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Thermodynamic Properties of Peptide Solutions 3. Partial Molar Volumes and Partial Molar Heat Capacities of Some Tripeptides in Aqueous Solution

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Partial molar volumes, V_2^o, and partial molar heat capacities, $C_{p,2}^o$, of the tripeptides glycylglycylglycine, glycylglycylalanine, glycylalanylglycine and alanylglycylglycine have been determined in aqueous solution at 25°C. For the three alanyl-containing tripeptides, the data indicate that the tripeptide-water interaction is influenced by the side chain position within the molecule. The results have been rationalized in terms of likely solute-solvent interactions. The V_2^o and $C_{p,2}^o$ data have also been used to calculate the contribution to these properties of a $-CH_3$ side chain.

KEY WORDS: Partial molar heat capacity; partial molar volume; tripeptide; aqueous solution; density; methyl group contribution.

1. INTRODUCTION

The various non-covalent interactions such as hydrogen bonding, electrostatic interactions, hydrophobic interactions and dispersion forces are very important in biological systems.[1-3] In aqueous protein solutions there are a large number of these non-covalent forces that occur among the different amino acid residues of the polypeptide chain with each other and with the surrounding aqueous solvent medium. The folded conformation adopted by a globular protein in aqueous solution results largely from a delicate balance of these non-covalent or non-bonding interactions.[1]

As proteins are particularly complex molecules, one useful approach that can be used to understand the conformational stability and unfolding behavior of proteins is to study model compounds.[4] A deter-

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mination of thermodynamic parameters characterizing solute-solute and solute-solvent interactions for low molecular weight solutes that incorporate some of the structural features associated with globular proteins, can provide information on the contribution of various non-covalent interactions in protein folding and stability.[1] One such group of model solutes comprises the amino acids, small peptides and their derivatives.

Two thermodynamic properties for aqueous solutions of these solutes that have received considerable attention are the limiting partial molar volume, V_2^o and the limiting partial molar heat capacity, $C_{p,2}^o$. For the amino acids, V_2^o and $C_{p,2}^o$ data have been determined by many workers.[5,6] One of the main objectives of these studies has been to assess the contribution of the amino acid side chain in the solute-solvent interaction and to investigate group additivity relationships. Aqueous solutions of peptides, in particular dipeptides, have received increasing attention in recent years.[7-13] From the V_2^o and $C_{p,2}^o$ data for the dipeptides it is apparent that the side chain contributions are different from those for the corresponding amino acids. In the dipeptides, the separated charged amino and carboxyl end groups have a greater influence on the peptide side chain solvation than in the α-amino acids where the charges are adjacent. There is some interest then in determining thermodynamic properties for aqueous solutions of tri- and tetra-peptides incorporating side chains that are further removed from the influence of the ionic end groups.

As part of a continuing study of peptide solutions we reported recently[14,15] the relative partial molar enthalpies at 25°C of aqueous solutions of the tripeptides glycylglycylglycine (GGG), DL-alanylglycylglycine (AGG), glycyl-L-alanylglycine (GAG) and glycylglycyl-L-alanine (GGA). The enthalpy pair interaction coefficients obtained indicated that solute-solute interaction is influenced by the position of the methyl side chain within the tripeptide. In order to examine the role of side chain position in solute-solvent interactions, we report here the V_2^o and $C_{p,2}^o$ data at 25°C for the above four tripeptides.

2. EXPERIMENTAL

2.1. Materials

The purification and analysis of each of the tripeptides GGG, AGG and GGA have been reported in a previous publication.[14] A redetermination of the relative molar mass of the hydrated AGG gave 239.6±1 g-mol^{-1}, in excellent agreement with 239.23 g-mol^{-1} expected for 2.00 H_2O of crystallization.

