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Hydration of diglycyl tripeptides with non-polar side chains: a volumetric study

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Abstract

We have determined the apparent molar volumes and the apparent molar adiabatic compressibilities at 25°C of 10 X–Gly–Gly and Gly–Gly–X tripeptides in which X represents a residue with a non-polar side chain. We also have determined the changes in volume and compressibility which accompany neutralization of the amino and carboxyl termini in these tripeptides. The mutual influence of the non-polar side chain of the X residue and the terminal amino and carboxyl groups on the hydration of each other depends on the chemical nature of the side chain and the state of ionization of the termini. We interpret our data in terms of the hydration of the component aliphatic, aromatic, and charged atomic groups, as well as the mutual interactions between these groups.

Keywords: Peptides; Hydration; Ionization of terminal groups; Side chains; Volume; Compressibility

1. Introduction

Volume and compressibility are among the most sensitive and informative thermodynamic characteristics that reflect solute hydration [1–6]. These two volumetric observables have been used extensively to characterize the hydration properties of proteins in their native and denatured conformational states [7–20]. Perhaps, the biggest challenge in these studies is to interpret the macroscopic experimental data in terms of microscopic results. In this respect, it is still a matter of controversy whether the patterns of hydration of surface atomic groups of proteins and small molecules are similar. In other words, it is not clear to what extent small molecule data can be used to rationalize volumetric results obtained for proteins. In our recent work [17], we have proposed that the hydration properties of hydrophobic and polar uncharged groups of proteins may differ significantly from those of small molecules. By contrast, the hydration properties

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of charged groups in proteins, as reflected by their volumetric observables, are similar to those of small molecules. Consequently, small molecules make good models for protein charged groups.

In recognition of this fact, many low molecular weight model compounds with ionizable amino and carboxyl groups, such as short oligopeptides, have been investigated by means of volumetric methods [21–30]. In spite of the wealth of information provided by these investigations, there are important questions yet to be addressed. In particular, we still have only limited understanding of the effects of different side chains on the hydration properties of adjacent ionizable groups in their charged and neutralized states.

To this end, in this paper, we report systematic data on the partial molar volume, V°, and the partial molar adiabatic compressibility, κ°_S, of 10 diglycyl tripeptides (Gly–Gly–X or X–Gly–Gly) with aliphatic and aromatic side chains. In addition, we report the changes in V° and κ°_S accompanying neutralization of the amino and carboxyl termini of these tripeptides. We interpret our results in terms of hydration properties of the charged and uncharged termini as a function of the proximity and chemical composition of the adjacent non-polar side chains.

Diglycyl tripeptides (Gly–Gly–X or X–Gly–Gly) are good models for such studies as (non-polar) side chains adjacent to one of the terminus are distantly located from the respective other terminus. Therefore in these tripeptides, the side chain (X) should not interact strongly with the non-adjacent terminus. In addition, our previous volumetric study of the hydration properties of oligoglycines [21] has revealed that the oppositely charged amino and carboxyl termini in triglycine and longer homologs do not interact with each other. In other words, our volumetric results [21] suggest that, in triglycine and longer homologs, turn like conformations in which the termini are brought into close proximity to each other are sparsely populated. By extension, it is reasonable to assume that, in diglycyl tripeptides too, the amino and carboxyl termini do not interact with each other and, consequently, may be considered to be independently hydrated. Hence, the microscopic interpretation of the experimental volumetric data on Gly–Gly–X and X–Gly–Gly tripeptides should not be complicated by the need to account for intercharge interactions between the termini and/or interactions between the side chain and the non-adjacent terminus.

Also, it is pertinent to note that in all probability there will be differences in the preferred backbone conformations of the diglycyl tripeptides studied here. However, for molecules as small as tripeptides, entropic factors should strongly favor only those ensembles of conformational states in which all constituent atomic groups are fully exposed to the solvent. Consequently, the hydration properties of the constituent atomic groups of a tripeptide should not depend significantly on its conformational state. Therefore below, when analyzing our volumetric data, we will ignore possible differences in the distribution of conformational states of the tripeptides.

2. Materials and methods

All 10 tripeptides (Gly–Gly–Gly, Ala–Gly–Gly, Val–Gly–Gly, Leu–Gly–Gly, Phe–Gly–Gly, Gly–Gly–Ala, Gly–Gly–Val, Gly–Gly–Leu, Gly–Gly–Ile, Gly–Gly–Phe) were purchased from Sigma Chemical (St Louis, MO) and were of the highest purity commercially available. The standard 1 M HCl and 1 M KOH solutions were obtained from J.T. Baker Inc. (Phillipsburg, NJ).

Solutions of the tripeptides were prepared using triply-distilled, degassed water. The concentrations of the samples were defined by weighing 15–20 mg of each tripeptide material with a precision ± 0.03 mg, and then dissolving the material in a known amount of water. Before weighing, all tripeptides were dried for 72 h under vacuum in the presence of phosphorus pentoxide.

All the densimetric and ultrasonic measurements reported here were conducted at 25°C. Solution sound velocities, U, and absorptions per wavelength, $\alpha\lambda$, were measured at a frequency of 7.5 MHz using a previously described resonator method [31–33]. The accuracy of all the relative sound velocity measurements achieved with this design is approx. $\pm 10^{-4}\%$, while the accuracy of the relative sound absorption measurements is

$\pm 2\%$ [34–36]. The differences between the sound velocities in the solvent and in the solution were measured, thereby allowing determination of the relative molar increments of sound velocity, $[U]$, which is equal to $(U - U_0)/(U_0 C)$, where C is the molar concentration of a solute; and U and U_0 are the sound velocities in the solution and the solvent, respectively.

All densities were measured with a precision of $\pm 1.5 \cdot 10^{-6}$ g cm^{-3} using a vibrating tube densimeter (DMA-60, Anton Paar, Austria). The apparent molar volumes, ϕV, of the tripeptides were calculated from the following well-known relationship [37]:

$$\phi V = M/\rho - (\rho - \rho_0)/(\rho \rho_0 m) \quad (1)$$

where M is the molecular weight of a solute (tripeptide); ρ and ρ_0 are the densities of the solution and the solvent (pure water), respectively; and m is the molal concentration of the solute.

Acoustic and densimetric titration experiments were performed according to the previously described protocols by adding equal aliquots of 1 M HCl or 1 M KOH solutions to the same volume of the tripeptide solution and water in sample and reference cells, respectively [15,16].

The relative molar sound velocity increment determined as described above, was used in conjunction with the apparent molar volume data to calculate the apparent molar adiabatic compressibility, $\phi\kappa_S$, of the tripeptides using the relationship [38,39]:

$$\phi\kappa_S = \beta_{S0}(2\phi V - 2[U] - M/\rho_0) \quad (2)$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent (water). The value of β_{S0} was calculated from data on the density [40] and sound velocity [41] of water using the well-known Laplace expression $\beta_{S0} = (\rho_0 U_0^2)^{-1}$.

Differentiating Eq. (2) yields the expression

$$\Delta\kappa_S = 2\beta_{S0}(\Delta V - \Delta[U]) \quad (3)$$

where ΔV and $\Delta[U]$ are, respectively, the changes in the volume and in the relative molar sound velocity increment of a tripeptide upon neutralization of its carboxyl or amino terminus. This relationship allows one to calculate the change in adiabatic compressibility, $\Delta\kappa_S$, accompanying neutralization of the carboxyl or the amino terminus in a tripeptide.

For each evaluation of $[U]$, ϕV, $\phi\kappa_S$, ΔV, and $\Delta\kappa_S$, three to five independent measurements were carried out within the concentration range 2–3 mg ml^{-1} for each of the tripeptides studied.

