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Partial molar heat capacities volumes and compressibilities of aqueous solutions of some peptides that model side-chains of proteins

Gavin R. Hedwig

Department of Chemistry and Biochemistry, Massey University,
Palmerston North, New Zealand

Abstract - Partial molar volumes, heat capacities and compressibilities at infinite dilution are presented for some tripeptides of sequence glycyl-X-glycine, where X is an amino acid, in aqueous solution at 25°C. These results are used to estimate the amino acid side-chain contributions to the thermodynamic properties. The side-chain contributions are compared with those determined using thermodynamic data for other model compounds.

INTRODUCTION

The determination of various thermodynamic properties that characterise solute-water interactions in aqueous solutions of amino acids, small peptides and their derivatives has been of interest in recent years (refs. 1,2). A considerable amount of this interest arises because of the importance of these interactions in protein chemistry. In aqueous solutions of globular proteins the interaction of the protein with the solvent is one important factor that contributes to the characteristic folded conformation adopted by the protein (ref. 3). As proteins are very large molecules, the overall protein-water interaction in aqueous solution is made up of many specific interactions between water and the various functional groups on the protein. One useful approach that can assist in our understanding of protein-water interactions is to study small molecule-water interactions in compounds that have structural features that mimic some aspects of the protein structure.

As the amino acids are the basic building blocks of proteins, it is not surprising that they have been used to model the side-chains in proteins (ref. 4). It has been recognised, however, that for the zwitterionic amino acids, the charged NH_3^+ and CO_2^- groups interfere with the solvation of the adjacent side-chain (refs. 4,5). For this reason the amino acids are not the preferred model compounds to calculate group contributions for non-interacting side-chains in proteins. One approach we have adopted is to use as model compounds small peptides of sequence glycyl-X-glycine (gly-X-gly) where the side-chain of interest is on the central amino acid X. The assumption made in using these peptides to estimate side-chain group contributions is that the interaction between the central side-chain and the solvated NH_3^+ and CO_2^- end groups is minimal (ref. 6). This paper reports partial molar volumes, V_2^∞, partial molar isentropic pressure coefficients, $K_{s,2}^\infty = -(\partial V_2^\infty/\partial p)_S$ ($K_{s,2}^\infty$ is sometimes referred to as the partial molar isentropic compressibility), and partial molar heat capacities, $C_{p,2}^\infty$ at infinite dilution for some tripeptides of sequence gly-X-gly. The peptides used are those with the amino acid X as glycine (gly), alanine (ala), valine (val), leucine (leu), isoleucine (ileu), serine (ser), threonine (thr), asparagine (asn), methionine (met), histidine (his) and phenylalanine (phe). These results are used to derive the contributions to the thermodynamic properties of the various amino acid side-chains.

PARTIAL MOLAR VOLUMES

Details of the procedures used to obtain the V_2^∞ results can be found in the original work (refs. 5,7). The V_2^∞ values together with their standard deviations are given in Table 1. The high uncertainties for the peptides glyhisgly and glyphegly result from the low solubility of these two compounds in water.

Semiempirical models are often used to rationalise the V_2^∞ data for organic solutes in aqueous solution (ref. 8). One such model is based on the equation

$$V_2^\infty = V_{int} + V_v + V_s \qquad (1)$$

where V_{int} is the intrinsic volume occupied by the solute, V_v is the void or empty volume that arise because of thermal motion and packing effects (ref. 9) and V_s is the contribution that arises from the interaction of the solute with the solvent.

TABLE 1. Values of V_2^∞, $K_{s,2}^\infty$ and $C_{p,2}^\infty$ for the tripeptides gly-X-gly in aqueous solution at 25°C.

X	$10^6V_2^\infty$ ($m^3\ mol^{-1}$)	$-10^{15}K_{s,2}^\infty$ ($m^3mol^{-1}Pa^{-1}$)	$C_{p,2}^\infty$ ($J\ K^{-1}mol^{-1}$)	X	$10^6V_2^\infty$ ($m^3\ mol^{-1}$)	$-10^{15}K_{s,2}^\infty$ ($m^3mol^{-1}\ Pa^{-1}$)	$C_{p,2}^\infty$ ($J\ K^{-1}\ mol^{-1}$)
gly	111.92(0.03)	44.9(0.1)	188.3(0.7)	thr	146.15(0.04)	45.90(0.08)	339.4(0.7)
ala	129.67(0.01)	41.2(0.1)	289.8(0.3)	asn	147.35(0.05)	46.90(0.08)	279.2(2.6)
val	160.38(0.03)	43.09(0.09)	443.6(0.7)	met	175.10(0.03)	43.56(0.09)	429.9(1.3)
leu	177.35(0.06)	43.62(0.09)	526.1(0.7)	his	169.9(0.2)	40.7(0.2)	358(7)
ileu	177.24(0.04)	40.6(0.1)	530.4(0.8)	phe	193.3(0.1)	39.1(0.2)	506.6(3.4)
ser	131.13(0.05)	43.56(0.08)	263(1)				

