Partial Molar Volumes and Adiabatic Compressibilities of a Series of Aliphatic Amino Acids and Oligoglycines in D_2O

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Abstract: This paper reports the first characterization of the hydration properties of some amino acids and oligoglycines, low molecular weight analogues of proteins, in D_2O. Specifically, the partial molar volumes, $V°$, and adiabatic compressibilities, $K°_S$, of five α-amino acids and five oligoglycines have been determined in D_2O at 25 °C. The resulting data have been used to estimate the volume and compressibility contributions of the component nonpolar (methylene group), polar (peptide group), and charged (oppositely charged amino and carboxyl terminal groups) chemical groups. It was found that the volume and compressibility contributions of these charged, polar, and nonpolar groups in D_2O are "measurably" distinct from those in H_2O. This distinction, in principle, may allow one to develop a method by which differential volumetric measurements of proteins in D_2O and H_2O can be used to gain insight into the nature of the solvent-exposed protein groups in the absence of detailed structural information.

Introduction

Hydration is widely acknowledged to be one of the major forces driving protein recognition events, in particular, protein folding/unfolding transitions.[1−3] Consequently, hydration properties of globular proteins in their native and denatured states have been under intensive scrutiny using various experimental and computational techniques.[4−7] In this connection, the volumetric characteristics of substances (e.g., the partial molar volume and adiabatic compressibility) have proven to be reflective of and sensitive to solute−solvent interactions.[8−10] Hence, volumetric properties represent useful observables for studying the hydration properties of proteins. In recognition of this fact, several laboratories have investigated the volumetric properties of proteins in aqueous solutions and have proposed different approaches for interpreting these macroscopic data in terms of protein hydration.[11−19] Such interpretations are not straightforward and always model-dependent. However, despite the difficulties in interpreting the volumetric data for systems as complex as proteins, experiments of this type have begun to provide important data against which different models of protein hydration can be evaluated.[20,21]

The microscopic interpretation of the measured volumetric properties in terms of protein hydration is usually performed in conjunction with structural data on the surface atomic groups.[11,13,18] Unfortunately, such structural data are not always available, especially for the denatured states of proteins which include molten globule and unfolded states. Consequently, the microscopic interpretation of the volumetric properties of denatured protein states remains highly speculative and subjective in nature.[14−17,19] This limitation is serious and prevents published results from being used with confidence for analysis of the hydration features of proteins as a function of their conformational states.

One potential way to derive information on the solvent-exposed atomic groups of a protein in solution in the absence of structural data is to conduct differential measurements of the partial molar volume, $V°$, and partial molar adiabatic compressibility, $K°_S$, in light (H_2O) and heavy (D_2O) water. Note that the physicochemical properties of D_2O are not very different from those of H_2O. Consequently, the substitution of H_2O with D_2O should cause only a very small perturbation of the structural preferences of a solute.

A differential solvent−isotope approach may prove viable if the volumetric contributions of the water-accessible atomic groups of different chemical nature (charged, polar, and nonpolar) in D_2O are distinct from those in H_2O. To this end, a systematic library of the hydration properties of charged, polar, and nonpolar groups in H_2O and D_2O should be established.

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As a first step toward this goal, this paper reports the partial molar volumes, $V°$, and adiabatic compressibilities, $K°_S$, for two homologous series in D_2O at 25 °C: amino acids with aliphatic side chains (glycine, alanine, α-aminobutyric acid, norvaline, and norleucine) and oligoglycines (glycine, diglycine, triglycine, tetraglycine, and pentaglycine). The corresponding values of the partial molar volume and adiabatic compressibility of the amino acids and oligoglycines in H_2O have been reported previously.[22−28]

Both homologous series consist of component chemical groups identical to those that can be found in proteins. Specifically, at neutral pH, the amino acids studied here consist of a zwitterionic skeleton of oppositely charged amino and carboxyl termini and a nonbranched chain of aliphatic groups. Oligoglycines contain oppositely charged amino and carboxyl terminal groups, one or more polar peptide groups, and aliphatic $-CH_2-$ groups. The main objective of this study is to characterize the hydration properties of the component chemical groups of amino acids and oligoglycines in D_2O and compare these values to those previously reported for the same groups in H_2O.

Materials and Methods

All chemicals used in this study were of the highest purity commercially available and were used without further purification. Specifically, the oligoglycines studied here (diglycine, triglycine, tetraglycine, and pentaglycine) were purchased from Fluka (Switzerland), and the α-amino acids (glycine, alanine, α-aminobutyric acid, norvaline, and norleucine) and D_2O (99.9%) were obtained from Sigma Chemical (St. Louis, MO). All of the amino acids were of the L-stereisomeric form.

Prior to the densimetric and ultrasonic velocimetric experiments, the amino acids and oligoglycines were dissolved in D_2O and lyophilized to exchange labile protons for deutrons. The concentrations of the samples were determined by weighing 10−20 mg of solute with a precision ±0.03 mg, and then dissolving the material in a known amount of D_2O. Before being weighed, amino acids glycine, alanine, α-aminobutyric acid, norvaline, and norleucine were dried at 110 °C for 24 h, while oligoglycines diglycine, triglycine, tetraglycine, and pentaglycine were dried for 72 h under vacuum at room temperature in the presence of phosphorus pentoxide.

Densities were measured at 25 °C with a precision $\pm 1.5 \times 10^{-6}$ g cm^{-3} using a vibrating tube densimeter (DMA-60, Anton Paar, Austria). These density values were used to calculate the apparent molar volume, ϕV, using the well-known equation[29]

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

where M is the solute molecular weight, m is the molal concentration, and ρ and ρ_0 are the densities of the solution and solvent, respectively. The requisite value for the density of D_2O, ρ_0, equal to 1.104449 g cm^{-3} at 25 °C, was taken from Kell.[30]

The apparent molar adiabatic compressibility, ϕK_S, was calculated from the densimetric and ultrasonic data using the expression[31,32]

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

where $[U] = (U - U_0)/(U_0 C)$ is the relative molar sound velocity increment, U and U_0 are the sound velocities in the solution and solvent, respectively, and β_{S0} is the coefficient of adiabatic compressibility of the solvent. The value of β_{S0}, equal to 46.2×10^{-6} bar^{-1} at 25 °C, was calculated from data on the density[30] and sound velocity[33] of D_2O using the expression $\beta_{S0} = (\rho_0 U_0^2)^{-1}$.

