Differential Hydration of α,*ω*-Aminocarboxylic Acids in D_2O and H_2O

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Abstract: We report the relative molar sound velocity increments, $[U]$, partial molar volumes, $V°$, expansibilities, $E°$, and adiabatic compressibilities, $K°_S$, for a homologous series of eight α,*ω*-aminocarboxylic acids in D_2O solution within the temperature range of 18−55 °C. We use the resulting data to estimate the volume, expansibility, and adiabatic compressibility contributions of the component aliphatic (methylene groups) and charged (oppositely charged amino and carboxyl termini) chemical groups. We compare these group contributions with similar group contributions for the same set of α,*ω*-aminocarboxylic acids in H_2O (Chalikian, T. V.; Sarvazyan, A. P.; Breslauer, K. J. *J. Phys. Chem.* **1993**, *97*, 13017−13026). We use these data to characterize quantitatively the differential hydration properties of charged and hydrophobic groups in D_2O and H_2O. Taken together, our results suggest that the hydration properties of hydrophobic and charged groups in D_2O, as reflected in their volume, expansibility, and compressibility contributions, are measurably distinct from those in H_2O. Significantly, these volumetric characteristics of the solute hydration differ not only in their absolute values but also in their temperature dependences. Such characteristics should prove useful in developing a better understanding of the role of differential D_2O/H_2O hydration in modulating thermal and thermodynamic stability of proteins. In addition, these results represent a further step in building up an empirical database of differential volumetric parameters of protein functional groups in D_2O and H_2O. Such a database is required for developing a methodology in which differential volumetric measurements in D_2O and H_2O can be employed to gain insight into the amount and chemical nature of solvent-exposed protein groups in the absence of structural information.

Introduction

Investigations of solvent-induced perturbations of protein stability provide one approach for studying the role of hydration in dictating the conformational preferences of a polypeptide chain at given experimental conditions. In this respect, differential studies of protein stability in light (H_2O) and heavy (D_2O) water are especially promising and have recently attracted considerable attention.[1−4] For all proteins studied so far, the thermal stability (T_M) increases in the presence of D_2O.[1−4] However, the thermodynamic stability ($\Delta G°$) in D_2O may either slightly decrease or slightly increase relative to H_2O as a result of entropy−enthalpy compensation that has been attributed to the differential hydration properties of protein groups in D_2O and H_2O.[1,2]

It should be noted that H_2O and D_2O represent an ideal pair for studying solvent-induced perturbations of protein stability, since they are chemically identical yet their physical properties differ significantly.[5] The differences between physical properties of H_2O and D_2O have been traditionally explained by the differential energetics of intermolecular hydrogen bonds. The lengths of the hydrogen bonds in D_2O (2.766 Å) and H_2O (2.765 Å) are essentially the same, while the energy of a hydrogen bond in D_2O is 0.24 kcal/mol (∼5%) higher than that in H_2O.[5] In addition, a larger degree of structural order exists in D_2O, although this order breaks down faster with increasing temperature.[5] These and other differences in physical properties of light and heavy water render them unequal as solvents. Consequently, the thermodynamics of solvation of various atomic groups in light and heavy water is considerably different, which is reflected in corresponding changes in Gibbs free energy, enthalpy, entropy, heat capacity, volume, compressibility, and other thermodynamic characteristics upon transfer of various substances from H_2O to D_2O.[6−10]

Volumetric measurements have proven useful for the quantitative characterization of hydration properties of biopolymers (proteins and nucleic acids) as well as their low molecular weight analogues.[11−15] Differential D_2O versus H_2O volumetric

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studies, performed on a systematic basis, will help us gain insight into the hydration properties of charged, polar, and nonpolar atomic groups. This knowledge is important for a better understanding of the role of water in determining the structural characteristics of proteins and nucleic acids as well as origins of increased thermal stability of proteins in heavy water. In addition, as discussed in our previous work, if the volumetric contributions of charged, polar, and nonpolar groups in D_2O are measurably different from those in H_2O, differential volumetric measurements in light and heavy water may offer one potential way to derive information on the amount and chemical nature of solvent-exposed protein groups in the absence of structural data.[9]

In our previous work, we have employed volumetric measurements to investigate the hydration properties of charged (oppositely charged amino and carboxyl termini), polar (a peptide group), and nonpolar (a methylene group) groups of α-amino acids and oligoglycines in D_2O at a single temperature of 25 °C.[9] At room temperature, judging by the volume and compressibility contributions, the hydration properties of these groups in D_2O are slightly yet "measurably" distinct from those in H_2O. However, since solute−solvent interactions are strongly temperature dependent, the information content of such single-temperature studies is rather limited. Clearly, further temperature-dependent investigations are required to better understand the differential hydration properties of various atomic groups in light and heavy water. In recognition of this need, we now expand our studies and report on the partial molar volume, $V°$, expansibility, $E°$, and adiabatic compressibility, $K°_S$, of a homologous series of α,ω-aminocarboxylic acids in D_2O over the temperature range 18−55 °C. At neutral pH, α,ω-aminocarboxylic acids are zwitterions, consisting of oppositely charged amino $-ND_3^+$ and carboxyl $-COO^-$ terminal groups separated by an unbranched chain of methylene $-CH_2-$ groups. Thus, only charged and nonpolar aliphatic groups contribute to the measured volumetric parameters of these solutes. We find that the dependences of $V°$, $E°$, and $K°_S$ of the α,ω-aminocarboxylic acids on the number of their constituent $-CH_2-$ groups are qualitatively similar to the same dependences previously reported for the same set of α,ω-aminocarboxylic acids in H_2O.[11] However, on a quantitative level, the $V°$, $E°$, and $K°_S$ contributions per $-CH_2-$ or a pair of charged groups in D_2O are considerably different from those in H_2O. Significantly, these volumetric characteristics of the solute hydration differ not only in their absolute values but also in their temperature dependences. We compare the volumetric results of this work with our previous data on the same set of α,ω-aminocarboxylic acids in H_2O.[11] We interpret the combined set of experimental data in terms of differential hydration properties of charged termini and aliphatic $-CH_2-$ groups in H_2O and D_2O. We also discuss the implications of our results for developing an understanding of the role of solvent in modulating conformational stability of proteins.

Materials and Methods

The α,ω-aminocarboxylic acids and D_2O (99.9%) used in our studies were purchased from Sigma-Aldrich Canada, Ltd. (Oakville, Ontario, Canada). The α,ω-aminocarboxilic acids were of the highest purity commercially available and were used without further purification. Prior to the densimetric and ultrasonic velocimetric experiments, the α,ω-aminocarboxylic acids 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 material with a precision of ±0.03 mg and then dissolving the material in a known amount of heavy water. Glycine, β-alanine, and 4-aminobutanoic acid were dried at 110 °C for 12 h prior to weighing. All other α,ω-aminocarboxylic acids were dried under vacuum in the presence of phosphorus pentoxide for 72 h prior to weighing. To prevent formation of air bubbles, all solutions were preheated in sealed Eppendorf tubes to 5 °C above the measurement temperature before filling the ultrasonic or densimetric cells.

Solution densities were measured using a vibrating tube densimeter (DMA-60/602, Anton Paar, Gratz, Austria) with a precision of $\pm 1.5 \times 10^{-6}$ g cm^{-3} at 18, 25, 40, and 55 °C. The apparent molar volume, ϕV, was calculated from these density values using the relationship:[16]

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

where M is the solute molecular weight, m is the molal concentration of a solute, and ρ and ρ_0 are the densities of the solution and solvent, respectively. The requisite values for the density of D_2O, ρ_0, were taken from Kell.[17] The values of ρ_0 are equal to 1.105599, 1.104449, 1.099958, and 1.093251 g cm^{-3} at 18, 25, 40, and 55 °C, respectively.

