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Isentropic and isothermal compressibilities of the backbone glycyl group of proteins in aqueous solution

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Abstract

The partial molar isentropic compressibilities at infinite dilution, $K_{S,2}^{o}$, have been determined for the peptides serylglycine, serylglycylglycine and serylglycylglycylglycine in aqueous solution at 25 °C. The partial molar volumes at infinite dilution, V_2^{o}, have also been determined for these peptides in aqueous solution at the temperatures 15, 30 and 40 °C. These results, along with those obtained previously at 25 °C, were used to derive the partial molar exansibilities, E_2^{o}, of the peptides at 25 °C, which in turn were used to convert the isentropic compressibilities into the partial molar isothermal compressibilities at infinite dilution, $K_{T,2}^{o}$. These $K_{S,2}^{o}$ and $K_{T,2}^{o}$ results were used to obtain the partial molar compressibilities of the glycyl group CH_2CONH at 25 °C. The results are compared with those obtained using data for other series of peptides of sequence ala(gly)$_n$, n=1–4, and (gly)$_n$, n=2–5.

Keywords: Partial molar isentropic compressibility; Partial molar isothermal compressibility; Speed of sound; Glycyl group; Aqueous solution; Peptide model compounds

1. Introduction

The partial specific (or molar) compressibilities of proteins in aqueous solution are thermodynamic properties of considerable interest, not only because of their fundamental importance in the quantitative assessment of the pressure stability of proteins [1–3], but also because of the direct link, through statistical thermodynamics, between the mean square volume fluctuations, $\langle \delta V^2 \rangle$, of a protein and its isothermal coefficient of compressibility, $\kappa_T \{\kappa_T = -(1/V)(\partial V/\partial p)_T\}$ [4,5],

$$\langle \delta V^2 \rangle = k_B T V \kappa_T \quad (1)$$

where k_B is Boltzmann's constant, T is the absolute temperature and V is the volume of the protein molecule. Protein compressibilities in solution are almost invariably derived from speed of sound measurements so the thermodynamic quantities obtained are isentropic rather than isothermal [6–8]. Although isentropic compressibilities can be converted into the more useful isothermal quantities [9,10], a dearth of reliable coefficient of thermal expansion data for proteins, α_P, required for the conversion has limited the widespread use of Eq. (1). However, with the recent development of pressure perturbation calorimetry [11–13], which enables α_P data to be readily determined, it is likely that the volume fluctuations of proteins will receive more attention than has been the case hitherto.

The partial molar compressibility of a protein is a thermodynamic property that is a particularly sensitive measure of protein hydration effects in aqueous solution [14,15]. Consequently, the determination of protein compressibilities provides some insight into both the conformational transitions of proteins [2,16] and the characterization of globular proteins in solution [17]. Since globular proteins are large complex molecules, the study of small compounds chosen to model the various functional groups of proteins is one approach that can contribute to a better understanding of the hydration contribution to the compressibility of a protein [14,18]. Several studies have used this model compound

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approach in recent years [14,19–24], generally with a focus on either the amino acid side-chain residues of a protein [19,21–23] or the repeating unit of the backbone chain [20,24].

The partial molar isentropic compressibility of the backbone glycyl group, CH_2CONH, of a protein was first determined using partial molar isentropic compressibilities at infinite dilution, $K^{o}_{S,2}$, for the oligoglycines, $(gly)_n$, $n=1$–5, in aqueous solution over the temperature range 18 to 55 °C [24]. For the higher members of the series, $(gly)_n$, $n=3$–5, $K^{o}_{S,2}$ was found to be a linear function of the number of glycyl groups, so the glycyl group contributions at the various temperatures were obtained from the slopes of these linear functions [24]. However, the results obtained do have high uncertainties because of the low solubility of tetra- and pentaglycine in water. In an attempt to overcome this difficulty, we determined [20] the partial molar isentropic and the partial molar isothermal compressibilities at infinite dilution, $K^{o}_{T,2}$, for aqueous solutions at 25 °C of the analogous series of peptides $ala(gly)_n$, $n=1$–4, for which the higher members are more soluble than the corresponding oligoglycines. The unexpected result obtained was that the glycyl group contribution based on $K^{o}_{S,2}$ data for the $(gly)_n$ peptides differed significantly from that obtained using data for the $ala(gly)_n$ peptides [20]. Significant differences were also noted between the isothermal compressibilities of the glycyl group at 25 °C derived using the two peptides series [20].

To further investigate this discrepancy, the partial molar isentropic compressibilities at infinite dilution have been determined for a new series of peptides, comprising serylglycine (sergly), serylglycylglycine ($ser(gly)_2$) and serylglycylglycylglycine ($ser(gly)_3$), in aqueous solution at 25 °C. The partial molar volumes at infinite dilution, V^{o}_2, have also been obtained for aqueous solutions of these peptides at the temperatures 15, 30 and 40 °C. These results, together with those reported previously [25] for the temperature of 25 °C, were used to derive the partial molar expansibilities at infinite dilution, E^{o}_2, for the peptides at 25 °C. These E^{o}_2 values and the partial molar heat capacities at infinite dilution, $C^{o}_{p,2}$, determined earlier [25] were used to convert the isentropic compressibilities into isothermal compressibilites. Both the isentropic and isothermal compressibilites for these $ser(gly)_n$ peptides were used to determine the respective glycyl group contributions at 25 °C.