Using a differential device[34] based on the resonator method,[34−38] ultrasonic velocities in the solutions of the amino acids and oligoglycines were measured at a frequency of about 7.5 MHz with a relative accuracy $\pm 10^{-4}$ % at 25 °C. These sound velocity measurements were performed as previously described.[28,39]

For each evaluation of ϕV or ϕK_S, three to five independent measurements were carried out within the concentration range of 3−4 mg/mL for each of the amino acids and oligoglycines studied, with the exception of pentaglycine. For this longer homologue, concentrations of about 1 mg/mL were used because of its low solubility.

Results

The relative molar sound velocity increments, $[U]$, apparent molar volumes, ϕV, and apparent molar adiabatic compressibilities, ϕK_S, at 25 °C for the amino acids and oligoglycines are shown in Tables 1 and 2, respectively. The reported errors include contributions from the solute concentration and apparatus limitation, as well as any temperature variability in the measuring cells. Previous studies have shown that, for amino acids and oligoglycines in H_2O, the apparent molar volumes and the apparent molar adiabatic compressibilities do not depend strongly on concentration.[22,23,26,27] By extension, one may assume that in D_2O also the concentration dependences of ϕV and ϕK_S are weak. In other words, the apparent molar volumes, ϕV, and adiabatic compressibilities, ϕK_S, of the amino acids and oligoglycines determined in the concentration range of 1−4 mg/mL can be assumed to coincide with the partial molar volumes, $V°$, and adiabatic compressibilities, $K°_S$, obtained by extrapolation to infinite dilution. Consequently, below, the apparent molar and partial molar characteristics of the amino acids and oligoglycines will be treated as equivalent.

Discussion

Partial Molar Volume. Theoretical Considerations. It is convenient to consider the dissolution of a solute as consisting of two steps: (i) the creation of a cavity in the solvent large enough to enclose the solute molecule; and (ii) the introduction into the cavity of a solute molecule that can interact with the solvent. Consequently, the partial molar volume of a solute at infinite dilution, $V°$, represents contributions from each step[40−43]

$$V° = V_M + V_T + V_I + \beta_{T0} RT \quad (3)$$

where V_M is the geometric volume occupied by the solute molecule itself; V_T is the "thermal" volume (the volume of the void space surrounding the solute molecule), which is due to

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Table 1. Molecular Weights, M, Relative Molar Sound Velocity Increments, $[U]$, Partial Molar Volumes, $V°$, and Partial Molar Adiabatic Compressibilities, $K°_S$, of the Amino Acids in D_2O at 25 °C

	M	$[U]$ ($cm^3\ mol^{-1}$)	$V°$ ($cm^3\ mol^{-1}$)	$K°_S$ ($10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$)
glycine	78.09	39.3 ± 0.3	42.9 ± 0.4 (43.2)[a]	29.3 ± 0.6 (−26.6)[b]
alanine	92.11	48.3 ± 0.3	60.2 ± 0.4 (60.4)	−27.5 ± 0.6 (−25.1)[b]
aminobutyric acid	106.12	63.0 ± 0.3	77.2 ± 0.4 (75.5)[a]	−31.3 ± 0.6 (−27.1)[b]
norvaline	120.12	76.8 ± 0.3	93.2 ± 0.4 (91.7)[a]	−35.1 ± 0.6 (−29.0)[b]
norleucine	134.22	89.7 ± 0.3	110.3 ± 0.4 (107.9)[a]	−37.2 ± 0.6 (−31.1)[b]

[a] Partial molar volumes, $V°$, of the amino acids in H_2O at 25 °C are from ref 23. [b] Partial molar adiabatic compressibilities, $K°_S$, of the amino acids in H_2O at 25 °C are from ref 25.

Table 2. Molecular Weights, M, Relative Molar Sound Velocity Increments, $[U]$, Partial Molar Volumes, $V°$, and Partial Molar Adiabatic Compressibilities, $K°_S$, of the Amino Acids in D_2O at 25 °C

	M	$[U]$ ($cm^3\ mol^{-1}$)	$V°$ ($cm^3\ mol^{-1}$)	$K°_S$ ($10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$)
glycine	78.09	39.3 ± 0.3	42.9 ± 0.4 (43.2)[a]	−29.3 ± 0.6 (−26.6)[a]
diglycine	136.1	62.2 + 0.3	76.6 ± 0.4 (76.2)[a]	−43.6 ± 0.6 (−40.8)[a]
triglycine	194.2	76.4 + 0.6	111.9 ± 0.5 (112.1)[a]	−48.4 ± 1.0 (−45.9)[a]
tetraglycine	252.2	92.1 ± 0.7	152.0 ± 0.6 (149.6)[a]	−50.1 ± 1.2 (−45.9)[a]
pentaglycine	310.2	108.2 ± 1.0	191.5 ± 1.0 (186.9)[a]	−52.9 ± 1.8 (−47.2)[a]

[a] Partial molar volumes, $V°$, and partial molar adiabatic compressibilities, $K°_S$, of the oligoglycines in H_2O at 25 °C are from ref 28.

the thermal motion of solute and solvent molecules; V_I is the volume change associated with solute−solvent interactions, the "interaction" volume; β_{T0} is the coefficient of isothermal compressibility of the solvent; R is the universal gas constant; and T is the absolute temperature. The term $\beta_{T0}RT$ describes the volume effect related to the kinetic contribution to the pressure of a solute molecule due to translational degrees of freedom.[41] For D_2O at 25 °C, the ideal term, $\beta_{T0}RT$, in eq 3 is equal to 1.15 $cm^3\ mol^{-1}$ compared to 1.12 $cm^3\ mol^{-1}$ for H_2O. Note that the sum $V_M + V_T$ in eq 3 represents the partial molar volume of the cavity, V_C, enclosing the solute.