The solution sound velocities required to calculate the apparent molar adiabatic compressibility, ϕK_S, of a solute were measured at 18, 25, 40, and 55 °C using the resonator method[18−20] at a frequency of about 7.5 MHz. The sample and reference resonator cells with minimum volumes of 0.8 cm^3 were thermostated with an accuracy of ±0.01 °C, and a previously described differential technique was employed for all measurements.[19] Theoretical analyses[21,22] have shown that, for the type of ultrasonic cells used in our studies, the accuracy of the sound velocity measurements is about $\pm 10^{-4}$% at frequencies between 6 and 8 MHz. The analyses of the frequency characteristics of the resonator cells were performed by a Hewlett-Packard Model HP4195A network/spectrum analyzer (Mississauga, Ontario, Canada).

The key characteristic of a solute directly derived from ultrasonic velocimetric measurements is the relative molar sound velocity increment, $[U]$:

$$[U] = (U - U_0)/(U_0 C) \qquad (2)$$

where U and U_0 are the sound velocities in solution and solvent, respectively; and C is the molar concentration of a solute.

The apparent molar adiabatic compressibility, ϕK_S, was calculated from the densimetric and ultrasonic data using the expression:[23,24]

$$\phi K_S = \beta_{S0}(2\phi V - 2[U] - M/\rho_0) \qquad (3)$$

where β_{S0} is the coefficient of adiabatic compressibility of D_2O. The requisite values of β_{S0} were calculated from data on the density, ρ_0,[17] and sound velocity, U_0,[25] of D_2O using the expression $\beta_{S0} = (\rho_0 U_0^2)^{-1}$. At 18, 25, 40, and 55 °C, the values of U_0 are equal to 1382, 1400, 1435, and 1443 ms^{-1}, respectively.[25] The calculated values of β_{S0} are equal to 47.36×10^{-6}, 46.20×10^{-6}, 44.15×10^{-6}, and 43.93×10^{-6} bar^{-1} at 18, 25, 40, and 55 °C, respectively.

For each evaluation of ϕV or ϕK_S, three to five independent measurements were carried out within a concentration range of 2−4 mg/mL for each of the α,ω-aminocarboxylic acids, with the exception of 11-aminoundecanoic acid. For this long homologue, concentrations of 1 mg/mL or less were used due to its low solubility.

Results

Tables 1, 2, and 3 show the relative molar sound velocity increments, $[U]$, apparent molar volumes, ϕV, and apparent

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Table 1. Molecular Weights, M, and Relative Molar Increments of Sound Velocity, $[U]$, as a Function of Temperature for the α,ω-Aminocarboxylic Acids in D_2O

		$[U]$ ($cm^3\,mol^{-1}$)			
	M, Da	18 °C	25 °C	40 °C	55 °C
glycine	78.09	41.5 ± 0.3	38.5 ± 0.3	37.5 ± 0.4	32.3 ± 0.5
β-alanine	92.11	50.4 ± 0.3	47.4 ± 0.3	44.4 ± 0.5	38.8 ± 0.6
4-aminobutanoic acid	106.14	65.7 ± 0.4	59.7 ± 0.4	55.3 ± 0.5	52.0 ± 0.7
5-aminopentanoic acid	120.17	84.1 ± 0.5	76.7 ± 0.5	66.9 ± 0.6	62.3 ± 0.7
6-aminohexanoic acid	134.19	96.4 ± 0.5	87.6 ± 0.5	80.2 ± 0.6	73.9 ± 0.8
7-aminoheptanoic acid	148.22	110.1 ± 0.6	103.1 ± 0.5	88.9 ± 0.7	81.9 ± 0.8
8-aminooctanoic acid	162.25	124.7 ± 0.6	115.7 ± 0.6	97.0 ± 0.8	87.2 ± 0.9
11-aminoundecanoic acid	204.33	164.1 ± 0.9	150.5 ± 0.9	120.3 ± 1.0	104.0 ± 1.2

Table 2. Apparent Molar Volumes, ϕV, as a Function of Temperature for the α,ω-Aminocarboxylic Acids in D_2O and H_2O[a]

	ϕV ($cm^3\,mol^{-1}$)			
	18 °C	25 °C	40 °C	55 °C
glycine	42.1 ± 0.3 (42.7)[a]	42.9 ± 0.3 (43.2)[a]	43.4 ± 0.4 (44.0)[a]	44.0 ± 0.5 (44.2)[a]
β-alanine	57.3 ± 0.3 (57.6)[a]	58.2 ± 0.3 (58.3)[a]	58.9 ± 0.5 (59.1)[a]	59.2 ± 0.5 (59.2)[a]
4-aminobutanoic acid	72.4 ± 0.5 (72.4)[a]	73.2 ± 0.4 (73.1)[a]	74.0 ± 0.6 (74.1)[a]	74.0 ± 0.7 (73.7)[a]
5-aminopentanoic acid	87.7 ± 0.5 (87.3)[a]	88.8 ± 0.5 (88.3)[a]	89.2 ± 0.6 (89.1)[a]	89.8 ± 0.7 (88.7)[a]
6-aminohexanoic acid	103.4 ± 0.5 (103.1)[a]	104.6 ± 0.6 (104.3)[a]	105.2 ± 0.7 (105.2)[a]	106.0 ± 0.8 (105.2)[a]
7-aminoheptanoic acid	118.6 ± 0.6 (118.6)[a]	120.4 ± 0.6 (120.0)[a]	121.2 ± 0.8 (121.3)[a]	122.1 ± 0.8 (121.4)[a]
8-aminooctanoic acid	134.4 ± 0.6 (134.2)[a]	136.3 ± 0.7 (135.6)[a]	137.2 ± 0.9 (136.9)[a]	138.3 ± 0.9 (137.7)[a]
11-aminoundecanoic acid	181.3 ± 0.9 (180.1)[a]	183.5 ± 0.9 (182.6)[a]	184.6 ± 1.1 (184.5)[a]	186.4 ± 1.3 (186.9)[a]

[a] The partial molar volumes, $V°$, of the α,ω-aminocarboxylic acids in H_2O are from ref. 11.

Table 3. Apparent Molar Adiabatic Compressibilities, ϕK_S, as a Function of Temperature for the α,ω-Aminocarboxylic Acids in D_2O and H_2O[a]

