represents one of the 20 amino acids, would be ideal compounds to model the various amino acid side-chains in proteins. In these peptides, the single side-chain of amino acid X is flanked by two peptide groups, which is structurally identical to that found in polypeptides and proteins. Additivity schemes utilizing group contributions derived from thermodynamic data for these tripeptides give reliable estimates of the partial molar volumes[1,6] and the partial molar heat capacities[1,7] of polypeptides over a wide temperature range.

The volumetric properties of protein model compounds have been determined, hitherto, primarily at a nominal pressure of 1 bar. For a quantitative assessment of the effect of pressure on proteins, it is desirable to have methods that enable volumetric properties to be determined over a wide pressure range. Such methods are now available. In recent work,[8,9] we described how the partial molar volumes, V_2°, the partial molar isentropic compressions, $K_{S,2}^\circ$, and the partial molar isothermal compressions, $K_{T,2}^\circ\{K_{T,2}^\circ = -(\partial V_2^\circ/\partial p)_T\}$, of solutes at infinite dilution in aqueous solution can be obtained from high precision sound speed measurements at high pressures. A prerequisite of the method is the availability of several thermodynamic properties at a pressure of 1 bar for solutions of the solutes of interest,[8] one of which is the partial molar isobaric expansion at infinite dilution, E_2° $\{E_2^\circ = (\partial V_2^\circ/\partial T)_p\}$. As part of previous work to determine protein side-chain heat capacities over a wide temperature range,[10] the partial molar volumes at infinite dilution for all the gly-X-gly peptides were determined over the temperature range (283.15 to 363.15) K using a differential scanning densimetric (DSD) method.[11] Although, in principle, these results could be used to derive partial molar expansions at infinite dilution, the relatively large uncertainties in V_2° that arise from the DSD method lead to values of E_2° with unacceptably large errors. The derivation of reliable volumetric properties of solutes from sound speed data at high pressures requires that the values of E_2° used are of high precision.[8] To achieve this objective, we report herein new solution densities at T = (288.15, 303.15, and 313.15) K for aqueous solutions of all the gly-X-gly peptides which have polar side-chains, viz., X = serine (ser), threonine (thr), asparagine (asn), glutamine (gln), cysteine (cys), histidine (his), and tyrosine (tyr). The V_2° results derived from these solution densities were combined with those determined previously[12,13] for T = 298.15 K to obtain E_2° values for the peptides at T = 298.15 K. For three of the peptides (X = ser, asn, and his), new V_2° values were also determined for T = 298.15 K. The partial molar expansions at infinite dilution for the gly-X-gly peptides which have nonpolar side-chains will be reported in a forthcoming publication.[14]

Experimental Section

The peptides glyhisgly and glytyrgly, which were samples recovered following previous experimental work,[11,15] were recrystallized from water + ethanol and dried under vacuum at room temperature. Since these peptides have been well characterized previously by both elemental and titrimetric analyses,[12,15] solution density was used as a criterion of purity in this study. The densities at T = 298.15 K for aqueous solutions of these peptides were in good agreement with those reported previously.[12,13] The peptide hydrate glythrgly·H_2O used was material remaining from a previous study.[11] The relative molar mass of the peptide hydrate, redetermined by alkalimetric titration,[16,17] was 252.1 ± 1.6 which is in excellent agreement with that for a monohydrate (M_r =

* Corresponding author. Tel.: +64 6 356 9099. Fax: +64 6 350 5682. E-mail: g.hedwig@massey.ac.nz.
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‡ Massey University.
§ University of Lethbridge.

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251.24). The densities at $T = 298.15$ K for aqueous solutions of the tripeptide hydrate were also in good agreement with previous results.[12] A new sample of glyglngly, purchased from Bachem Feinchemikalien, was recrystallized from water + ethanol to give a monohydrate, as determined by titrimetric analysis. As noted in previous work,[13] this hydrated peptide is very hygroscopic. The approach used to obtain reliable solution densities using this hygroscopic material is outlined vide infra. The sample of glycysgly (Bachem Feinchemikalien) was used as supplied to avoid possible decomposition during further purification steps. Aqueous solution densities at $T = 298.15$ K for the sample were in good agreement with those reported in previous work.[13]

The peptide glysergly was synthesized using the following procedure. The protected dipeptide *N*-benzyloxycarbonylglycylserine (4.1 g, 0.014 mol, Bachem) was dissolved in DMF (15 cm^3). To this solution was added glycine benzyl ester *p*-toluenesulfonate (4.7 g, 0.014 mol), which was prepared as described previously[5] and dissolved in dichloromethane (10 cm^3) and triethylamine (1.4 g, 0.014 mol). *N*-Hydroxysuccinimide (1.7 g, 0.015 mol), which acts as a trapping agent,[18] was dissolved in a mixture of dichloromethane (80 cm^3) and DMF (2 cm^3), and the resulting solution was added to the reaction mixture along with the coupling agent[19] *N*,*N*′-dicyclohexylcarbodiimide (3.1 g, 0.015 mol) dissolved in dichloromethane (10 cm^3). The reaction mixture was stirred overnight at room temperature. The precipitated dicyclohexylurea was removed by filtration, and the filtrate was washed with water, then 2 $mol \cdot dm^{-3}$ HCl(aq). Following the acid washing step, a white solid began to separate from the mixture. This solid, along with the organic phase, was chilled in an ice-bath for about 20 min, after which the solid was separated by filtration, then washed successively with water, half-saturated $NaHCO_3$(aq), and water. The crude protected tripeptide *N*-benzyloxycarbonylglycylserylglycine benzyl ester (ZglyserglyOBz) was then recrystallized from ethanol + diethylether. The overall yield was 3.1 g, 52 %. The protecting groups on the tripeptide were removed by catalytic hydrogenation using a Paar low-pressure shaker-type hydrogenator with 5 % Pd/C (Aldrich Chemical Co.) as the catalyst. A sample of ZglyserglyOBz (ca. 1.5 g) was dissolved in ethanol (ca. 150 cm^3) to which was added the catalyst suspended in a small amount of water. As the peptide formed during the hydrogenation is not soluble in ethanol, water was added during the course of the hydrogenation. On completion of the hydrogenation, the Pd/C was removed by filtration, and the filtrate was evaporated to dryness to give a white solid. The products from all the batch hydrogenations were combined and recrystallized from water + ethanol and dried under vacuum at room temperature. The overall yield was 1.25 g, 82 %. The relative molar mass determined by alkalimetric titrimetry was 220.3 ± 1.3 which is in good agreement with that expected for the anhydrous glysergly ($M_r = 219.20$). Elemental analyses gave the mass fractions: C, 0.383; H, 0.059; N, 0.189; cf. calculated composition for $C_7H_{13}N_3O_5$: C, 0.384; H, 0.060; N, 0.192.