Scaled particle theory,[40−42,44] by employing simple statistical mechanical and geometric arguments to describe the dissolution of a solute, allows one to evaluate the intrinsic and thermal contributions of a spherical solute to the partial molar volume, $V°$. A characteristic feature of scaled particle theory is that it considers dissolution thermodynamics in terms of geometric properties of the solute and thermodynamic properties of the solvent. Scaled particle theory has often been used to calculate the partial molar volumes, $V°$, of nonpolar solutes (such as gases and hydrocarbons) in aqueous solutions.[40,42] It is worthy to note that remarkably good quantitative agreement has been observed between the calculated and experimental values of $V°$.[40,42]

On the basis of the concepts of scaled particle theory, the cavity volume, V_C, of a spherical solute is given by the expression[40,42]

$$V_C = 82.054\beta_{T0}H_C/\alpha_0 RT + \pi d_2^{\,3}N_A/6 \quad (4)$$

where α_0 is the volume coefficient of thermal expansion of the solvent; N_A is Avogadro's number; d_2 is the effective diameter of the solute molecule; and H_C is the partial molar enthalpy of the cavity formation which is given by the relationship

$$H_C = \alpha_0 RT^2[6By/(1-y)^2 + 36Cy^2/(1-y)^3 + y/(1-y)] \quad (5)$$

where $y = \pi d_1^{\,3}N_A/(6V°_0)$ is the packing density of the solvent; d_1 is the effective hard-sphere diameter of the solvent molecules; $V°_0$ is the partial molar volume of the solvent; $B = 2(d_{12}/d_1)^2 - d_{12}/d_1$; $C = (d_{12}/d_1)^2 - d_{12}/d_1 + 0.25$; and $d_{12} = (d_1 + d_2)/2$.

Edward and Farrell[45] have proposed a model in which the thermal volume, V_T, is considered as consisting of a layer of "empty" volume of a thickness Δ surrounding a solute molecule. On the basis of this model, the following expression for the partial molar volume, V_C, of the cavity enclosing the solute can be derived: $V_C = \pi N_A(d_2 + 2\Delta)^3/6$. Consequently, for a spherical solute, the value of Δ can be calculated as follows:

$$\Delta = 0.5[(6V_C/\pi N_A)^{1/3} - d_2] \quad (6)$$

Implicit in this model is the assumption that the thickness, Δ, of the thermal volume depends primarily on the thermodynamic and structural characteristics of the solvent (such as the coefficient of isothermal compressibility, β_{T0}, and the packing density, y) while only secondarily depending on the diameter, d_2, of the solute molecule. This assumption can be used to evaluate the effective hard-sphere diameter, d_1, of the solvent molecule from eqs 4−6. More specifically, if the optimum value of d_1 is used, then the calculations performed using eqs 4−6 should result in the weakest dependence of Δ on the solute diameter, d_2. Such calculations revealed that, for both H_2O and D_2O, the optimum value of d_1 is 2.74 Å, in good agreement with previous estimates.[43,46,47] Figure 1 shows the dependencies of Δ on d_2 in H_2O and D_2O, calculated from eqs 4−6 by using a d_1 value of 2.74 Å. As can be seen in Figure 1, for any value of d_2, the thickness, Δ, in D_2O is slightly larger than in H_2O. For solutes with a diameter, d_2, of 2 Å and larger, the values of Δ in D_2O and H_2O are equal to 0.56 and 0.55 Å, respectively.

Figure 2a shows how the partial molar volume of the cavity, V_C, enclosing a spherical solute depends on the solute diameter, d_2, in D_2O (Δ) and H_2O (▼), respectively. Inspection of Figure 2a reveals that the values of V_C in D_2O and H_2O are quite similar. To illustrate this point at a greater resolution, Figure 2b shows the difference, ΔV_C, between the values of V_C for a spherical solute in D_2O and H_2O as a function of V_C. As can be seen in Figure 2b, V_C in D_2O is slightly larger than in H_2O. More specifically, when V_C increases from 0 to 500 $cm^3\ mol^{-1}$, the difference, ΔV_C, increases from 0 to 2 $cm^3\ mol^{-1}$. Note that, since the intrinsic volume, V_M, of a solute is the same in D_2O and H_2O, the value of ΔV_C is determined solely by the differential value of V_T in D_2O and H_2O. Consequently, for solutes with a value of V_C between 50 and 200 $cm^3\ mol^{-1}$ (which roughly corresponds to the range of values of V_C of the amino acids and oligoglycines studied here), the thermal volume, V_T, in D_2O is larger than in H_2O by 0.3 to 1.0 $cm^3\ mol^{-1}$.

Amino Acids. The intrinsic volume, V_M, of a simple solute molecule can be approximated by its van der Waals volume, V_W. Thus, by setting $V_M = V_W$ in eq 3, the difference between

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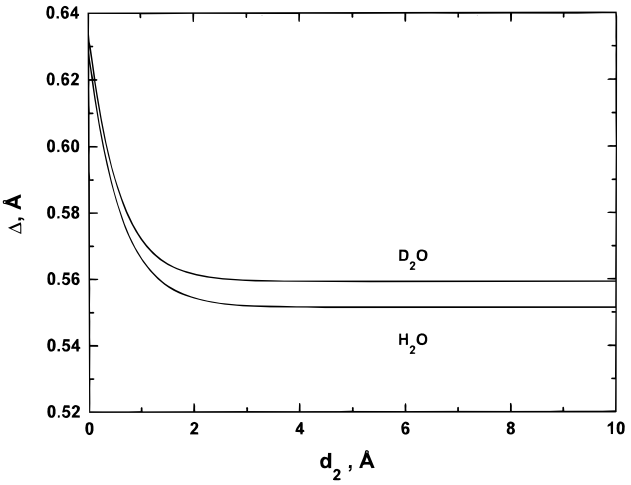

Figure 1. The thickness, Δ, of the thermal volume as a function of the diameter of a solute, d_2, in D_2O and H_2O.

