Biophysical Chemistry, 49 (1994) 175–181
Elsevier Science B.V., Amsterdam

BIOCHE 1814

Thermodynamic properties of peptide solutions. Part 11. Partial molar isentropic pressure coefficients in aqueous solutions of some tripeptides that model protein side-chains

Gavin R. Hedwig [a,*] and Harald Høiland [b]

[a] *Department of Chemistry and Biochemistry, Massey University, Palmerston North (New Zealand)*
[b] *Department of Chemistry, University of Bergen, N-5007 Bergen (Norway)*

(Received 16 April 1993; accepted 24 August 1993)

Abstract

The partial molar isentropic pressure coefficients at infinite dilution $K_{s,2}^{\infty}$ ($K_{s,2}^{\infty} = -(\partial V_2^{\infty}/\partial p)_s$, where V_2^{∞} is the partial molar volume at infinite dilution) have been determined for nine tripeptides in aqueous solution at 25°C. The tripeptides are all of sequence glycyl-X-glycine, where X is an amino acid. These $K_{s,2}^{\infty}$ results, in conjunction with those for glycylglycylglycine, were used to estimate the amino acid side-chain contributions to $K_{s,2}^{\infty}$ of peptides. These side-chain contributions are critically compared with previous estimates based on $K_{s,2}^{\infty}$ data for the amino acids. The variation in the side-chain contributions derived using the peptide $K_{s,2}^{\infty}$ results has been rationalized in terms of likely peptide–solvent interactions.

Keywords: Partial molar isentropic pressure coefficient; Speed of sound; Tripeptide; Aqueous solution; Side-chain solvation

1. Introduction

A knowledge of the interactions responsible for stabilizing the native state of a globular protein in aqueous solution is essential to the understanding of its structure and function. As proteins are large complex molecules, small solutes that incorporate some of the structural features found in globular proteins have been used by numerous investigators to study the important interactions that determine protein stability [1–6].

One objective in some of our recent work has been to model the amino acid side-chains of proteins [5,7,8]. For this purpose we have chosen to use as model compounds a series of tripeptides of sequence glycyl-X-glycine (gly-X-gly), where X is one of the amino acids. These peptides, in which the single side-chain of amino acid X is attached to the central carbon atom within the molecule, are reasonable models for investigating side-chain effects in proteins [5]. In earlier papers in this series we reported the partial molar vol-

* Corresponding author.

SSDI 0301-4622(93)E0068-G

umes V_2^∞ and the partial molar heat capacities $C_{p,2}^\infty$ at infinite dilution in aqueous solution of some tripeptides of sequence gly-X-gly [7,8]. These results were used to estimate the contributions of the amino acid side-chains to the thermodynamic properties of peptides. As an extension of this work, we report here the partial molar isentropic pressure coefficients at infinite dilution $K_{s,2}^\infty = -(\partial V_2^\infty/\partial p)_s$ in aqueous solution at 25°C for the nine tripeptides: glycyl-L-leucylglycine (glyleugly), glycyl-DL-serylglycine (glysergly), glycyl-L-isoleucylglycine (glyileugly), glycyl-DL-threonylglycine (glythrgly), glycyl-L-asparagylglycine (glyasngly), glycyl-L-valylglycine (glyvalgly), glycylhistidylglycine (glyhisgly), glycylphenylalanylglycine (glyphegly) and glycylmethionylglycine (glymetgly). These $K_{s,2}^\infty$ results, in conjunction with the $K_{s,2}^\infty$ value for glycylglycylglycine (glyglygly) [9], can be used to estimate the side-chain contributions to the isentropic pressure coefficient of a peptide.

The partial specific isentropic pressure coefficients of the extended states of some proteins in aqueous solution have been derived by Iqbal and Verrall [10] using simple additivity of protein constituent groups. The contributions of the amino acid side-chains used by Iqbal and Verrall were obtained from $K_{s,2}^\infty$ data for the amino acids taken from the literature. These side-chain contributions are critically compared with those derived using the $K_{s,2}^\infty$ data for the tripeptides determined in this study.

2. Materials and methods

Samples of the tripeptides used were from the same batches of material prepared for earlier studies. The preparation, purification and analyses of these peptides have been reported in detail elsewhere [7,8]. The water used to prepare solutions was glass distilled. Solutions were prepared by mass and corrections were made for air buoyancy.