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. Values of V_w can be obtained by the addition of the van der Waals increments for the individual atoms that make up the molecule (refs. 10,11). A plot of V_2^∞ versus V_w, calculated using the van der Waals increments of Edward (ref. 11), for the various tripeptides of sequence gly-X-gly is shown in Fig 1. For triglycine and the peptides with hydrophobic side-chains (X = ala, val, leu, ileu and phe) there is a reasonable linear relationship of the form

$$V_2^\infty = aV_w + b \qquad (2)$$

The slope of the line ($a = 1.56 \pm 0.02$) is the same as that obtained in the analogous plot for a series of n-alcohols (ref. 12). As the value of a is not unity, it follows from eqs (1) and (2) that the sum ($V_v + V_s$) is also a linear function of V_w.

Figure 1 also shows that the points that correspond to the peptides with hydrophilic side-chains lie, to varying degrees, below the straight line. In other words, the V_2^∞ value for each of these peptides is smaller than that of a hypothetical peptide having a nonpolar side-chain and with a V_w value the same as that of the peptide itself. These smaller V_2^∞ values are due to volume decreases arising from polar group water interactions, presumably hydrogen bonding. For the seryl and threonyl side-chains, the volume decreases of $4.5 \times 10^{-6}\ m^3\ mol^{-1}$ and $5.4 \times 10^{-6}\ m^3\ mol^{-1}$ respectively are similar to previous estimates of the shrinkage due to hydrogen bonding between hydroxyl functional groups and water (refs. 8,12). The large deviation from the line in Fig. 1 for glyasngly is consistent with the large hydrogen bonding propensity of amide functional groups. Although hydrogen bonding between water and thioether groups is not expected to be extensive, the slight deviation of the V_2^∞ value for glymetgly from the line in Fig. 1 suggests that there may be some interaction of the sulphur atom with water that is not typically hydrophobic.

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

$$V^\infty(R) = V_2^\infty(\text{gly-X-gly}) - V_2^\infty(\text{glyglygly}) \qquad (3)$$

It should be stressed that $V^\infty(R)$ 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. Values of $V^\infty(R)$ calculated using the V_2^∞ results in Table 1 and also those derived using V_2^∞ data for the amino acids are given in Table 2.

There are small but significant differences between the side-chain $V^\infty(R)$ values derived using amino acid and peptide V_2^∞ data. The differences range from about $0.6 \times 10^{-6}\ m^3\ mol^{-1}$ for the alanyl and threonyl side chains to $3.1 \times 10^{-6}\ m^3\ mol^{-1}$ for the isoleucyl side-chain. These differences can be attributed to the different side-chain environment in amino acids compared with the tripeptides. The mutual interaction between the solvated side-chain and the charged end groups and their associated cospheres will be more significant in amino acids than in tripeptides of sequence gly-X-gly.

Ionic end group effects are manifested in the $V^\infty(R)$ results for the isomeric leucyl and isoleucyl side chains. The $V^\infty(R)$ values determined using V_2^∞ data for the tripeptides are the same, within the combined experimental errors, whereas a difference of $2.1 \times 10^{-6}\ m^3\ mol^{-1}$ is observed using amino acid data. Some isomeric butanols, which have hydrocarbon chains that model those of leucine and isoleucine, have very similar V_2^∞ values (ref. 13) which is consistent with the results obtained using tripeptide data.

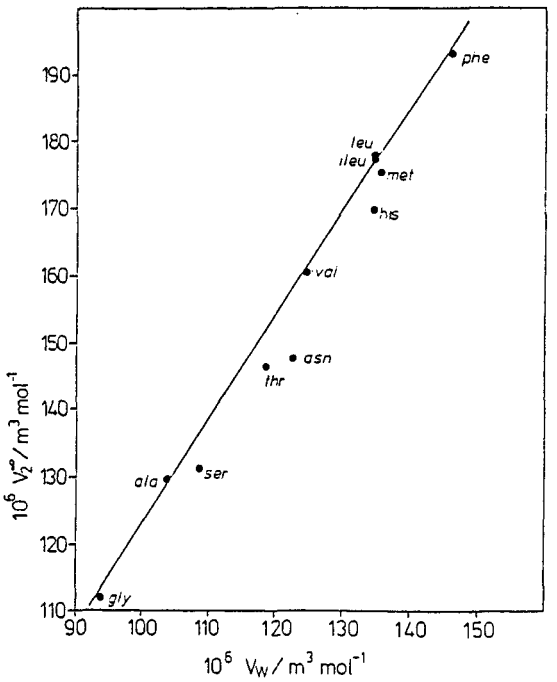

Fig. 1. Correlation between V_2^∞ and V_W for peptides of sequence gly-X-gly.