The peptide glyasngly was also synthesized using the carbodiimide method.[19] The procedures used for the preparation of the starting material *N*-benzyloxycarbonylglycylasparagine, the coupling reaction, and the hydrogenation to remove the protecting groups have been described in detail elsewhere.[12] The combined product obtained following several batch hydrogenations was recrystallized from water + ethanol then dried under vacuum at room temperature. The relative molar mass determined by alkalimetric titrimetry was 247.1 ± 1.5 which is in good agreement with that expected for the anhydrous glyasngly ($M_r = 246.22$). Elemental analyses gave the mass fractions: C, 0.389; H, 0.058; N, 0.225; cf. calculated composition for $C_8H_{14}N_4O_5$: C, 0.390; H, 0.057; N, 0.228. The densities at $T = 298.15$ K for two aqueous solutions at molalities of ca. 0.03 $mol \cdot kg^{-1}$ were smaller by $6 \cdot 10^{-3}$ $kg \cdot m^{-3}$ than those determined in previous work[12] using a different batch of synthesized material.

Table 1. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycylserylglycine at $T = (288.15, 298.15, 303.15, \text{and } 313.15)$ K

m / ($mol \cdot kg^{-1}$)	ρ / ($kg \cdot m^{-3}$)	V_ϕ / ($cm^3 \cdot mol^{-1}$)	m / ($mol \cdot kg^{-1}$)	ρ / ($kg \cdot m^{-3}$)	V_ϕ / ($cm^3 \cdot mol^{-1}$)
		T/K = 288.15			
0.06526	1004.947[a]	128.90 ± 0.08	0.04543	1003.183	$128.8_7 \pm 0.1_1$
0.06026	1004.509	128.80 ± 0.08	0.04107	1002.788	$128.9_9 \pm 0.1_2$
0.05524	1004.055	128.91 ± 0.09	0.03542	1002.284	$128.9_7 \pm 0.1_4$
0.05019	1003.609	$128.8_4 \pm 0.1_0$	0.03014	1001.815	$128.8_4 \pm 0.1_7$
		T/K = 298.15			
0.06564	1002.841	130.30 ± 0.08	0.04024	1000.613	$130.2_4 \pm 0.1_3$
0.05980	1002.335	130.20 ± 0.08	0.03505	1000.158	$130.1_6 \pm 0.1_4$
0.05493	1001.905	130.25 ± 0.09	0.03060	999.768	$130.0_5 \pm 0.1_6$
0.05166	1001.615	$130.3_1 \pm 0.1_0$	0.02500	999.264	$130.3_4 \pm 0.2_0$
0.04521	1001.047	$130.3_2 \pm 0.1_1$			
		T/K = 303.15			
0.06473	1001.336	130.79 ± 0.08	0.04530	999.640	$130.7_8 \pm 0.1_1$
0.06095	1001.005	130.82 ± 0.08	0.03496	998.733	$130.7_9 \pm 0.1_4$
0.05550	1000.528	130.85 ± 0.09	0.02992	998.289	$130.8_4 \pm 0.1_7$
0.05003	1000.051	$130.8_4 \pm 0.1_0$	0.02533	997.881	$131.0_0 \pm 0.2_0$
		T/K = 313.15			
0.06498	997.808	132.80 ± 0.08	0.04401	996.025	$132.5_7 \pm 0.1_2$
0.05935	997.320	132.93 ± 0.09	0.03976	995.655	$132.6_7 \pm 0.1_3$
0.05419	996.891	132.72 ± 0.09	0.03329	995.098	$132.6_8 \pm 0.1_5$
0.04969	996.506	$132.7_0 \pm 0.1_0$	0.02969	994.793	$132.5_0 \pm 0.1_7$

[a] The repeatability for ρ is $\pm 5 \cdot 10^{-3}$ $kg \cdot m^{-3}$.

The water used to prepare solutions or as the reference solvent was either obtained from an Osmonics model Aries High-purity D. I. Loop that can produce water with a resistivity of typically $1.8 \cdot 10^5$ $\Omega \cdot m$ or was deionized and glass distilled. All solutions were prepared by mass, and corrections were made for the effect of air buoyancy.

Solution densities were measured either at the University of Lethbridge using a Sodev O2D vibrating-tube densimeter or at Massey University using an Anton Paar digital density meter (model DMA 60/602). The reproducibility of an individual density measurement was to within $\pm 5 \cdot 10^{-3}$ $kg \cdot m^{-3}$ for the Sodev instrument and $\pm 3 \cdot 10^{-3}$ $kg \cdot m^{-3}$ for the DMA instrument. Details of the procedures used have been outlined in previous work.[5,20−22]

Results and Discussion

Partial Molar Volumes. Densities at $T = (288.15, 303.15, \text{and } 313.15)$ K for aqueous solutions of the gly-X-gly peptides, X = (ser, thr, asn, gln, cys, his, and tyr), are given in Tables 1 to 7. Although satisfactory analyses were obtained for the synthesized glysergly used in our first studies at $T = 298.15$ K,[5] subsequent work[6,15] using a further recrystallized sample suggested that there may have been an impurity in the original material. Consequently, new solution densities at $T = 298.15$ K for glysergly are given in Table 1. Although the batch of glyhisgly used in this work gave solution densities at $T = 298.15$ K that differed only slightly ($\Delta\rho \leq 6 \cdot 10^{-3}$ $kg \cdot m^{-3}$) from those determined in earlier work,[12] new data determined for $T = 298.15$ K are included in Table 6. Solution densities at $T = 298.15$ K for the peptide glyasngly are also included in Table 3. These new data were determined because the V_2° value reported previously[12] was lower than expected, based on the V_2° results for the other temperatures.