	ϕK_S ($10^{-4}\,cm^3\,mol^{-1}\,bar^{-1}$)			
	18 °C	25 °C	40 °C	55 °C
glycine	−32.9 ± 0.5 (−29.6)[a]	−28.6 ± 0.5 (−26.6)[a]	−26.1 ± 0.7 (−22.4)[a]	-21.1 ± 0.7 (−20.4)[a]
β-alanine	−32.9 ± 0.5 (−30.0)[a]	−28.5 ± 0.5 (−26.3)[a]	−24.2 ± 0.9 (−21.0)[a]	-19.1 ± 1.0 (−18.7)[a]
4-aminobutanoic acid	−39.1 ± 0.8 (−35.1)[a]	−31.9 ± 0.7 (−30.2)[a]	−26.1 ± 1.0 (−22.8)[a]	-23.3 ± 1.2 (−20.9)[a]
5-aminopentanoic acid	−48.0 ± 0.9 (−41.4)[a]	−39.0 ± 0.9 (−34.3)[a]	−28.5 ± 1.0 (−24.7)[a]	-24.1 ± 1.2 (−22.2)[a]
6-aminohexanoic acid	−50.8 ± 0.9 (−45.6)[a]	−40.4 ± 1.0 (−37.1)[a]	−31.8 ± 1.1 (−27.1)[a]	-25.7 ± 1.4 (−21.4)[a]
7-aminoheptanoic acid	−55.4 ± 1.1 (−49.1)[a]	−46.0 ± 1.0 (−39.6)[a]	−31.0 ± 1.3 (−26.1)[a]	-24.2 ± 1.4 (−18.4)[a]
8-aminooctanoic acid	−60.3 ± 1.0 (−52.1)[a]	−48.8 ± 1.1 (−41.1)[a]	−29.6 ± 1.5 (−24.9)[a]	-20.3 ± 1.6 (−16.1)[a]
11-aminoundecanoic acid	−71.1 ± 1.6 (−61.9)[a]	−55.0 ± 1.6 (−45.1)[a]	−25.1 ± 1.9 (−21.2)[a]	−9.6 ± 2.2 (−6.9)[a]

[a] The partial molar adiabatic compressibilities, $K°_S$, of the α,ω-aminocarboxylic acids in H_2O are from ref 11.

molar adiabatic compressibilities, ϕK_S, of eight α,ω-aminocarboxylic acids at 18, 25, 40, and 55 °C, respectively. Due to space limitations, we intentionally omit our primary experimental data on solution densities, ρ, sound velocities, U, and solute concentrations required for calculation of the apparent molar volumes, ϕV, and relative molar sound velocity increments, $[U]$, from eqs 1 and 2, respectively. These primary data are relatively uninformative and, if needed, can be easily obtained from the values of ϕV and $[U]$ using eqs 1 and 2. Errors reported represent maximum uncertainties due to the concentration determination, temperature drifts, and apparatus limitations. Previous studies have shown that, for the α,ω-aminocarboxylic acids in H_2O, the apparent molar volumes and the apparent molar adiabatic compressibilities do not depend strongly on concentration.[26,27] By extension, one may plausibly assume that, in D_2O too, the concentration dependences of ϕV and ϕK_S are weak. In other words, within experimental error, the apparent molar volumes, ϕV, and adiabatic compressibilities, ϕK_S, of the α,ω-aminocarboxylic acids determined in the concentration range of 1−4 mg/mL can be assumed to coincide with the partial

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Table 4. Partial Molar Expansibilities, $E°$, as a Function of Temperature for the α,ω-Aminocarboxylic Acids in D_2O and H_2O[a]

	$E°$ (10^{-2} cm^3 mol^{-1} K^{-1})			
	18 °C	25 °C	40 °C	55 °C
glycine	8.0 ± 1.5	6.8 ± 1.5	4.4 ± 1.4	1.9 ± 1.5
	(8.5)[a]	(6.8)[a]	(3.2)[a]	(0)[a]
β-alanine	11.0 ± 1.5	8.7 ± 1.5	3.7 ± 1.5	−1.2 ± 1.5
	(10.6)[a]	(8.2)[a]	(3.1)[a]	(−2.0)[a]
4-aminobutanoic acid	11.8 ± 2.5	8.9 ± 2.5	2.8 ± 2.5	3.3 ± 2.5
	(13.6)[a]	(9.8)[a]	(1.8)[a]	(−6.3)[a]
5-aminopentanoic acid	9.7 ± 3.5	7.9 ± 3.5	4.1 ± 3.5	0.4 ± 3.5
	(14.9)[a]	(10.6)[a]	(1.5)[a]	(−7.6)[a]
6-aminohexanoic acid	11.1 ± 4.0	9.3 ± 4.0	5.5 ± 4.0	1.7 ± 4.0
	(15.8)[a]	(11.9)[a]	(3.4)[a]	(−5.0)[a]
7-aminoheptanoic acid	17.1 ± 4.5	13.8 ± 4.5	6.9 ± 4.5	0 ± 4.5
	(19.6)[a]	(15.0)[a]	(5.0)[a]	(−5.0)[a]
8-aminooctanoic acid	17.8 ± 6.0	14.7 ± 6.0	8.0 ± 6.0	1.3 ± 6.0
	(17.2)[a]	(14.1)[a]	(7.5)[a]	(1.0)[a]
11-aminoundecanoic acid	19.2 ± 7.5	16.7 ± 7.5	11.3 ± 7.5	6.0 ± 7.5
	(24.6)[a]	(21.8)[a]	(15.9)[a]	(9.9)[a]

[a] The partial molar expansibilities, $E°$, of the α,ω-aminocarboxylic acids in H_2O are from ref 11.

molar volumes, $V°$, and adiabatic compressibilities, $K°_S$, obtained by extrapolation to infinite dilution. Therefore, below, we do not discriminate between the apparent molar and partial molar characteristics of the α,ω-aminocarboxylic acids.

We have approximated our measured temperature dependences of the partial molar volumes, $V°$, by second-order polynomials. The temperature derivatives of $V°$ then were determined analytically by differentiation of the approximating functions at each of the temperatures studied. Table 4 presents the resulting data as the temperature slopes of the partial molar volume [equal to the partial molar expansibility, $E°$, since $E° = (\partial V°/\partial T)_P$] at 18, 25, 40, and 55 °C.

Discussion

Partial Molar Volume. The partial molar volume of a solute at infinite dilution, $V°$, can be interpreted in terms of hydration based on the following relationship:

$$V° = V_M + \Delta V_h = V_M + n_h(V_h - V_0) \qquad (4)$$

where ΔV_h is the volume effect of hydration, that is the solute-induced change in the solvent volume; V_0 and V_h are the partial molar volumes of water in the bulk state and in the hydration shell of a solute, respectively; and n_h is the "hydration number", that is, the number of water molecules in the hydration shell of a solute.

Scaled particle theory (SPT), originally formulated for a system of hard spheres, has been subsequently extended with great success to a description of aqueous solvation of both polar and nonpolar solutes.[28−33] Based on SPT theory, the hydration contribution, ΔV_h, in eq 4 can be presented as the sum of three terms:[29−34]

$$\Delta V_h = V_T + V_I + \beta_{T0}RT \qquad (5)$$

where V_M is the intrinsic volume of the solute molecule; V_T is the "thermal" volume, which 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 (hydrogen bonding) and charged (electrostriction) 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 ideal term $\beta_{T0}RT$ is small and does not strongly depend on temperature. For D_2O, the value of $\beta_{T0}RT$ is equal to 1.15 cm^3 mol^{-1} at 18 °C and increases to 1.23 cm^3 mol^{-1} at 55 °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 eight α,ω-aminocarboxylic acids based on the group contribution data of Bondi.[35] To derive the interaction volume, V_I, which represents the electrostriction of oppositely charged amino and carboxyl termini of the α,ω-aminocarboxylic acids, one needs to estimate the thermal volume, V_T. As previously discussed, the thermal volume, V_T, is a linear function of S_W, the van der Waals surface area:[9,11,34,36]

$$V_T = AS_W + B \qquad (6)$$

where the coefficients A and B are the same for a homologous series of solutes.