GAG was synthesized using the classical carbodiimide method[16] followed by hydrogenation. The *p*-toluenesulfonate salt of glycine benzyl ester (27.7 g, 0.082 mol), prepared by the standard azeotrope distillation method,[17] was coupled with 23.0 g (0.082 mol) of the protected dipeptide N-carbobenzoxyglycyl-L-alanine (Sigma Chemical) using N,N'-dicyclohexylcarbodiimide as the coupling reagent. The protected tripeptide N-carbobenzoxyglycyl-L-alanylglycine benzyl ester was isolated using procedures similar to those described for other small peptides.[16] The yield was 21.5 g (62%). The protecting groups were removed by hydrogenation in acetone-water using a Parr low pressure shaker-type hydrogenator and 5% Pd/C as the catalyst. The hydrogenation was carried out batchwise using 3-4 g amounts of the protected tripeptide; yields were in the range 80-90%. The products obtained from each hydrogenation were combined together and recrystallized from water-ethanol by diffusing ethanol vapor into an aqueous solution of the tripeptide. The purity of the tripeptide was checked using a number of methods. Thin-layer chromatograms of the tripeptide gave a single spot with the chromatographic solvent mixtures propanol-formic acid-water and butanol-pyridine-water; a single peak was observed using HPLC (Waters Radial-pak C_{18} reversed phase column, NH_4HCO_3 / $CH_3CH(OH)CH_3$ as the solvent). Optical rotation measurements (Optical Activity Ltd. AA-100 polarimeter) gave $[\alpha]_D^{(23)} = -65.9°$, in good agreement with the literature result,[18] $[\alpha]_D^{(24)} = -65.3°$. Alkalimetric titration[14,19] confirmed that the peptide was anhydrous and elemental analyses gave: found, C 41.3, H 6.2, N 20.5%; calculated for $C_7H_{13}N_3O_4$, C 41.4, H 6.5, N 20.7%.

Samples of GGG, GGA and GAG were dried under vacuum at room temperature before use. AGG dihydrate, which was stored in a desiccator over silica gel, was used without vacuum drying. In calculating the molalities of AGG solutions, the molar mass used was that corresponding exactly to a dihydrate. All water used, both to prepared 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.

2.2. Apparatus and Methods

Density measurements were made at 25°C using an Anton Paar vibrating-tube digital density meter (Model DMA 60/602). The temperature of the fluid surrounding the density cell was maintained to ±0.002°C (Sodev, Model CT-L Circulating Thermostat). A thermistor probe was used to monitor the temperature of the density meter cell.

The thermistor was calibrated against a Quartz Thermometer (Hewlett-Packard 2801A) the accuracy of which has been recently certified to be better than 0.01°C.[20] All solution densities were measured relative to that for pure water. The calibration constant of the density meter was determined daily using the known densities of water[21] (0.997047 g-cm^{-3} at 25°C) and air.[22] The reproducibility of an individual density measurement was to better than 3×10^{-6} g-cm^{-3}. The accuracy of the density meter was checked by determining the densities of some aqueous sodium chloride solutions. The results obtained for solutions in the range 0.1-1.0 mol-kg^{-1} differed from literature data[23,24] by less than 5 ppm.

Heat capacity measurements were made using a Sodev Inc. Model CP-C Picker flow microcalorimeter housed in a room controlled at 24.4 ± 0.5°C.[25,26] The calorimeter thermostat (Sodev Inc. Model CT-L) was set so that during a heat capacity measurement, the mean temperature was 25°C. The temperature of the thermostat fluid was monitored using a calibrated thermistor. The heating power setting of about 21 mW gave a temperature increment of about 0.5°C. The calorimeter vacuum jacket was maintained at about 3×10^{-3} Pa (Metrovac oil diffusion pump, Precision G.C.A. rotary pump). Solution transport was achieved by gravity flow; flow rates were normally about 0.5 cm^3-min^{-1}. All heat capacity measurements were made relative to the solvent. The value used for the specific heat capacity of pure water at 25°C was 4.1793 J-g^{-1}-K^{-1}.[27]

Small systematic errors have been reported[28-31] in heat capacities determined using some Picker flow microcalorimeters. These errors probably arise because of a power loss between the calorimeter heating element and the calorimeter jacket.[28] To check whether systematic errors are significant for our instrument, the heat capacities of solutions of the recommended chemical standard,[28] NaCl, were determined at 25°C. The apparent molar heat capacity, $C_{p,\phi}$ of electrolytes can be expressed as a function of the molality using

$$C_{p,\phi} = C^{o}_{p,\phi} + A_c\sqrt{d_o m} + B_c m \qquad (1)$$

where A_c is the Debye-Huckel limiting slope and d_o is the solvent density. Using the limiting apparent molar heat capacity, $C^{o}_{p,\phi}$, and the parameter B_c reported by Desnoyers *et al.*,[22] ($C^{o}_{p,\phi}$ = -84.4 J-K^{-1}-mol^{-1}, B_c = 15.6 J-kg-K^{-1}-mol^{-2}) values of $C_{p,\phi}$ calculated using Eq(1) were in good agreement with those calculated from the experimental specific heat capacities for ten aqueous NaCl solutions in the molality range 0.2 to 1.1 mol-kg^{-1}. The average deviation of the observed value from that calculated using Eq(1) was 0.3% which is less than the typical ex-

perimental uncertainty of 0.5%. Hence, under the conditions used in this work, the performance of the calorimeter is satisfactory, and no corrections have been made to any of the specific heat capacities.

