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Volumetric Characterization of Homopolymeric Amino Acids

Abstract: *We have determined the partial molar volumes, expansibilities, and adiabatic compressibilities for poly(L-alanine), poly(L-proline), and poly(L-threonine) within the temperature range of 18–55°C. In addition, we have determined at 25°C changes in volume, ΔV, and adiabatic compressibility, ΔK_S, associated with the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) and the α-to-β transition of poly(L-lysine). We have interpreted our volumetric data as suggesting that poly(L-alanine) and poly(L-proline) are not fully unfolded and, probably, retain some solvent-inaccessible core. Further, we propose that poly(L-threonine) is fully unfolded with the majority of its atomic groups being solvent-exposed. Near zero changes in volume and compressibility accompanying the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) suggest that, in the absence of fortuitous compensations, the coil-to-helix transitions of the polypeptides do not result in any significant enhancement of solute hydration. By contrast, the α-to-β transition of poly(L-lysine) causes slight but statistically significant increases in volume and compressibility, an observation that may suggest that the β-sheet conformation of poly(L-lysine) is slightly less hydrated than its α-helical conformation. In general, our results provide a quantitative volumetric description of the hydration properties of the homopolymeric polypeptides investigated. Such characterizations should prove useful in developing an understanding of the role that solvent plays in the stabilization/destabilization of folded protein structures and protein complexes.* Biopolymers 73: 563–574, 2003

Keywords: *polypeptides; conformational transitions; hydration; volume; compressibility; ultrasonics*

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INTRODUCTION

Understanding the hydration properties of the protein in its native and denatured conformational states is an important step in the quest for unveiling thermodynamic origins of protein recognition such as folding and binding events.[1–3] The differential energetics of protein-water interactions (commonly referred to as hydration) in the folded and unfolded states or in the ligand-free and ligand-bound states represent a major determinant of the affinity and specificity of protein folding and binding. In recognition of this fact, protein hydration has been under intensive scrutiny, employing a variety of structural and thermodynamic techniques.[1–3] Volumetric methods, including ultrasonic velocimetry and high precision densimetry, have proven useful in characterizing the hydration properties of proteins and their low molecular weight model compounds.[4–11] Density and sound velocity measurements have been applied to studying transitions between the native and denatured conformations of globular proteins, including native-to-unfolded and native-to-molten globule transitions.[7,9–18] Such studies enable one to gain insight into hydration, compactness, and intrinsic packing of the native and fully and partially unfolded protein states, thereby facilitating a better understanding of intermediates on protein folding pathways. In a recent article, we proposed a relatively simple theoretical-empirical model of solute hydration.[19] This model is based on a two-state structural representation of liquid water and enables one to interpret volume and adiabatic compressibility data in terms of the number and thermodynamic state of water molecules solvating external atomic groups of a solute.[19] The proposed approach was used to investigate the hydration properties of globular proteins in their native states.[19] It was found that the hydration shell of a native protein comprises at least two layers of water molecules.[19] From a structural point of view, these water molecules are similar to waters solvating polar atomic groups in small molecules (e. g., hydroxyl groups of simple sugars).[19]

For protein folding studies, denatured forms of proteins are as important as the native state.[20] One problem of volumetric investigations of proteins is related to the question of what is the best system for modeling an unfolded polypeptide chain.[10,11] The problem is aggravated by the fact that, in contrast to the native state, structures of denatured protein states cannot be accurately resolved by conventional structural techniques, such as X-ray crystallography and NMR spectroscopy. The lack of structural information renders thermodynamic characterizations of denatured protein states assumption- and/or model-dependent. There are currently a variety of approaches that have been put forward to model the volumetric properties of unfolded polypeptide chains. For example, in one approach, the partial molar volumes and adiabatic compressibilities of unfolded polypeptide chains have been estimated by performing additive calculations using group contributions derived from amino acid[21,22] and oligopeptide[23,24] data. However, it is reasonable to expect that longer peptides, such as homopolymeric peptides, might represent a better model of unfolded protein states.