2. Materials and methods

The preparation, purification and analyses of the peptides of sequence $ser(gly)_n$, $n=1$–3, have been described in detail elsewhere [25]. The samples used in this work were either from the same batches of solids prepared previously, or were recrystallized solids recovered from the aqueous solutions used in this earlier study [25]. For the recovered materials, the reproducibility of solution densities at 25 °C [25] was used as a criterion of purity. The peptide $ser(gly)_3$, which is slightly hygroscopic, was dried under vacuum at 50 °C before use. The remaining peptides were routinely dried under vacuum at room temperature before the preparation of solutions. The water used to prepare solutions and as the reference solvent was deionized, glass distilled and thoroughly degassed immediately prior to use. All solutions were prepared by mass and corrections were made for the effect of air buoyancy.

Densities of solutions were measured using an Anton Paar digital density meter (model DMA 60/602) as outlined previously [26,27]. The reproducibility of an individual density measurement was to within $\pm 3.0\times10^{-6}$ g cm^{-3}. Sound speed measurements for the peptide solutions were carried out during a visit by the author to the University of Bergen. Details of the rubidium clock sound velocity meter used have been described elsewhere [28]. The temperature of the thermostat bath was maintained to ±0.001 °C using the methods outlined by Horvat-Szabo et al. [29]. The reproducibility of a speed of sound measurement was to better than ±0.005 m s^{-1}.

3. Results

3.1. Partial molar volumes

Densities at the temperatures 15, 30 and 40 °C for aqueous solutions of the peptides sergly, $ser(gly)_2$ and $ser(gly)_3$ are given in Tables 1–3, respectively. The apparent molar volumes of the solutes, V_ϕ, were calculated from the solution densities, ρ, using the equation

$$V_\phi = (M_2/\rho)-(\rho-\rho^{o}_1)/(\rho\rho^{o}_1 m) \quad (2)$$

where M_2 is the solute molar mass, m is the solution molality and ρ^{o}_1 is the density of the solvent. The ρ^{o}_1 values used for water were those reported by Kell [30] (0.999101, 0.995650 and 0.992219 g cm^{-3} at 15, 30 and 40 °C, respectively). The V_ϕ values, together with their uncertainties estimated using the procedures outlined in previous work [27], are also given in

Table 1
Densities and apparent molar volumes for aqueous solutions of serylglycine at the temperatures 15, 30 and 40 °C

m (mol kg^{-1})	ρ (g cm^{-3})	V_ϕ (cm^3 mol^{-1})	m (mol kg^{-1})	ρ (g cm^{-3})	V_ϕ (cm^3 mol^{-1})
15 °C					
0.03193	1.001258	94.41±0.09[a]	0.06963	1.003782	94.51±0.04
0.03602	1.001533	94.43±0.08	0.07980	1.004457	94.55±0.04
0.04091	1.001862	94.42±0.07	0.08979	1.005117	94.61±0.03
0.04113	1.001876	94.45±0.07	0.09883	1.005712	94.66±0.03
0.04990	1.002463	94.49±0.06	0.11270	1.006633	94.63±0.03
0.05993	1.003131	94.55±0.05			
30 °C					
0.02605	0.997375	95.90±0.12	0.08462	1.001200	96.16±0.04
0.03760	0.998135	95.94±0.08	0.09459	1.001846	96.18±0.03
0.04604	0.998690	95.96±0.07	0.10373	1.002434	96.23±0.03
0.05693	0.999399	96.07±0.05	0.11492	1.003154	96.27±0.03
0.06595	0.999994	96.00±0.05	0.12299	1.003666	96.34±0.03
0.07388	1.000502	96.14±0.04			
40 °C					
0.02390	0.993787	96.64±0.13	0.08475	0.997735	96.77±0.04
0.03483	0.994500	96.68±0.09	0.08960	0.998044	96.82±0.03
0.04589	0.995222	96.67±0.07	0.10024	0.998728	96.83±0.03
0.05673	0.995927	96.67±0.05	0.11172	0.999465	96.83±0.03
0.07868	0.997343	96.77±0.04			