3. RESULTS

Densities of aqueous solutions of the four tripeptides at 25°C are given in Table I. The apparent molar volumes V_ϕ of the solutes were calculated from these solution densities d using the equation

$$V_\phi = M/d - 1000(d-d_o)/mdd_o \quad (2)$$

where M is the solute molar mass, and m is the solution molality. V_ϕ varied linearly with solute molality over the concentration range investigated. Hence, the results were fitted by least squares methods to an equation of the form

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

where V_ϕ^o is the apparent molar volume at infinite dilution and S_v is the experimental slope. The quantity V_ϕ^o is just the limiting partial molar volume of the solute, V_2^o.

The error in a solution molality is low (typically 0.03%) and its contribution to the uncertainty in V_ϕ is much smaller than that arising from the error in density. Considering only the error in density, the uncertainty δV_ϕ was calculated using

$$\delta V_\phi = -(M + 1000/m)\delta d/d^2 \quad (4)$$

where δd is the uncertainty in the solution density (2×10^{-6} g-cm^{-3}). Typical values of δV_ϕ at molalities of 0.20, 0.10 and 0.03 mol-kg^{-1} were 0.01, 0.02 and 0.06 cm^3-mol^{-1}, respectively. Weighting factors inversely proportional to δV_ϕ^2 were included in the least squares analysis using Eq. (3). Values of V_2^o and S_V together with their standard deviations are given in Table IV.

For each tripeptide, the density data in Table I were also analyzed using a power series in the solution molality

$$d = d_o + p_1 m + p_2 m^2 \quad (5)$$

where p_1 and p_2 are adjustable parameters. All the density data were weighted at unity. The parameters p_i along with their standard deviations are given in Table III. In the analysis of the calorimetric data, Eq. (5) was used to convert volumetric heat capacities into specific heat capacities.

$C_{p,\phi}$ were calculated from the specific heat capacities using

Table I. Densities of Aqueous Solutions of Tripeptides at 25°C[a]

m	*d*	*m*	*d*	*m*	*d*
		Glycylglycylglycine			
0.20196	1.012181	0.12695	1.006667	0.05791	1.001487
0.18311	1.010800	0.11940	1.006110	0.04549	1.000541
0.17454	1.010180	0.11097	1.005479	0.02986	0.999346
0.15733	1.008920	0.10063	1.004708	0.02139	0.998695
0.15184	1.008519	0.08566	1.003584	0.01197	0.997973
		Glycylalanylglycine			
0.20129	1.011387	0.12321	1.005939	0.04468	1.000315
0.17993	1.009914	0.10839	1.004889	0.04039	1.000004
0.16477	1.008853	0.09756	1.004119	0.03160	0.999363
0.16072	1.008572	0.09039	1.003609	0.02247	0.998697
0.14703	1.007615	0.08039	1.002890	0.02014	0.998526
0.14371	1.007385	0.06074	1.001477	0.01217	0.997944
0.12381	1.005985	0.05359	1.000960		
		Glycylglycylalanine			
0.20834	1.012032	0.13175	1.006643	0.05704	1.001258
0.20435	1.011750	0.11295	1.005303	0.05113	1.000827
0.18494	1.010402	0.10207	1.004528	0.04211	1.000165
0.17176	1.009479	0.09245	1.003831	0.03325	0.999512
0.15929	1.008602	0.08130	1.003025	0.02234	0.998706
0.14519	1.007600	0.06891	1.002125		
		Alanylglycylglycine			
0.21138	1.012008	0.12680	1.006144	0.06679	1.001886
0.19091	1.010601	0.11093	1.005025	0.05260	1.000865
0.17821	1.009728	0.08914	1.003481	0.03507	0.999600
0.16384	1.008733	0.07931	1.002777	0.02535	0.998895
0.14320	1.007290				

[a] Units: molality, mol-kg^{-1}; density, g-cm^{-3}.

$$C_{p,\phi} = Mc_p + 1000\,(c_p - c_p^o)/m \tag{6}$$

where c_p and c_p^o are, respectively, the specific heat capacities of the solution and solvent.