In this article, we have employed ultrasonic velocimetry and high precision densimetry to measure the partial molar volume, V°, expansibility, E°, and adiabatic compressibility, K°_S, of poly(L-alanine), poly(L-proline), and poly(L-threonine) as a function of temperature between 18 and 55°C. We use these parameters to derive the volumetric contributions of the alanine, proline, and threonine residues. We compare these contributions with additive estimates using the group contributions of the peptide group and side chains derived from low molecular weight model compounds. We discuss the differences between the experimental values and the additive estimates in terms of differential hydration of amino acid residues in the polypeptides and small molecules. Further, we have measured the changes in volume and adiabatic compressibilities accompanying the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) and the α-to-β transition of poly(L-lysine) at 25°C. These polypeptides may exist in their coil, α-helical, or β-sheet conformations depending on the solution pH and temperature.[25–27] Specifically, poly(L-glutamic acid) undergoes a coil-to-helix transition when the solution pH decreases from neutral to pH 4, while poly(L-lysine) undergoes the same transition upon an increase in pH from neutral to pH 11.[25–27] In addition, an irreversible α-to-β transition of poly(L-lysine) can be induced by incubation of the initially α-helical polypeptide at 51°C and pH 11.1 for at least 4 h.[26] We discuss our pH-dependent volumetric data in terms of the differential hydration properties of the lysine and glutamic acid residues in their coil, α-helix, and β-sheet conformations.

MATERIALS AND METHODS

Materials

All the polypeptides used were of the highest purity commercially available and used without further purification. Specifically, poly(L-alanine), poly(L-proline), poly(L-threonine), poly(L-lysine) · HCl, and sodium salt of poly(L-

glutamic acid) were purchased from Sigma-Aldrich Canada (Oakville, Ontario, Canada). These peptides were highly polymerized, with poly(L-alanine) consisting of 1000 to 5000 monomeric units, poly(L-proline) consisting of 1000 to 10,000 monomeric units, poly(L-threonine) consisting of 5000 to 15,000 monomeric units, poly(L-lysine) consisting of 15,000 to 30,000 monomeric units, and poly(L-glutamic acid) consisting of 5000 to 15,000 monomeric units. Solutions of HCl and NaOH were purchased from BDH Inc. (Toronto, Ontario, Canada) and Fisher Scientific Canada (Nepean, Ontario, Canada), respectively. The polypeptides were dissolved in doubly distilled water rather than buffers to avoid the need to correct for volume and compressibility changes due to the ionization-neutralization equilibria of the buffer.

The concentrations of the samples were determined by weighing, with a precision ± 0.02 mg, of ≈ 20 mg of each solute material, and then dissolving the material in a known amount of water. Prior to weighing, all polypeptides were dried for several days under vacuum in the presence of phosphorus pentoxide. Throughout the article, all concentrations and molecular weights, used for determining the volumetric properties and molar ellipticities of the polypeptides, are expressed per mole of monomeric unit. The molecular weights of poly(L-alanine), poly(L-proline), poly(L-threonine), poly(L-lysine) · HCl, and sodium salt of poly(L-glutamic acid) are 71.07, 97.12, 101.11, 164.70, and 151.09 Da, respectively (if expressed per monomeric unit).

Acoustic Measurements

Solution sound velocities and absorptions were measured using a differential technique and a previously described resonator method at a frequency of 7.2 MHz.[28–31] In this technique, two identical resonator cells (sample and reference) are placed in a common thermostated environment, and the difference in ultrasound velocities between the two cells is then measured. We employed ultrasonic resonator cells with lithium niobate piezotransducers and a minimum sample volume of 0.8 mL.[29] The accuracy of all the sound velocity relative measurements achieved with this design is approximately $\pm 10^{-4}$%, while the accuracy of the relative sound absorption measurements is ± 2%.[29,32,33] The analysis of the frequency characteristics of the resonator was performed by a Hewlett Packard model HP4195A network/spectrum analyzer (Mississauga, Ontario, Canada).

The key acoustic characteristics of a solute that can be derived directly from ultrasonic measurements are the relative molar sound velocity increment, $[U]$, and the molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$. The relative molar sound velocity increment, $[U]$, of a solute is equal to $(U - U_0)/(U_0C)$, 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. The molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$, is equal to $\Delta(\alpha\lambda)/C$, where α is the coefficient of sound absorption, λ is the sound wavelength, $\Delta(\alpha\lambda)$ is the difference in the ultrasonic absorption per wavelength between the solution and the solvent.