The coefficient B in eq 6 represents the volume of the cavity containing a point particle of zero radius and can be obtained readily from SPT theory.[28−31] For D_2O *and* H_2O based solutions, the value of B is 0.6 cm^3 mol^{-1} and practically does not depend on temperature within the 18−55 °C range. The coefficient A in eq 6 can be determined using a previously described approach[11] in which the contribution of nonpolar groups to the interaction volume, V_I, is assumed to be negligible.[11,34,36] With this assumption, inspection of eqs 4−6 reveals that any two homologues which are distinct with respect to the size of their nonpolar moieties will have ($V° - V_W$) values which differ only by the difference between the thermal volumes, V_T. Hence, the coefficient A in eq 6 can be determined as the slope of a $\Delta(V° - V_W)$ versus ΔS_W plot. Figure 1 shows such a plot for the α,ω-aminocarboxylic acids studied here.

Comparison of data presented in Figure 1 with similar data obtained earlier for the same set of α,ω-aminocarboxylic acids in H_2O[11] reveals a great deal of similarity. In particular, in both H_2O and D_2O, the dependences of ($V° - V_W$) on S_W at each temperature exhibit slight yet noticeable breaks at the point corresponding to 5-aminopentanoic acid. For the α,ω-aminocar-

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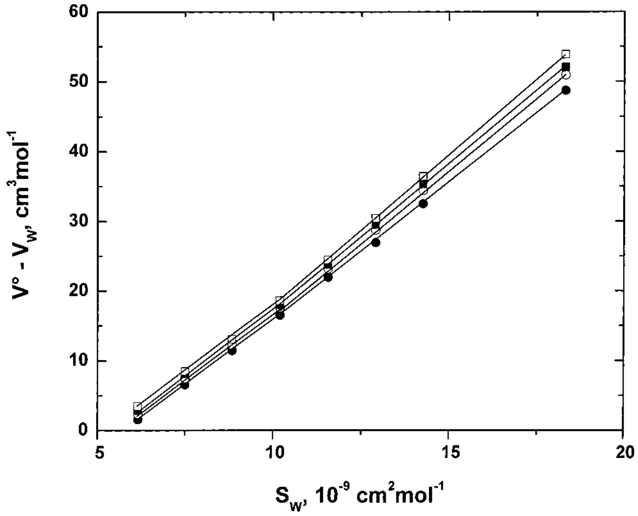

Figure 1. The difference between the partial molar volume of the α,*ω*-aminocarboxylic acids, *V*°, in D_2O and their van der Waals volume, V_W, as a function of the van der Waals surface area, S_W, at 18 (●), 25 (○), 40 (■), and 55 °C (□).

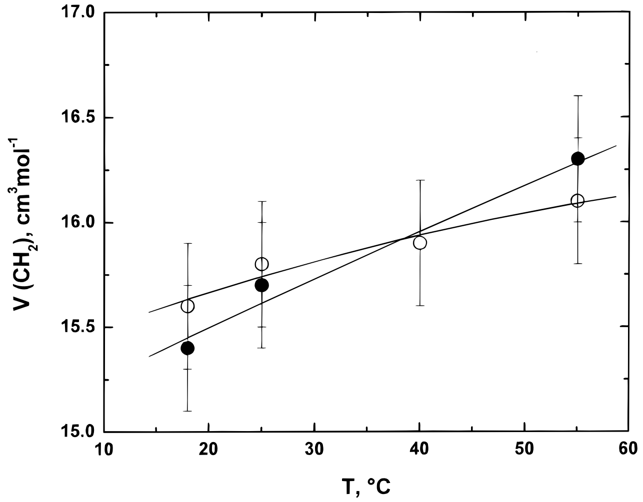

Figure 2. The temperature dependences of the contributions of the independently hydrated $-CH_2-$ group to the partial molar volume of the α,*ω*-aminocarboxylic acids in H_2O (●) (from ref 11) and D_2O (○).

boxylic acids in H_2O, we have rationalized these breaks by proposing that the character of solute−solvent interactions in long and short homologues (before and beyond 5-aminopentanoic acid) is qualitatively different.[11] In the short homologues (glycine, *β*-alanine, and 4-aminobutanoic acids), the hydration shell is electrostatic in nature and is predominantly determined by the mutually interacting oppositely charged terminal groups. For 5-aminopentanoic acid and longer homologues, the charged termini stop interacting with each other and each added $-CH_2-$ link becomes independently hydrated. Thus, any incremental change in the partial molar volume, *V*°, of the “long” α,*ω*-aminocarboxylic acids is determined by the volume contribution of an independently hydrated $-CH_2-$ group, $V(CH_2)$.

Figure 2 presents the temperature dependences of $V(CH_2)$ in H_2O (●) and D_2O (○). Inspection of Figure 2 reveals that the values of $V(CH_2)$ in the two solvents practically coincide within ±0.3 $cm^3\ mol^{-1}$, although the temperature dependence of $V(CH_2)$ may appear to be somewhat steeper in H_2O relative to D_2O. In D_2O at 25 °C, the volume contribution of an independently hydrated methylene group, $V(CH_2)$, in α,*ω*-aminocarboxylic acids equals 15.8 ± 0.3 $cm^3\ mol^{-1}$, which is somewhat lower than 16.7 ± 0.3 $cm^3\ mol^{-1}$, our estimate of $V(CH_2)$ in α-amino acids with nonbranched aliphatic side chain.[9] It is difficult to assess if this discrepancy is statistically significant or to provide a reliable explanation for its origins. At present, we just make notice of this fact for our future investigations.

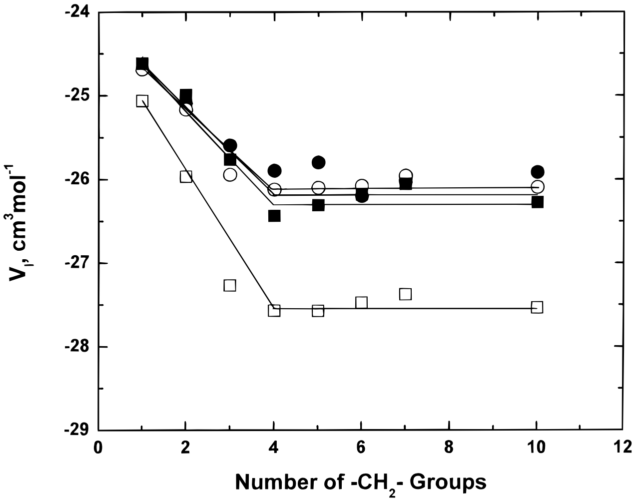

Figure 3. The dependence of the interaction volume, V_I, on the number of methylene groups in the α,*ω*-aminocarboxylic acids in D_2O at 18 (●), 25 (○), 40 (■), and 55 °C (□).