The uncertainty in the apparent molar heat capacity $\delta C_{p,\phi}$ was calculated using the equation

$$\delta C_{p,\phi} = (M + 1000/m)\delta c_p \tag{7}$$

Table II. Apparent Molar Heat Capacities of Some Tripeptides in Aqueous Solution at 25°C[a]

m	$C_{p,\phi}$	m	$C_{p,\phi}$	m	$C_{p,\phi}$
		Glycylglycylglycine			
0.18843	198.4(3.2)	0.11228	192.8(1.6)	0.05346	191.1(3.0)
0.17193	197.6(1.6)	0.10615	195.4(1.9)	0.04194	190.3(3.4)
0.15700	195.6(1.6)	0.08908	191.9(2.5)	0.02969	189.1(2.7)
0.13603	195.7(1.7)	0.07754	193.5(2.0)	0.01991	189.7(2.5)
0.12292	194.5(1.9)	0.06689	191.2(2.4)		
		Glycylalanylglycine			
0.17882	296.6(1.4)	0.12078	293.9(1.7)	0.06357	292.0(2.1)
0.16859	296.3(2.1)	0.11126	294.0(1.7)	0.05252	291.9(2.7)
0.15921	296.3(1.5)	0.09871	293.0(1.6)	0.03184	291.6(1.9)
0.13827	296.0(1.9)	0.08282	293.1(2.2)	0.02289	290.9(2.2)
0.12872	294.1(2.1)	0.07386	292.4(1.9)		
		Glycylglycylalanine			
0.15933	305.7(1.4)	0.09081	302.9(1.5)	0.04406	297.6(1.4)
0.14988	304.8(1.2)	0.08384	300.1(1.5)	0.03745	298.5(2.4)
0.13806	306.2(1.2)	0.06936	300.7(1.6)	0.02961	298.5(1.4)
0.12280	304.2(1.0)	0.06195	299.5(2.0)	0.02961	297.8(1.4)
0.10919	302.1(1.4)	0.05354	299.2(2.8)	0.02222	298.2(1.4)
0.10048	301.9(1.2)				
		Alanylglycylglycine			
0.18933	292.2(1.5)	0.11712	290.3(1.5)	0.05291	286.5(2.1)
0.16557	290.9(2.0)	0.09401	288.8(1.7)	0.04101	287.6(1.5)
0.14302	290.3(2.3)	0.08401	289.7(1.8)	0.03717	287.9(1.6)
0.12936	290.6(1.5)	0.06701	287.6(2.1)	0.02426	286.7(2.1)

[a] Units: apparent molar heat capacity J-K^{-1}-mol^{-1}. Estimated uncertainty for each $C_{p,\phi}$ is in parenthesis. Refer to text.

where δc_p is the estimated error in the determination of the specific heat capacity. In deriving Eq. (7), it has been assumed that the contribution of the error in the molality is negligible. Values of the apparent molar heat capacities together with their estimated uncertainties are given in Table II.

Over the concentration range studied, $C_{p,\phi}$ was found to vary linearly with solution molality. The $C_{p,\phi}$ results were analyzed by a weighted least squares method using an equation of the form

Table III. Parameters of Eq. (5)

Tripeptide	$10^{-3}p_1$	$-10^{-3}p_2$
GGG	0.07733±0.00003	11.9_5±0.2
GAG	0.07367±0.0001	12.10±0.09
GGA	0.07455±0.00002	12.6_4±0.1
AGG	0.07318±0.00001	11.40±0.08

Table IV. Standard Partial Molar Volumes and Heat Capacities of Some Tripeptides in Aqueous Solution at 25°C[a]

Solute	V_2°	S_v	$C_{p,2}^\circ$	S_c
GGG	111.92±0.03	3.6_3±0.2	188.3±0.7	52±6
GAG	129.67±0.01	2.96±0.09	289.8±0.3	38±3
GGA	128.79±0.02	3.4_8±0.1	296.0±0.5	63±5
AGG	130.16±0.01	2.29±0.08	286.2±0.4	32±3

[a] Units: See Table II; partial molar volume, cm^3-mol^{-1}.

$$C_{p,\phi} = C_{p,\phi}^\circ + S_c m \tag{8}$$

where $C_{p,\phi}^\circ$ is the apparent molar heat capacity at infinite dilution (equivalent to the limiting partial molar heat capacity of the solute, $C_{p,2}^\circ$) and S_c is the experimental slope. Values of $C_{p,2}^\circ$ and S_c for each tripeptide are given in Table IV.