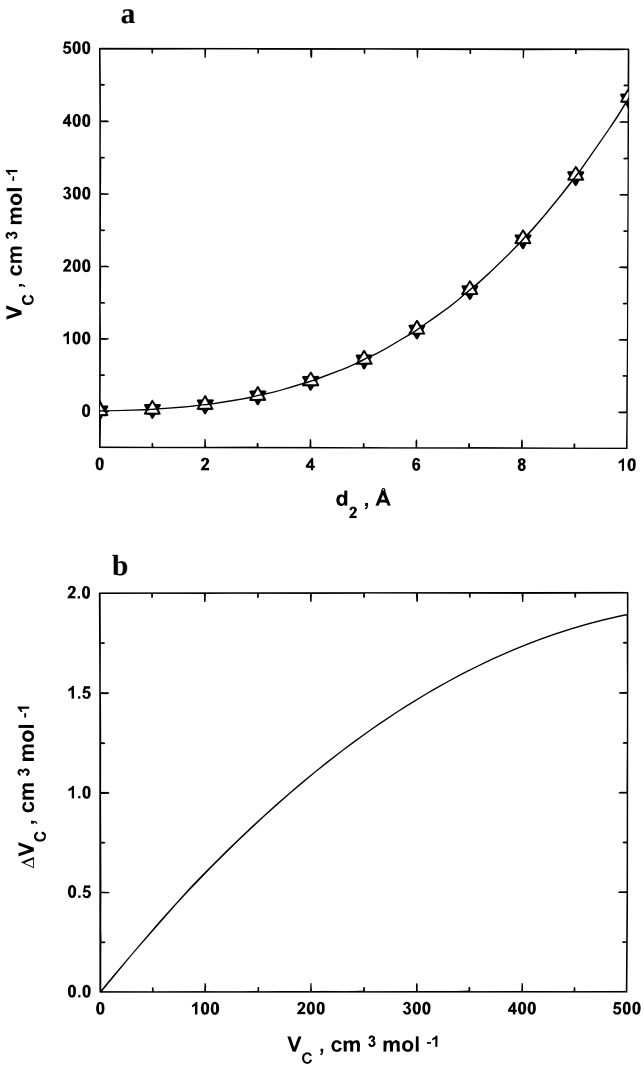

Figure 2. (a) The dependence of the partial molar volume, V_C, of the cavity enclosing a spherical solute on its diameter, d_2, in D_2O (Δ) and H_2O (▼); (b) The difference, ΔV_C, in the partial molar volume, V_C, of the cavity enclosing a spherical solute in D_2O and H_2O as a function of V_C.

the partial molar volume of a solute and its van der Waals volume, ($V° - V_W$), becomes equal to the sum ($V_T + V_I + \beta_{T0}RT$), which reflects the volume effect of solute−solvent interactions. The requisite values of V_W for the amino acids

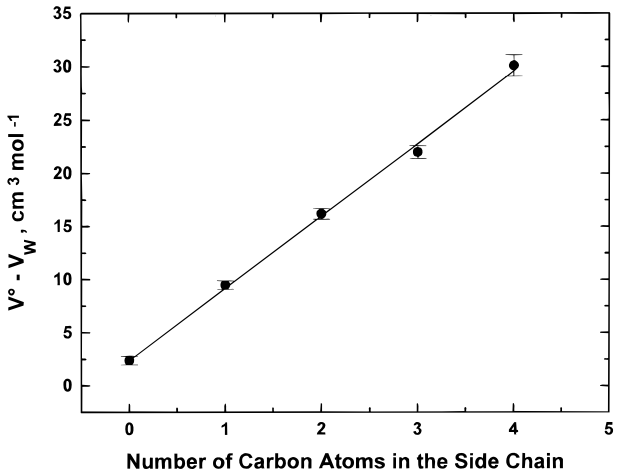

Figure 3. The difference between the partial molar volume, $V°$, and the van der Waals volume, V_W, for the amino acids as a function of the number of carbon atoms in the side chain.

studied here can be calculated using the additive approach of Bondi.[48] However, it should be noted that the choice of a specific scheme to divide the solution volume into the solute and solvent components (e.g., if one uses excluded volume instead of van der Waals volume) may affect the interpretation of the origin of volume changes associated with various biomolecular processes.[49−51]

Figure 3 shows how the difference between the partial molar volume, $V°$, and van der Waals volume, V_W, of the amino acids depends on the number of carbon atoms in the side chain. Note that the plot in Figure 3 is linear. This observation can be interpreted to mean that each methylene group added to the molecule causes the same alteration in the volume effect of hydration. Thus, the partial molar volume observable does not detect interactions, if any, between the aliphatic side chain and the zwitterionic skeleton of the amino acid molecules.

On the basis of the above discussion, the volume contribution of an independently hydrated methylene group, $V(CH_2)$, can be estimated from the data presented in the fourth column of Table 1 as the average increment of the partial molar volume per $-CH_2-$ group in alanine, α-aminobutyric acid, norvaline, and norleucine. This estimate yields a value of $V(CH_2)$ in D_2O equal to 16.7 ± 0.3 cm^3 mol^{-1} which is somewhat higher than the value of $V(CH_2)$ in H_2O, 15.9 ± 0.4 cm^3 mol^{-1}.[23,24,39] Recall that, for nonpolar methylene groups, the volume contribution, $V(CH_2)$, is the sum of its intrinsic volume, V_M, and the thermal volume, V_T, with the interaction volume, V_I, being negligibly small. The intrinsic volume, V_M, of the $-CH_2-$ group is the same in D_2O and H_2O. Consequently, a small positive difference in the $V(CH_2)$ values in D_2O and H_2O suggests that the thermal volume, V_T, in D_2O may be slightly higher than that in H_2O. This experiment-based conclusion is in qualitative agreement with the theoretical results presented in Figure 2b. For the methylene group with the value of V_C on the order of 10−20 cm^3 mol^{-1}, the calculated thermal volume, V_T, in D_2O is about 0.2 cm^3 mol^{-1} larger than in H_2O (see Figure 2b).