Table 2. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycylthreonylglycine at T = (288.15, 303.15, and 313.15) K

m	ρ	V_ϕ	m	ρ	V_ϕ
(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})	(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})
		T/K = 288.15			
0.09131	1007.080[a]	144.74 ± 0.03	0.04925	1003.435	144.65 ± 0.06
0.07941	1006.047	144.81 ± 0.04	0.04017	1002.641	144.65 ± 0.08
0.07023	1005.256	144.75 ± 0.04	0.03395	1002.098	144.56 ± 0.09
0.05962	1004.342	144.61 ± 0.05	0.02986	1001.736	$144.6_5 \pm 0.1_0$
0.05602	1004.030	144.58 ± 0.05	0.02134	1000.987	$144.6_4 \pm 0.1_4$
		T/K = 303.15			
0.09143	1003.460	146.93 ± 0.03	0.05481	1000.357	146.92 ± 0.06
0.08055	1002.544	146.90 ± 0.04	0.04506	999.529	146.84 ± 0.07
0.07099	1001.730	146.95 ± 0.04	0.03031	998.267	$146.7_7 \pm 0.1_0$
0.06908	1001.574	146.87 ± 0.04	0.02019	997.395	$146.7_9 \pm 0.1_5$
0.05146	1000.078	146.80 ± 0.06			
		T/K = 313.15			
0.09325	1000.066	148.41 ± 0.03	0.04187	995.778	148.19 ± 0.07
0.08221	999.148	148.41 ± 0.04	0.03494	995.189	148.28 ± 0.09
0.07257	998.353	148.29 ± 0.04	0.02986	994.770	$147.9_0 \pm 0.1_0$
0.06044	997.339	148.25 ± 0.05	0.02395	994.263	$148.0_7 \pm 0.1_3$
0.04964	996.431	148.23 ± 0.06			

[a] The repeatability for ρ is ± 3·10^{-3} kg·m^{-3}.

Table 3. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycylasparagylglycine at T = (288.15, 298.15, 303.15, and 313.15) K

m	ρ	V_ϕ	m	ρ	V_ϕ
(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})	(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})
		T/K = 288.15			
0.06951	1005.982[a]	146.27 ± 0.07	0.04516	1003.597	$146.0_6 \pm 0.1_1$
0.06457	1005.500	146.22 ± 0.08	0.03979	1003.071	$145.9_0 \pm 0.1_3$
0.05974	1005.028	146.18 ± 0.08	0.03248	1002.340	$146.0_8 \pm 0.1_5$
0.05155	1004.231	$146.0_0 \pm 0.1_0$	0.02634	1001.735	$145.8_6 \pm 0.1_9$
		T/K = 298.15			
0.05892	1002.802[b]	147.84 ± 0.05	0.03370	1000.352	147.80 ± 0.09
0.05367	1002.289	147.91 ± 0.06	0.03159	1000.147	$147.7_7 \pm 0.1_0$
0.04860	1001.800	147.87 ± 0.06	0.02795	999.790	$147.8_2 \pm 0.1_1$
0.04282	1001.244	147.74 ± 0.07	0.02484	999.489	$147.7_0 \pm 0.1_2$
0.03757	1000.729	147.81 ± 0.08	0.02287	999.295	$147.7_5 \pm 0.1_3$
		T/K = 303.15			
0.06187	1001.639[b]	148.76 ± 0.05	0.03190	998.757	$148.5_8 \pm 0.1_0$
0.05540	1001.019	148.74 ± 0.06	0.02783	998.360	$148.6_7 \pm 0.1_1$
0.04971	1000.473	148.70 ± 0.06	0.02508	998.095	$148.6_0 \pm 0.1_2$
0.04290	999.814	148.76 ± 0.07	0.02228	997.821	$148.6_6 \pm 0.1_4$
0.03772	999.316	148.71 ± 0.08			
		T/K = 313.15			
0.06187	998.140[b]	150.05 ± 0.05	0.03190	995.290	$149.9_0 \pm 0.1_0$
0.05540	997.526	150.05 ± 0.06	0.02783	994.898	$149.9_7 \pm 0.1_1$
0.04971	996.987	150.00 ± 0.06	0.02508	994.635	$149.9_5 \pm 0.1_2$
0.04290	996.336	150.05 ± 0.07	0.02228	994.368	$149.8_4 \pm 0.1_4$
0.03772	995.842	150.05 ± 0.08			

[a] The repeatability for ρ is ± 5·10^{-3} kg·m^{-3}. [b] The repeatability for ρ is ± 3·10^{-3} kg·m^{-3}.

In a previous study of the hygroscopic peptide glyglngly,[13] particular care was taken to monitor the water content of the solid sample during the density data collection at T = 298.15 K. To avoid using this rather laborious procedure at the new temperatures chosen in this work, densities of the glyglngly solutions were measured at both T = 298.15 K and the new temperature T. The power series in solution molality, m, reported previously[13]

$$\rho = \rho_1^\circ + (p_1 m) + (p_2 m^2) \quad (1)$$

where ρ and ρ_1° are, respectively, the densities of the solution and solvent and p_1 and p_2 are parameters determined by least-squares fitting to the density data at T = 298.15 K, was then used to calculate the molality for each glyglngly solution from its density at T = 298.15 K.