3. Results

Table 1 lists the relative molar sound velocity increments, $[U]$, the apparent molar volumes, ϕV, and the apparent molar adiabatic compressibilities, $\phi\kappa_S$, of the tripeptides at 25°C that we have measured or calculated along with the available literature values given in parentheses. Note that our data are in good agreement with the available literature values. The errors indicated include contributions from the determination of solute concentration, from instrumental limitations, as well as from any temperature variability in the measuring cells.

Previous studies have shown that, for short peptides, the apparent molar volumes [24,26,27] and the apparent molar adiabatic compressibilities [22,28,29] do not depend strongly on concentration. For example, the estimated difference between the apparent molar volumes, ϕV, measured in this study (where solute concentrations fall in the range of 2–3 mg ml^{-1}) and the corresponding partial molar volumes, $V°$, obtained by extrapolation to infinite dilution does not exceed 0.1–0.2 cm^3 mol^{-1}, a variance that falls well within the experimental error. The estimated difference between the apparent molar adiabatic compressibilities, $\phi\kappa_S$, of the tripeptides at the concentrations used in this study and the partial molar adiabatic compressibilities, $\kappa°_S$, is $<$ 0.1–0.2 10^{-4} cm^3 mol^{-1} bar^{-1}, which also falls well within the experimental error. These estimates show that any solute–solute interactions that may exist at our concentrations should not appreciably affect the measured volumetric properties of the tripeptides. Thus, hereafter, we will not discriminate between the apparent molar

Table 1
The relative molar sound velocity increments, $[U]$, the apparent molar volumes, ϕV, and the apparent molar adiabatic compressibilities, $\phi\kappa_S$, of the tripeptides

	$[U]$ ($cm^3\ mol^{-1}$)	ϕV ($cm^3\ mol^{-1}$)	$\phi\kappa_S$ ($10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$)
$^+$Gly–Gly–Gly$^-$	67.5 ± 0.3[a]	112.1 ± 0.3[a]	-45.0 ± 0.5[a]
$^+$Ala–Gly–Gly$^-$	71.5 ± 0.3	130.0 ± 0.3 (130.16[b]; 129.9[c])	-38.9 ± 0.5 (-39.0[c]; -39.1[d])
$^+$Val–Gly–Gly$^-$	90.0 ± 0.4	161.3 ± 0.4 (161.1[c])	-40.0 ± 0.7 (-40.3[c])
$^+$Leu–Gly–Gly$^-$	101.4 ± 0.4	178.2 ± 0.4 (178.5[c])	-41.4 ± 0.7 (-40.7[c])
$^+$Phe–Gly–Gly$^-$	100.8 ± 0.4	192.0 ± 0.4	-43.8 ± 0.7
$^+$Gly–Gly–Ala$^-$	75.6 ± 0.3	129.6 ± 0.3 (128.79[b]; 129.2[c])	-42.9 ± 0.5 (-43.2[c]; -44.6[d])
$^+$Gly–Gly–Val$^-$	92.7 ± 0.3	161.2 ± 0.3 (160.5[c])	-42.5 ± 0.5 (-42.5[c])
$^+$Gly–Gly–Leu$^-$	104.8 ± 0.5	177.8 ± 0.5	-44.8 ± 0.9
$^+$Gly–Gly–Ile$^-$	102.0 ± 0.3	176.5 ± 0.4	-43.4 ± 0.6
$^+$Gly–Gly–Phe$^-$	100.7 ± 0.3	191.2 ± 0.3	-44.4 ± 0.5

[a] Chalikian et al. [21].
[b] Hedwig [24].
[c] Nikitin [25].
[d] Hedwig and Høiland [23].

and partial molar characteristics of the tripeptides.

Fig. 1a,b show the pH dependences of the relative molar sound velocity increment, $[U]$, for triglycine in the acidic and alkaline pH regions. The pH dependences of $[U]$ for all other tripeptides studied here have similar shapes and, therefore, are not shown. Previously [42,43], it has been demonstrated that changes in $[U]$ with pH can be rationalized if one takes into account two effects: (i) the hydration changes in the solvent which occur as a result of neutralization of the amino or carboxyl group; and (ii) the relaxation process caused by periodic shifts in chemical equilibria of the proton-transfer reactions due to changes in temperature and pressure in the ultrasonic wave field. In the absence of the relaxation contribution to $[U]$ (when only the hydration changes contribute), the pH dependence of $[U]$ can be described by a symmetrical S-shaped function [42,43]. In this paper, we consider only the hydration contribution by subtracting the relaxation contribution from the measured values of $[U]$. The relaxation contributions were determined from our measured data on the ultrasonic absorption (not shown) according to standard procedure described previously [43].

Tables 2 and 3 present the changes in $[U]$, $V°$, and $\kappa°_S$ accompanying neutralization of the $-NH_3^+$ and $-COO^-$ termini, respectively, for the 10 tripeptides studied here. As far as we know, no such data are reported in the literature so our results cannot be compared directly with published data.

4. Discussion

4.1. Partial molar volumes of zwitterionic tripeptides

As shown below in Eq. (4), the partial molar volume, $V°$, of a solute can be considered to be the sum of four terms [44,45]:

$$V° = V_M + V_T + V_I + \beta_{T0}RT \quad (4)$$

where V_M is the geometric volume occupied by the solute molecule itself; V_T is the volume of the void space surrounding the solute molecule, which

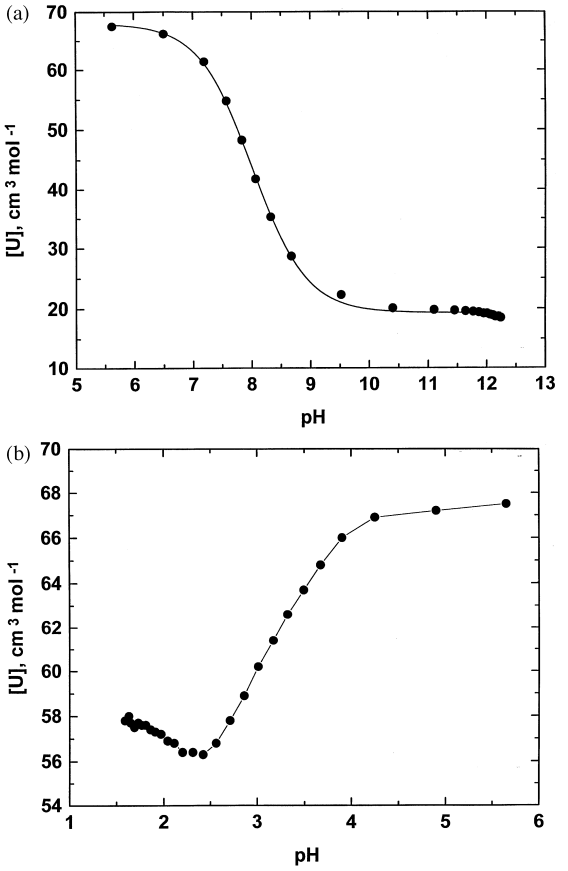

Fig. 1. The pH dependence of the relative molar sound velocity increment, $[U]$, of triglycine: (a) at alkaline pH range; (b) at acidic pH range.

results from the mutual thermal motion of solute and solvent molecules; V_I is the 'interaction volume', which accounts for solvent contraction under the influence of polar and charged groups of the solute; β_{T0} is the coefficient of isothermal compressibility of the solvent; R is the universal gas constant; and T is the absolute temperature. The final term in Eq. (4), $\beta_{T0}RT$, describes the volume effect of the ideal part of solute dissolution and is equal to 1.1 cm^3 mol^{-1} at 25°C.

For low molecular weight substances, the value of V_M can be approximated by the van der Waals volume, V_W. We have calculated V_W for the 10 tripeptides using the group contribution data of Bondi [46]. To derive the interaction volume, V_I, which is determined by the solute–solvent interactions, one needs to estimate the thermal volume, V_T. As previously discussed, the thermal volume, V_T, is proportional to S_A, the solvent accessible surface area of a solute molecule. For low molecular weight compounds, S_A can be approximated by S_W, the van der Waals surface area [44,45,47]. Hence, we find:

$$V_T = AS_W + B \quad (5)$$

where the coefficients A and B are the same for a homologous series of solutes, but, in general, can differ for different classes of substances.