Ultrasonic measurements reported here for poly(L-alanine), poly(L-proline), and poly(L-threonine) were conducted at temperatures of 18, 25, 40, and 55°C at ≈pH 5.5. For the solutions of poly(L-lysine) and poly(L-glutamic acid), ultrasonic pH-titrations were carried out at a single temperature of 25°C. In addition, the solution of poly(L-lysine) at pH 11.1 was divided into two batches. One batch (in α-helical conformation) was kept at room temperature, while the second batch was incubated at 51°C for 5 h and then annealed to 25°C (to obtain the β-sheet conformation). The relative molar sound velocity increments, $[U]$, of poly(L-lysine) from the two batches were measured and compared at 25°C.

Acoustic titration experiments on poly(L-lysine) and poly(L-glutamic acid) were performed by adding equal aliquots of 0.1 or 1 *M* HCl [for titration of poly(L-glutamic acid)] or NaOH [for titration of poly(L-lysine)] solutions to both the sample and the reference cells filled with the same volume of 0.80 cm^3 of the solution and solvent, respectively. Additions were made using Hamilton syringes equipped with a Chaney adapter (Hamilton Co., Reno, NV). When calculating the relative molar sound velocity increment, $[U]$, and the molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$, we took into account the changes in sound velocity in the solvent, U_0, and in the molar concentration of the solute, C, that result from addition of acid or base.

Densimetric Measurements

All densities were measured with a precision of $\pm 1.5 \times 10^{-6}$ g/cm^3 using a vibrating tube densimeter (DMA-60/602; Anton Paar, Graz, Austria). The apparent molar volumes, ϕV, of polypeptides were then calculated from the following relationship[34]:

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

Table I Relative Molar Sound Velocity Increments, $[U]$ ($cm^3 mol^{-1}$), as a Function of Temperature, *T*, for the Polypeptides

Compounds	18°C	25°C	40°C	55°C
Poly-L-alanine	25.5 ± 0.4	22.3 ± 0.4	18.0 ± 0.4	14.6 ± 0.5
Poly-L-proline	32.9 ± 0.4	30.1 ± 0.4	24.4 ± 0.4	20.3 ± 0.5
Poly-L-threonine	25.3 ± 0.4	23.6 ± 0.4	19.7 ± 0.4	17.0 ± 0.5

Table II Partial Molar Volumes, V° (cm^3mol^{-1}), as a Function of Temperature, T, for the Polypeptides

Compounds	18°C	25°C	40°C	55°C
Poly-L-alanine	49.8 ± 0.4	50.3 ± 0.4	51.5 ± 0.4	52.1 ± 0.5
Poly-L-proline	71.9 ± 0.4	72.2 ± 0.4	73.1 ± 0.4	74.1 ± 0.5
Poly-L-threonine	71.0 ± 0.4	72.1 ± 0.4	73.3 ± 0.4	74.8 ± 0.5

where M is the molecular weight of the solute, ρ and ρ_0 are the densities of the solution and the solvent, respectively, and m is the molar concentration of the solute.

Analogous to ultrasonic measurements, all densimetric measurements reported here for poly(L-alanine), poly(L-proline), and poly(L-threonine) were conducted at temperatures of 18, 25, 40, and 55°C and ≈pH 5.5, while densimetric pH-titrations were carried out in the solutions of poly(L-lysine) and poly(L-glutamic acid) at 25°C. The apparent molar volumes, ϕV, of poly(L-lysine) at pH 11.1 from the two batches (the batch left at room temperature and the one preheated to 51°C) were measured and compared at 25°C.

The volume changes, ΔV, accompanying pH-titrations of poly(L-lysine) and poly(L-glutamic acid) were calculated using the following relationship:

$$\Delta V = [(\rho - \rho_0) - (\rho_{solution} - \rho_{solvent})(1 + V'/V_0)]/(\rho_0 C_0) \quad (2)$$

where V_0 is the initial volume of the polypeptide solution with an initial concentration of C_0 and/or the pure water in which the same volume of the HCl or NaOH solution, V', is added; ρ and ρ_0 are the densities of the initial polypeptide solution and water, respectively; $\rho_{solution}$ and $\rho_{solvent}$ are, respectively, the densities of the polypeptide solution and the solvent to which the same volume of the HCl or NaOH solution has been added.