Table 4. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycylglutaminylglycine at T = (288.15, 303.15, and 313.15) K

m	ρ	V_ϕ	m	ρ	V_ϕ
(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})	(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})
		T/K = 288.15			
0.06734	1005.830[a]	159.31 ± 0.05	0.04216	1003.333	159.26 ± 0.07
0.05780	1004.881	159.38 ± 0.05	0.03159	1002.275	$159.3_2 \pm 0.1_0$
0.04626	1003.744	159.19 ± 0.07	0.02725	1001.836	$159.5_0 \pm 0.1_1$
		T/K = 303.15			
0.05656	1001.186	161.75 ± 0.05	0.04087	999.656	161.85 ± 0.07
0.05226	1000.764	161.85 ± 0.06	0.03622	999.207	161.75 ± 0.08
0.04429	999.990	161.84 ± 0.07	0.03193	998.782	$161.9_3 \pm 0.1_0$
0.04323	999.880	161.99 ± 0.07	0.03003	998.601	$161.7_9 \pm 0.1_0$
		T/K = 313.15			
0.05656	997.683	163.26 ± 0.05	0.03622	995.724	163.43 ± 0.08
0.05226	997.267	163.35 ± 0.06	0.03193	995.319	$163.1_6 \pm 0.1_0$
0.04323	996.398	163.41 ± 0.07	0.03003	995.134	$163.2_3 \pm 0.1_0$

[a] The repeatability for ρ is ± 3·10^{-3} kg·m^{-3}.

Table 5. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycylcysteinylglycine at T = (288.15, 303.15, and 313.15) K

m	ρ	V_ϕ	m	ρ	V_ϕ
(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})	(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})
		T/K = 288.15			
0.04953	1003.707[a]	141.66 ± 0.06	0.02984	1001.884	$141.6_4 \pm 0.1_0$
0.04442	1003.233	141.69 ± 0.07	0.02582	1001.513	$141.5_3 \pm 0.1_2$
0.04097	1002.915	141.67 ± 0.07	0.02087	1001.053	$141.4_9 \pm 0.1_4$
0.03950	1002.783	141.56 ± 0.08	0.01633	1000.629	$141.5_2 \pm 0.1_9$
0.03474	1002.336	141.72 ± 0.09			
		T/K = 303.15			
0.05296	1000.429	144.56 ± 0.06	0.03394	998.731	144.26 ± 0.09
0.05003	1000.168	144.53 ± 0.06	0.03046	998.417	$144.2_6 \pm 0.1_0$
0.04358	999.593	144.44 ± 0.07	0.02788	998.184	$144.2_3 \pm 0.1_1$
0.04008	999.279	144.42 ± 0.08	0.02497	997.920	$144.2_5 \pm 0.1_2$
0.03740	999.043	144.29 ± 0.08	0.02103	997.568	$144.0_0 \pm 0.1_4$
		T/K = 313.15			
0.05751	997.331	146.06 ± 0.05	0.03941	995.739	145.85 ± 0.08
0.05218	996.867	145.93 ± 0.06	0.03597	995.437	145.75 ± 0.08
0.05002	996.672	146.02 ± 0.06	0.03109	995.001	$145.8_0 \pm 0.1_0$
0.04508	996.242	145.87 ± 0.07	0.02094	994.099	$145.6_2 \pm 0.1_5$

[a] The repeatability for ρ is ± 3·10^{-3} kg·m^{-3}.

The solution densities were used to calculate the apparent molar volumes of the tripeptides, V_ϕ, using the equation

$$V_\phi = (M_2/\rho) - (\rho - \rho_1^\circ)/(m\rho\rho_1^\circ) \quad (2)$$

where M_2 is the molar mass of the solute and the other symbols are as defined for eq 1. The values of ρ_1° for water at the various temperatures used were those reported by Kell[23] {ρ_1° = (999.101, 997.047, 995.650, and 992.219) kg·m^{-3} at T = (288.15, 298.15, 303.15, and 313.15) K, respectively}. The V_ϕ values, together with their uncertainties estimated using the procedures outlined earlier,[20] are given in Tables 1 to 7. For the dilute solutions used in this work, the molality dependence of V_ϕ for the tripeptides at the various temperatures can be represented by the linear equation

$$V_\phi = V_2^\circ + (S_v m) \quad (3)$$

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, and their standard errors obtained from weighted least-squares analyses of the V_ϕ data using eq 3, are given in Table 8. In these analyses, the weighting factors used were the inverse squares of the uncertainties of the apparent molar volumes. For several systems, in particular for glyhisgly, which is sparingly soluble in water, and for the hygroscopic peptide glyglngly, the values of S_v obtained from the

Table 6. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycylhistidylglycine at T = (288.15, 298.15, 303.15, and 313.15) K

m	ρ	V_ϕ	m	ρ	V_ϕ
(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})	(mol·kg^{-1})	(kg·m^{-3})	(cm^3·mol^{-1})
		T/K = 288.15			
0.01205_0	1000.307[a]	$169.0_3 \pm 0.4_2$	0.00705_1	999.802	$169.7_8 \pm 0.7_1$
0.01088_4	1000.191	$168.9_9 \pm 0.4_6$	0.00607_2	999.708	$169.2_5 \pm 0.8_3$
0.01011_9	1000.112	$169.2_4 \pm 0.4_9$	0.00501_0	999.600	$169.6_3 \pm 1.0$
0.00901_0	1000.003	$169.0_6 \pm 0.5_6$	0.00401_9	999.501	$169.7_3 \pm 1.3$
0.00812_7	999.911	$169.5_2 \pm 0.6_2$			
		T/K = 298.15			
0.01226_7	998.254	$170.8_7 \pm 0.4_1$	0.00804_1	997.838	$170.9_7 \pm 0.6_3$
0.01109_1	998.142	$170.5_6 \pm 0.4_5$	0.00722_6	997.762	$170.4_0 \pm 0.7_0$
0.01009_0	998.038	$171.0_9 \pm 0.5_0$	0.00400_9	997.442	$170.8_9 \pm 1.3$
0.00901_0	997.939	$170.3_1 \pm 0.5_6$			
		T/K = 303.15			
0.01224_7	996.842	$172.0_4 \pm 0.4_1$	0.00610_3	996.247	$171.6_5 \pm 0.8_3$
0.01105_8	996.730	$171.7_3 \pm 0.4_6$	0.00498_0	996.138	$171.5_0 \pm 1.0$
0.00797_5	996.427	$172.0_2 \pm 0.6_3$	0.00404_0	996.046	$171.4_9 \pm 1.3$
0.00711_0	996.346	$171.5_6 \pm 0.7_1$			
		T/K = 313.15			
0.01232_2	993.400	$173.8_1 \pm 0.4_1$	0.00693_6	992.890	$173.0_0 \pm 0.7_3$
0.01138_4	993.309	$173.9_3 \pm 0.4_5$	0.00605_1	992.804	$173.0_7 \pm 0.8_4$
0.01022_4	993.207	$173.0_4 \pm 0.5_0$	0.00510_8	992.711	$173.4_5 \pm 0.9_9$
0.00820_0	993.012	$173.0_1 \pm 0.6_2$			