Table 2
Densities and apparent molar volumes for aqueous solutions of serylglycylglycine at the temperatures 15, 30 and 40 °C

m (mol kg^{-1})	ρ (g cm^{-3})	V_ϕ (cm^3 mol^{-1})	m (mol kg^{-1})	ρ (g cm^{-3})	V_ϕ (cm^3 mol^{-1})
15 °C					
0.02400	1.001248	129.50±0.13	0.06978	1.005297	129.64±0.04
0.03191	1.001951	129.56±0.09	0.08021	1.006214	129.63±0.04
0.03980	1.002652	129.56±0.08	0.08837	1.006926	129.67±0.03
0.05143	1.003680	129.61±0.06	0.09952	1.007901	129.67±0.03
0.05976	1.004419	129.56±0.05			
30 °C					
0.02349	0.997705	131.62±0.13	0.06890	1.001636	131.73±0.04
0.03031	0.998303	131.52±0.10	0.07989	1.002588	131.64±0.04
0.03966	0.999116	131.55±0.08	0.08930	1.003382	131.80±0.03
0.04980	0.999992	131.63±0.06	0.09764	1.004086	131.89±0.03
0.05961	1.000842	131.61±0.05			
40 °C					
0.02368	0.994267	132.80±0.13	0.06962	0.998191	132.99±0.04
0.03410	0.995162	132.86±0.09	0.07963	0.999036	133.05±0.04
0.03854	0.995547	132.77±0.08	0.08720	0.999674	133.08±0.04
0.04880	0.996419	132.93±0.06	0.09948	1.000703	133.16±0.03
0.05996	0.997368	133.00±0.05			

Tables 1–3. For the dilute solutions used in this study, the molality dependence of V_ϕ for the peptides can be represented by the linear equation

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

where V_2^o is the partial molar volume of the solute at infinite dilution and S_v is the experimental slope. Values of V_2^o and S_v, and their standard errors obtained from weighted least-squares analyses of the V_ϕ data, are given in Table 4. In these analyses,

Table 3
Densities and apparent molar volumes for aqueous solutions of serylglycylglycylglycine at the temperatures 15, 30 and 40 °C

m (mol kg^{-1})	ρ (g cm^{-3})	V_ϕ (cm^3 mol^{-1})	m (mol kg^{-1})	ρ (g cm^{-3})	V_ϕ (cm^3 mol^{-1})
15 °C					
0.02505	1.001872	165.22±0.12	0.06265	1.005969	165.54±0.05
0.03096	1.002518	165.36±0.10	0.07024	1.006801	165.40±0.04
0.03926	1.003435	165.18±0.08	0.08011	1.007857	165.55±0.04
0.04753	1.004333	165.35±0.06	0.08625	1.008522	165.51±0.04
0.05461	1.005103	165.40±0.06	0.09333	1.009289	165.45±0.03
30 °C					
0.02587	0.998447	167.90±0.12	0.05290	1.001338	168.02±0.06
0.03124	0.999026	167.89±0.10	0.06147	1.002247	168.08±0.05
0.03662	0.999600	167.97±0.08	0.07151	1.003312	168.08±0.05
0.04642	1.000647	168.03±0.07	0.08284	1.004500	168.19±0.04
40 °C					
0.02401	0.994791	169.19±0.13	0.05651	0.998225	169.43±0.05
0.02699	0.995107	169.24±0.11	0.06183	0.998785	169.42±0.05
0.03124	0.995558	169.29±0.10	0.07535	1.000200	169.46±0.04
0.03980	0.996466	169.31±0.08	0.08573	1.001278	169.54±0.04
0.04839	0.997376	169.29±0.06			

Table 4
Partial molar volumes at infinite dilution and the S_v values for the ser(gly)$_n$ peptides in aqueous solution at various temperatures

t (°C)	V_2^o (cm^3 mol^{-1})	S_v (cm^3 kg mol^{-2})	$10^{15}K_{S,2}^o$ (m^3 mol^{-1} Pa^{-1})	$10^{15}S_k$ (m^3 kg mol^{-2} Pa^{-1})
Sergly				
15	94.34±0.03	2.8±0.4		
25	95.31±0.04[a]	1.8±0.5[a]	−39.19±0.07	15.1±0.9
30	95.77±0.03	4.5±0.4		
40	96.56±0.03	2.6±0.3		
Serglygly				
15	129.49±0.03	1.9±0.4		
25	130.75±0.09[a]	2.8±0.9[a]	−44.45±0.08	18.5±1.1
30	131.34±0.09	5.1±1.1		
40	132.67±0.04	4.9±0.5		
Serglyglygly				
15	165.22±0.10	3.1±1.3		
25	167.17±0.04[a]	4.3±0.5[a]	−47.11±0.09	27.1±1.3
30	167.77±0.04	4.9±0.6		
40	169.10±0.04	5.0±0.5		

[a] From Wise and Hedwig [25].

the weighting factors used were the inverse squares of the uncertainties for the apparent molar volumes.

The V_2^o results for the three peptides, along with those at 25 °C reported in earlier work [25], are displayed in Fig. 1.