A comparison of the present results for GGG with available literature data is shown in Table V. The value of V_2° determined in this work is in reasonable agreement with two previous determinations[7,8] where densities were determined using the vibrating-tube technique. However, the V_2° reported recently by Iqbal and Verrall[10] is 0.6 cm^3-mol^{-1} higher than the value in this work. The different values of V_2° obtained by Bonincontro *et al.*[32] and by Cohn *et al.*[33] probably result from the lower precision of their density data.

Although the value of $C_{p,2}^\circ$ determined in the present study is in reasonable agreement with that determined by Jolicoeur and Boileau,[7] the discrepancy between the S_c values far exceeds the combined uncertainties. We can find no reason for this large difference. The $C_{p,2}^\circ$ determined by Prasad and Ahluwalia[9] is probably less reliable than the value in this work as the heat capacity of pure crystalline GGG required in

Table V. A Comparison of V_ϕ and $C_{p,\phi}$ data for GGG at 25°C with Literature Values[a]

V_2°	S_v	$C_{p,2}^\circ$	S_c	Ref.
111.92±0.03	3.6_3±0.2	188.3±0.7	52±6	This Work
111.82±0.01[b]	3.3_7±0.1[b]	186.0±0.9[b]	102±7[b]	7
112.11±0.03	5.4±0.4			8
112.51±0.03	4.1_6±0.5			10
110.6	--			32
113.5[c]	--			33
		239±8[d]	--	9
		218±13	--	13

[a] For units, see Table IV. [b] These values were obtained from an analysis of the V_ϕ and $C_{p,\phi}$ data (deposited with CISTI, NRC, Canada) using Eqs. (5,10). [c] This value is actually V_ϕ determined at 0.25 mol-kg^{-1}. [d] At 30°C.

their determination was only an estimated quantity. The values of $C_{p,2}^\circ$ determined by Cabani *et al.*[13] is much higher than that determined by flow calorimetry. From their study it is not clear whether the apparent molar heat capacity is actually the value at infinite dilution. In their earlier studies,[34,35] the quantities determined were apparent molar heat capacities averaged over a concentration range. Assuming their value of $C_{p,\phi}$, GGG was determined using the same procedure, then a value higher than that determined in this work would be expected.

4. DISCUSSION

4.1. Limiting Partial Molar Volumes

In the analysis of V_2° data for solutes in solution, there has been considerable interest in developing various models[36,37] and additivity schemes[38] that can be used to predict solute partial molar volumes. Although these predictive methods have met with some success,[39,40] they cannot account for the subtle effects of solute-solvent interactions. For example, based on the predictive methods, isomeric solutes will have the same V_2° values. However, significant differences have been observed among V_2° data for various isomeric compounds such as alcohols,[41] amides[12] and dipeptides.[8,42] For the three isomeric alanyl-containing tripeptides in this study, there is a significant variation in the V_2° data. The difference between the smallest and largest values is 1.37

cm^3-mol^{-1} and although this is only about 1% of the mean value of V_2°, it is nevertheless well outside the combined uncertainties of the results. The largest V_2° value occurs for the tripeptide where the methyl side group is attached to the carbon adjacent to the $-NH_3^+$ end group while the tripeptide with the methyl group near the $-CO_2^-$ group has the smallest V_2° value. This trend is consistent with the partial molar volume data for some isomeric butylcarboxylate, RCO_2^-, and butylammonium, RNH_3^+, ions.[43,44] For the RCO_2^- isomers, V_2° (R = *sec*-butyl) < V_2° (R = *n*-butyl) while for the RNH_3^+ series, V_2° (R = *sec*-butyl) > V_2° (R = *n*-butyl).