[a] The repeatability for ρ is $\pm 5 \cdot 10^{-3}$ kg·m^{-3}.

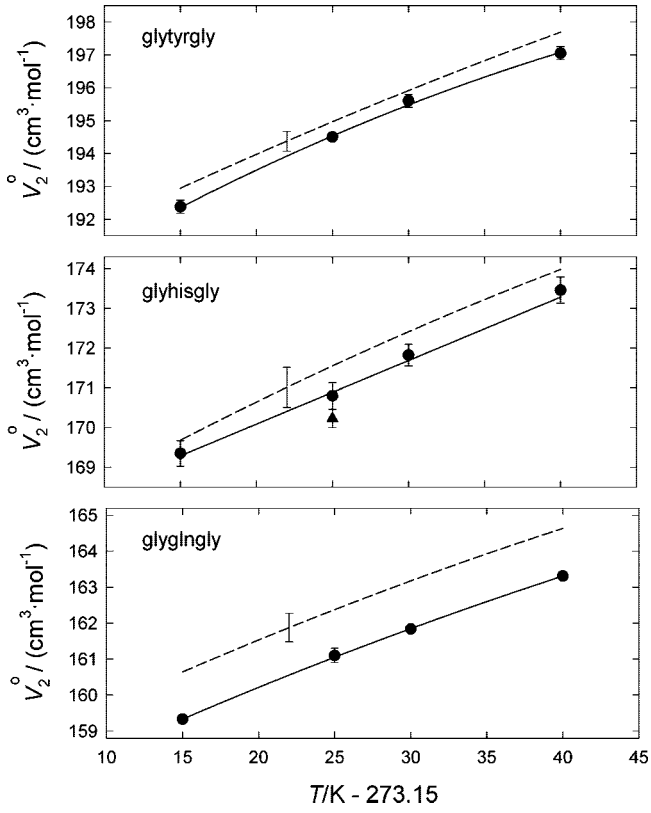

Figure 1. Temperature dependences of the partial molar volumes for the tripeptides glytyrgly, glyhisgly, and glyglngly: ●, this work; ▲, from ref 12; - - -, results obtained using DSD, glytyrgly from ref 15, glyhisgly and glyglngly from ref 11.

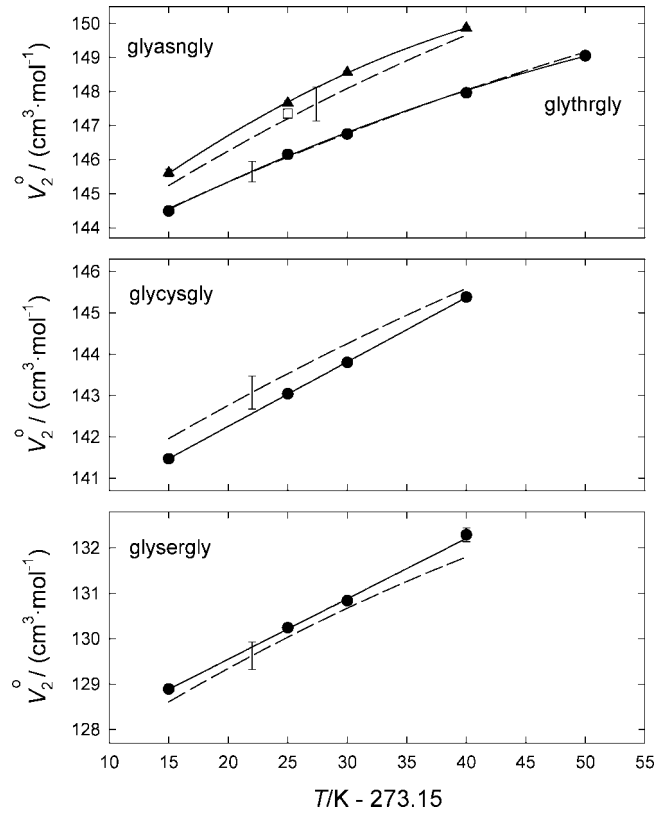

Figure 2. Temperature dependences of the partial molar volumes for the tripeptides glyasngly, glythrgly, glycysgly, and glysergly: ●, this work; □, from ref 12; - - -, results obtained using DSD, glyasngly from ref 6, glythrgly and glycysgly from ref 11, glysergly from ref 15.