TABLE 2. Contributions of the amino acid side chains to V_2^∞ of peptides in aqueous solution

Side-chain (R)	$10^6 V^\infty(R)/m^3\ mol^{-1}$	
	tripeptide	amino acid[a]
ala ($-CH_3$)	17.75 (0.04)	17.20 (0.02)
val ($-CH(CH_3)_2$)	48.46 (0.06)	47.54 (0.02)
leu ($-CH_2CH(CH_3)_2$)	65.43 (0.09)	64.32 (0.06)
ileu ($-CH(CH_3)CH_2CH_3$)	65.32 (0.07)	62.20 (0.06)
ser ($-CH_2OH$)	19.21 (0.08)	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 ($-CH_2$-imidazole)	58.0 (0.2)	55.89 (0.05)
phe ($-CH_2$-phenyl)	81.4 (0.2)	78.67 (0.03)

[a] From ref. 4.

PARTIAL MOLAR ISENTROPIC PRESSURE COEFFICIENTS

The $K_{s,2}^\infty$ results for the tripeptides were determined from analyses of the measured sound speeds of the aqueous solutions at 25°C using the methods described previously (ref. 14). As shown in Table 1 the values of $10^{15}\ K_{s,2}^\infty$ for the peptides are all negative. These negative values arise because the electrostricted water in the solvation sheaths around the NH_3^+ and CO_2^- functional groups in the zwitterionic peptides is less compressible than that in the bulk solvent (ref. 15). The variation in the $K_{s,2}^\infty$ values, which range from -39.1 x 10^{-15} m^3 mol^{-1} Pa^{-1} for glyphegly to -46.9 x 10^{-15} m^3 mol^{-1} Pa^{-1} for glyasngly indicates that side-chain hydration also makes a significant contribution to the isentropic pressure coefficient. The side-chain contributions to $K_{s,2}^\infty$ can be estimated using an equation analogous to eq. (2)

$$K_s^\infty(R) = K_{s,2}^\infty\ (\text{gly-X-gly}) - K_{s,2}^\infty\ (\text{glyglygly}) \qquad (4)$$

Values of $K_s^\infty(R)$ obtained using the $K_{s,2}^\infty$ results in Table 1 along with values of $K_s^\infty(R)$ derived from $K_{s,2}^\infty$ data for the amino acids are given in Table 3.

TABLE 3. Contributions of the amino acid side-chains R to $K_{s,2}^\infty$ of peptides in aqueous solution

Side-chain (R)	$10^{15}K_s^\infty(R)/m^3\ mol^{-1}\ Pa^{-1}$		Side-chain (R)	$10^{15}K_s^\infty(R)\ /m^3\ mol^{-1}\ Pa^{-1}$	
	tripeptide	amino acid[a]		tripeptide	amino acid[a]
ala	3.7(0.2)	1.5(0.5)	thr	-1.0(0.2)	-4.2(0.5)
val	1.8(0.2)	-3.6(0.6)	asn	-2.0(0.2)	-3.9(0.5)
leu	1.3(0.2)	-4.8(1.0)	met	1.3(0.2)	-4.2(0.7)
ileu	4.3(0.2)	-	his	4.2(0.3)	-4.8(0.7)
ser	1.3(0.2)	-2.9(0.5)	phe	5.8(0.3)	-7.5(1.9)

[a] From ref. 16.

Large differences are observed between the $K_s^\infty(R)$ values derived using the $K_{s,2}^\infty$ data for tripeptides and the data for the amino acids. The differences are larger for the side-chains with larger volumes e.g. phe, his, leu and met. This is probably due to a shielding effect that occurs for the zwitterionic amino acids. The electrostriction of the NH_3^+ and CO_2^- groups for an amino acid which has a bulky side chain will be smaller than that for glycine. Consequently, the $K_s^\infty(R)$ value derived using a relationship analogous to eq. (4) will give a more negative result compared with that obtained using peptides where the mutual interaction between the charged end groups and the central side-chain is minimal. Thus, the isentropic compressibilities of side-chains in polypeptides will be better represented by the $K_s^\infty(R)$ values determined using the model tripeptides rather than using amino acids.