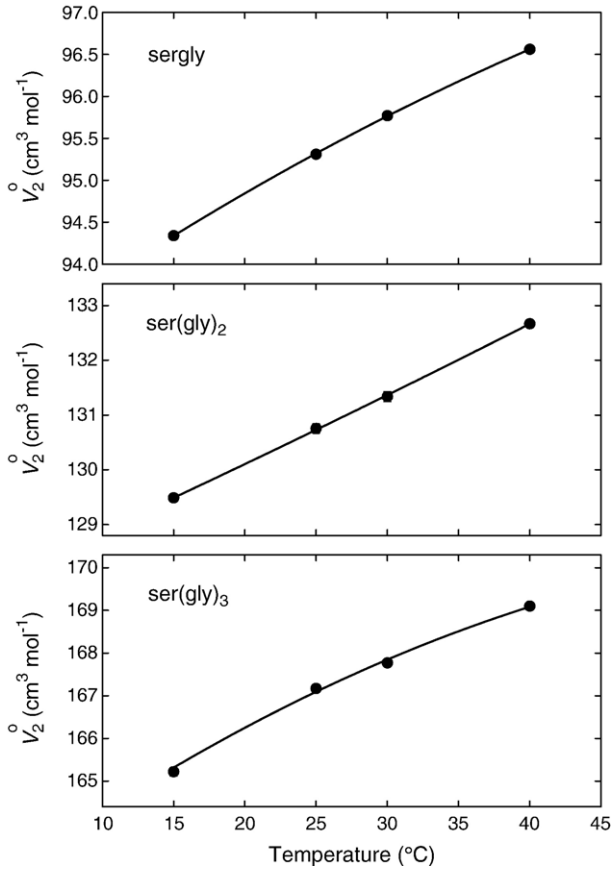

Fig. 1. Temperature dependences of the partial molar volumes at infinite dilution for the peptides ser(gly)$_n$, n=1–3.

For each peptide, the smooth curve drawn through the data was derived from a weighted least-squares analysis of the V_2^o results using the equation

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

where T_m represents the mid-point temperature of the range used, and a, b and c are the fitted coefficients. The weighting factor for each V_2^o value was taken as $1/(\delta V_2^o)^2$, where δV_2^o is the standard error of V_2^o. The polynomial coefficients, together with their standard deviations obtained from the least-squares analyses, are given in Table 5.

For the peptide ser(gly)$_2$, the coefficient c, which is barely statistically significant, has a positive value. This result is unexpected as the corresponding c coefficients for a wide variety of di-, tri-, tetra- and pentapeptides are all negative [20,31,32]. The positive c value observed for ser(gly)$_2$ is simply a manifestation of the scatter in the V_2^o values. Presumably, if the V_2^o data for ser(gly)$_2$ were determined over a wider temperature range, then a negative value of c would ensue. Since the primary objective of the current $V_2^o(T)$ study is to obtain partial molar expansibilities (vide infra) at 25 °C, the current V_2^o results are deemed sufficient for this purpose. The uncertainties for the polynomial coefficients for ser(gly)$_3$ are higher than is desirable. This is probably a reflection of the difficulties associated with determining densities for solutions of a hygroscopic peptide.

3.2. Partial molar expansibilities

The partial molar expansibility of each peptide at infinite dilution, $E_2^o = (\partial V_2^o/\partial T)_p$, can be obtained using the polynomial coefficients given in Table 5. Differentiation of Eq. (4) with respect to temperature at constant pressure leads to

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

Since in this work the value of T_m is 27.5 °C, it follows from Eq. (5) that the quantity $(b-5c)$ is equivalent to E_2^o at a temperature of 25 °C. Values of E_2^o at 25 °C for the peptides ser(gly)$_n$, n=1–3, are given in Table 6. The uncertainty for each E_2^o was estimated by the application of propagation of errors [33] to Eq. (5). It is worth noting that, if the V_2^o data for ser(gly)$_2$ are analysed using Eq. (4) with c=0, then the value of E_2^o at 25 °C is 0.1270± 0.0008 cm^3 mol^{-1} K^{-1}, which is in agreement, within the combined uncertainties, with the result given in Table 6. Also included in Table 6 is the E_2^o value at 25 °C for the amino acid serine obtained from an analysis of literature V_2^o data [34]. The actual V_2^o values used in the calculation (76.21±0.06, 76.92±0.07, 77.92±0.19 and 78.53±0.07 cm^3 mol^{-1} at the temperatures 15, 25, 40 and 55 °C, respectively) were obtained from a reanalysis (A.W. Hakin, personal communication) of the reported density data [34] using an updated version of the original software package.

Table 5
Coefficients of Eq. (4)

Peptide	a (cm^3 mol^{-1})	b (cm^3 K^{-1} mol^{-1})	10^3c (cm^3 K^{-2} mol^{-1})
Sergly	95.546±0.008	0.0889±0.0005	-0.61_5±0.07
Ser(gly)$_2$	131.05±0.03	0.1271±0.0008	0.2_0±0.1_8
Ser(gly)$_3$	167.48±0.09	0.15_1±0.01_2	-1.8_0±1.2

Table 6
Partial molar expansibilities, heat capacities and compressibilities at infinite dilution for the ser(gly)$_n$ peptides, and the partial molar compressibilities for the oligopeptides X(gly)$_n$, X=ala or ser, in aqueous solution at 25 °C