The measured sound speeds for aqueous solutions of the peptides were determined during two visits to Bergen by one author (GRH). For five peptides (the first five listed in table 1) the ultrasonic speeds were determined using the "sing-around" method [11]. The apparatus used was the same as that reported elsewhere [12]. The pulse transit time for a solution was calculated from the measurement of the pulse repetition frequency associated with a single pass of the pulse between the two transducers in the cell. The system was calibrated as described previously [12]. The sound speeds for aqueous solutions of the remaining peptides were determined by the "pulse-echo-overlap" method [13] using a Panametrics Ultrasonic Time Intervalometer (Pulsing Module 5053A). The cylindrical ultrasonic cell was made of gold-plated brass with a single piezoceramic transducer and a reflector separated by a distance of about 4 cm. The cell volume was ≈ 5 cm^3. The cell was thermostated using a modified LKB 7600 precision thermostat with coolant supplied from a Heto (Birkerød, Denmark) thermostat bath. Temperature control during a series of measurements was to better than ± 0.0005°C. The water bath temperature was measured using a Hewlett-Packard 2804A quartz thermometer. The pulse-echo-overlap method involves the determination of the time t between two echoes. The signals of interest are applied to the vertical axis of an oscilloscope (Tektronics model 465) with the horizontal axis driven at a frequency whose period is t. Thus, the two signals will appear to overlap on the cathode ray tube. The frequency measurement was made using a Philips PM5192 programmable synthesizer/function generator. The precision of a speed of sound measurement made using this pulse-echo-overlap method was to ± 0.01 m s^{-1}. In order to check the accuracy of the system, sound speeds were determined for some aqueous solution of the peptide glyasngly. The results obtained were the same, within the combined experimental uncertainties, as those determined earlier using the sing-around method.

3. Results

The measured sound speeds u for aqueous solutions of the nine tripeptides at 25°C are given in table 1. The isentropic compressibilities β_s

Table 1

Sound speeds and apparent molar isentropic pressure coefficients of some tripeptides in aqueous solution at 25°C

m (mol kg^{-1})	u (m s^{-1})	$-10^{15} K_{s,\phi}$ (m^3 mol^{-1} Pa^{-1})	m (mol kg^{-1})	u (m s^{-1})	$-10^{15} K_{s,\phi}$ (m^3 mol^{-1} Pa^{-1})
glycyl-L-leucylglycine					
0.04471	1503.48 [a]	42.08 (0.53) [b]	0.09881	1511.54	40.68 (0.24) [b]
0.05552	1505.12	41.96 (0.43)	0.10897	1513.03	40.39 (0.22)
0.06621	1506.72	41.66 (0.36)	0.12213	1514.92	39.88 (0.19)
0.07455	1507.97	41.48 (0.32)	0.12990	1516.07	39.78 (0.18)
0.08681	1509.77	41.00 (0.27)	0.13979	1517.48	39.43 (0.17)
glycyl-DL-serylglycine					
0.03507	1500.35 [a]	43.36 (0.68)	0.07951	1504.94	42.58 (0.30)
0.03615	1500.44	42.97 (0.66)	0.08580	1505.59	42.51 (0.28)
0.04527	1501.39	42.93 (0.53)	0.10928	1507.99	42.11 (0.22)
0.05417	1502.31	42.81 (0.44)	0.11879	1508.98	42.06 (0.20)
0.06537	1503.47	42.69 (0.36)			
glycyl-L-isoleucylglycine					
0.02037	1499.70 [a]	39.9 (1.2)	0.06964	1506.85	38.46 (0.34)
0.02992	1501.10	39.60 (0.80)	0.07598	1507.75	38.21 (0.31)
0.04108	1502.73	39.43 (0.58)	0.08372	1508.87	38.14 (0.28)
0.04666	1503.53	39.06 (0.51)	0.09156	1509.97	37.83 (0.26)
0.05153	1504.26	39.20 (0.46)	0.09741	1510.81	37.72 (0.24)
0.06114	1505.62	38.60 (0.39)	0.10890	1512.41	37.25 (0.22)
glycyl-DL-threonylglycine					
0.02108	1499.22 [a]	45.7 (1.1)	0.07111	1505.12	44.32 (0.33)
0.03000	1500.27	45.22 (0.80)	0.08509	1506.73	43.90 (0.28)
0.03789	1501.20	45.02 (0.63)	0.09617	1508.01	43.59 (0.25)
0.04506	1502.05	44.80 (0.53)	0.10789	1509.38	43.43 (0.22)
0.04959	1502.58	44.67 (0.48)	0.11833	1510.58	43.20 (0.20)
0.06260	1504.13	44.56 (0.38)			
glycyl-L-asparagylglycine					
0.01611	1498.52 [a]	46.7 (1.5)	0.04069	1501.28	45.65 (0.59)
0.01860	1498.79	46.2 (1.3)	0.04351	1501.60	45.63 (0.55)
0.02383	1499.39	46.4 (1.0)	0.04772	1502.06	45.43 (0.50)
0.02397	1499.40	46.2 (1.0)	0.05193	1502.52	45.23 (0.46)
0.02791	1499.84	46.05 (0.86)	0.05372	1502.74	45.38 (0.44)
0.03280	1500.40	46.01 (0.73)	0.05542	1502.91	45.11 (0.43)
0.03699	1500.86	45.71 (0.65)	0.05917	1503.33	45.04 (0.40)
0.03805	1500.97	45.64 (0.63)	0.06438	1503.92	45.04 (0.37)
glycyl-L-valylglycine					
0.01387	1498.60 [c]	42.91 (0.43)	0.05992	1504.85	41.51 (0.10)
0.03045	1500.87	42.41 (0.20)	0.07005	1506.21	41.26 (0.09)
0.03947	1502.09	42.00 (0.15)	0.07957	1507.50	41.12 (0.07)
0.05179	1503.77	41.79 (0.12)	0.09073	1508.97	40.75 (0.07)
glycylhistidylglycine					
0.00401	1497.17 [c]	40.7 (1.5)	0.00800	1497.64	39.81 (0.75)
0.00443	1497.21	40.0 (1.4)	0.00896	1497.75	39.76 (0.67)
0.00500	1497.28	39.9 (1.2)	0.00994	1497.87	39.53 (0.60)
0.00540	1497.33	40.4 (1.1)	0.01102	1497.99	39.52 (0.54)
0.00599	1497.41	40.5 (1.0)	0.01207	1498.12	39.60 (0.50)
0.00696	1497.52	39.90 (0.86)			