Since the interaction volume, V_I, of aliphatic groups is negligible, an increase in the value of ($V° - V_W$) with increasing solvent accessible surface area, S_W, of 5-aminopentanoic acid and longer homologues can be ascribed solely to an increase in the thermal volume, V_T. As is seen from Figure 1, the dependence ($V° - V_W$) on S_W beyond 5-aminopentanoic acid is linear. As mentioned above, the value of the coefficient *A* in eq 6 can be evaluated as the slope, $\Delta(V° - V_W)/\Delta S_W$, of this straight line. We estimate the coefficient *A* in eq 6 to be equal to 3.98×10^{-9}, 4.11×10^{-9}, 4.18×10^{-9}, and 4.35×10^{-9} cm at 18, 25, 40, and 55 °C, respectively. These results are very close to our previous estimates of the coefficient *A* for α,*ω*-aminocarboxylic acids in H_2O: 3.87×10^{-9}, 4.05×10^{-9}, 4.19×10^{-9}, and 4.54×10^{-9} cm at 18, 25, 40, and 55 °C, respectively.[11] This similarity suggests that the thermal volumes, V_T, for the α,*ω*-aminocarboxylic acids in D_2O do not differ strongly from those in H_2O. This conclusion is in agreement with our SPT-based calculations for the partial molar volume, V_C, of cavities enclosing spherical solutes in D_2O and H_2O.[9] Recall that, at 25 °C, the cavity volumes, V_C, for solutes with diameters up to 10 Å were estimated to be similar in D_2O and in H_2O (the difference was on the order of 1 $cm^3\ mol^{-1}$).[9] In the present study, we repeat these calculations for 18, 40, and 55 °C and find that, at these temperatures too, the cavity volumes are similar to within ±1 $cm^3\ mol^{-1}$ (data are not shown). Note that the cavity volume, V_C, represents the intrinsic volume, V_M, of a solute plus its thermal volume, V_T.[34,36] Importantly, since the intrinsic volume, V_M, of a solute in D_2O is equal to that in H_2O, the differential value of ΔV_C is determined solely by the differential value of V_T. Thus, within the 18−55 °C temperature range, the thermal volume, V_T, for solutes approximately of the size of the eight α,*ω*-aminocarboxylic acids studied here is very close in D_2O and H_2O.

Armed with the value of *A*, we can now use eqs 5 and 6 to calculate the interaction volumes, V_I, for the α,*ω*-aminocarboxylic acids. The resulting data are plotted in Figure 3 against the number of $-CH_2-$ groups. Inspection of Figure 3 reveals

Table 5. The Interaction Volume, V_I, for the Independently Hydrated Amino and Carboxyl Termini as a Function of Temperature for the α,ω-Aminocarboxylic Acids in D_2O and H_2O^a

	V_I ($cm^3\,mol^{-1}$)			
	18 °C	25 °C	40 °C	55 °C
D_2O	-26.2 ± 0.5	-26.1 ± 0.5	-26.3 ± 0.5	-27.5 ± 0.5
H_2O^a	-25.2 ± 0.5	-25.7 ± 0.5	-26.2 ± 0.5	-30.2 ± 0.5

[a] The interaction volumes, V_I, for the independently hydrated amino and carboxyl termini of the α,ω-aminocarboxylic acids in H_2O are from ref 11.

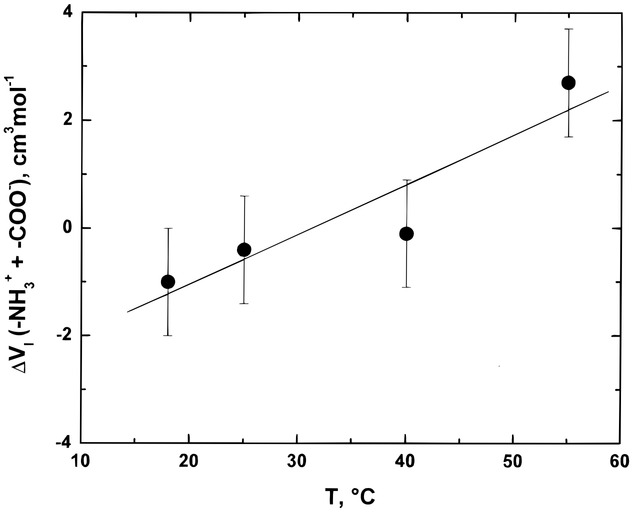

Figure 4. The difference between the interaction volumes, V_I, of the independently hydrated amino and carboxyl termini of the α,ω-aminocarboxylic acids in D_2O and H_2O as a function of temperature.

that the interaction volume, V_I, decreases (becomes more negative) going from glycine to 5-aminopentanoic acid and becomes constant for the longer α,ω-aminocarboxylic acids, when four or more $-CH_2-$ groups separate the charged end groups. As shown in Figure 3, the value of V_I at 55 °C is somewhat more negative than that at lower temperatures. We have made a similar observation of V_I for the α,ω-aminocarboxylic acids in H_2O.[11] These observations simply reflect nonlinear, parabolic (concave downward) temperature dependences of V_I for charged groups in H_2O and D_2O. The average value of V_I for the "longer" α,ω-aminocarboxylic acids can be taken as a measure of the total electrostriction of independently hydrated ND_3^+ and COO^- groups.

Table 5 compares the values of V_I for the independently hydrated amino and carboxyl termini of the α,ω-aminocarboxylic acids in D_2O with those in H_2O. Figure 4 presents the difference between the interaction volumes, V_I, of the independently hydrated amino and carboxyl termini of the α,ω-aminocarboxylic acids in D_2O and H_2O as a function of temperature. It is tempting to separate individual volumetric contributions of the amino and carboxyl termini. It should be noted that there are some indications in the literature that the positively charged amino group may be solvated significantly less than the negatively charged carboxyl group (e.g., see refs 37 and 38). However, any attempt to separate the hydration contributions of the amino and carboxyl termini would be highly speculative and require the use of nonthermodynamic assumption(s). Therefore, in our analysis below, we treat the pair of charged termini of α,ω-aminocarboxylic acid as a single thermodynamic entity.

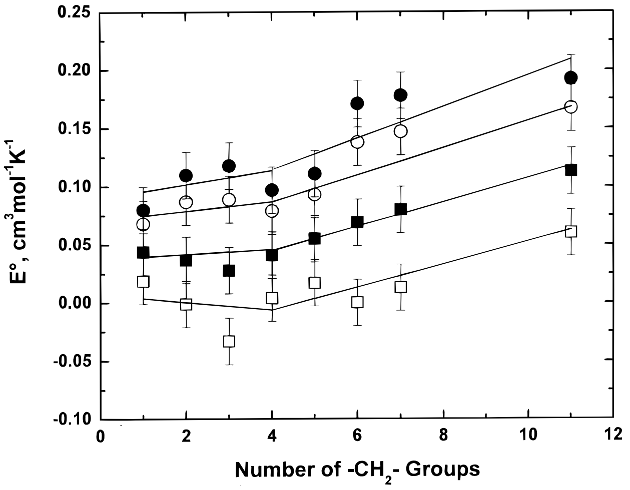

Figure 5. The dependence of the partial molar expansibility of the α,ω-aminocarboxylic acids in D_2O on the number of methylene groups at 18 (●), 25 (○), 40 (■), and 55 °C (□).

Inspection of Figure 4 reveals that the electrostriction of oppositely charged amino and carboxyl groups is stronger (more negative) in D_2O at low temperatures. However, above 35 °C, the situation is opposite: the electrostriction of charged groups becomes stronger in H_2O. For example, at 18 °C, ΔV_I is negative and equals -1.0 ± 1.0 $cm^3\,mol^{-1}$ (or ~5% of V_I), while at 55 °C, ΔV_I becomes positive and equals 2.7 ± 1.0 $cm^3\,mol^{-1}$ (or 10% of V_I). Note that, at 25 °C, ΔV_I is small and equals -0.4 ± 1.0 $cm^3\,mol^{-1}$. This result is in qualitative agreement with our previous evaluation of the interaction volume, V_I, of glycine.[9] Specifically, we found that, at 25 °C, the interaction volumes, V_I, of the charged termini of glycine in D_2O and H_2O coincide within ± 0.5 $cm^3\,mol^{-1}$.[9]

Partial Molar Expansibility. Differentiating eq 4 with respect to temperature and assuming that the hydration number, n_h, does not strongly depend on temperature, one obtains the following relationship for the partial molar expansibility:

$$E° = E_M + \Delta E_h = E_M + n_h(E_h - E_0) \qquad (7)$$

where E_M is the intrinsic expansibility of a solute molecule, ΔE_h is the expansibility effect of hydration, and E_0 and E_h are the partial molar expansibilities of water in the bulk state and in the hydration shell of a solute, respectively.