Table 2
The changes in the relative molar sound velocity increment, $\Delta[U]$, volume, ΔV, and adiabatic compressibility, $\Delta\kappa_S$, accompanying neutralization of the amino terminus in the tripeptides at 25°C

	$\Delta[U]$ (cm^3 mol^{-1})	ΔV (cm^3 mol^{-1})	$\kappa_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})
Gly–Gly–Gly	-49.0 ± 0.5	26.5 ± 0.5	67.5 ± 0.9
Ala–Gly–Gly	-42.2 ± 0.5	26.3 ± 0.5	61.3 ± 0.9
Val–Gly–Gly	-41.5 ± 0.5	26.4 ± 0.5	60.8 ± 0.9
Leu–Gly–Gly	-42.1 ± 0.5	26.2 ± 0.5	61.2 ± 0.9
Phe–Gly–Gly	-46.1 ± 0.5	26.4 ± 0.5	64.9 ± 0.9
Gly–Gly–Ala	-46.8 ± 0.5	26.5 ± 0.5	65.6 ± 0.9
Gly–Gly–Val	-46.1 ± 0.5	25.4 ± 0.5	64.1 ± 0.9
Gly–Gly–Leu	-46.0 ± 0.5	25.0 ± 0.5	63.6 ± 0.9
Gly–Gly–Ile	-46.3 ± 0.5	25.3 ± 0.5	64.1 ± 0.9
Gly–Gly–Phe	-46.6 ± 0.5	25.8 ± 0.5	64.8 ± 0.9

Table 3
The changes in the relative molar sound velocity increment, $\Delta[U]$, volume, ΔV, and adiabatic compressibility, $\Delta\kappa_S$, accompanying neutralization of the carboxyl terminus in the tripeptides at 25°C

	$\Delta[U]$ (cm^3 mol^{-1})	ΔV (cm^3 mol^{-1})	$\Delta\kappa_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})
Gly–Gly–Gly	-10.0 ± 0.5	10.5 ± 0.5	18.4 ± 0.9
Ala–Gly–Gly	-8.8 ± 0.5	10.2 ± 0.5	17.0 ± 0.9
Val–Gly–Gly	-9.0 ± 0.5	10.0 ± 0.5	17.0 ± 0.9
Leu–Gly–Gly	-9.2 ± 0.5	10.5 ± 0.5	17.6 ± 0.9
Phe–Gly–Gly	-9.4 ± 0.5	9.6 ± 0.5	17.0 ± 0.9
Gly–Gly–Ala	-11.9 ± 0.5	11.5 ± 0.5	21.0 ± 0.9
Gly–Gly–Val	-14.0 ± 0.5	12.3 ± 0.5	23.6 ± 0.9
Gly–Gly–Leu	-16.9 ± 0.5	12.7 ± 0.5	26.5 ± 0.9
Gly–Gly–Ile	-14.5 ± 0.5	13.5 ± 0.5	25.1 ± 0.9
Gly–Gly–Phe	-11.0 ± 0.5	12.4 ± 0.5	21.0 ± 0.9

The coefficients A and B in Eq. (5) serve to take into account the shape of a solute molecule since solutes with the same van der Waals surface areas, S_W, but different shapes may have different thermal volumes, V_T. However, it is reasonable to assume that the closely related X–Gly–Gly and Gly–Gly–X tripeptide isomers have similar thermal volume, V_T. In a previous study [47], we have proposed that the value of the coefficient B in Eq. (5) does not strongly depend on the type of solute and estimated its value to be 0.6 cm^3 mol^{-1} at 25°C.

The coefficient A in Eq. (5) can be determined using an approach previously described for homologous series of α-amino [44] and α,ω-aminocarboxylic acids [47]. In this treatment, the contribution of non-polar groups to the interaction volume, V_I, is assumed to be negligible [44,45]. With these assumptions and estimates, inspection of Eq. (4) reveals that any two homologues which are distinct with respect to the size of their non-polar domains will have $(V^\circ - V_W)$ values which differ only by the difference between the thermal volumes, V_T. By combining Eq. (4) with Eq. (5), the coefficient A in Eq. (5) can be derived from the slope of a $\Delta(V^\circ - V_W)$ vs. ΔS_W plot. Fig. 2 shows such a plot for the tripeptides studied here. Note that this dependence is practically linear. From the slope, $\Delta(V^\circ - V_W)/\Delta S_W$, of this straight line, the coefficient A in Eq. (5) can be estimated to be 4.2×10^{-9} cm. This value is in good agreement with 4.05×10^{-9} cm, our previous estimate of the coefficient A for α,ω-aminocarboxylic acids [47].

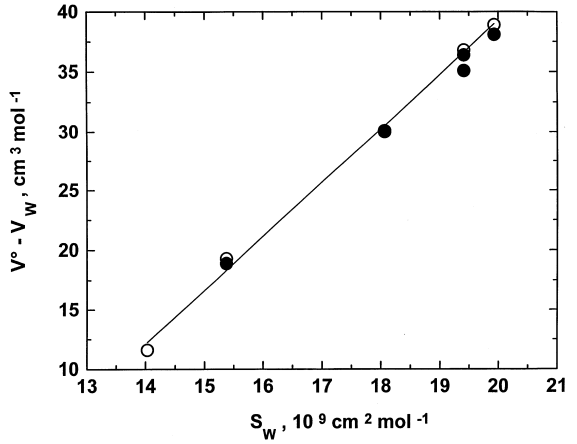

Fig. 2. Difference between the partial molar volume, V°, of the tripeptides and their van der Waals volume, V_W, vs. the van der Waals surface area, S_W; X–Gly–Gly (○), Gly–Gly–X (●).

Armed with the value of A, we can now use Eqs. (4) and (5) to calculate the interaction volumes, V_I, for the tripeptides. The resulting data listed in Table 4 reveal that, on average, for the diglycyl tripeptides with a non-polar side chain at the either end, V_I is equal to -47.2 ± 0.6 cm^3 mol^{-1}, while for triglycine with no side chain, V_I is equal to -49.0 cm^3 mol^{-1}. Thus, the interactions between the non-polar side chains and the adjacent charged amino or carboxyl terminus result in a decrease in V_I by 1.8 cm^3 mol^{-1}. Note that this decrease does not depend on the size or chemical nature (aromatic or aliphatic) of the side chain as well as on the type of termini (amino or carboxyl) to which the non-polar side chain is adjacent.

4.2. Partial molar adiabatic compressibilities of zwitterionic tripeptides

Inspection of the data presented in Table 1 reveals that the pairs of tripeptide isomers (X–Gly–Gly and Gly–Gly–X) with the same non-polar side chain exhibit essentially different partial molar adiabatic compressibilities, κ°_S. This observation suggests that differential interactions exist between the non-polar side chain and the adjacent $-NH_3^+$ and $-COO^-$ termini and that

Table 4
The interaction volume, V_I (cm^3 mol^{-1}), for the tripeptides in zwitterionic and neutralized forms

	Zwitterionic	Neutralized
Gly–Gly–Gly	−49.0	−33.8
Ala–Gly–Gly	−47.0	−32.2
Val–Gly–Gly	−47.5	−32.7
Leu–Gly–Gly	−46.4	−31.5
Phe–Gly–Gly	−46.5	−32.3
Gly–Gly–Ala	−47.4	−31.1
Gly–Gly–Val	−47.6	−31.6
Gly–Gly–Leu	−46.8	−30.9
Gly–Gly–Ile	−48.1	−31.1
Gly–Gly–Phe	−47.3	−30.9

the compressibility observable is more sensitive to these differential interactions than the volume observable. In general, as can be seen from Table 1, tripeptides with non-polar side chains located adjacent to the N-terminus exhibit greater partial molar adiabatic compressibility, κ°_S, than isomers with non-polar side chains located adjacent to the C-terminus.