Table 1 (continued)

m (mol kg^{-1})	u (m s^{-1})	$-10^{15} K_{s,\phi}$ (m^3 mol^{-1} Pa^{-1})	m (mol kg^{-1})	u (m s^{-1})	$-10^{15} K_{s,\phi}$ (m^3 mol^{-1} Pa^{-1})
glycylphenylalanylglycine					
0.00593	1497.54 [c]	38.3 (1.1)	0.01397	1498.69	38.07 (0.43)
0.00706	1497.70	38.45 (0.85)	0.01581	1498.96	38.46 (0.38)
0.00810	1497.86	39.22 (0.74)	0.01752	1499.20	38.26 (0.34)
0.00891	1497.98	38.99 (0.67)	0.01994	1499.54	37.93 (0.30)
0.01007	1498.14	38.60 (0.60)	0.02132	1499.75	38.12 (0.28)
0.01199	1498.41	38.35 (0.50)	0.02313	1500.00	37.90 (0.26)
glycylmethionylglycine					
0.01589	1498.86 [c]	43.31 (0.38)	0.06938	1506.02	41.34 (0.09)
0.02567	1500.19	42.89 (0.23)	0.07998	1507.44	41.16 (0.07)
0.03025	1500.80	42.71 (0.20)	0.08911	1508.65	40.94 (0.07)
0.03836	1501.89	42.34 (0.16)	0.09999	1510.05	40.46 (0.06)
0.04813	1503.21	42.12 (0.12)	0.10960	1511.32	40.23 (0.05)
0.05656	1504.32	41.76 (0.11)			

[a] The uncertainty in u is ± 0.04 m s^{-1}.
[b] The estimated uncertainty of each $K_{s,\phi}$ is given in parentheses.
[c] The uncertainty in u is ± 0.01 m s^{-1}.