Differentiating eq 3 results in the following relationship for the difference, $\Delta V°$, between the partial molar volumes of the amino acids in D_2O and H_2O:

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$$\Delta V^\circ = \Delta V_M + \Delta V_T + \Delta V_I + \Delta(\beta_{T0}RT) \qquad (7)$$

The V_M and $\beta_{T0}RT$ components of the partial molar volume of the amino acids are similar in D_2O and H_2O. Inspection of the data in Table 1 reveals that the partial molar volume, V°, of glycine in D_2O practically coincides with that in H_2O ($\Delta V^\circ = -0.3 \pm 0.5$ cm^3 mol^{-1}). Furthermore, the thermal volume, V_T, of glycine in D_2O is only 0.3 cm^3 mol^{-1} larger than in H_2O (see Figure 2b). Hence, as is seen from eq 7, within our experimental error, $\Delta V^\circ \approx \Delta V_I \approx 0$. We conclude that, within ± 0.5 cm^3 mol^{-1}, the interaction volume, V_I, of glycine in D_2O is similar to that in H_2O. The interaction volume, V_I, of glycine reflects the water contraction due to electrostatic solute−solvent interactions in the vicinity of the zwitterionic skeleton of the molecule.[24] Such a contraction of water in the vicinity of charged groups is usually referred to as electrostriction. Thus, in D_2O, the oppositely charged amino and carboxyl termini of the amino acids cause a contraction of water which is very close to that in H_2O.

In α-amino acids, the closely located charged termini interact with each other via overlapping hydration shells.[22,24,25,28,39] Therefore, the value of ΔV_I determined above may not represent the true difference in the electrostriction of independently hydrated oppositely charged amino and carboxyl groups in D_2O and H_2O. In this respect, long oligoglycines represent a better model for determining the electrostriction of independently hydrated amino and carboxyl termini.

Oligoglycines. Figure 4 shows how the difference between the partial molar volume, V°, and the van der Waals volume, V_W, of the oligoglycines depends on the number of peptide bonds in the molecules. Note that two pronounced breaks are observed at points corresponding to diglycine and triglycine. After triglycine, the plot in Figure 4 becomes linear. Similar observation has been made for the partial molar volumes, V°, of oligoglycines in H_2O.[28] This observation has been interpreted as indicating that, in glycine and diglycine, the oppositely charged amino and carboxyl termini interact via overlapping hydration shells.[28] Significantly, the degree of overlap should be smaller in diglycine than in glycine, which accounts for the break point corresponding to diglycine. The amino and carboxyl termini in triglycine, tetraglycine, and pentaglycine cease to interact and become independently hydrated as reflected by the constant slope of the line in Figure 4 after triglycine. This interpretation implies that, in "long" oligoglycines, the glycyl units, $-CH_2COND-$, begin to exert an independent and significant influence on the characteristics of the adjacent water molecules. Hence, any incremental increase in the partial molar volumes, V°, of these "long" oligoglycines reflects the contribution of independently hydrated $-CH_2COND-$ atomic group.

The volume contribution of a single $-CH_2COND-$ group can be determined from the data presented in the fourth column of Table 2 as the increment of the partial molar volume, V°, per glycyl unit in triglycine, tetraglycine, and pentaglycine. This estimate yields a value for the volume contribution of the glycyl unit, $V(CH_2COND)$, in D_2O equal to 39.8 ± 0.8 cm^3 mol^{-1}. The volume contribution of the peptide group, $V(COND)$, can be calculated by subtracting the value of $V(CH_2)$ from that of $V(CH_2COND)$: $V(COND) = 39.8 - 16.7 = 23.1 \pm 1.1$ cm^3 mol^{-1}. This value is 1.5 cm^3 mol^{-1} larger than 21.6 cm^3 mol^{-1}, the estimate for the volume contribution of the peptide group, V(CONH), in H_2O.[28] The difference between V(COND) and V(CONH) can be ascribed solely to the difference between the interaction volumes, V_I, of the polar peptide group in D_2O and H_2O.

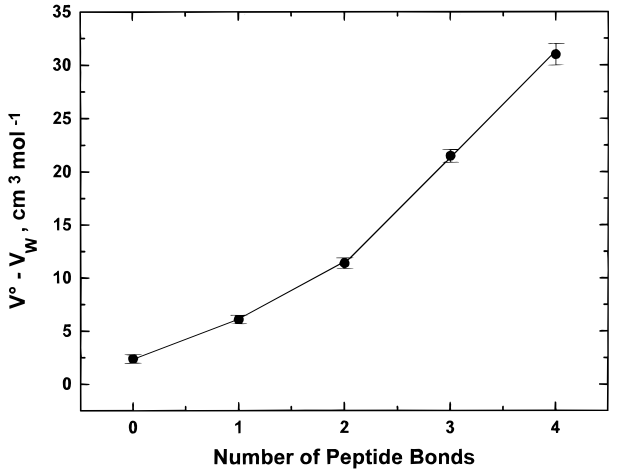

Figure 4. The difference between the partial molar volume, V°, and the van der Waals volume, V_W, for the oligoglycines as a function of the number of peptide bonds.

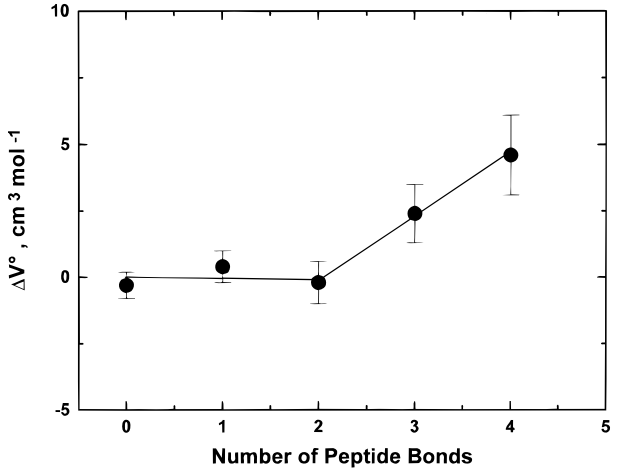

Figure 5. The difference, ΔV°, between the partial molar volumes, V°, of the oligoglycines in D_2O and H_2O as a function of the number of peptide bonds. The partial molar volumes, V°, of the oligoglycines in H_2O are from ref 28.