As previously shown [48], partial molar adiabatic compressibility data can be interpreted in terms of hydration by using the following relationship:

$$\kappa^\circ_S = \kappa_M + \Delta\kappa_{Sh} = \kappa_M + n_h(\kappa^\circ_{Sh} - \kappa^\circ_{S0}) \quad (6)$$

where κ_M is the intrinsic compressibility of a solute; $\Delta\kappa^\circ_{Sh}$ is the compressibility effect of hydration; κ°_{S0} and κ°_{Sh} are the partial molar adiabatic compressibilities of water in the bulk state and in the hydration shell of a solute, respectively; and n_h is the 'hydration number', which refers to the number of water molecules in the hydration shell of a solute.

For small molecules, such as tripeptides, the intrinsic compressibility term, κ_M, in Eq. (8) can be neglected as it is determined primarily by the small compressibility of covalent bonds and external electron shells [1,6,49]. Thus, the partial molar adiabatic compressibility of low molecular weight substances can be considered to primarily reflect solvent hydration changes:

$$\kappa^\circ_S = \Delta\kappa_{Sh} = n_h(\kappa^\circ_{Sh} - \kappa^\circ_{S0}) \quad (7)$$

As, usually, the solute surface is not homogeneous, a more complete form of Eq. (7) is the following:

$$\Delta\kappa_{Sh} = \Sigma n_{hi}(\kappa^\circ_{Shi} - \kappa^\circ_{S0}) = \Sigma n_{hi}\kappa^\circ_{Shi} - n_h\kappa^\circ_{S0} \quad (8)$$

where n_{hi} is the hydration number for the i-th solvent exposed atomic group of a solute ($\Sigma n_{hi} = n_h$); and κ°_{Shi} is the partial molar adiabatic compressibility of water molecules solvating the i-th atomic group.

4.2.1. Tripeptides with side chains adjacent to the amino terminus

The data presented in Table 1 suggest that the presence of a methyl group in the β-position of the Ala–Gly–Gly tripeptide leads to a 15% increase [by $(6.1 \pm 1) \times 10^{-6}$ cm^3 mol^{-1} bar^{-1}] in κ°_S relative to triglycine. There are two reasons for such large increase in κ°_S. First, as discussed by Hedwig and Høiland [50], the solvent accessibility of the positively charged amino terminus may be reduced by the adjacent β-methyl group. This reduction in solvent accessibility ('shielding') should result in a decrease in n_{hi}, the total number of waters solvating the amino terminus, thereby increasing the negative $\Delta\kappa_h$ term in Eqs. (6) and (7). Second, the inductive effect of the methyl group can reduce the effective charge on the adjacent amino terminus, thereby decreasing electrostatic solute–solvent interactions.

Further inspection of the data in Table 1 reveals that the increase in the number of carbon atoms in the aliphatic side chain beyond the β-position (going from Ala–Gly–Gly to Val–Gly–Gly and further to Leu–Gly–Gly) leads to a steady decrease in the value of κ°_S. Fig. 3 shows the dependence of κ°_S for the tripeptides with the non-polar side chains adjacent to the amino terminus (○) on the van der Waals surface area, S_W, of the side chain. Note that κ°_S decreases almost linearly with increasing van der Waals surface area of the side chain. The slope, $\Delta\kappa^\circ_S/\Delta S_W$, of -0.6×10^{-13} cm bar^{-1} corresponds to a κ°_S change of -0.8×10^{-4} cm^3 mol^{-1} bar^{-1} per aliphatic carbon atom. This value is almost twice as high (less negative) as the compressibility contribution of an independently hydrated methylene group in the long aliphatic chain of α,ω-aminocarboxylic acids (-1.6×10^{-4} cm^3 mol^{-1} bar^{-1} per aliphatic carbon atom) [47] or the aliphatic side chain of α-amino acids (-1.9×10^{-4} cm^3 mol^{-1} bar^{-1} per aliphatic carbon atom) [49]. This disparity suggests that the aliphatic side chains in the X–Gly–Gly tripeptides may not be hydrated independently and may interact with the rest of the molecule.

Inspection of Fig. 3 reveals that Phe–Gly–Gly deviates by -2.5×10^{-4} cm^3 mol^{-1} bar^{-1} from

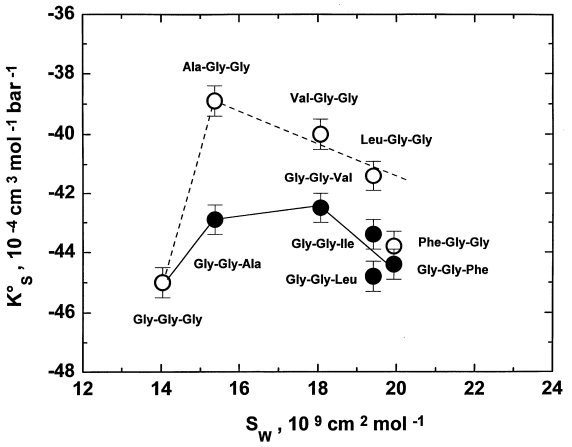

Fig. 3. The partial molar adiabatic compressibility, κ°_S, of the tripeptides vs. the van der Waals surface area, S_W: X–Gly–Gly (○), Gly–Gly–X (●).

the straight line which passes through the points corresponding to the Ala–Gly–Gly, Val–Gly–Gly, and Leu–Gly–Gly tripeptides. This observation suggests that the compressibility contribution of the benzene ring in the Phe–Gly–Gly tripeptide is more negative than that of aliphatic groups of equivalent surface area, a conclusion consistent with the compressibility data on α-amino acids [49]. However, this generalization should be viewed with caution as the benzene ring in the Phe–Gly–Gly tripeptide with its negative cloud of π-electrons may diminish the inductive effect of the adjacent β-methylene group thereby enhancing the hydration of the positively charged amino terminus. In addition, water molecules which are exposed simultaneously to the positively charged amino group and the negative cloud of the benzene π-electrons may become additionally oriented, and, as a result, may manifest reduced mobility and compressibility [6]. Finally, because of electrostatic attraction between the negative cloud of the π-electrons and the positively charged amino group, the benzene ring may become polarized, thereby exhibiting greater hydration.

4.2.2. Tripeptides with side chains adjacent to the carboxyl terminus

Further inspection of the data in Table 1 reveals that, relative to triglycine, the presence of a methyl group in Gly–Gly–Ala causes an increase of $(2.1 \pm 1) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} in κ°_S. This increase is three times as small as 6.1×10^{-4} cm^3 mol^{-1} bar^{-1}, the corresponding increase in the value of κ°_S caused by a methyl group adjacent to the amino terminus in Ala–Gly–Gly. As discussed previously by Hedwig and Høiland [50], this disparity suggests that the influence of a methyl group on the hydration of an adjacent negatively charged carboxyl terminus is weaker than its influence on the hydration of the adjacent positively charged amino terminus. In part, this observation may be accounted for by the greater solvent accessible surface area of the carboxyl terminus (by approx. 55%) relative to the amino terminus [51] which means that the relative reduction in solvent exposure ('shielding') of the amino terminus in Ala–Gly–Gly should be higher than that of the carboxyl terminus in Gly–Gly–Ala. Alternatively (or in addition) the inductive effect of the methyl group may result in an increase in the effective charge of the adjacent carboxyl terminus, thereby partially neutralizing the effect of the 'shielding'.