The small differences in V_2° for the three isomeric tripeptides can be interpreted in terms of the changes in hydration that arise from the mutual interaction of the methyl side group and its cosphere with a solvated ionic end group. From partial molar volume[5,45] and heat capacity[46] data for aqueous solutions of α,ω-amino acids, it appears that the charged end groups and their cospheres act independently when separated by at least four methylene groups.[5,8,45] It also appears[45] that this effect occurs independently of the nature of the interposing atoms so for tripeptides, where there are seven atoms separating the $-NH_3^+$ from the $-CO_2^-$ group, the solvated ionic end groups will not interfere with each other to any great extent. In addition, the interaction of each end group with the central carbon should be minimal. Consequently, for the tripeptide GAG, the methyl side chain, with its cosphere, and each solvated ionic end group, should make contributions to V_2° which are to a first approximation independent of one another.

Table VI. Partial Molar Volume of a Methyl Side Chain

Solutes	$V^\circ(CH_3)^a$	Ref.
$NH_3^+CH_2CONHCH(R)CONHCH_2CO_2^-$	17.75±0.05	This Work
$CH_3CONH(R)$	18.2	38
$CH_3CONHCH(R)CONH_2$	17.50±0.06	12
$NH_2CONH(R)$	18.0	38
$NH_3^+CH(R)CO_2^-$	17.20±0.02	6

[a] Calculated using Eq. (9). Units: see Table IV.

For GGA and AGG, the methyl side group is no longer independent of the adjacent charged group. As outlined by Zana,[45], there are two antagonistic effects to consider: (1) a positive contribution to V_2° resulting from a reduction in the electrostriction of the charged group

because of the presence of the adjacent methyl group, and (2) a negative contribution to V_2° due to the disruption of the hydration of the alkyl group by the charged group. The electrostriction of an $-NH_3^+$ group has been shown to be about 10 times larger than that for the $-CO_2^-$ group.[37,43] This presumably is associated with the lower charge density on the $-CO_2^-$ group because of electron delocalization. Hence, the positive contribution to V_2° from the reduction in electrostriction will be much more significant when the alkyl group is adjacent to the $-NH_3^+$ group than when adjacent to the $-CO_2^-$ group. This is reflected in the V_2° data for the three peptides. The V_2° value for AGG is greater than that for GAG because of the reduced electrostriction of the charged $-NH_3^+$ group. For GGA, it is the effect of the $-CO_2^-$ on the hydration of the adjacent methyl group that dominates leading to a smaller value of V_2° compared with that for GAG. Similar effects have also been observed for some dipeptides containing an alkyl side group.[8,42]

The contribution to V_2° of a methyl side group can be estimated from the difference between the V_2° for GGG and GAG.

$$V^\circ(CH_3) = V_2^\circ(GAG) - V_2^\circ(GGG) \tag{9}$$

The quantity $V^\circ(CH_3)$ gives the contribution to V_2° of the peptide on replacing C-H by C-CH_3. Table VI gives a comparison of this result with estimates obtained from other model compounds. The value obtained in this work is similar to those obtained from molar volume data of uncharged amides. The slightly lower value of $V^\circ(CH_3)$ obtained using V_2° for glycine and alanine presumably arises because of the close proximity of the charged functional groups in the α-amino acids.

4.2. Concentration Dependence of Apparent Molar Volumes

The concentration dependence of the apparent molar volume V_ϕ can be interpreted in terms of solute-solute interactions. The parameter S_v is the volumetric virial coefficient that characterizes the pairwise interaction of solvated solute species in solution.[12] The sign of S_v is determined by the nature of the interaction between the solute species. The overlap of the solvent cospheres when two charged centers, such as the peptide end groups $-NH_3^+$ and $-COO^-$, interact in solution will result in a positive volume change as some electrostricted water returns to the bulk solvent.[8] On the other hand, the overlap of two hydrophobic hydration cospheres when a polar groups interact leads to a negative volume change, and hence a negative S_v.

In the pairwise interaction of peptides, all these interactions can

occur. For the zwitterionic tripeptides, the positive S_v values suggest that the pairwise interaction is dominated by the interaction of the charged functional groups. However, the variation in S_v with side chain position indicates that the methyl group modulates the interaction of the charged end groups in the pairwise interaction. The smallest value of S_v is observed for the peptide with the $-CH_3$ substituent adjacent to the $-NH_3^+$ group. This is consistent with the fact that the electrostriction of water by $-NH_3^+$ is much greater than by $-CO_2^-$[37] so the shielding of an adjacent $-CH_3$ group will be more significant for $-NH_3^+$ than for $-CO_2^-$.