least-squares analyses were not statistically significant. The V_2° results for these systems, reported in Table 8, are actually the mean values of the V_ϕ data, and the uncertainties given are the standard deviations. Included in Table 8 are the V_2° and S_v results for the tripeptides obtained previously[5,12,13] at T = 298.15 K and also at T = 323.15 K for the peptide glythrgly.[24] The V_2° value at T = 298.15 K for glysergly obtained in this work is 0.9 cm^3·mol^{-1} smaller than that reported in our first study,[5] but it is in excellent agreement with that obtained in subsequent work[15] using the less precise DSD method, viz., (130.0 ± 0.3) cm^3·mol^{-1}. The previous[12] V_2° value for glyhisgly at T = 298.15 K was obtained from a weighted least-squares analysis of the V_ϕ data. With the benefit of hindsight, the molality range accessible for this sparingly soluble peptide is too narrow for reliable results to be obtained from an analysis using eq 3. The mean value of the V_ϕ data reported previously[12] and its standard deviation are 170.2_3 and 0.2_3 cm^3·mol^{-1}, respectively. This estimate of the V_2° value is certainly in better agreement with that obtained herein. The value of V_2° for glyasngly at T = 298.15 K obtained in this study is 0.31 cm^3·mol^{-1} larger than that reported previously.[12] This is due, in part, to the smaller value for S_v obtained in this work. In this context, it is worth noting that at the higher molalities two of the V_ϕ values are in agreement, within the combined uncertainties, with the V_ϕ values calculated using eq 3 and the V_2° and S_v results given in previous work.[12]

Partial Molar Expansions. The temperature dependences of V_2° for the gly-X-gly peptides are shown in Figures 1 and 2. For each tripeptide, the equation

$$V_2^\circ = a + b(T - T_m) + c(T - T_m)^2 \quad (4)$$

where T_m represents the midpoint temperature of the range used (T_m = 300.65 K for all peptides except glythrgly, for which T_m = 305.65 K), was fitted to the V_2° data using a weighted least-squares procedure, with the weights taken as the inverse squares of the V_2° uncertainties. The polynomial coefficients of eq 4, together with their uncertainties obtained from the least-squares analyses, are given in Table 9. For each of the peptides gly-X-gly, X = ser, cys, and his, the value of the coefficient c was not statistically

Table 7. Densities and Apparent Molar Volumes for Aqueous Solutions of Glycyltyrosylglycine at T = (288.15, 303.15, and 313.15) K

m / (mol·kg^{-1})	ρ / (kg·m^{-3})	V_ϕ / (cm^3·mol^{-1})	m / (mol·kg^{-1})	ρ / (kg·m^{-3})	V_ϕ / (cm^3·mol^{-1})
		T/K = 288.15			
0.02909	1002.058[a]	$193.1_6 \pm 0.1_0$	0.02384	1001.531	$192.9_6 \pm 0.1_3$
0.02790	1001.946	$192.8_8 \pm 0.1_1$	0.02300	1001.449	$192.8_5 \pm 0.1_3$
0.02705	1001.853	$193.1_0 \pm 0.1_1$	0.02117	1001.261	$192.9_3 \pm 0.1_4$
0.02575	1001.724	$193.0_2 \pm 0.1_1$	0.01938	1001.081	$192.8_4 \pm 0.1_6$
0.02504	1001.650	$193.0_7 \pm 0.1_2$	0.01708	1000.848	$192.7_9 \pm 0.1_8$
		T/K = 303.15			
0.02882	998.496	$196.4_1 \pm 0.1_1$	0.02301	997.932	$196.0_8 \pm 0.1_3$
0.02792	998.415	$196.1_5 \pm 0.1_1$	0.02087	997.719	$196.1_9 \pm 0.1_5$
0.02701	998.323	$196.2_2 \pm 0.1_1$	0.01900	997.535	$196.1_2 \pm 0.1_6$
0.02604	998.224	$196.3_5 \pm 0.1_2$	0.01781	997.420	$195.9_8 \pm 0.1_7$
0.02501	998.127	$196.1_7 \pm 0.1_2$	0.01685	997.325	$196.0_0 \pm 0.1_8$
0.02396	998.023	$196.2_3 \pm 0.1_3$			
		T/K = 313.15			
0.02902	995.047	$198.0_6 \pm 0.1_1$	0.02288	994.456	$197.8_8 \pm 0.1_3$
0.02763	994.913	$198.0_3 \pm 0.1_1$	0.02160	994.332	$197.8_2 \pm 0.1_4$
0.02688	994.842	$197.9_9 \pm 0.1_1$	0.02086	994.259	$197.8_7 \pm 0.1_5$
0.02586	994.742	$198.0_0 \pm 0.1_2$	0.01889	994.065	$198.0_0 \pm 0.1_6$
0.02492	994.655	$197.8_4 \pm 0.1_2$	0.01785	993.972	$197.5_3 \pm 0.1_7$
0.02376	994.546	$197.6_9 \pm 0.1_3$	0.01700	993.889	$197.5_0 \pm 0.1_8$

[a] The repeatability for ρ is $\pm 3 \cdot 10^{-3}$ kg·m^{-3}.

Table 8. Partial Molar Volumes at Infinite Dilution, and the S_v Values, for the Tripeptides in Aqueous Solution at Various Temperatures