($\beta_s = -(1/V)(\partial V/\partial p)_s$) of the solutions were determined from the sound speeds using the relation

$$\beta_s = 1/u^2\rho, \tag{1}$$

where ρ is the solution density. Densities of the aqueous solutions were calculated using a power series in the solution molality m of the form

$$\rho = \rho_1^* + p_1 m + p_2 m^2, \tag{2}$$

where ρ_1^* is the density of pure solvent (0.997047 g cm^{-3} at 25°C [14]) and p_1 and p_2 are parameters obtained by least-squares fitting of density data reported in previous papers [7,8]. The isentropic compressibilities β_s were used to obtain values for the apparent isentropic pressure coefficient $K_{s,\phi} = -(\partial V_{2,\phi}/\partial p)_s$, where $V_{2,\phi}$ is the apparent molar volume, using the equation:

$$K_{s,\phi} = M_2\beta_s/\rho - (\beta_{s,1}^*\rho - \beta_s\rho_1^*)/m\rho\rho_1^*. \tag{3}$$

In eq. (3) M_2 is the solute molar mass and $\beta_{s,1}^*$ is the isentropic compressibility of the pure solvent (4.47736×10^{-10} Pa^{-1} [15]). The $K_{s,\phi}$ values for aqueous solutions of the tripeptides are given in table 1. The uncertainties in the $K_{s,\phi}$values, which were estimated using propagation of errors methods as outlined previously [12], are also given in table 1.

For each solute, $K_{s,\phi}$ was a linear function of the solution molality over the range studied. Hence, the isentropic pressure coefficient at infinite dilution $K_{s,2}^{\infty}$ was obtained by least-squares methods using the equation

$$K_{s,\phi} = K_{s,2}^{\infty} + S_k m, \tag{4}$$

where S_k is the experimental slope. In each analysis the $K_{s,\phi}$ values were weighted using the procedures described in a previous paper [12]. The $K_{s,2}^{\infty}$ and S_k values together with their standard deviations are given in table 2. For the purposes of comparison, the results for the tripeptides glyglygly and glyalagly that were reported in earlier work [9,16] are also given in table 2.

For the peptides glyphegly and glyhisgly the standard deviations for S_k are rather large. This is because these peptides have a low solubility in water. The molality ranges over which the $K_{s,\phi}$ values were determined are too narrow to enable reliable values of S_k to be obtained. If the concentration dependence of $K_{s,\phi}$ is ignored for the dilute solutions studied, values of $K_{s,2}^{\infty}$ can be

Table 2

Partial molar isentropic pressure coefficients of some tripeptides in aqueous solution at 25°C

Peptide	$-10^{15} K_{s,2}^{\infty}$ (m^3 mol^{-1} Pa^{-1})	$10^{15} S_k$ (m^3 kg mol^{-2} Pa^{-1})
glyglygly [a]	44.9 (0.1) [b]	18.9 (1.0) [b]
glyalagly [c]	41.2 (0.1)	16.3 (1.0)
glyvalgly	43.09 (0.09)	25.6 (1.2)
glyleugly	43.62 (0.09)	29.9 (0.7)
glyileugly	40.6 (0.1)	30.0 (1.2)
glysergly	43.56 (0.08)	12.8 (0.9)
glythrgly	45.90 (0.08)	23.1 (0.8)
glyasngly	46.9 (0.1)	30.8 (2.0)
glyhisgly	40.7 (0.2)	101 (22) [d]
glymetgly	43.56 (0.09)	30.4 (1.0)
glyphegly	39.1 (0.2)	51 (12) [d]

[a] Ref. [9].
[b] Standard deviations are in parentheses.
[c] Ref. [16].
[d] See text.

estimated by averaging the $K_{s,\phi}$ results. The means and standard deviations of the $K_{s,\phi}$ data for the peptides glyphegly and glyhisgly are $-(38.4 \pm 0.4) \times 10^{-15}$ m^3 mol^{-1} Pa^{-1} and $-(40.0 \pm 0.4) \times 10^{-15}$ m^3 mol^{-1} Pa^{-1} respectively. These values are almost the same, within the combined standard deviations, as the values of $K_{s,2}^{\infty}$ obtained by analyses of the $K_{s,\phi}$ data using eq. (4).

4. Discussion

For all of the tripeptides, the $K_{s,2}^{\infty}$ values are large and negative. The negative values are due to the presence of the charged $-NH_3^+$ and $-CO_2^-$ functional groups in the zwitterionic tripeptides. The electrostricted water in the regions surrounding these charged groups is less compressible than water in the bulk solvent [17,18]. As a result of this effect, $K_{s,2}^{\infty}$ values for zwitterionic amino acids and small peptides in aqueous solution at 25°C are always negative [12,19,20].