For small molecules, such as α,ω-aminocarboxylic acids, the intrinsic expansibility, E_M, in eq 7 is small and can be neglected. Consequently, only the hydration changes contribute to the partial molar expansibility, $E°$, of low molecular weight substances: $E° = \Delta E_h$.

Figure 5 shows the dependence on the number of methylene groups of the partial molar expansibility, $E°$, of the α,ω-aminocarboxylic acids at 18, 25, 40, and 55 °C. Even though the experimental errors in $E°$ are relatively high, pronounced breaks can be observed at a point corresponding to 5-aminopentanoic acid. It should be noted that similar breakpoints have been observed for the dependence of the partial molar expansibility, $E°$, of the α,ω-aminocarboxylic acids on the number of methylene groups in H_2O.[11] Analogous to volume, the breaks observed in Figure 5 suggest that the nature of hydration of the

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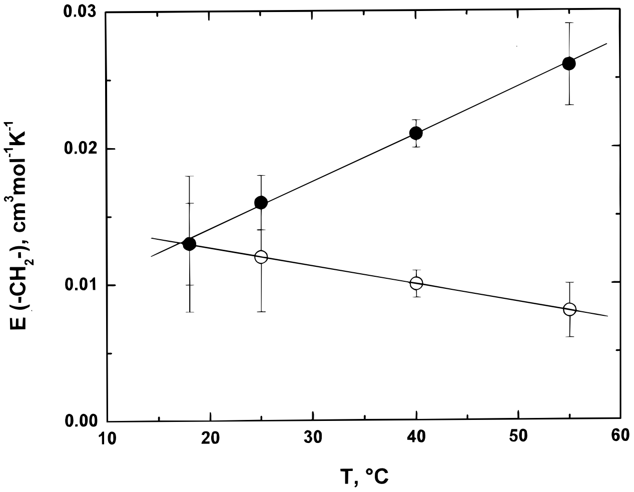

Figure 6. The temperature dependences of the contributions of the independently hydrated $-CH_2-$ group to the partial molar expansibility of the α,*ω*-aminocarboxylic acids in H_2O (●) (from ref 11) and D_2O (○).

short α,*ω*-aminocarboxylic acids qualitatively differs from that of the longer homologues. The incremental change in the partial molar expansibility, *E*°, of 5-aminopentanoic acid and the longer homologues represents the expansibility contribution per independently hydrated $-CH_2-$ group, *E*(CH_2). Figure 6 shows how the value of *E*(CH_2) depends on temperature in H_2O (●) (calculated from our previous data presented in ref 11) and D_2O (○). Inspection of Figure 6 reveals that the temperature dependences of *E*(CH_2) in H_2O and D_2O are significantly different. In H_2O, the value of *E*(CH_2) is positive and linearly *increases* with temperature from 0.013 ± 0.003 at 18 °C to 0.026 ± 0.003 at 55 °C. By contrast, in D_2O, the value of *E*(CH_2) is still positive but linearly *decreases* with temperature from 0.013 ± 0.005 at 18 °C to 0.008 ± 0.002 at 55 °C. This striking discrepancy reflects the differential hydration of aliphatic groups in H_2O and D_2O. On the basis of eq 7, the molar expansibility of water solvating aliphatic groups, E_h, is greater than that of bulk solvent, E_0. However, in H_2O, E_h increases faster with temperature than E_0, while in D_2O, E_0 increases faster than E_h.

The partial molar expansibility, *E*°, of 5-aminopentanoic acid can be assumed to be roughly equal to the contribution of noninteracting amino and carboxyl groups. On the basis of this assumption, the differential expansibility contribution of charged amino and carboxyl groups in D_2O and H_2O can be obtained by comparing the partial molar expansibility, *E*°, of 5-aminopentanoic acid in light and heavy water. Figure 7 shows how the expansibility contributions of charged amino and carboxyl groups depends on temperature in H_2O [$E(NH_3^+ + COO^-)$] (●) and D_2O [$E(ND_3^+ + COO^-)$] (○). Inspection of Figure 7 reveals that the expansibility contributions of the charged termini, $E(NH_3^+ + COO^-)$ and $E(ND_3^+ + COO^-)$, in both solvents are positive at low temperatures but become negative at higher temperatures: at ~40 °C in H_2O and ~55 °C in D_2O. On the basis of eq 7, this observation suggests that, at low temperatures, the molar expansibility of water solvating charged groups, E_h, is larger than that of bulk H_2O or D_2O. However, at higher temperatures, E_0 becomes larger than E_h. Further inspection of Figure 7 reveals that, in H_2O, the value of $E(NH_3^+ + COO^-)$ diminishes with temperature faster than $E(ND_3^+ + COO^-)$ in D_2O. As a result, at low temperatures, the expansibility contribution of charged groups is smaller in D_2O, while at high temperatures (above ~35 °C), the contribution of charged groups in D_2O becomes larger than that in H_2O.

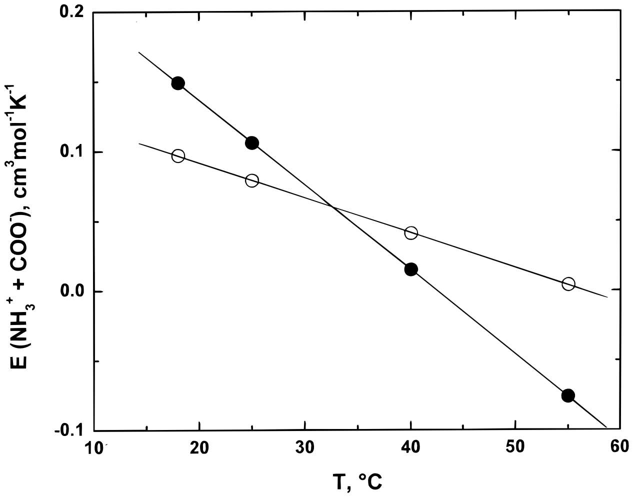

Figure 7. The temperature dependences of the contributions of the independently hydrated amino and carboxyl termini to the partial molar expansibility of the α,*ω*-aminocarboxylic acids in H_2O (●) (from ref 11) and D_2O (○).

Partial Molar Adiabatic Compressibility. Analogous to volume and expansibility, the partial molar adiabatic compressibility, $K°_S$, of a solute can be described as the sum of intrinsic and hydration contributions:

$$K°_S = K_M + \Delta K_h = K_M + n_h(K_h - K_0) \qquad (8)$$

where K_M is the intrinsic compressibility of a solute molecule; 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 a solute, respectively; and n_h, the hydration number, has the same meaning as in eqs 4 and 7.