Note that the value of κ°_S for Gly–Gly–Val is somewhat higher than that for Gly–Gly–Ala (see Table 1), despite the negative compressibility contribution of independently hydrated aliphatic groups [6,47,49]. This observation suggests that, in Gly–Gly–Val, the interactions between the carboxyl terminus and the methylene groups in the γ-position persist. By contrast, in Gly–Gly–Leu and Gly–Gly–Ile, the methylene or methyl group(s) in the δ-position are hydrated independently. We draw this conclusion based on the comparison between the values of κ°_S corresponding to Gly–Gly–Leu (Gly–Gly–Ile) and Gly–Gly–Val. As can be seen from Fig. 3, the slope, $\Delta\kappa^\circ_S/\Delta S_W$, of the straight line passing through the points corresponding to Gly–Gly–Val, Gly–Gly–Leu, and Gly–Gly–Ile (●) is equal to -1.2×10^{-13} cm^{-1} bar^{-1}. This slope corresponds to a κ°_S change of -1.6×10^{-4} cm^3 mol^{-1} bar^{-1} per aliphatic carbon which coincides with the compressibility contribution of an independently hydrated methylene group in α,ω-aminocarboxylic acids [47].

It should be noted that -1.5×10^{-4} cm^3 mol^{-1}

bar^{-1}, the difference between the κ°_S values corresponding to Gly–Gly–Phe and Gly–Gly–Ala is substantially higher (less negative) than the corresponding differences between the tripeptides Phe–Gly–Gly and Ala–Gly–Gly (-4.9×10^{-4} cm^3 mol^{-1} bar^{-1}) or α-amino acids phenylalanine and alanine (-8.6×10^{-4} cm^3 mol^{-1} bar^{-1}) [49]. As discussed above, this discrepancy may be related to the hydration enhancement due to the interactions between the positively charged amino terminus and the closely located aromatic ring.

4.3. Neutralization of the amino terminus

Neutralization of the positively charged amino terminus at alkaline pH follows the reaction:

$$^{-}OOC{-}R{-}NH_3^+ + OH^- = {}^{-}OOC{-}R{-}NH_2 + H_2O \quad (9)$$

Consequently, the measured changes in volume, ΔV, and adiabatic compressibility, $\Delta\kappa_S$, accompanying neutralization of the amino terminus can be presented as follows:

$$\Delta V = \delta V^+ + V^\circ_0 - V^\circ(OH^-) \quad (10)$$

$$\Delta\kappa_S = \delta\kappa_S^+ + \kappa^\circ_{S0} - \kappa^\circ_S(OH^-) \quad (11)$$

where δV^+ is the difference between the partial molar volumes of species with the neutralized ($-NH_2$) and charged ($-NH_3^+$) amino termini; $\delta\kappa_S^+$ is the difference between the partial molar adiabatic compressibilities of species with the neutralized and charged amino termini; $V^\circ_0 = 18.07$ cm^3 mol^{-1} and $\kappa^\circ_{S0} = 8.09\times10^{-4}$ cm^3 mol^{-1} bar^{-1} are, respectively, the partial molar volume and the partial molar adiabatic compressibility of water; $V^\circ(OH^-)$ and $\kappa^\circ_S(OH^-)$ are, respectively, the partial molar volume and the partial molar adiabatic compressibility of a hydroxyl ion. At 25°C, the average values of $V^\circ(OH^-)$ and $\kappa^\circ_S(OH^-)$ are equal to 1.2 ± 0.6 cm^3 mol^{-1} and $-(51.8\pm0.5)\times10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively [43].

Inspection of Table 2 reveals that neutralization of the independently hydrated amino terminus of triglycine causes increases in volume, ΔV, and adiabatic compressibility, $\Delta\kappa_S$, equal to 26.5 ± 0.5 cm^3 mol^{-1} and $(67.5\pm0.9)\times10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. From Eqs. (10) and (11), we calculate for the independently hydrated amino terminus of triglycine values of δV^+ and $\delta\kappa_S^+$ equal to 9.6 cm^3 mol^{-1} and 7.6×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.

As can be seen from Table 2, the presence of the aliphatic side chain adjacent to the amino terminus does not strongly affect the value of ΔV relative to triglycine but results in a decrease in $\Delta\kappa_S$, which, on average, is equal to $6.4\pm0.3\times10^{-4}$ cm^3 mol^{-1} bar^{-1}. This value is close to 6.1×10^{-4} cm^3 mol^{-1} bar^{-1}, the difference in κ°_S values between triglycine and Ala–Gly–Gly. This similarity suggests that the difference, $\Delta\Delta\kappa_S$, between the compressibility changes, $\Delta\kappa_S$, accompanying the neutralization of the amino terminus in triglycine and Ala–Gly–Gly, is predominantly determined by the differential hydration of the terminus in its charged rather than its neutralized state.

Within the experimental error, the values of $\Delta\kappa_S$ for Ala–Gly–Gly, Val–Gly–Gly, and Leu–Gly–Gly are, practically, the same suggesting that only the alkyl group in the β-position interacts with the charged amino terminus. Interestingly, the presence of the aromatic ring in Phe–Gly–Gly causes an increase in $\Delta\kappa_S$ by $(3.6\pm1.8)\times10^{-4}$ cm^3 mol^{-1} bar^{-1} as compared to Ala–Gly–Gly. This observation further supports our assumption that interactions between the aromatic ring and the positively charged amino terminus enhance the Phe–Gly–Gly hydration.

4.4. Neutralization of the carboxyl terminus

Neutralization of the negatively charged carboxyl terminus at acidic pH follows the reaction:

$$^{-}OOC{-}R{-}NH_3^+ + H^+ = HOOC{-}R{-}NH_3^+ \quad (12)$$

Consequently, the measured changes in volume, ΔV, and adiabatic compressibility, $\Delta\kappa_S$, accompanying neutralization of the carboxyl terminus can be presented as follows:

$$\Delta V = \delta V^{-} - V^{\circ}(H^{+}) \quad (13)$$

$$\Delta \kappa_S = \delta \kappa_S^{-} - \kappa^{\circ}{}_S(H^{+}) \quad (14)$$

where δV^{-} is the difference in the partial molar volumes of the species with neutralized (–COOH) and charged ($-COO^{-}$) carboxyl termini; $\delta \kappa_S^{-}$ is the difference in the partial molar adiabatic compressibilities of the species with neutralized and charged carboxyl group; $V^{\circ}(H^{+})$ and $\kappa^{\circ}{}_S(H^{+})$ are, respectively, the partial molar volume and the partial molar adiabatic compressibility of a proton. At 25°C, the average values of $V^{\circ}(H^{+})$ and $\kappa^{\circ}{}_S(H^{+})$ are equal to -5.2 ± 0.6 cm^3 mol^{-1} and $(8.6 \pm 0.5) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively [43].

Inspection of Table 3 reveals that neutralization of the independently hydrated carboxyl terminus of triglycine causes increases in volume, ΔV, and compressibility, $\Delta \kappa_S$, equal to 10.5 ± 0.5 cm^3 mol^{-1} and $(18.4 \pm 0.9) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. For the independently hydrated carboxyl terminus of triglycine, we calculate, from Eqs. (13),(14), and values of δV^{-} and $\delta \kappa_S^{-}$ equal to 5.3 cm^3 mol^{-1} and 27.0×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.

Further inspection of Table 3 reveals that the changes in volume, ΔV, and adiabatic compressibility, $\Delta \kappa_S$, accompanying neutralization of the carboxyl terminus increase when the adjacent aliphatic side chain becomes bulkier [going from triglycine to Gly–Gly–Leu(Ile)]. In fact, as can be seen from Fig. 4a,b, the ΔV and $\Delta \kappa_S$ values increase almost linearly with the increase in the number of carbon atoms in the aliphatic side chain. This observation cannot be explained by either the shielding of the carboxyl terminus by the adjacent non-polar side chain or the inductive effect of the side chains. Shielding should cause diminution in the measured values of ΔV and $\Delta \kappa_S$ when the non-polar side chain increases in size. On the other hand, the inductive effect should not strongly depend on the size of the aliphatic side chain. Further systematic studies are required to account for this intriguing observation which probably reflects differential interactions between the aliphatic side chain and the adjacent carboxyl terminus in its charged and uncharged states.