The $K_{s,2}^{\infty}$ values for the tripeptides range from -39.1×10^{-15} m^3 mol^{-1} Pa^{-1} for glyphegly to -46.9×10^{-15} m^3 mol^{-1} Pa^{-1} for glyasngly. This variation indicates that the interaction of each side-chain with the solvent and also the mutual interaction between the solvated side-chain and other functional groups in the peptide make significant contributions to the value of $K_{s,2}^{\infty}$ of the peptide.

The side-chain contributions to $K_{s,2}^{\infty}$ can be derived from the difference between the $K_{s,2}^{\infty}$ value for each peptide and that for the corresponding peptide without a side-chain

$$K_s^{\infty}(R) = K_{s,2}^{\infty}(\text{gly-X-gly}) - K_{s,2}^{\infty}(\text{glyglygly}). \quad (5)$$

The quantity $K_s^{\infty}(R)$ is not the absolute value of the partial molar isentropic pressure coefficient for the side-chain, but it gives the contribution to $K_{s,2}^{\infty}$ on replacing a C—H group by a C—R group. Values of K_s^{∞}(R) calculated using the $K_{s,2}^{\infty}$ results for the peptides are given in table 3. Table 3 also gives K_s^{∞}(R) values calculated using amino acid $K_{s,2}^{\infty}$ data taken from the literature [10,17,21,22]. For all the side-chains except ala and asn, these K_s^{∞}(R) results are the same as those reported by Iqbal and Verrall [10]. The K_s^{∞}(ala) and K_s^{∞}(asn) values were derived using alternative amino acid $K_{s,2}^{\infty}$ results to those used by Iqbal and Verrall [10]. The uncertainty in the value of K_s^{∞}(ileu) does not appear in table 3

Table 3

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

Side-chain (R)	$10^{15} K_s^{\infty}$(R)/m^3 mol^{-1} Pa^{-1}	
	tripeptide	amino acid [a]
ala ($-CH_3$)	3.7 (0.2) [b]	2.0 (0.5) [b]
val ($-CH(CH_3)_2$)	1.8 (0.2)	−3.6 (0.6)
leu ($-CH_2CH(CH_3)_2$)	1.3 (0.2)	−4.8 (1.0)
ileu ($-CH(CH_3)CH_2CH_3$)	4.3 (0.2)	−4.8 [c]
ser ($-CH_2OH$)	1.3 (0.2)	−2.9 (0.5)
thr ($-CH(OH)CH_3$)	−1.0 (0.2)	−4.2 (0.5)
asn ($-CH_2CONH_2$)	−2.0 (0.2)	−6.0 (0.5)
met ($-CH_2CH_2SCH_3$)	1.3 (0.2)	−4.2 (0.7)
his ($-CH_2$-imidazole)	4.2 (0.3)	−4.8 (0.7)
phe ($-CH_2$-C_6H_5)	5.8 (0.3)	−7.5 (1.9)

[a] Based on $K_{s,2}^{\infty}$ data for the amino acids from refs. [10,17,21,22].
[b] Estimated uncertainties are in parentheses.
[c] See text.

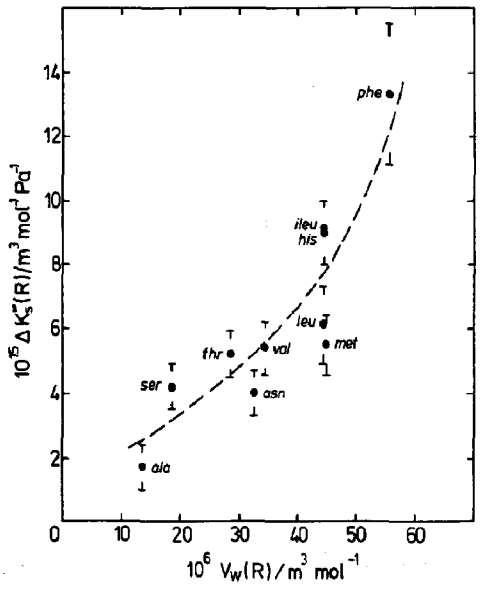

Fig. 1. A plot of the quantity $\Delta K_s^\infty(R)$ against the van der Waals volume of the side-chain $V_w(R)$.

because no error was given for the $K_{s,2}^\infty$ value for the amino acid isoleucine [10].