For low molecular weight substances, the intrinsic compressibility, K_M, is predominantly determined by the small compressibility of covalent bonds and external electron shells, and, therefore, can be neglected.[12-15] Hence, only the hydration changes contribute to the partial molar adiabatic compressibility of low molecular weight substances:

$$K°_S = \Delta K_h = n_h(K_h - K_0) \qquad (9)$$

Figure 8 shows the dependences on the number of $-CH_2-$ groups of the partial molar adiabatic compressibilities, K_S, of the α,*ω*-aminocarboxylic acids. Compared to volume and expansibility (see Figures 1 and 5), the dependence of the partial molar adiabatic compressibility, $K°_S$, on the number of methylene groups is more complex. Inspection of Figure 8 reveals that, at 40 and 55 °C, the interaction between the termini continues to influence the value of $K°_S$ up to a point corresponding to 6-aminohexanoic acid when the charged groups become separated by five $-CH_2-$ links. This observation indicates that compressibility may be a more sensitive parameter with respect to subtle features of solute solvation as compared to volume and expansibility. We have made a similar observation earlier for the partial molar adiabatic compressibility, $K°_S$, of the α,*ω*-aminocarboxylic acids in H_2O.[11] Note that the intercharge distance in 6-aminohexanoic acid in H_2O determined from dielectric constant measurements is between 6.3 and 7.1 Å,[37] which corresponds to 2 to 2.5 diameters of a water molecule. Thus, based on our compressibility data, the hydration shell of

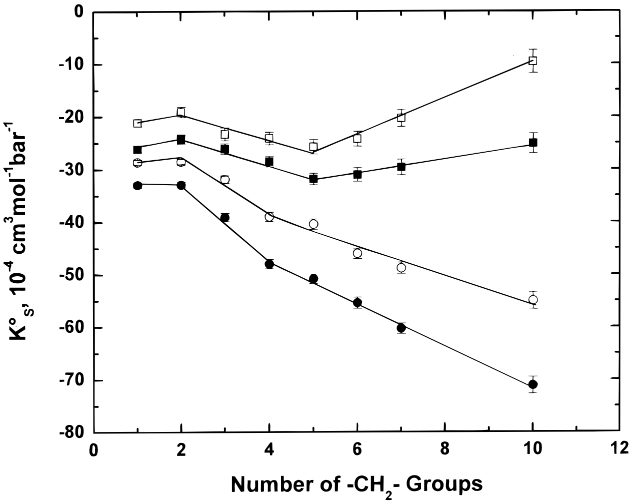

Figure 8. The dependence of the partial molar adiabatic compressibility of the α,ω-aminocarboxylic acids in D_2O on the number of methylene groups at 18 (●), 25 (○), 40 (■), and 55 °C (□).

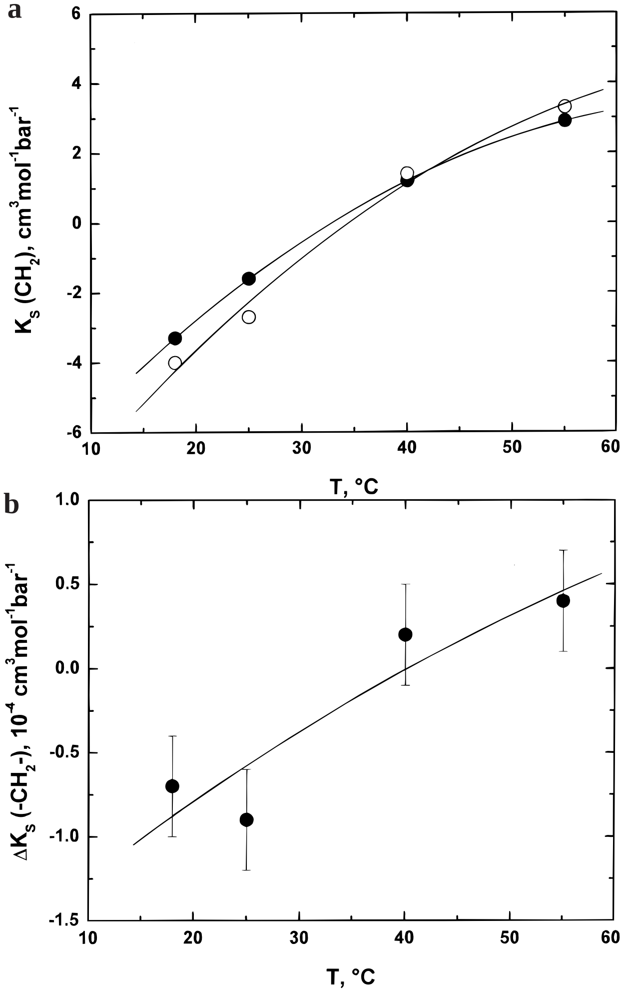

Figure 9. (a) The temperature dependences of the contributions of the independently hydrated $-CH_2-$ group to the partial molar adiabatic compressibility of the α,ω-aminocarboxylic acids in H_2O (●) (from ref 11) and D_2O (○). (b) The difference between the contributions of the $-CH_2$- group to the partial molar adiabatic compressibility of the α,ω-aminocarboxylic acids in D_2O and H_2O as a function of temperature.

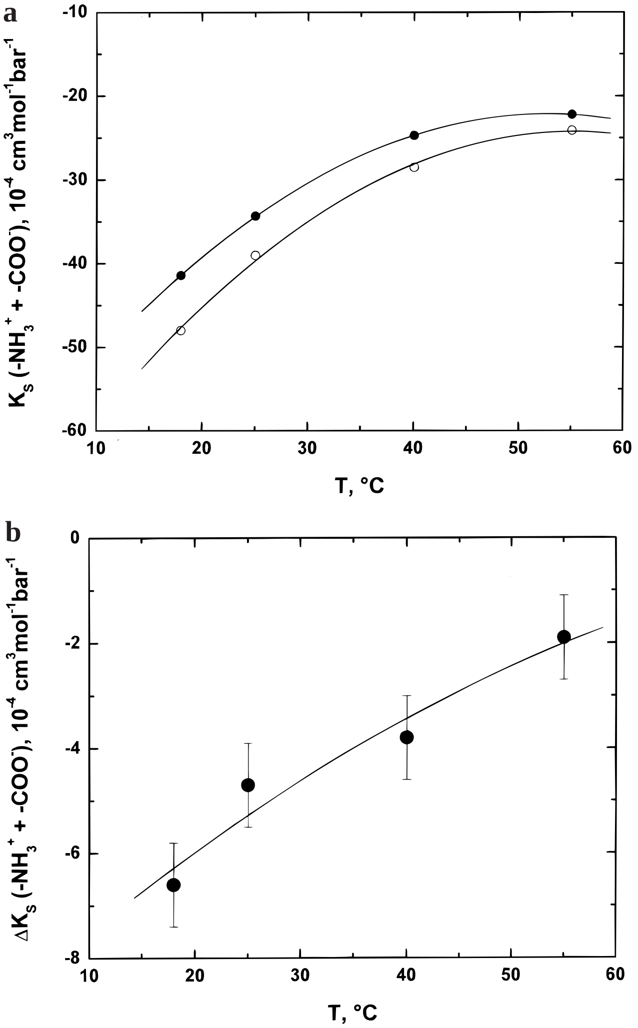

Figure 10. (a) The temperature dependences of the contributions of the independently hydrated amino and carboxyl termini to the partial molar adiabatic compressibility of the α,ω-aminocarboxylic acids in H_2O (●) (from ref 11) and D_2O (○). (b) The difference between the contributions of the independently hydrated amino and carboxyl termini to the partial molar adiabatic compressibility of the α,ω-aminocarboxylic acids in D_2O and H_2O as a function of temperature.

the charged group in α,ω-aminocarboxylic acids in D_2O roughly involves 1−1.5 effective layers of water molecules, which coincides with earlier estimations of the "thickness" of hydration shell of charged groups in H_2O.[11−14,38]