T / (K)	V_2° / (cm^3·mol^{-1})	S_v / (cm^3·kg·mol^{-2})	T / (K)	V_2° / (cm^3·mol^{-1})	S_v / (cm^3·kg·mol^{-2})
	glycysgly			glythrgly	
288.15	$141.4_7 \pm 0.1_0$	$4._2 \pm 2._4$	288.15	144.49 ± 0.07	$3._1 \pm 1._0$
298.15	143.04 ± 0.05[a]	$8._7 \pm 1._1$[a]	298.15	146.15 ± 0.04[b]	2.3 ± 0.3[b]
303.15	143.80 ± 0.06	$14._5 \pm 1._3$	303.15	146.75 ± 0.06	2.0 ± 0.8
313.15	145.38 ± 0.09	$11._6 \pm 1._8$	313.15	147.96 ± 0.07	$5._0 \pm 1._0$
			323.15	149.05 ± 0.04[c]	4.3 ± 0.4[c]
	glytyrgly			glyglngly	
288.15	$192.3_8 \pm 0.2_3$	$24._5 \pm 9$	288.15	$159.3_3 \pm 0.1_1$	[d]
298.15	$194.5_2 \pm 0.1_1$[a]	$23._4 \pm 5$[a]	298.15	161.1 ± 0.2[a]	[d]
303.15	$195.6_0 \pm 0.2_0$	$24._7 \pm 8$	303.15	161.84 ± 0.08	[d]
313.15	$197.0_5 \pm 0.2_3$	$34._8 \pm 9$	313.15	$163.3_1 \pm 0.1_1$	[d]
	glysergly			glyhisgly	
288.15	128.89 ± 0.07	[d]	288.15	$169.3_6 \pm 0.3_1$	[d]
298.15	130.24 ± 0.09	[d]	298.15	$170.7_3 \pm 0.3_0$	[d]
	131.13 ± 0.05[e]	1.9 ± 0.4[e]		169.9 ± 0.2[b]	
303.15	130.84 ± 0.07	[d]	303.15	$171.7_2 \pm 0.2_3$	[d]
313.15	$132.2_9 \pm 0.1_5$	$8._7 \pm 2._9$	313.15	$173.3_3 \pm 0.4_0$	[d]
	glyasngly				
288.15	$145.6_1 \pm 0.1_1$	$9._3 \pm 1._8$			
298.15	147.66 ± 0.07	$3._6 \pm 1._4$			
	147.35 ± 0.05[b]	$8._0 \pm 1._0$[b]			
303.15	148.56 ± 0.05	$3._3 \pm 1._1$			
313.15	149.86 ± 0.06	$3._3 \pm 1._1$			

[a] From ref 13. [b] From ref 12. [c] From ref 24. [d] See text. [e] From ref 5.

Table 9. Coefficients of Equation 4 and the Partial Molar Expansions at Infinite Dilution and T = 298.15 K for the Tripeptides in Aqueous Solution

tripeptide	a / (cm^3·mol^{-1})	b / (cm^3·mol^{-1}·K^{-1})	$10^4 c$ / (cm^3·mol^{-1}·K^{-2})	E_2° / (cm^3·mol^{-1}·K^{-1})
glysergly	130.55 ± 0.03	$0.133_1 \pm 0.003_1$		0.133 ± 0.003
glythrgly	147.13 ± 0.05	$0.128_6 \pm 0.002_9$	$-11._1 \pm 2._7$	0.145 ± 0.003
glyasngly	148.13 ± 0.02	$0.170_8 \pm 0.002_7$	$-25._9 \pm 2._7$	0.184 ± 0.003
glyglngly	161.46 ± 0.02	$0.159_0 \pm 0.001_6$	$-8._7 \pm 1._9$	0.163 ± 0.002
glycysgly	143.423 ± 0.006	$0.156_0 \pm 0.001_0$		0.156 ± 0.001
glyhisgly	171.28 ± 0.06	$0.159_9 \pm 0.008_1$		0.160 ± 0.008
glytyrgly	195.02 ± 0.07	$0.188_8 \pm 0.007_9$	$-19._2 \pm 8._1$	0.198 ± 0.009

significant, hence a linear function was used in the least-squares analysis. The solid-line curves drawn in Figures 1 and 2 are those calculated using eq 4 and the coefficients given in Table 9. For the purposes of comparison, the temperature dependences of V_2° for the tripeptides, obtained previously[6,11,15] using the less precise DSD method, are also included in Figures 1 and 2. With the exception of the peptide glyglngly, the results obtained using DSD and those obtained in this study are, in general, in satisfactory agreement. The larger molar volumes for glyglngly obtained by DSD over the temperature range (288.15 to 313.15) K are consistent with possible water adsorption by the very hygroscopic solid during the preparation of the solutions used in the DSD study.[11] The V_2° (T) curve obtained using DSD does, however, lie parallel to that obtained in this work.

The partial molar isobaric expansion for each peptide at infinite dilution, E_2°, can be derived from the polynomial coefficients given in Table 9. Differentiation of eq 4 with respect to temperature at constant pressure gives

$$E_2^\circ = (\partial V_2^\circ/\partial T)_p = b + 2c(T - T_m) \quad (5)$$

It follows from eq 5 that the quantity $\{b + 2c(298.15 - T_m)\}$ is equivalent to E_2° at a temperature of $T = 298.15$ K. These E_2° values for the tripeptides are given in Table 9. The uncertainty for each E_2° was estimated by the application of propagation of errors[25] to eq 5.

Various semiempirical models are often used to rationalize the volumetric properties of small solutes in aqueous solution.[26−29] One such model that involves an interpretation in terms of hydration effects is based on the relationship[26]

$$V_2^\circ = V_{int} + n_h(V_h - V_1^\circ) \quad (6)$$

where V_{int} is the intrinsic volume of the solute molecule; n_h is the "hydration number", i.e., the number of water molecules in the hydration shell of the solute; and V_h and V_1° are, respectively, the partial molar volumes of water in the hydration shell and in the bulk solvent. The value of n_h, which is determined largely by the number of water molecules in the first hydration shell, should not vary significantly with temperature, at least over the moderate temperature changes considered in this work.[29] Consequently, differentiating eq 6 with respect to temperature at constant pressure gives

$$E_2^\circ = (\partial V_2^\circ/\partial T)_p = (\partial V_{int}/\partial T)_p + n_h(E_h - E_1^\circ) \quad (7)$$

where E_h $\{E_h = (\partial V_h/\partial T)_p\}$ is the partial molar expansion of water in the hydration shell of the solute and E_1° $\{E_1^\circ = (\partial V_1^\circ/\partial T)_p\}$ is the partial molar volume of water in the bulk solvent. For solutes of low molar mass, the temperature dependence of the intrinsic volume can be neglected, at least to a first approximation, because it essentially involves the expansion of covalent bonds,[26,30] hence eq 7 reduces to

$$E_2^\circ = n_h(E_h - E_1^\circ) \quad (8)$$

It is immediately apparent from eq 8 that the thermodynamic property E_2° for any solute ought to be a sensitive measure of solute−solvent interactions. A perusal of the E_2° results presented in Table 9 confirms that this is indeed the case. The insertion of a methylene unit in the side-chain of glysergly to give glythrgly results in an increase in the value of E_2°, whereas the same insertion in the side-chain of glyasngly to give glyglngly leads to a decrease in the value of E_2°.