Peptide groups contain two polar entities −CO− and −NH− (or −ND−). These polar entities are capable of forming hydrogen bonds with adjacent water molecules thereby causing solvent contraction (which is reflected in the value of V_I). In our previous paper,[28] the value of V_I per peptide group in H_2O was estimated to be equal to -10.5 ± 0.5 cm^3 mol^{-1}, with this value being temperature-independent. On average, each of the polar groups (−CO− or −NH−) of a peptide moiety in H_2O, is characterized by an interaction volume of $V_I = -5.3 \pm 0.3$ cm^3 mol^{-1}.[28] In D_2O, the value of V_I of a peptide group is equal to $-10.5 + 1.5 = -9.0 \pm 1.1$ cm^3 mol^{-1}. On average, each of the polar groups (−CO− or −ND−) of a peptide moiety in D_2O is characterized by an interaction volume of $V_I = 0.5(-9.0 \pm 1.1) = -4.5 \pm 0.5$ cm^3 mol^{-1}. Note that the contraction of water caused by polar groups in D_2O, as reflected in the values of V_I, is about 15% smaller than in H_2O.

Figure 5 shows the difference, in partial molar volumes, ΔV°, of the oligoglycines in D_2O and H_2O plotted against the number of peptide bonds in the molecule. In fact, the values of ΔV° represent the volume change of transferring the oligoglycines from H_2O to D_2O. These data can be discussed in the framework of eq 7. The intrinsic volume, V_M, of the oligoglycines and the ideal term, $\beta_{T0}RT$, in D_2O and H_2O are similar. Furthermore, for oligoglycines, the thermal volume, V_T, in D_2O is higher than

in H_2O by less than 1 cm^3 mol^{-1} (see Figure 2b) which is within the experimental uncertainty of the data in Figure 5. Consequently, for the oligoglycines the value of $\Delta V°$ predominantly reflects the difference in the interaction volume, V_I, in D_2O and H_2O: $\Delta V° = V_I$ (see eq 7).

Inspection of Figure 5 reveals a pronounced break at the point corresponding to triglycine. Before triglycine, $\Delta V°$ practically remains constant and close to zero as the number of peptide bonds increases. By contrast, after triglycine, $\Delta V°$ increases proportional to the number of peptide bonds. The break point in Figure 5 indicates the different character of the solute−solvent interactions "before and beyond" triglycine. Specifically, as discussed above, in glycine, diglycine, and triglycine, the hydration shell is predominantly determined by electrostatic solute−solvent interactions. Furthermore, due to a decrease of the overlapping hydration shells of the amino and carboxyl termini from glycine to triglycine, the effective hydration of the charged groups increases. However, since charged groups in D_2O and H_2O exhibit similar V_I (as observed above in the case of glycine), the value of $\Delta V°$ for the oligoglycines does not change and remains close to zero when the intercharge distance increases from glycine to triglycine. As noted above, in triglycine and longer homologues, the charged termini cease to interact with each other, and the hydration of the peptide groups begins to influence the partial molar volume, $V°$. As the V_I of a glycyl unit ($-CH_2COND-$) in D_2O is higher (less negative) than in H_2O, after triglycine, $\Delta V°$ increases as the number of peptide bonds increases.

To a first approximation, the value of $\Delta V°$ corresponding to triglycine can be viewed as a measure of the difference in the electrostriction of an independently hydrated pair of the oppositely charged amino and carboxyl groups in D_2O and H_2O. As is seen from Figure 5, this difference is negligibly small (-0.2 ± 0.8 cm^3 mol^{-1}). Therefore, one may reasonably conclude that the electrostriction of an independently hydrated pair of the oppositely charged amino and carboxyl groups in D_2O is, within ± 0.8 cm^3 mol^{-1}, similar to that in H_2O. In H_2O, the electrostriction of an independently hydrated pair of the charged amino and carboxyl groups has been estimated to be -26.0 cm^3 mol^{-1}.[39] Consequently, electrostriction of the independently hydrated amino and carboxyl termini in D_2O is -26.0 ± 0.8 cm^3 mol^{-1}.

Partial Molar Adiabatic Compressibilities. The partial molar adiabatic compressibility, $K°_S$, of a solute can be represented as the sum of intrinsic and hydration contributions[10]

$$K°_S = K_M + \Delta K_h = K_M + n_h(K°_h - K°_0) \qquad (8)$$

where K_M is the intrinsic compressibility of the solute molecules; ΔK_h is the compressibility effect of hydration; $K°_0$ and $K°_h$ are the partial molar adiabatic compressibilities of water in the bulk state and in the hydration shell of the solute, respectively; and n_h, the "hydration number", is the number of water molecules within the hydration shell of a solute.

For low molecular weight substances, the intrinsic compressibility term, K_M, in eq 8, is negligibly small since it is determined by the compressibilities of covalent bonds and external electron shells, which are close to zero.[10] Thus, the partial molar adiabatic compressibility, $K°_S$, of low molecular weight substances primarily reflects solvent hydration changes as reflected by the reduced form of eq 8

$$K°_S = n_h(K°_h - K°_0) \qquad (9)$$

Amino Acids. Figure 6 shows the partial molar adiabatic compressibility, $K°_S$, of the amino acids in D_2O (○) and H_2O (●) plotted against the number of aliphatic carbons in the side chain. As can be seen in Figure 6, there is a pronounced break at the point corresponding to alanine in both plots. After alanine, the plot in Figure 6 becomes linear. This observation can be interpreted to mean that only the alkyl group in the β-position interacts with the zwitterionic skeleton of the molecule, perhaps, via overlapping hydration shells. After alanine (in α-aminobutyric acid, norvaline, and norleucine), each methylene group added to the molecule is independently hydrated and, consequently, contributes to the same extent to the amino acid compressibility. This result should be viewed with caution when applied to the amino acids in proteins which are branched at the β-carbon, such as valine, isoleucine, and threonine. Such β-branched compounds, whose bulk side chains beyond the β-carbon are quite close to the main chain, and therefore their hydration shells might well overlap those of the adjacent backbone regions.