Solute	E_2^o (cm^3 mol^{-1} K^{-1})	$C_{p,2}^o$ (J K^{-1} mol^{-1})	$-10^{15}K_{S,2}^o$ (m^3 mol^{-1} Pa^{-1})	$-10^{15}K_{T,2}^o$ (m^3 mol^{-1} Pa^{-1})
Ser	0.082_5±0.002[a]	114.1±0.4[b]	30.5±0.2[c]	27.6±0.2
Sergly	0.0920 ±0.0006	190.0±1.1[d]	39.19±0.07	36.02±0.07
Ser(gly)$_2$	0.126_1±0.001	275.3±1.1[d]	44.45±0.08	40.12±0.09
Ser(gly)$_3$	0.16_0±0.01_3	360.3±1.6[d]	47.11±0.09	41.6_4±0.5
Ala	0.068_3±0.006[a]	141.2±0.6[e]	25.0±0.1[e]	22.7±0.2
Alagly			34.2±0.1[e]	31.1±0.2[e]
Ala(gly)$_2$			39.1±0.1[e]	34.9±0.1[e]
Ala(gly)$_3$			41.80±0.09[e]	35.9±0.4[e]
Ala(gly)$_4$			44.6±0.1[e]	38.0±0.1[e]
Gly			27.0±0.4[e]	24.7±0.4[e]
Glygly			40.2±0.1[e]	36.8±0.2[e]
(Gly)$_3$			44.9±0.1[e]	39.4±0.2[e]
(Gly)$_4$			45.9±0.7[e]	40.0±0.7[e]
(Gly)$_5$			47.2±1.4[e]	40.1±1.8[e]

[a] Derived using updated V_2^o data. See text.
[b] Value from Hakin et al. [34]; uncertainty from A.W. Hakin, personal communication.
[c] From Mizuguchi et al. [21]. See text.
[d] From Wise and Hedwig [25].
[e] From Hakin et al. [20].

3.3. Partial molar compressibilities

The measured sound speeds, u, for aqueous solutions of the three peptides ser(gly)$_n$, n=1–3, at 25 °C are given in Table 7. The isentropic coefficient of compressibility, $\kappa_S\{\kappa_S=-(1/V)(\partial V/\partial p)_S\}$, for each solution was obtained using the Newton–Laplace equation [35]

$$\kappa_S = 1/(u^2\rho) \quad (6)$$

The density for each solution was calculated using a power series in the solution molality, m, of the form

$$\rho = \rho_1^o + p_1(m/m^o) + p_2(m/m^o)^2 \quad (7)$$

where p_1 and p_2 are adjustable parameters, ρ_1^o is the density of water at 25 °C (0.997047 g cm^{-3}) [30], and m^o=1.0 mol kg^{-1}. The parameters and their estimated uncertainties, which were obtained from least-squares analyses of density data at 25 °C reported in a previous paper [25], are given in Table 8.

The values of κ_S were used to calculate the apparent molar isentropic compressibility, $K_{S,\phi}$, which is defined by the relation [9,36]

$$K_{S,\phi} = M_2\kappa_S/\rho - (\kappa_{S,1}^o\rho - \kappa_S\rho_1^o)/(m\rho\rho_1^o) \quad (8)$$

where $\kappa_{S,1}^o$ is the isentropic coefficient of compressibility for solvent water ($\kappa_{S,1}^o$=4.47736×10^{-10} Pa^{-1} at 25 °C) [37], and

Table 7
Sound speeds and apparent molar isentropic compressibilities for aqueous solutions of the ser(gly)$_n$ peptides

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})
Sergly					
0.03051	1499.306	−38.77±0.10	0.06804	1502.486	−38.17±0.04
0.03646	1499.816	−38.71±0.08	0.07793	1503.308	−37.94±0.04
0.04372	1500.432	−38.58±0.07	0.08708	1504.081	−37.85±0.03
0.05133	1501.072	−38.39±0.06	0.09544	1504.790	−37.80±0.03
0.05874	1501.699	−38.28±0.05			
Serglygly					
0.02801	1499.622	−43.93±0.11	0.06328	1503.285	−43.29±0.05
0.03715	1500.576	−43.78±0.08	0.07490	1504.476	−43.01±0.04
0.04717	1501.619	−43.62±0.06	0.08228	1505.247	−42.97±0.04
0.05508	1502.434	−43.41±0.05			
Serglyglygly					
0.02538	1499.754	−46.50±0.12	0.06178	1504.083	−45.36±0.05
0.03582	1501.003	−46.17±0.08	0.07013	1505.081	−45.24±0.04
0.04389	1501.961	−45.87±0.07	0.08179	1506.451	−44.90±0.04
0.05343	1503.104	−45.70±0.06			

the remaining symbols are as defined for Eq. (2). The $K_{S,\phi}$ results along with their uncertainties, which were estimated using the propagation of errors methods as outlined previously [38], are given in Table 7.

For the dilute solutions of peptides used in this work, the molality dependence of $K_{S,\phi}$ can be represented by the linear equation

$$K_{S,\phi} = K^{o}_{S,2} + S_k m \quad (9)$$

where $K^{o}_{S,2}$ is the partial molar isentropic compressibility of the solute at infinite dilution and S_k is the experimental slope. Values of $K^{o}_{S,2}$ and S_k and their standard deviations obtained from weighted least-squares analyses of the apparent molar isentropic compressibilities using Eq. (9) are given in Table 4.