Figure 9 (panel a) shows the temperature dependences of the compressibility contributions of independently hydrated $-CH_2-$ groups, $K_S(CH_2)$, of the α,ω-aminocarboxylic acids in H_2O (●) (from ref 11) and D_2O (○). Inspection of Figure 9a reveals that, in both solvents, the value of $K_S(CH_2)$ is negative at low temperatures but becomes positive roughly above 35 °C. On the basis of eq 9, this observation suggests that, at low temperatures, water solvating aliphatic groups is less compressible than bulk H_2O or D_2O but becomes more compressible at higher temperatures. Note that, at 25 °C, the compressibility contribution of an independently hydrated methylene group, $K_S(CH_2)$, in the α,ω-aminocarboxylic acid in D_2O equals $-(2.8 \pm 0.3) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, which is in good agreement with $-(3.2 \pm 0.4) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, our previous estimate for $K_S(CH_2)$ in α-amino acids with nonbranched aliphatic side chains.[9] Further inspection of Figure 9a reveals that, below 40 °C, the value of $K_S(CH_2)$ in D_2O is smaller than that in H_2O. However, at higher temperatures, the value of $K_S(CH_2)$ in D_2O becomes larger than that in H_2O. This trend is more clearly shown in Figure 9 (panel b), which depicts the temperature dependence of the difference between the compressibility contributions of the independently hydrated methylene groups, $\Delta K_S(CH_2)$, in D_2O and H_2O. In fact, the data shown in Figure 9b represent the temperature dependence of the adiabatic compressibility change accompanying the transfer of an independently hydrated methylene group from H_2O to D_2O.

Analogous to volume and expansibility, the compressibility contribution of the independently hydrated amino and carboxyl terminal groups, $K_S(ND_3^+ + COO^-)$, can be considered to be roughly equal to the partial molar adiabatic compressibility, $K°_S$, of 5-aminopentanoic acid. Figure 10 (panel a) shows the temperature dependences of $K_S(NH_3^+ + COO^-)$ in H_2O (●) (from ref 11) and $K_S(ND_3^+ + COO^-)$ in D_2O (○). Inspection of Figure 10a reveals that, in both solvents, the compressibility contribution of charged groups is negative within the whole temperature range studied although it becomes less negative with increasing temperature. In agreement with conventional wisdom, this observation suggests that, within the entire temperature range studied, water dipoles experiencing strong electrostatic influence of charged groups exhibit reduced compressibility relative to bulk H_2O or D_2O. Further inspection of Figure 10a reveals that, over the whole temperature range studied, the value of $K_S(ND_3^+ + COO^-)$ in D_2O is smaller (more negative) than the value of $K_S(NH_3^+ + COO^-)$ in H_2O. Figure 10 (panel b) depicts the temperature dependence of the difference between the compressibility contributions of an independently hydrated pair of amino and carboxyl termini in D_2O and H_2O. It should be noted that the data presented in Figure 10b represent the temperature dependence of the compressibility change accompanying the transfer of charged groups from H_2O to D_2O. At 18 °C, the compressibility contribution of a pair of independently hydrated charged amino and carboxyl groups in D_2O is $(6.6 \pm 1.4) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [~15% of $K_S(NH_3^+ + COO^-)$] smaller (more negative) than that in H_2O. At 55 °C, this difference decreases to $(1.9 \pm 1.9) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [~10% of $K_S(NH_3^+ + COO^-)$].

Conclusion

We have determined the relative molar sound velocity increments, $[U]$, partial molar volumes, $V°$, expansibilities, $E°$, and adiabatic compressibilities, $K°_S$, for eight α,ω-aminocarboxylic acids in D_2O solution within the temperature range 18–55 °C. We have used the resulting data to estimate the volume, expansibility, and adiabatic compressibility contributions of the component aliphatic (methylene groups) and charged (oppositely charged amino and carboxyl termini) chemical groups.

The volume contributions of an independently hydrated methylene group, $V(CH_2)$, in D_2O and H_2O are similar to within ± 0.3 cm^3 mol^{-1}, although the temperature dependence of $V(CH_2)$ may be somewhat steeper in H_2O relative to D_2O. In D_2O, the electrostriction, V_I, of an independently hydrated pair of charged amino and carboxyl terminal groups decreases (becomes more negative) with increasing temperature. Specifically, the value of V_I is equal to -26.2 ± 0.5 cm^3 mol^{-1} at 18 °C and decreases to -27.5 ± 0.5 cm^3 mol^{-1} at 55 °C. Compared to H_2O, the electrostriction of the charged termini is stronger (more negative) in D_2O below 35 °C. At higher temperatures, the situation is opposite: the solvent contraction in the vicinity of the charged termini is stronger in H_2O.

In D_2O, the expansibility contribution of methylene groups, $E(CH_2)$, linearly *decreases* with temperature from 0.013 ± 0.005 at 18 °C to 0.008 ± 0.002 at 55 °C. By contrast, in H_2O, $E(CH_2)$ *increases* with temperature from 0.013 ± 0.003 at 18 °C to 0.026 ± 0.003 at 55 °C. This discrepancy reflects the fact that, in H_2O, the molar expansibility of water solvating aliphatic groups, E_h, increases faster with temperature than the expansibility of the bulk solvent, E_0, while, in D_2O, E_0 increases faster than E_h. In D_2O, the expansibility contributions of the charged termini, $E(ND_3^+ + COO^-)$, decreases from $(9.7 \pm 3.5) \times 10^{-2}$ cm^3 mol^{-1} K^{-1} at 18 °C to $(0.4 \pm 3.5) \times 10^{-2}$ cm^3 mol^{-1} K^{-1} at 55 °C. Compared to H_2O, the expansibility contribution of charged groups is smaller in D_2O at low temperatures, while, above ~35 °C, the contribution of charged groups in D_2O becomes larger than that in H_2O.

In D_2O, the compressibility contribution of an independently hydrated methylene group, $K_S(CH_2)$, increases from $(-4.0 \pm 0.3) \times 10^{-4}$ cm^{3}mol^{-1}bar^{-1} at 18 °C to $(3.3 \pm 0.3) \times 10^{-4}$ cm^{3}mol^{-1}bar^{-1} at 55 °C. Compared to H_2O, the value of $K_S(CH_2)$ in D_2O is smaller below 40 °C. However, at higher temperatures, the value of $K_S(CH_2)$ in D_2O becomes larger than that in H_2O. In D_2O, the compressibility contribution of charged groups, $K_S(ND_3^+ + COO^-)$, is negative within the whole temperature range studied although it becomes less negative with increasing temperature: it increases from $(-48 \pm 0.9) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} at 18 °C to $(-24.1 \pm 1.2) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} at 55 °C. Over the whole temperature range studied, the value of $K_S(ND_3^+ + COO^-)$ in D_2O is smaller (more negative) than the value of $K_S(NH_3^+ + COO^-)$ in H_2O, but the difference diminishes when the temperature increases.

Taken together, our data suggest that, in D_2O, the hydration properties of hydrophobic and charged groups, as reflected in their volume, expansibility, and compressibility contributions, are measurably distinct from those in H_2O. Significantly, these volumetric characteristics of solute hydration differ not only in their absolute values but also in their temperature dependences. These results provide a detailed quantitative description of the hydration of nonpolar and charged protein groups in D_2O. Such characteristics should prove useful in developing a better understanding of the role that differential D_2O/H_2O hydration of atomic groups plays in modulating thermal and thermodynamic stability of proteins. In addition, these results represent one further step in building an empirical database of differential volumetric parameters of protein functional groups in D_2O and H_2O. Such a database is required for a possible application of differential volumetric measurements in protein solutions in D_2O and H_2O to gaining insight into the amount and chemical nature of solvent-exposed protein groups in the absence of structural information.

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