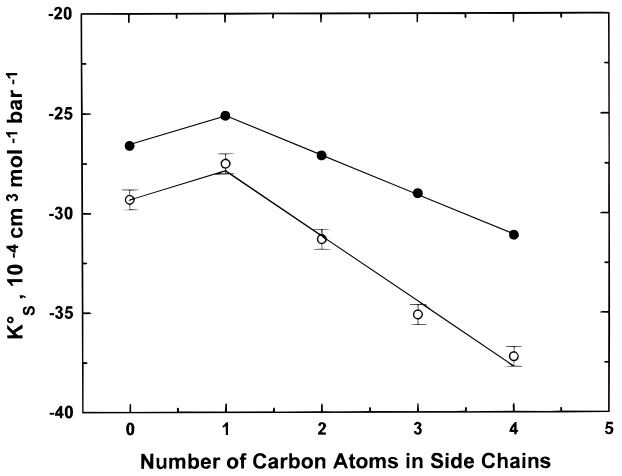

Figure 6. The partial molar adiabatic compressibility, $K°_S$, of the amino acids in D_2O (○) and H_2O (●) as a function of the number of carbon atoms in the side chain. The partial molar adiabatic compressibilities, $K°_S$, of the amino acids in H_2O are from ref 25.

The compressibility contribution of an independently hydrated methylene group, $K(CH_2)$, can be determined as the increment of the partial molar adiabatic compressibility per $-CH_2-$ group in alanine, α-aminobutyric acid, norvaline, and norleucine. This estimate yields a value of $K(CH_2)$ in D_2O equal to $-(3.2 \pm 0.4) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} which is significantly smaller (more negative) than -1.9×10^{-4} cm^3 mol^{-1} bar^{-1}, the value of $K(CH_2)$ in H_2O.[25] Thus, at 25 °C, aliphatic groups in D_2O cause a decrease in the solvent compressibility which is about 60% larger than in H_2O.

The compressibility contribution of a methylene group in H_2O is known to be strongly dependent on temperature: it is negative at low temperatures but becomes positive at high temperatures passing through zero at 30 °C. It is reasonable to expect that $K(CH_2)$ in D_2O exhibits an equally strong temperature dependence. Therefore, one cannot rule out the possibility that the relationship between the values of $K(CH_2)$ in D_2O and H_2O at other temperatures may be qualitatively *and* quantitatively different from that observed at 25 °C. Clearly, further temperature-dependent studies are required to address this important issue.

Further inspection of Figure 6 reveals that the partial molar adiabatic compressibility, $K°_S$, of glycine in D_2O is $(2.7 \pm 0.8) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} more negative than in H_2O. Since the hydration properties of glycine are dominated by electrostatic solute−solvent interactions, one may reasonably suggest that the compressibility contribution of charged groups in D_2O is

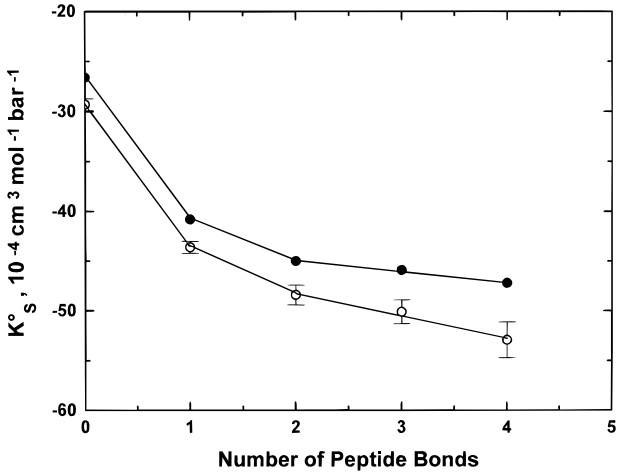

Figure 7. The partial molar adiabatic compressibility, $K°_S$, of the oligoglycines in D_2O (○) and H_2O (●) as a function of the number of peptide bonds. The partial molar adiabatic compressibilities, $K°_S$, of the oligoglycines in H_2O are from ref 28.

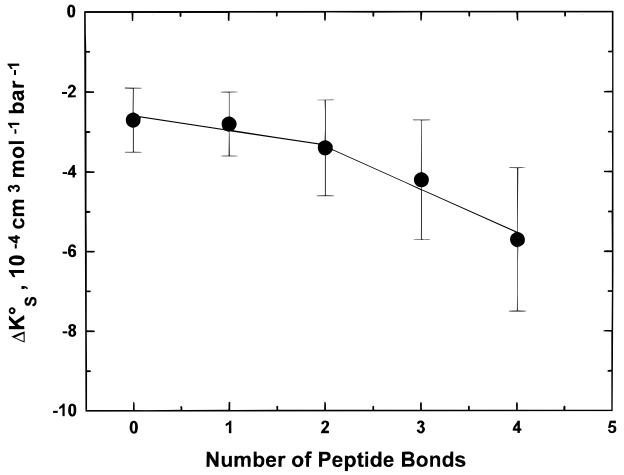

Figure 8. The difference, $\Delta K°_S$, between the partial molar volumes, $K°_S$, of the oligoglycines in D_2O and H_2O as a function of the number of peptide bonds. The partial molar adiabatic compressibilities, $K°_S$, of the oligoglycines in H_2O are from ref 28.

lower (more negative) than in H_2O. This is in agreement with the previous data on the partial molar adiabatic compressibility, $K°_S$, of salts in H_2O and D_2O.[47] Specifically, Desrosiers and Lucas[47] found that the partial molar adiabatic compressibilities, $K°_S$, of 1−1 electrolytes NaCl, KCl, CsCl, and NaF in D_2O are smaller than those in H_2O by 4.4×10^{-4}, 2.8×10^{-4}, 1.7×10^{-4}, and 5.5×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.