The partial molar isentropic pressure coefficient at infinite dilution is a quantity that is a sensitive indicator of the subtlties of solute-solvent interactions. This is manifested in the side-chain contributions derived using the peptide $K_{s,2}^\infty$ data. For apolar groups in aqueous solution, the hydrophobic hydration results in the formation of water that is less compressible than that in the bulk solvent (ref. 17). The intrinsic contribution of these groups to $K_{s,2}^\infty$ of a solute is therefore negative. As the $K_s^\infty(R)$ value for the alanyl side-chain is positive, it follows therefore that there must be some other process that overrides the intrinsic contribution. This process is probably a disruption of the hydrogen bonding between water and the peptide(-CONH-) groups that are adjacent to the side-chain. Hydrogen bonding interactions make a negative contribution to $K_{s,2}^\infty$, the extent of which depends on the strength of the bond and the resulting structures (ref. 17). Disruption of hydrogen bonding interactions will therefore result in a positive contribution to $K_{s,2}^\infty$.

Although the $V^\infty(R)$ values are the same for the isomeric side-chains of leucine and isoleucine the $K_{s,2}^\infty(R)$ values are quite different. As the solvent-accessible surface areas of the two side-chains are about the same (ref. 10), the hydrophobic hydration of the side-chains also should be similar. Perhaps the larger $K_{s,2}^\infty(R)$ value for the isoleucyl side-chain results from a greater degree of disruption of the peptide group hydration.

PARTIAL MOLAR HEAT CAPACITIES

The $C_{p,2}^\infty$ values for the tripeptides in Table 1 were derived from the experimental specific heat capacities using the procedures described previously (ref. 5). For the sparingly soluble peptides glyphegly and glyhisgly the $C_{p,2}^\infty$ values were taken as the mean values of the apparent molar heat capacities (ref. 7). The side-chain contributions to the heat capacity $C_p^\infty(R)$ were derived using a relationship analogous to eqs. (3) and (4) above. Values of $C_p^\infty(R)$ are given in Table 4, along with results based on data for the amino acids. The $C_{p,2}^\infty$ values over a wide temperature range were reported recently (ref. 18) for a variety of small solutes chosen as models for selected side-chains of proteins. For example, methanol was used as a model for the side-chain of serine and methane was used as an analogue for the alanyl side-chain. Absolute values of the side-chain heat capacities were derived by subtracting an estimated value for the heat capacity of the H atom from the $C_{p,2}^\infty$ values for the solutes. Values of $C_p^\infty(R)$ derived from these results along with the estimated uncertainties are also given in Table 4. The high uncertainties are due to the large standard deviation associated with the estimated value of the heat capacity of the H atom (ref. 18).

The results in Table 4 show that, in general, the $C_p^\infty(R)$ values derived using $C_{p,2}^\infty$ data for tripeptides are less than those determined using amino acid data although in some cases the differences are small. Given the mutual interactions that exist between the side-chain and the adjacent NH_3^+ and CO_2^- groups in the amino acids, these small differences are somewhat surprising. As the side-chain in a peptide of sequence gly-X-gly is adjacent to two peptide functional groups, which is structurally analogous to that found in proteins, the $C_p^\infty(R)$ 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 $C_p^\infty(R)$ values in columns 2 and 4 of Table 4 are the same, within the admittedly large uncertainties. This is consistent with the observation that the partial molar heat capacity of a hydrocarbon chain R in a compound R-X is not too dependent on the nature of the group X (ref. 19). For the hydrophilic side-chains of serine, threonine and asparagine the differences between the results in columns 2 and 4 are quite large. This suggests that for these side-chains the hydration of the group R in each of the analogue compounds R-H is quite different from that when R is part of a peptide chain.

From the differences between side-chain heat capacities determined using amino acids and peptides in water and those determined using the side-chain analogues and also tripeptides in a buffered solution at pH 4, it has been suggested (ref. 20) that the central side-chain in the tripeptides may be influenced by the ionic end groups. In Table 5 the contribution of the alanyl side-chain $C_p^\infty(CH_3)$ determined using the model tripeptides is compared with that determined using a variety of neutral peptide derivatives (refs. 20, 21).

TABLE 4. Contributions of the amino acid side-chains to $C_{p,2}^{\infty}$ of peptides in aqueous solution

Side-chain (R)	$C_p^{\infty}(R)$ / J K^{-1} mol^{-1}		
	tripeptide[a]	amino acid[b]	analogue[c]
ala	102(1)	102.2(0.6)	89(23)
val	253(1)	263(1)	236(23)
leu	338(1)	359(2)	304(29)
ileu	343(2)	344.1(0.8)	324(43)
ser	75(2)	78.2(0.5)	2(23)
thr	151(1)	171(2)	107(24)
asn	91(3)	86(1)	11(26)
met	242(2)	254(29)	-
his	170(8)	202(1)	-
phe	318(4)	345(2)	305(50)

[a] From refs. 5,7. [b] From ref. 4.
[c] Based on $C_{p,2}^{\infty}$ data from ref. 18.