4.3. Limiting Partial Molar Heat Capacities

A comparison of $C^\circ_{p,2}$ for GGG with the results for the alanyl containing tripeptides shows that there is a large contribution to the partial molar heat capacity of a peptide on the replacement of a C-H by a C-CH_3. The contribution of the CH_3 side chain, $C^\circ_{p,2}(CH_3)$, can be estimated using the $C^\circ_{p,2}$ values for GGG and GAG (where the side chain is well separated from the ionic end groups),

$$C^\circ_{p,2}(CH_3) = C^\circ_{p,2}(GAG) - C^\circ_{p,2}(GGG) \qquad (10)$$

Using the data in Table IV, $C^\circ_{p,2}(CH_3) = 102 \pm 1$ J-K^{-1}-mol^{-1}. This result compares favorably with the estimated value of 105 J-K^{-1}-mol^{-1} obtained from data for oligoglycines and oligoalanines.[7] Also, it is interesting to note that the value of $C^\circ_{p,2}(CH_3)$ obtained using $C^\circ_{p,2}$ for the α-amino acids glycine and alanine is identical (102 ± 0.6 J-K^{-1}-mol^{-1}) to that calculated from the tripeptide data. However, this agreement is probably fortuitous. For alanine there may be a balance between the decreased contribution to $C^\circ_{p,2}$ from the $-CH_3$ group because of the adjacent charged functional groups and the increased contribution from the ionic groups arising from shielding by the adjacent CH_3 substituent.

For the three isomeric alanyl-containing tripeptides, there is a small but significant variation in the $C^\circ_{p,2}$ values. A shift of the $-CH_3$ side chain from the central carbon to be adjacent to the $-NH_3^+$ group leads to a decrease in $C^\circ_{p,2}$ of 4 J-K^{-1}-mol^{-1}. Presumably, this effect arises from the mutual interaction between the apolar side chain, and its associated solvent cosphere, with the solvated $-NH_3^+$ end group. With an adjacent charged functional group the heat capacity of the $-CH_3$ group will not be fully developed and $C^\circ_{p,2}$ should be smaller. On the other hand, the shielding effect of the $-CH_3$ will reduce the electrostrictive effect of the $-NH_3^+$ which should result in an increase in $C^\circ_{p,2}$. For AGG, it appears that the former effect dominates. The same effect also occurs

for α-amino acids, $NH_3^+CH(R)CO_2^-$, with isomeric apolar side chains. When the side chain is branched, the partial molar heat capacity is lower than for the straight chain analogue ($C^\circ_{p,2}$(R = Ileu) < $C^\circ_{p,2}$(R = Leu),[6] $C^\circ_{p,2}$(R = Val) < $C^\circ_{p,2}$(R = Norval)).[48]

A comparison of $C^\circ_{p,2}$ for GAG and GGA indicates that a shift in the $-CH_3$ group from the central carbon to the carbon adjacent to the $-CO_2^-$ group results in an increase in $C^\circ_{p,2}$ of 6 J-K^{-1}-mol^{-1}. This increase may arise because the mutual interaction of the $-CH_3$ and $-CO_2^-$ groups and their associated solvent cospheres produces a slight increase in hydrogen bonding in the carboxyl terminal region of GGA. It would be useful to see whether this effect is observed in other systems. It appears that $C^\circ_{p,2}$ data are not yet available for carboxylate salts M^+ RCO_2^-, with isomeric hydrocarbon chains. It is interesting to note, however, that the partial molar heat capacity for *sec*-butyl alcohol ($C^\circ_{p,2}$ = 449.1 J-K^{-1}-mol^{-1}), where there is a $-CH_3$ group adjacent to the -OH functional group, is greater than that for *iso*-butyl alcohol ($C^\circ_{p,2}$ = 432.5 J-K^{-1}-mol^{-1}).[41]

In summary, the results of this study show that a tripeptide with a side chain in the central position within the molecule is a reasonable model for investigating side chain effects in polypeptides. Also, the results for the isomeric alanyl-containing peptides indicate that the V°_2 and $C^\circ_{p,2}$ data depend on the side group position within the molecule.

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