Oligoglycines. Figure 7 shows how the partial molar adiabatic compressibility, $K°_S$, of the oligoglycines depends in D_2O (○) and H_2O (●) on the number of peptide bonds in the molecule. As discussed above, the charged termini do not interact with each other in triglycine and longer homologues. Consequently, the compressibility contribution of an independently hydrated $-CH_2COND-$ group can be determined as the increment of the partial molar adiabatic compressibility, $K°_S$, per glycyl unit in triglycine, tetraglycine, and pentaglycine. The estimated value of $K(CH_2COND)$ in D_2O is equal to $(-2.2 \pm 0.5) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} which is somewhat smaller (more negative) than $(-1.1 \pm 0.5) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, a value of $K(CH_2CONH)$ in H_2O.[28] The compressibility contribution of the peptide group, K(COND), in D_2O can be calculated by subtracting $K(CH_2)$ from $K(CH_2COND)$: $K(COND) = -2.2 \times 10^{-4} + 3.2 \times 10^{-4} = (1.0 \pm 1.0) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. This value is somewhat larger than $(0.5 \pm 0.8) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, our previous estimate for the compressibility contribution of the peptide group, K(CONH), in H_2O.[28] Unfortunately, the large experimental/computational uncertainty does not allow us to determine whether the observed differential compressibility contribution of the peptide group is statistically significant.

Figure 8 shows the dependence of the difference in partial molar adiabatic compressibility, $\Delta K°_S$, of the oligoglycines in D_2O and H_2O on the number of peptide bonds in the molecule. In fact, the values of $\Delta K°_S$ represent the adiabatic compressibility change of transferring the oligoglycines from H_2O to D_2O. Inspection of Figure 8 reveals a pronounced break at the point corresponding to triglycine. In the context of the foregoing discussion, this break is related to the fact that, in triglycine and longer homologues, the charged termini cease to interact with each other. As a first approximation, the value of $\Delta K°_S$ corresponding to triglycine, $-(3.4 \pm 1.0) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, can be considered to be the difference between the compressibility contributions of an independently hydrated pair of the oppositely charged amino and carboxyl groups in D_2O and H_2O. In H_2O, the compressibility contribution of an independently hydrated pair of the charged amino and carboxyl groups has been estimated to be -34.0×10^{-4} cm^3 mol^{-1} bar^{-1}.[39] Consequently, the compressibility contribution of the independently hydrated amino and carboxyl termini in D_2O can be calculated to be $-34.0 \times 10^{-4} - 3.4 \times 10^{-4} = -(37.4 \pm 1.0) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. Note that charged groups in D_2O cause a decrease in solvent compressibility which is by about 10% larger than in H_2O.

Concluding Remarks

This paper presents the first study of the hydration properties of amino acids and oligoglycines in D_2O. Specifically, the partial molar volumes, $V°$, and partial molar adiabatic compressibilities, $K°_S$, of five amino acids and five oligoglycines have been determined in D_2O at 25 °C. The resulting data have been used to estimate the volume and compressibility contributions of the component nonpolar (methylene group), polar (peptide group), and charged (oppositely charged amino and carboxyl termini) chemical groups.

The volume contribution of a nonpolar methylene group, $V(CH_2)$, in D_2O is equal to 16.7 ± 0.3 cm^3 mol^{-1}. This value is slightly higher than 15.9 ± 0.4 cm^3 mol^{-1}, the volume contribution of a methylene group in H_2O. The compressibility contribution of a methylene group, $K(CH_2)$, in D_2O is equal to $-(3.2 \pm 0.4) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} which is significantly smaller (more negative) than -1.9×10^{-4} cm^3 mol^{-1} bar^{-1}, the value of $K(CH_2)$ in H_2O.

The volume contribution of a polar peptide group, V(COND), in D_2O is equal to 23.1 ± 1.1 cm^3 mol^{-1}. This value is 1.5 cm^3 mol^{-1} larger than 21.6 cm^3 mol^{-1}, the volume contribution, V(CONH), of a peptide group in H_2O. This difference suggests that hydrogen bonding between polar groups of a solute and solvent molecules brings about a slightly weaker contraction of water in D_2O than in H_2O. The compressibility contribution of the peptide group, K(COND), in D_2O has been calculated to be $(1.0 \pm 1.0) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} which is somewhat higher than $(0.5 \pm 0.8) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, the compressibility contribution, K(CONH), of the peptide group in H_2O.

The electrostriction of an independently hydrated pair of amino and carboxyl termini in D_2O practically coincides with the corresponding value in H_2O and has been estimated to be -26 ± 0.8 cm^3 mol^{-1}. The compressibility contribution of an independently hydrated pair of amino and carboxyl termini in

D_2O is equal to $-(37.4 \pm 1.0) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} which is 10% smaller (more negative) than the corresponding value in H_2O.

In the aggregate, the volume and compressibility contributions of charged, polar, and nonpolar groups in D_2O are "measurably" distinct from those in H_2O. This distinction, in principle, may allow one to apply differential volumetric measurements to protein solutions in D_2O and H_2O to gain insight into the nature of the solvent-exposed protein groups in the absence of structural information. A prerequisite for such applications of volumetric measurements is the accumulation of an empirical database large enough to permit reliable interpretation of the differential D_2O/H_2O volumetric results in terms of solvent-exposed atomic groups. To achieve this goal, more systematic investigations of low-molecular weight model compounds and proteins including temperature-dependent studies should be conducted. Such work is in progress.

Finally, this work raises fundamental questions about the molecular nature of the differential hydration properties of charged, polar, and nonpolar groups in D_2O and H_2O. Further experimental and theoretical studies are required to answer these questions.

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