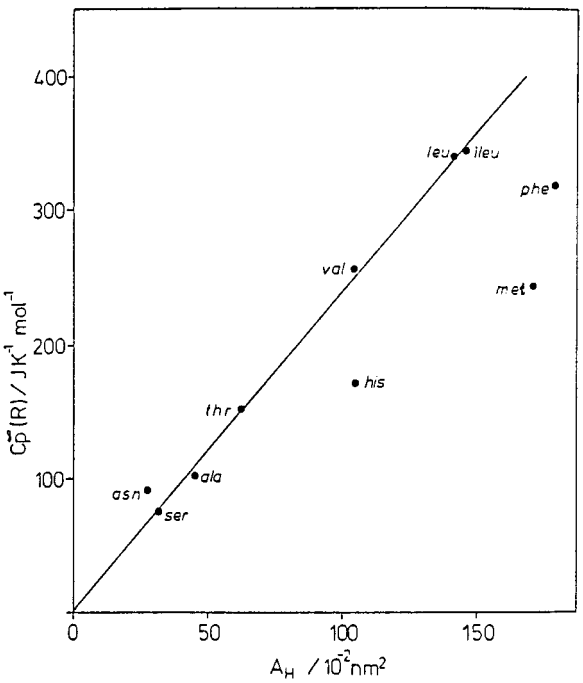

Fig. 2. $C_p^{\infty}(R)$ as a function of the side-chain hydrophobic solvent-accessible surface area, A_H

Given the variation in the $C_p^{\infty}(CH_3)$ values obtained using the different combinations of neutral peptide derivatives, the result based on the zwitterionic tripeptides is not unreasonable. It would appear that the interaction between the NH_3^+ and CO_2^- groups and the central side-chain in the tripeptide does not significantly affect the value of $C_p^{\infty}(CH_3)$.

For nonpolar solutes in aqueous solution the partial molar heat capacities generally correlate with the solvent-accessible surface area (ref. 22). Fig. 2 shows the correlation between the $C_p^{\infty}(R)$ values obtained using the tripeptides and the solvent-accessible surface areas of the hydrophobic component of the side-chain, A_H. The A_H values used were those given by Jolicoeur et al. (ref. 4). For the nonpolar hydrocarbon side-chains and for the side-chains of serine and threonine, there is a reasonably good linear correlation that passes 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 that at 25°C the -OH groups contribute little to the $C_p^{\infty}(R)$ 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 amide (—$CONH_2$) moiety. Significant deviations

TABLE 5. Comparison of $C_p^{\infty}(CH_3)$ derived using various peptide model compounds

Solutes[a]	$C_p^{\infty}(CH_3)$ (J K^{-1} mol^{-1})	Solutes	$C_p^{\infty}(CH_3)$ (J K^{-1} mol^{-1})	Solutes	$C_p^{\infty}(CH_3)$ J K^{-1} mol^{-1}
glyalagly-glyglygly	102 (1)	c(aa) - c(ga)	86 (13)[b]	AG - GG	109(1)[c]
c(ga) - c(gg)	97 (11)[b]	A - G	108 (1)[c]	{AA - GG}/2	108(2)[c]
{c(aa) - c(gg)}/2	92 (12)[b]	GA - GG	100 (2)[c]		

[a] Abbreviations used: c(gg) = 2,5-diketopiperazine; c(ga) = 3-methyl-2,5-diketopiperazine; c(aa) = 3,6-dimethyl-2,5-diketopiperazine; G = N-acetylglycinamide; A = N-acetyl-L-alaininamide; GA = N-acetylglycyl-L-alaninamide; GG = N-acetylglycylglycinamide; AG = N-acetyl-L-alanylglycinamide; AA = N-acetyl-L-alanyl-L-alaninamide. [b] From ref. 20. [c] From ref. 21.

from the line occur for the side-chains containing ring systems (phe and his) and also for that containing a sulphur atom (met). The met side-chain is generally regarded as being hydrophobic. (ref.18). However, recent $C^{\infty}_{p,2}$ data for some alkylsulphides in water suggest that the contribution of the —S— group to $C^{\infty}_{p,2}$ is negative (ref. 23). This finding is certainly consistent with the result for methionine given in Fig. 2.

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