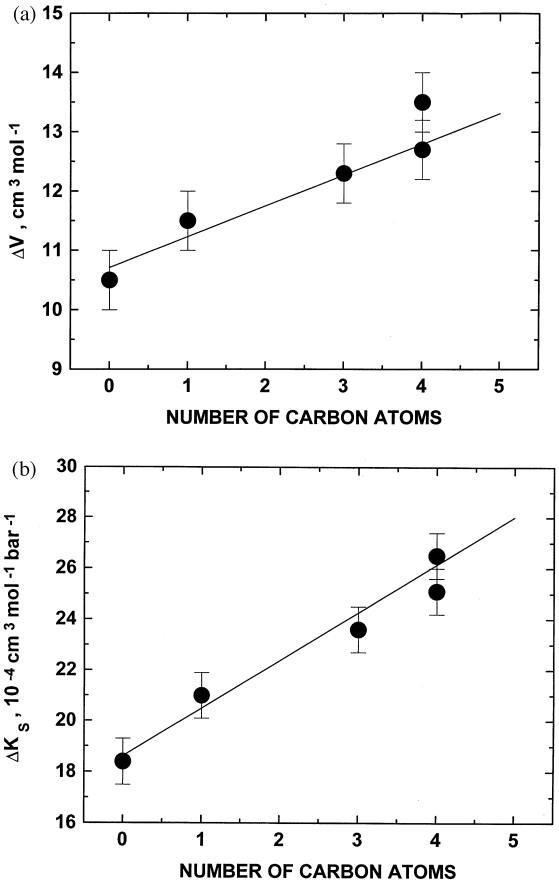

Fig. 4. (a) Dependence of the change in volume, ΔV, accompanying neutralization of the carboxyl terminus of the tripeptides with the aliphatic side chain adjacent to the carboxyl terminus on the number of carbon atmos in the side chain; (b) dependence of the change in adiabatic compressibility, $\Delta \kappa_S$, accompanying neutralization of the carboxyl terminus of the tripeptides with the aliphatic side chain adjacent to the carboxyl terminus on the number of carbon atoms in the side chain.

Finally, note that, within the experimental error, the values of ΔV and $\Delta \kappa_S$ for Gly–Gly–Phe coincide with those for Gly–Gly–Ala (see Table 3) suggesting that the aromatic ring in Gly–Gly–Phe does not interact with the adjacent carboxyl terminus.

4.5. Individual hydration properties of constituent groups

4.5.1. Uncharged amino and carboxyl termini

The partial molar volume, V°(neut), of the

tripeptides with the neutralized amino and carboxyl termini can be calculated as follows:

$$V^{\circ}(\text{neut}) = V^{\circ} + \Delta V(\text{carboxyl}) + \Delta V(\text{amino}) + \Delta V^{\circ}(\text{ion}) \quad (15)$$

where V° is the partial molar volume of zwitterionic tripeptides; ΔV(amino) and ΔV(carboxyl) are the volume changes that accompany neutralization of the amino and the carboxyl termini, respectively; and ΔV°(ion) is the volume change for the ionization of water:

$$\Delta V^{\circ}(\text{ion}) = V^{\circ}(H^{+}) + V^{\circ}(OH^{-}) - V^{\circ}_{0} \quad (16)$$

At 25°C, ΔV°(ion) is equal to -21.7 cm^3 mol^{-1} [52].

Analogously, the partial molar adiabatic compressibility, κ°_S(neut), of the tripeptides with the neutralized amino and carboxyl termini equals:

$$\kappa^{\circ}_S(\text{neut}) = \kappa^{\circ}_S + \Delta\kappa_S(\text{carboxyl}) + \Delta\kappa_S(\text{amino}) + \Delta\kappa^{\circ}_S(\text{ion}) \quad (17)$$

where κ°_S is the partial molar adiabatic compressibility of zwitterionic tripeptides; $\Delta\kappa_S$(amino) and $\Delta\kappa_S$(carboxyl) are the compressibility changes accompanying neutralization of the amino and the carboxyl termini, respectively; $\Delta\kappa^{\circ}_S$(ion) is the compressibility change for the ionization of water:

$$\Delta\kappa^{\circ}_S(\text{ion}) = \kappa^{\circ}_S(H^{+}) + \kappa^{\circ}_S(OH^{-}) - \kappa^{\circ}_{S0} \quad (18)$$

At 25°C, $\Delta\kappa^{\circ}_S$(ion) is equal to -49.1×10^{-4} cm^3 mol^{-1} bar^{-1} [52].

Table 5 lists the calculated values V°(neut) and κ°_S(neut) for the tripeptides with neutralized amino and carboxyl termini. Comparison between the data presented in Tables 1 and 5 reveals that the neutralization of the termini in triglycine brings about an increase in V° and κ°_S of 15.2 ± 1.0 cm^3 mol^{-1} and $(36.8 \pm 1.8) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. These values correspond to the volume and compressibility contributions of the net 'pure electrostriction' of the independently hydrated charged amino and carboxyl termini.

Table 5
The partial molar volumes, V°(neut), and the partial molar adiabatic compressibilities, κ°_S(neut), of the tripeptides with the neutralized termini

	V° (cm^3 mol^{-1})	κ°_S (10^{-4} cm^3 mol^{-1} bar^{-1})
Gly–Gly–Gly	127.4 ± 1.3	-8.2 ± 2.3
Ala–Gly–Gly	144.8 ± 1.3	-9.7 ± 2.3
Val–Gly–Gly	176.0 ± 1.4	-11.3 ± 2.5
Leu–Gly–Gly	193.2 ± 1.5	-11.7 ± 2.7
Phe–Gly–Gly	206.3 ± 1.4	-11.0 ± 2.5
Gly–Gly–Ala	145.9 ± 1.5	-5.4 ± 2.5
Gly–Gly–Val	177.2 ± 1.3	-3.9 ± 2.3
Gly–Gly–Leu	193.8 ± 1.5	-3.8 ± 2.7
Gly–Gly–Ile	193.6 ± 1.5	-3.3 ± 2.7
Gly–Gly–Phe	207.7 ± 1.3	-7.7 ± 2.3

The net contribution of the independently hydrated charged amino and carboxyl termini to the interaction volume, V_I, was estimated to be -26 cm^3 mol^{-1} [47]. The difference between this value and -15.2 cm^3 mol^{-1}, the above estimated net volume contribution of pure electrostriction, is equal to -10.8 cm^3 mol^{-1}, which represents the sum of the interaction volumes, V_I, for the uncharged amino ($-NH_2$) and carboxyl ($-COOH$) termini.

Application of Eqs. (4) and (5) to the volume data presented in Table 5 yields the interaction volumes, V_I, for the tripeptides with the uncharged termini. The results of these calculations are presented in the third column of Table 4. Triglycine with its independently hydrated uncharged amino and carboxyl termini is characterized by the interaction volume, V_I, of -33.8 cm^3 mol^{-1}. If there is any non-polar side chain adjacent to the amino terminus of the X–Gly–Gly tripeptide, the interaction volume, V_I, becomes equal to -32.4 ± 0.5 cm^3 mol^{-1}. Thus, the interactions between the non-polar side chains and the adjacent uncharged amino terminus result in an increase of 1.4 cm^3 mol^{-1} in the interaction volume, V_I. If there is a non-polar side chain adjacent to the carboxyl terminus of the Gly–Gly–X tripeptide, the interaction volume, V_I, becomes equal to -31.1 ± 0.3 cm^3 mol^{-1}. Thus, the interactions between the non-polar side

chain and the adjacent uncharged carboxyl terminus result in an increase of 2.7 cm^3 mol^{-1} in the interaction volume, V_I.