For every side-chain, the $K_s^\infty(R)$ value derived using tripeptide $K_{s,2}^\infty$ data is more positive than that derived using amino acid data. However the difference between these two $K_s^\infty(R)$ values

$$\Delta K_s^\infty(R) = K_s^\infty(R)(\text{tripeptide}) - K_s^\infty(R)(\text{amino acid}), \quad (6)$$

where $K_s^\infty(R)$ (Y) represents the value of $K_s^\infty(R)$ derived using $K_{s,2}^\infty$ data for species Y, does vary significantly. This variation is illustrated in fig. 1 which shows the quantity $\Delta K_s^\infty(R)$ plotted against $V_w(R)$, the van der Waals volume of the side-chain. The $V_w(R)$ values, which give a measure of side-chain sizes, were calculated using the van der Waals atomic increments given by Edward [23]. Although the data in fig. 1 are rather scattered, it is apparent that the value of $\Delta K_s^\infty(R)$ increases with increasing side-chain size. This is probably a result of a side-chain shielding effect present in zwitterionic amino acids. For amino acids with large side-chains, the electrostriction around the $-NH_3^+$ and $-CO_2^-$ groups adjacent to the side-chain will be less extensive than that for glycine. Consequently, the charged groups in the amino acid will make a less negative contribution to the value of $K_{s,2}^\infty$ than they do for glycine. This difference will be incorporated into the values of $K_s^\infty(R)$ when these quantities are evaluated using a relationship analogous to eq. (5) and the $K_{s,2}^\infty$ data for the amino acids. As there is a reasonable separation between the side-chain and the charged end groups in a tripeptide of sequence gly–X–gly, there will be a more effective cancellation of charged group contributions when estimating $K_s^\infty(R)$ values using $K_{s,2}^\infty$ data for peptides. Thus, the $K_s^\infty(R)$ values based on tripeptide data are more reliable estimates of the contributions of solvated side-chains in proteins than are those based on amino acid data.

It is interesting to note that for seven of the side-chains, the $K_s^\infty(R)$ values in columns (2) and (3) of table 2 have opposite signs. It follows therefore that the estimation of protein isentropic pressure coefficients using additivity schemes with side-chain contributions based on $K_{s,2}^\infty$ data for the amino acids will give results that differ significantly from those with side-chain contributions estimated using $K_{s,2}^\infty$ data for the peptides in this study.

As the intrinsic volume of a solute can be regarded as being incompressible, at least to a first approximation [20, 21], the $K_s^\infty(R)$ results in column 2 of table 3 can be rationalized in terms of changes in peptide-water interactions that occur when the C—H group of the peptide is replaced by the C—R group. For the aliphatic side-chains the $K_s^\infty(R)$ values are all positive. Hydrophobic hydration of apolar groups results in the formation of water in the solvation sheath that is less compressible than that in the bulk solvent [24,25]. Thus, the intrinsic contribution of an apolar group to the value of $K_s^\infty(R)$ will be negative. The positive $K_s^\infty(R)$ values suggest that there must be some other effect that outweighs the contribution of the hydrophobic hydration of the side-chain itself. This is probably a disruption of the hydrogen bonding between the adjacent peptide groups and water. Hydrogen bonding interactions tend to make a negative contribution to the value of $K_{s,2}^\infty$ of a solute in aqueous solution [26]. Consequently, any disruption or distortion of hydrogen bonding between water and the

peptide functional groups will make a positive contributions to the value of $K_s^\infty(R)$. The $K_s^\infty(R)$ values for these aliphatic side-chains reflect a balance between the intrinsic contribution of the hydrocarbon chains and the disruption of hydrogen bonding of the adjacent peptide functional groups.

Although the isomeric side-chains leu and ileu have very similar side-chain volumes and heat capacities [8], there is a significant difference between the values of K_s^∞(leu) and K_s^∞(ileu). This difference is not easy to interpret. The hydrophobic hydration for the two side-chains ought to be similar because the solvent accessible surface areas of the side-chain are almost identical [2,4]. Perhaps the peptides glyleugly and glyileugly fold in slightly different ways such that there is a difference in the peptide group-water hydrogen bonding in the two species. Large differences between $K_{s,2}^\infty$ values for structurally isomeric solutes are not uncommon. For example, the $K_{s,2}^\infty$ values for the isomers 2-methyl-1-propanol and 2-butanol are 9.4×10^{-15} m^3 mol^{-1} Pa^{-1} and 3.5×10^{-15} m^3 mol^{-1} Pa^{-1}, respectively [20].