If eqs 6 and 8 are combined to eliminate n_h, then E_2° can be expressed as

$$E_2^\circ = (V_2^\circ - V_{int})(E_h - E_1^\circ)/(V_h - V_1^\circ) \quad (9)$$

This relationship is explored in Figure 3 in which a plot of E_2° versus the quantity $(V_2^\circ - V_w)$ is displayed. The van der Waals volume, V_w, which was used as an estimate of the intrinsic volume for each tripeptide, was calculated as a sum of atomic and bond contributions, using the procedure outlined by Zhao et al.[31] The scatter of the data given in Figure 3 indicates that the interpretation of E_2° for these gly-X-gly peptides is certainly not simple. Clearly, the value of the quantity $(E_h - E_1^\circ)/(V_h - V_1^\circ)$ for each peptide does depend on the nature of the hydrophilic side-chain. However, there does appear to be, perhaps somewhat fortuitously, an approximate correlation between E_2° and $(V_2^\circ - V_w)$ for the tripeptides glysergly, glythrgly, glycysgly, and glytyrgly. The common feature among these peptides is the presence in the side-chain of the −OH functional group, or in the case of glycysgly

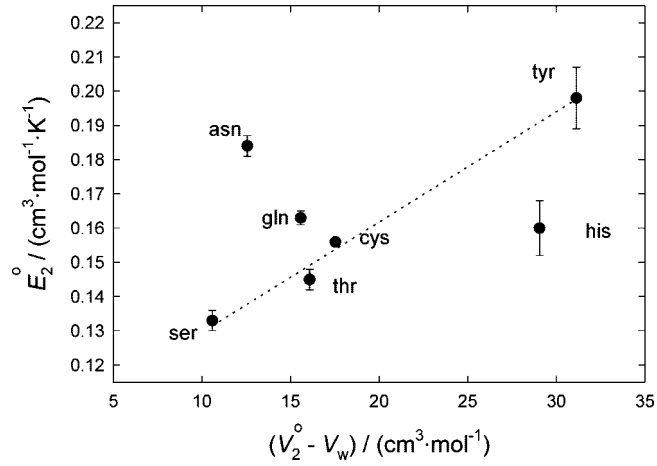

Figure 3. Plot of E_2° at $T = 298.15$ K for the tripeptides as a function of the difference between the partial molar volume, V_2°, and the van der Waals volume, V_w: ●, this work; ····, see text.

Table 10. Amino Acid Side-Chain Contributions to E_2° at $T = 298.15$ K Derived Using Tripeptides and *N*-Acetyl Amino Acid Amides as Model Compounds

	E°(R)/(cm^3·mol^{-1}·K^{-1})	
side-chain (R)	gly-X-gly	$AcXNH_2$
ser	-0.022 ± 0.004	0.043 ± 0.007^a
thr	-0.010 ± 0.004	0.0320 ± 0.0009^a
asn	0.028 ± 0.004	0.038 ± 0.002^b
gln	0.008 ± 0.004	0.046 ± 0.002^b
cys	0.000 ± 0.003	
his	0.004 ± 0.009	
tyr	$0.04_3 \pm 0.01_0$	$0.07_7 \pm 0.01_0{}^b$

[a] E_2° values for $AcXNH_2$ were derived using $V_2^\circ(T)$ data from ref 36. [b] E_2° values for $AcXNH_2$ from ref 35.

the closely related −SH moiety. Thus, similar aspects of side-chain hydration for these four tripeptides would not be unexpected.

Various empirical group additivity schemes have been used successfully to provide reliable estimates of the partial molar volumes of compounds from the knowledge of their molecular structure and the respective group contributions.[1−4,28,32] However, to date, the application of group additivity methods to both the temperature and pressure derivatives of the partial molar volume, i.e., the partial molar expansion and the partial molar compression, has been less successful.[30,33,34] In previous work,[6,12,13] the partial molar volume of an amino acid side-chain R, V°(R), was derived using the expression

$$V^\circ(R) = V_2^\circ(\text{gly-X-gly}) - V_2^\circ(\text{glyglygly}) \quad (10)$$

where V_2°(gly-X-gly) and V_2°(glyglygly) are the partial molar volumes at infinite dilution for the species in parentheses. These V°(R) values that are used in our group additivity scheme to calculate the partial molar volumes of unfolded proteins[1,11] actually give the contributions to the partial molar volume of polypeptide on replacing a methylene H atom of the glycyl group by the side-chain R. Using an expression analogous to eq 10, the contributions to the partial molar expansion of a peptide of some side-chains were derived using the E_2° results given in Table 9 and the E_2° value for triglycine,[33] (E_2°(glyglygly) = $(0.155_5 \pm 0.003)$ cm^3·mol^{-1}·K^{-1}). These E°(R) results are given in Table 10. Included in Table 10 are the side-chain contributions derived using E_2° values reported[35,36] for some *N*-acetyl amino acid amides ($AcXNH_2$) and for *N*-acetylglycinamide ($AcglyNH_2$).[30] It is clear from these E°(R) results that the side-chain contributions to E_2° of a peptide derived using tripeptides as model compounds differ significantly

from those based on the *N*-acetyl amino acid amides. These *N*-acetyl amino acid amides were shown in previous work[35] to be a realistic set of model compounds that gave side-chain volumes that are in good agreement with those obtained using the tripeptides. In other words, the group contributions recommended for use in the additivity scheme to calculate partial molar volumes of polypeptides are not model dependent.[30] In contrast, this is not the case for the partial molar expansion. Even though in both sets of model compounds the single side-chain is flanked by two peptide groups, there must be interactions between the hydrated side-chain and other functional groups beyond these peptide groups that make significant contributions to the partial molar expansion. For any additivity scheme to have predictive utility for E_2°, these more complex interactions would, somehow, have to be considered.

Acknowledgment

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