One can use an additive approach and present the partial molar adiabatic compressibility of triglycine, κ°_S(neut), (see Table 5) with its neutralized termini as the sum of the contributions of the constituent atomic groups:

$$\kappa^\circ_S(\text{neut}) = \kappa^\circ_S(NH_2) + \kappa^\circ_S(CH_2) + 2\kappa^\circ_S(CONHCH_2) + \kappa^\circ_S(COOH) \quad (19)$$

Substituting $\kappa_S(CH_2) = -1.6 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [31] and $\kappa_S(CONHCH_2) = -1.1 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [21] in Eq. (19), one obtains the net compressibility contribution of the independently hydrated uncharged amino and carboxyl termini, $[\kappa_S(NH_2) + \kappa_S(COOH)]$, equal to $-4.4 \pm 2.3 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}.

As is seen from Table 5, the presence of a non-polar side chain at the amino end causes a decrease in κ°_S(neut) consistent with the negative compressibility contributions of independently hydrated hydrophobic groups. By contrast, the presence of a non-polar side chain at the carboxyl end causes an increase in κ°_S(neut) relative to triglycine. This increase may suggest that the aliphatic side chain in the Gly–Gly–X tripeptides reduces solvent accessibility of the adjacent uncharged carboxyl terminus thereby decreasing its hydration. Based on this observation, we conclude that the compressibility contribution of the uncharged carboxyl terminus should be a negative non-zero value.

Unfortunately, the large uncertainty of the calculated values of κ°_S(neut) does not permit us to perform a more detailed analysis. However, it should be noted that, in contrast to zwitterionic species, the X–Gly–Gly tripeptides with the uncharged termini have lower compressibility than the Gly–Gly–X tripeptides.

4.5.2. Methyl group and hydrogen atom

At 25°C, the compressibility contributions of a methyl group and an aliphatic hydrogen atom have been previously estimated to be -3.1×10^{-4} cm^3 mol^{-1} bar^{-1} and -1.1×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively [49]. The volume contributions of methyl group, $V^\circ(CH_3)$, and hydrogen atom, V°(H), can be calculated from Eq. (5). Specifically, according to Eq. (5), the volume contribution of methyl group can be presented as follows:

$$V^\circ(CH_3) = AS_W(CH_3) + V_W(CH_3) \quad (20)$$

where $S_W(CH_3)$, the van der Waals surface area of a methyl group, is equal to 2.12×10^9 cm^2 mol^{-1}; $V_W(CH_3)$, the van der Waals volume of a methyl group, is equal to 17.12 cm^3 mol^{-1} [46].

From Eq. (20), we calculate $V^\circ(CH_3)$ of 26.0 cm^3 mol^{-1}. This value is in good agreement with 25.2 cm^3 mol^{-1}, the value of $V^\circ(CH_3)$ previously estimated by Kharakoz [45]. The volume contribution of an aliphatic hydrogen atom can be calculated as the difference between $V^\circ(CH_3)$ and $V^\circ(CH_2)$, the volume contribution of the methylene group:

$$V^\circ(H) = V^\circ(CH_3) - V^\circ(CH_2) \quad (21)$$

where $V^\circ(CH_2)$ is equal to 15.7 cm^3 mol^{-1} [47].

From Eq. (21), we calculate V°(H) equal 10.3 cm^3 mol^{-1}. It should be noted that Makhatadze et al. [53] have derived a similar value for V°(H) from the comparison of the partial molar volumes of methanol, triglycine, and the tripeptide Gly–Ser–Gly.

4.5.3. Aromatic ring

Our data can be used to evaluate the volume, V°(arom), and the compressibility, κ°_S(arom), contributions of the aromatic ring in two different ways: (i) by subtracting V° or κ°_S of Ala–Gly–Gly from those of Phe–Gly–Gly; and (ii) by subtracting V° or κ°_S of Gly–Gly–Ala from those of Gly–Gly–Phe. In both cases, the volume and the compressibility contributions of hydrogen atom should be taken into account:

$$V^\circ(\text{arom}) = V^\circ(\text{R–Phe}) - V^\circ(\text{R–Ala}) + V^\circ(H) \quad (22)$$

$$\kappa^\circ_S(\text{arom}) = \kappa^\circ_S(\text{R–Phe}) - \kappa^\circ_S(\text{R–Ala}) + \kappa^\circ_S(H) \quad (23)$$

where the compressibility contribution of hydrogen atom is equal to -1.1×10^{-4} cm^3 mol^{-1} bar^{-1} [49], and the volume contribution of hydrogen atom is equal to 10.3 cm^3 mol^{-1}.

For the tripeptides with uncharged termini (see Table 5), the two ways just described yield similar values of V°(arom) and κ°_S(arom). On average, V°(arom) is equal to 72.0 cm^3 mol^{-1}, while κ°_S(arom) is equal to -2.9×10^{-4} cm^3 mol^{-1} bar^{-1}.

Analogous calculations performed for zwitterionic tripeptides (using the data in Table 1) and for amino acids alanine and phenylalanine [44] yield close values for V°(arom), equal to 72.6 cm^3 mol^{-1} and 71.6 cm^3 mol^{-1}, respectively.

The value of -2.9×10^{-4} cm^3 mol^{-1} bar^{-1} we calculate for κ°_S(arom) from the data on the tripeptides with the uncharged termini is in close agreement with -2.6×10^{-4} cm^3 mol^{-1} bar^{-1}, the value which can be calculated from the κ°_S data on the zwitterionic Gly–Gly–Ala and Gly–Gly–Phe tripeptides. By contrast, calculations performed using the κ°_S data on the zwitterionic Ala–Gly–Gly and Phe–Gly–Gly tripeptides yield more negative value of -6×10^{-4} cm^3 mol^{-1} bar^{-1} for κ°_S(arom). An even more negative value of -9.7×10^{-4} cm^3 mol^{-1} bar^{-1} can be calculated for κ°_S(arom) from the partial molar adiabatic compressibilities, κ°_S, of zwitterionic amino acids alanine and phenylalanine [49]. These differences further support the assumption (see Section 4.2.1) that interactions exist between the charged amino terminus and the adjacent aromatic benzene ring of the phenylalanine side chain and that these interactions influence the solute hydration.

5. Concluding remarks

We report the partial molar volumes, V°, and the partial molar adiabatic compressibilities, κ°_S, of the Gly–Gly–Gly, Ala–Gly–Gly, Val–Gly–Gly, Leu–Gly–Gly, Phe–Gly–Gly, Gly–Gly–Ala, Gly–Gly–Val, Gly–Gly–Leu, Gly–Gly–Ile, and Gly–Gly–Phe tripeptides at 25°C. In addition, we have determined the changes in volume and compressibility accompanying neutralization of the amino and the carboxyl termini of these tripeptides. Based on these data, the following results have been obtained:

1. The net contribution to the interaction volume, V_I, of the independently hydrated neutralized amino and carboxyl termini is equal to -10.8 cm^3 mol^{-1}.
2. The net contribution to the partial molar adiabatic compressibility, κ°_S, of the independently hydrated neutralized amino and carboxyl termini is equal to -4.4×10^{-4} cm^3 mol^{-1} bar^{-1}.
3. Volume changes associated with neutralization of the independently hydrated amino and carboxyl termini are equal to 9.6 cm^3 mol^{-1} and 5.3 cm^3 mol^{-1}, respectively.
4. Compressibility changes associated with neutralization of the independently hydrated amino and carboxyl termini are equal to 7.6×10^{-4} cm^3 mol^{-1} bar^{-1} and 27.0×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.
5. The volume and compressibility contributions of the independently hydrated benzene ring are equal to 72.0 cm^3 mol^{-1} and -2.9×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.
6. The volume contributions of methyl group and aliphatic hydrogen atom are equal to 26.0 cm^3 mol^{-1} and 10.3 cm^3 mol^{-1}, respectively,
7. In tripeptides with the charged termini, the presence of a non-polar side chain adjacent to the amino or carboxyl termini results in an increase in the interaction volume, V_I, by 1.8 cm^3 mol^{-1}.
8. In the X–Gly–Gly tripeptides, only a methylene (methyl) group in the β-position interacts with the adjacent positively charged amino terminus which causes an increase in the partial molar adiabatic compressibility, κ°_S, by 6.1×10^{-4} cm^3 mol^{-1} bar^{-1}.
9. In the Gly–Gly–X tripeptides, methylene (methyl) groups in the β- and γ-positions of the aliphatic side chain interact with the adjacent negatively charged carboxyl terminus. The interaction between the β-methylene (methyl) group and the charged carboxyl terminus causes an increase of 2.1×10^{-4}

$cm^3\ mol^{-1}\ bar^{-1}$ in the partial molar adiabatic compressibility, κ°_S.