The negative values for K_s^∞(thr) and K_s^∞(asn) suggest that the hydrogen bonding between water and the –OH and $-CONH_2$ moieties in these peptides is a significant feature of the peptide–water interactions. Given that the side-chain ser also has an –OH group, the positive value for K_s^∞(ser) is somewhat surprising. However, based on results for some alcohols the difference between the K_s^∞(ser) and K_s^∞(thr) values does not look unreasonable. The two alcohols $R'CH(OH)CH_3$ and $R'CH_2OH$ ($R' = CH_3CH_2$), which contain the fragments that model the side-chains thr and ser, have $K_{s,2}^\infty$ values of 3.5×10^{-15} m^3 mol^{-1} Pa^{-1} and 6.2×10^{-15} m^3 mol^{-1} Pa^{-1}, respectively. The difference between these values is similar to that between K_s^∞(ser) and K_s^∞(thr).

The values of $K_s^\infty(R)$ for the bulky side-chains his and phe are relatively large and positive. It is likely that these side-chains disrupt the solvent structure to form some "unbound" water. Water in this form is more compressible than water in the bulk solvent [24]. This effect will make positive contribution to the values of $K_s^\infty(R)$ for these side-chains.

Acknowledgement

One of us (GRH) is grateful for the financial support of the New Zealand Vice-Chancellors' Committee through the award of a Claude McCarthy Fellowship. We thank Einar Høgseth for his technical skill in the design and maintenance of the speed of sound equipment.

References

1 T.H. Lilley, in: Biochemical thermodynamics, 2nd Ed., ed. M.N. Jones (Elsevier, Amsterdam, 1988) ch. 1.
2 C. Jolicoeur, B. Riedl, D. Desrochers, L.L. Lemelin, R. Zamojska and O. Enea, J. Solution Chem. 15 (1986) 109.
3 K.P. Murphy and S.J. Gill, J. Mol. Biol. 222 (1991) 699.
4 G.I. Makhatadze and P.L. Privalov, J. Mol. Biol. 213 (1990) 375.
5 G.R. Hedwig, Biopolymers 32 (1992) 537.
6 R. Bhat and J.C. Ahluwalia, J. Phys. Chem. 89 (1985) 1099.
7 J.F. Reading and G.R. Hedwig, J. Chem. Soc. Faraday Trans. 86 (1990) 3117.
8 G.R. Hedwig, J. Chem. Soc. Faraday Trans, 89 (1993) 2761.
9 G.R. Hedwig and H. Høiland, J. Chem. Thermodyn. 23 (1991) 1029.
10 M. Iqbal and R.E. Verrall, J. Biol. Chem. 263 (1988) 4159.
11 R. Garnsey, R.J. Boe, R. Mahoney and T.A. Litovitz, J. Chem. Phys. 50 (1969) 5222.
12 G.R. Hedwig and H. Høiland J. Solution Chem. 20 (1991) 1113.
13 E.P. Papadakis, J. Acoust. Soc. Am. 52 (1972) 843.
14 G.S. Kell, J. Chem. Eng. Data 12 (1967) 66.
15 V.A. Del Grosso and C.W. Mader, J. Acoust. Soc. Am. 52 (1972) 1442.
16 G.R. Hedwig and H. Høiland, J. Chem. Thermodyn. 25 (1993) 349.
17 T. Ogawa, M. Yasuda and K Mizutani, Bull. Chem. Soc. Japan 57 (1984) 662.
18 J.G. Mathieson and B.E. Conway, J. Solution Chem. 3 (1974) 455.
19 D.P. Kharakoz, J. Phys. Chem. 95 (1991) 5634.
20 H. Høiland, in: Thermodynamic data for biochemistry and biotechnology, ed. H.-J. Hinz (Springer, Berlin, 1986) ch. 4.
21 F.J. Millero, A. Lo Surdo and C. Shin, J. Phys. Chem. 82 (1978) 784.
22 G.R. Hedwig, J. Chem. Thermodyn. 23 (1991) 123.
23 J.T. Edward, J. Chem. Ed. 47 (1970) 261.
24 B.E. Conway and R.E. Verrall, J. Phys. Chem. 70 (1966) 3952.
25 S. Cabani, G. Conti and E. Matteoli, J. Solution Chem. 8 (1979) 11.
26 B.E. Conway and E. Ayranci, J. Chem. Thermodyn. 20 (1988) 9.