The partial molar heat capacities at infinite dilution, $C^{o}_{p,2}$, for the ser(gly)$_n$ peptides at 25 °C have been determined in recent work [25]. These $C^{o}_{p,2}$ results, which are given in Table 6, the E^{o}_2 values determined herein, and various properties of the solvent at 25 °C were used to convert the partial molar isentropic compressibilities at infinite dilution into the more useful partial molar isothermal compressibilities at infinite dilution, $K^{o}_{T,2}\{K^{o}_{T,2}=-(\partial V^{o}_2/\partial p)_T\}$. The expression used for this conversion is [9,10]

$$K^{o}_{T,2} = K^{o}_{S,2} + \delta^{o}_1(2E^{o}_2/\alpha^{o}_1 - C^{o}_{p,2}/\sigma^{o}_1) \quad (10)$$

where the quantities δ^{o}_1, α^{o}_1 and σ^{o}_1 are all properties of the pure solvent: σ^{o}_1 is the heat capacity per unit volume, σ^{o}_1=4.1670 J K^{-1} cm^{-3} at 25 °C [39]; α^{o}_1 is the isobaric expansibility $\{\alpha^{o}_1=(\partial V^{o}_1/\partial T)_p/V^{o}_1\}$, $10^6\alpha^{o}_1$=257.21 K^{-1} at 25 °C [40]; δ^{o}_1 is the difference between the isothermal coefficient of compressibility $\kappa^{o}_{T,1}$ $\{\kappa^{o}_{T,1}=-(\partial V^{o}_1/\partial p)_T/V^{o}_1\}$ and the isentropic coefficient of compressibility $\kappa^{o}_{S,1}$ $\{\kappa^{o}_{S,1}=-(\partial V^{o}_1/\partial p)_S/V^{o}_1\}$, $\delta^{o}_1=\kappa^{o}_{T,1}-\kappa^{o}_{S,1}=0.4736\times10^{-11}$ Pa^{-1} at 25 °C [37,40]. The values of $K^{o}_{T,2}$ for serine and the ser(gly)$_n$ peptides, and their uncertainties estimated using propagation of errors methods, are given in Table 6. For serine, the $K^{o}_{S,2}$ value used was obtained from a weighted least-squares analysis of literature $K_{S,\phi}$ data [21] with the uncertainty in the speed of sound taken as ±0.01 m s^{-1}. The requirement of precise E^{o}_2 data in the calculation of partial molar isothermal compressibilities using Eq. (10) is clearly illustrated by the high uncertainty of the $K^{o}_{T,2}$ result for ser(gly)$_3$.

For the purposes of comparison, $K^{o}_{S,2}$ and $K^{o}_{T,2}$ results obtained previously [20] for the oligoglycines, (gly)$_n$, n=1–5, and the peptide of sequence ala(gly)$_n$, n=1–4, are also given in Table 6. For the amino acid alanine, the $K^{o}_{T,2}$ value given in Table 6 has been recalculated using an updated E^{o}_2 value derived using Eqs. (4), (5) and V^{o}_2 data (59.64±0.03, 60.47±0.02, 61.14±0.05 and 61.53±0.07 cm^3 mol^{-1} at the temperatures 15, 25, 40 and 55 °C, respectively) obtained from a reanalysis (A.W. Hakin, personal communication) of published density data [34] as described for serine in Section 3.2.

4. Discussion

The partial molar isentropic and isothermal compressibilities for the amino acids serine and alanine, and the two series of peptides ser(gly)$_n$, n=1–3, and ala(gly)$_n$, n=1–4, are plotted as a function of the number of glycyl groups in Fig. 2. In the interests of clarity, the compressibilities for the oligoglycines have not been included in Fig. 2 because, as is evident from Table 6, the $K^{o}_{S,2}$ and $K^{o}_{T,2}$ values for di- and triglycine are very similar to those for the corresponding seryl peptides. Moreover, a detailed comparison between the compressibilities for the oligoglycines and the ala(gly)$_n$ peptides has been reported in earlier work [20]. What is immediately obvious from Fig. 2 is that it would have been desirable to have $K^{o}_{S,2}$ and $K^{o}_{T,2}$ data for the pentapeptide ser(gly)$_4$. This peptide was indeed synthesized as part of our earlier study [25], but the material was far too hygroscopic for reliable solution density and sound speed data to be obtained.