10. The benzene ring of the phenylalanine side chain of Phe–Gly–Gly interacts with the adjacent positively charged amino terminus which enhances the solute hydration as can be judged from compressibility data.

In the aggregate, our results suggest that care must be exercised when the hydration properties of complex molecules, such as proteins, are modeled based on additive calculations using low molecular weight model compound data.

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References

[1] A.P. Sarvazyan, Annu. Rev. Biophys. Biophys. Chem. 20 (1991) 321–342.
[2] A.A. Zamyatnin, Annu. Rev. Biophys. Bioeng. 13 (1984) 145–165.
[3] H. Høiland, Thermodynamic data for biochemistry and biotechnology, in: H.-J. Hinz (Ed.), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986, pp. 17–44.
[4] H. Durchschlag, Thermodynamic data for biochemistry and biotechnology, in: H.-J. Hinz (Ed.), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986, pp. 45–128.
[5] H. Høiland, Thermodynamic data for biochemistry and biotechnology, in: H.-J. Hinz (Ed.), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986, pp. 129–147.
[6] T.V. Chalikian, A.P. Sarvazyan, K.J. Breslauer, Biophys. Chem. 51 (1994) 89–109.
[7] K. Gekko, H. Noguchi, J. Phys. Chem. 83 (1979) 2706–2714.
[8] A.P. Sarvazyan, P. Hemmes, Biopolymers 18 (1979) 3015–3024.
[9] B. Gavish, E. Gratton, C.J. Hardy, Proc. Natl. Acad. Sci. U.S.A. 80 (1983) 750–754.
[10] K. Gekko, Y. Hasegawa, Biochemistry 25 (1986) 6563–6571.
[11] M. Iqbal, R.E. Verrall, J. Biol. Chem. 263 (1988) 4159–4165.
[12] K. Gekko, Y. Hasegawa, Phys. Chem. 93 (1989) 426–429.
[13] D.P. Kharakoz, A.P. Sarvazyan, Biopolymers 33 (1993) 11–26.
[14] Y. Tamura, K. Gekko, Biochemistry 34 (1995) 1878–1884.
[15] T.V. Chalikian, V.S. Gindikin, K.J. Breslauer, J. Mol. Biol. 250 (1995) 291–306.
[16] T.V. Chalikian, V.S. Gindikin, K.J. Breslauer, FASEB J. 10 (1996) 164–170.
[17] T.V. Chalikian, M. Totrov, R. Abagyan, K.J. Breslauer, J. Mol. Biol. 260 (1996) 588–603.
[18] T.V. Chalikian, K.J. Breslauer, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 1012–1014.
[19] T.V. Chalikian, J. Völker, D. Anafi, K.J. Breslauer, J. Mol. Biol. 274 (1997) 237–252.
[20] B. Nölting, S.G. Sligar, Biochemistry 32 (1993) 12319–12323.
[21] T.V. Chalikian, A.P. Sarvazyan, Th. Funck, K.J. Breslauer, Biopolymers 34 (1994) 541–553.
[22] G.R. Hedwig, H. Høiland, J. Solution Chem. 20 (1991) 1113–1127.
[23] G.R. Hedwig, H. Høiland, J. Chem. Thermodyn. 25 (1993) 349–354.
[24] G.R. Hedwig, J. Solution Chem. 17 (1988) 383–397.
[25] S. Ya. Nikitin, Ph.D. Thesis, Inst. Biol. Physics. Acad. Sci. USSR, Pushchino, Russia, 1984.
[26] J.F. Reading, G.R. Hedwig, J. Chem. Soc. Faraday Trans. 86 (1990) 3117–3123.
[27] G.R. Hedwig, J. Chem. Soc. Faraday Trans. 89 (1993) 2761–2768.
[28] G.R. Hedwig, H. Høiland, J. Chem. Thermodyn. 23 (1991) 1029–1035.
[29] G.R. Hedwig, H. Høiland, Biophys. Chem. 49 (1994) 175–181.
[30] N. Poklar, M.M. Cenegacnik, F. Šveglj, S. Lapanje, Int. J. Pept. Protein Res. 39 (1992) 415–418.
[31] F. Eggers, Th. Funck, Rev. Sci. Instrum. 44 (1973) 969–978.
[32] F. Eggers, Acustica 76 (1992) 231–240.
[33] A.P. Sarvazyan, Ultrasonics 20 (1982) 151–154.
[34] A.P. Sarvazyan, E.E. Selkov, T.V. Chalikian, Sov. Phys. Acoust. 34 (1988) 631–634.
[35] A.P. Sarvazyan, T.V. Chalikian, Ultrasonics 29 (1991) 119–124.
[36] T.V. Chalikian, A.P. Sarvazyan, Sensors and probes for physico-chemical biology (in Russian), in: A.I. Gaziev, (Ed.),Pushchino, Pushchino Research Center Press, 1991, pp. 104–114.
[37] F.J. Millero, Water and aqueous solutions, in: R.A. Horne (Ed.), New York, John Wiley and Sons, Inc., 1972, pp. 519–595.
[38] S. Barnatt, J. Chem. Phys. 20 (1952) 278–279.
[39] B. Owen, H.L. Simons, J. Phys. Chem. 61 (1957) 479–482.
[40] V.A. Del Grosso, C.W. Mader, J. Acoust. Soc. Am. 52 (1972) 1442–1446.
[41] G.S. Kell, J. Chem. Eng. Data 15 (1970) 119.
[42] A.P. Sarvazyan, D.P. Kharakoz, P. Hemmes, J. Phys. Chem. 83 (1979) 1796–1799.
[43] T.V. Chalikian, D.P. Kharakoz, A.P. Sarvazyan, et al., J. Phys. Chem. 96 (1992) 876–883.
[44] D.P. Kharakoz, Biophys. Chem. 34 (1989) 115–125.

[45] D.P. Kharakoz, J. Solution Chem. 21 (1992) 569–595.
[46] A. Bondi, J. Phys. Chem. 68 (1964) 441–451.
[47] T.V. Chalikian, A.P. Sarvazyan, K.J. Breslauer, J. Phys. Chem. 97 (1993) 13017–13026.
[48] H. Shiio, T. Ogawa, H. Yoshihashi, J. Am. Chem. Soc. 77 (1955) 4980–4982.
[49] D.P. Kharakoz, J. Phys. Chem. 95 (1991) 5634–5642.
[50] G.R. Hedwig, H. Høiland, J. Chem. Thermodyn. 27 (1995) 745–750.
[51] C. Jolicoeur, B. Riedl, D. Desrochers, L.L. Lemelin, R. Zamojska, O. Enea, J. Solution Chem. 15 (1986) 109–128.
[52] J.P. Hershey, R. Damesceno, F.J. Millero, J. Solution Chem. 13 (1984) 825–848.
[53] G.I. Makhatadze, V.N. Medvedkin, P.L. Privalov, Biopolymers 30 (1990) 1001–1010.