As the number of glycyl groups increases from n=0 to n=2, the values for both $K^{o}_{S,2}$ and $K^{o}_{T,2}$ become more negative but the decrease is significantly larger from n=0 to n=1 (ca. 8.7×10^{-15} m^3 mol^{-1} Pa^{-1}) than it is from n=1 to n=2 (ca. 4.5×10^{-15} m^3 mol^{-1} Pa^{-1}). This trend can be rationalized in terms of the separation of the charged NH_3^+ and CO_2^- functional groups as the peptide chain length increases. Electrostricted water in the hydration shells surrounding these charged groups is less compressible than water in the bulk solvent [14,41,42]. The increased separation of the charged centres in a dipeptide compared with that in an amino acid leads to a significant reduction in the overlap of the hydration shells of the NH_3^+ and CO_2^- groups, with a concomitant increase in electrostriction and

Table 8
Coefficients of Eq. (7)

Peptide	p_1 (g cm^{-3})[a]	p_2 (g cm^{-3})[a]
Sergly	0.06692±0.00004	−0.0080±0.0004
Ser(gly)$_2$	0.08856±0.00009	−0.0141±0.0009
Ser(gly)$_3$	0.10924±0.00004	−0.0220±0.0005

[a] Calculated using density data from Wise and Hedwig [25].

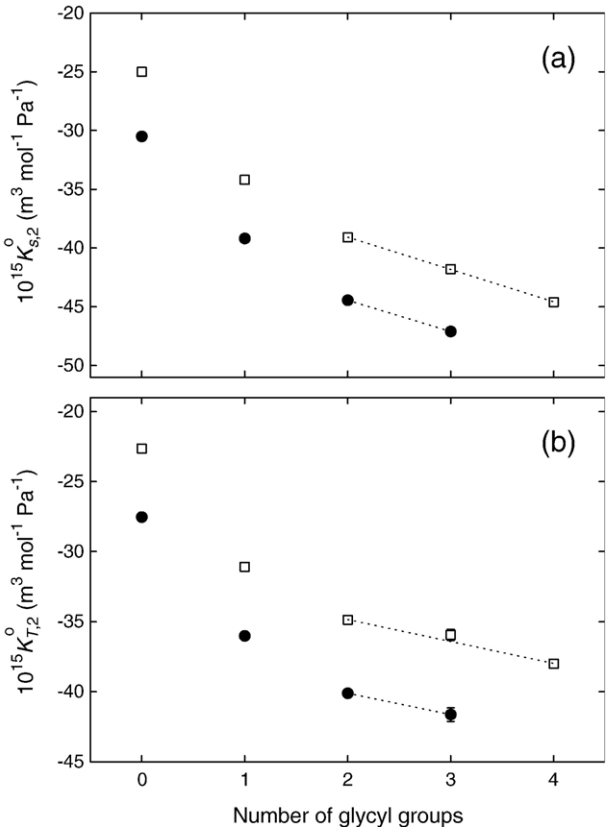

Fig. 2. Partial molar isentropic (a) and isothermal (b) compressibilities at infinite dilution and at 25 °C for ala(gly)$_n$, n=0–4, and for ser(gly)$_n$, n=0–3, as a function of the number of glycyl groups: (●) ser(gly)$_n$, (□) ala(gly)$_n$, (·····) limiting slope.

hence a decrease in the values of $K^{o}_{S,2}$ and $K^{o}_{T,2}$. With the addition of second glycyl group to form a tripeptide, the charged centres are now far enough apart that their hydration spheres no longer overlap [14,24]. Consequently, there is a further, but smaller, decrease in the values of $K^{o}_{S,2}$ and $K^{o}_{T,2}$ when the value of n increases from n=1 to n=2.

For homologous series of peptides, such as the oligoglycines [20,24], the α,ω-aminocarboxylic acids [43] and the ala(gly)$_n$ peptides [20], the values of $K^{o}_{S,2}$ for the tripeptides and beyond are approximate linear functions of the number of glycyl groups in the chain. This suggests that the central glycyl groups in these peptides are independently hydrated [24] and, as a consequence of this, the glycyl group contribution to $K^{o}_{S,2}$, K^{o}_{S}(CH_2CONH), can be obtained from the slope of the linear plot of $K^{o}_{S,2}$ against the number of glycyl groups. This linearity, which is illustrated for the peptides ala(gly)$_n$, n=2–4, in Fig. 2(a), also extends to the isothermal compressibility [20] as shown in Fig. 2(b). Values of K^{o}_{S}(CH_2CONH) and the glycyl group contribution to the isothermal compressibility, K^{o}_{T}(CH_2CONH), were obtained from weighted linear least-squares analyses of the $K^{o}_{S,2}$ and $K^{o}_{T,2}$ data [20]. In the absence of a pentapeptide for the ser(gly)$_n$ peptides, the glycyl group contribution was estimated simply from the difference between the partial molar compressibilites of the tri- and tetrapeptides, i.e. the slope of the line joining the two points as shown in Fig. 2. Values of K^{o}_{S}(CH_2CONH) and K^{o}_{T}(CH_2CONH), and their estimated uncertainties, obtained from results for the ser(gly)$_n$ series, along with those obtained previously [20] using $K^{o}_{S,2}$ and $K^{o}_{T,2}$ data for the ala(gly)$_n$ and (gly)$_n$ peptides are given in Table 9.

The results given in Table 9 indicate that there is good agreement, within the combined uncertainties, between the K^{o}_{S}(CH_2CONH) values obtained using the ser(gly)$_n$ and ala(gly)$_n$ peptides. Similarly, the K^{o}_{T}(CH_2CONH) values obtained from these two series of peptides are concordant, although, unfortunately, the estimated uncertainty for the value of K^{o}_{T}(CH_2CONH) obtained in this work is high (vide supra). This agreement between the two sets of K^{o}_{S}(CH_2CONH) and K^{o}_{T}(CH_2CONH) results is not unexpected. The replacement of a hydrogen atom by the –OH moiety on the methyl side-chain of an N-terminal amino acid should not have any significant effect on the hydration of the amino acids further down the chain. In general, for any series of peptides of sequence X(gly)$_n$, where X is any amino acid, the hydration of the repeating glycyl unit as the chain length increases should be independent of the nature of X, assuming that the basic chain configurations are the same for all peptides. Given this expectation, it is difficult, therefore, to find a satisfactory explanation for the significant differences that are observed between the K^{o}_{S}(CH_2CONH) and K^{o}_{T}(CH_2-$CONH$) results based on data for the (gly)$_n$ peptides [20] and those for the ser(gly)$_n$ and ala(gly)$_n$ peptides. The uncertainties for the $K^{o}_{S,2}$ and $K^{o}_{T,2}$ values of tetra- and pentaglycine are indeed high but, as noted previously [20], even if the data are analysed in such a way to maximize the limiting slopes, the results obtained are still not in satisfactory agreement with those based on the ala(gly)$_n$ series or the ser(gly)$_n$ series. Since all the simple peptides used in these studies are expected to have a high degree of conformational flexibility in aqueous solution, it is unlikely that the different K^{o}_{S}(CH_2CONH) and K^{o}_{T}(CH_2CONH) results based on the (gly)$_n$ series can be accounted for in terms of some subtle change in the hydration of the glycyl group. The agreement between the glycyl group compressibilities obtained using the ser(gly)$_n$ and ala(gly)$_n$ peptides provides additional support for our previous suggestion [20] that the K^{o}_{S}(CH_2CONH) and K^{o}_{T}(CH_2CONH) values that best represent the glycyl group contributions in polypeptides are those derived using data for the ala(gly)$_n$ peptides.

It is evident from Fig. 2 that the dependences of both $K^{o}_{S,2}$ and $K^{o}_{T,2}$ on the number of glycyl groups for the ser(gly)$_n$ peptides closely parallel those for the ala(gly)$_n$ peptides. The differences between the $K^{o}_{S,2}$ values for the corresponding members of the series are very similar (5.5±0.2, 5.0±0.1, 5.4±0.1 and 5.3±0.1×10^{-15} m^3 mol^{-1} Pa^{-1} for n=0 to 3, respectively), while the

Table 9
Partial molar isentropic and isothermal compressibilities of the glycyl group in aqueous solution at 25 °C

Peptide series	$10^{15}K^{o}_{S}$(CH_2CONH) (m^3 mol^{-1} Pa^{-1})	$10^{15}K^{o}_{T}$(CH_2CONH) (m^3 mol^{-1} Pa^{-1})
Ser(gly)$_n$, n=2–3	-2.6_6±0.1_2	-1.5_2±0.5
Ala(gly)$_n$, n=2–4	−2.75±0.03	−1.57±0.09
(Gly)$_n$, n=3–5	−1.08±0.08 [a]	-0.4_9±0.1_0 [a]

[a] From Hakin et al. [20].

variation is slightly greater for $K^{o}_{T,2}$ (4.9±0.3, 4.9±0.2, 5.2±0.1 and 5.7±0.6×10^{-15} m^3 mol^{-1} Pa^{-1} for $n=0$ to 3, respectively). These results indicate that the change in hydration that occurs when a hydrogen atom of the methyl side-chain is replaced by the –OH moiety is essentially independent of the adjacent peptide chain.

The contribution that a polar group makes to the compressibility of a solute depends on its position relative to other polar or charged functional groups [2,14,44]. For example, the isolated –OH group in the *n*-alcohols makes a positive contribution to $K^{o}_{S,2}$, but the contribution to $K^{o}_{S,2}$ of an –OH group in sugars is negative [14,44]. This negative contribution arises because of cooperative hydrogen bonding effects, which produce water in the hydration shell that is less compressible than that in the bulk solvent. The more negative $K^{o}_{S,2}$ and $K^{o}_{T,2}$ values for the ser(gly)$_n$ peptides compared with those for the ala(gly)$_n$ peptides suggests that there is significant cooperative hydrogen bonding within the hydration shells of the terminal NH_3^+ group and the adjacent –CH_2OH side-chain for each of the ser(gly)$_n$ peptides.

In conclusion, the new $K^{o}_{S,2}$ and $K^{o}_{T,2}$ results for the ser(gly)$_n$ compounds confirm the values of the glycyl group compressibilities obtained previously [20] using data for the ala(gly)$_n$ peptides. Consequently, these K^{o}_{S}(CH_2CONH) and K^{o}_{T}(CH_2CONH) values are likely to be a good representation of the glycyl group contributions to the compressibilities of polypeptides in aqueous solution.

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