Determination of the Partial Molar Adiabatic Compressibility

The relative molar sound velocity increments, $[U]$, were used in conjunction with the apparent molar volumes, ϕV, to calculate the apparent molar adiabatic compressibility, ϕK_S, of the polypeptides using the relationship[35,36]

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

where β_{S0} is the coefficient of adiabatic compressibility of the solvent.

Differentiating Eq. (3) yields the expression

$$\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U]) \quad (4)$$

where ΔV and $\Delta[U]$ are, respectively, the changes in the volume and in the relative molar sound velocity increment of poly(L-lysine) or poly(L-glutamic acid) upon their pH-titrations. This relationship allows one to calculate the adiabatic compressibility change, ΔK_S, accompanying pH-induced changes of poly(L-lysine) or poly(L-glutamic acid).

The pH of the poly(L-lysine) and poly(L-glutamic acid) solutions was measured separately using the same amounts and concentrations of solutions and titrant as for the ultrasonic measurements. The absolute error of the pH measurements was ±0.01 pH units. For each evaluation of $[U]$, ϕV, ϕK_S, ΔV, and ΔK_S, three to five independent measurements were carried out within the concentration range 1 to 2 mg/mL for each of the solutes studied.

CD Spectroscopic Measurements

CD spectra were recorded at 25°C in the far UV range using an Aviv model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ). The CD spectroscopic measurements were carried out in a 1 mm path-length cuvette. For all CD spectroscopic measurements, the polypeptides' concentrations were on the order of 0.3 mg/mL.

RESULTS

Tables I, II, and III show the relative molar increments of sound velocity, $[U]$, apparent molar volumes, ϕV, and apparent molar adiabatic compressibilities, ϕK_S, of poly(L-alanine), poly(L-proline), and

Table III Partial Molar Adiabatic Compressibilities, K°_S (10^{-4} $cm^3mol^{-1}bar^{-1}$), as a Function of Temperature, T, for the Polypeptides

Compounds	18°C	25°C	40°C	55°C
Poly-L-alanine	−10.3 ± 0.7	−7.0 ± 0.7	−1.9 ± 0.7	1.0 ± 0.8
Poly-L-proline	−8.9 ± 0.7	−6.2 ± 0.7	0.6 ± 0.7	4.2 ± 0.8
Poly-L-threonine	−3.7 ± 0.7	−1.3 ± 0.7	2.9 ± 0.7	4.9 ± 0.8

Table IV Partial Molar Expansibilities, E° ($cm^3mol^{-1}K^{-1}$), as a Function of Temperature, T, for the Polypeptides

Compounds	18°C	25°C	40°C	55°C
Poly-L-alanine	0.10 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.04 ± 0.02
Poly-L-proline	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
Poly-L-threonine	0.12 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.08 ± 0.02

poly(L-threonine) at 18, 25, 40, and 55°C, respectively. Errors were estimated by taking into account uncertainties due to the concentration determination, temperature drifts, and apparatus limitations. The concentration dependencies of the apparent molar volumes and adiabatic compressibilities of proteins, peptides, and amino acids are negligible in the range of specific concentrations used in the present work.[37–47] By extension, we assume that the concentration dependencies of $[U]$, ϕV, and ϕK_S of polypeptides are also negligible. Therefore, below, we do not discriminate between the apparent and molar volumetric characteristics of the polypeptides.

We have approximated the temperature dependencies of the partial molar volumes, V°, of poly(L-alanine), poly(L-proline), and poly(L-threonine) by second order polynomial functions. A correlation coefficient of each approximation was higher than 0.99. The temperature derivatives of V° [equal to the partial molar expansibility, because $E^\circ = (\partial V^\circ/\partial T)_P$] then were determined at 18, 25, 40, and 55°C by analytical differentiation of the approximating functions. Table IV presents the resulting data for the partial molar expansibility, E°, of poly(L-alanine), poly(L-proline), and poly(L-threonine).

To independently verify that, at the experimental conditions investigated, poly(L-lysine) and poly(L-glutamic acid) undergo pH- and/or temperature-induced coil-to-helix and α-to-β conformational transitions, we have measured the CD spectra of the two polypeptides as a function of pH. Figure 1 presents the CD spectra of poly(L-lysine) at pH 5.6 (●) and pH 11.9 (○) and poly(L-glutamic acid) at pH 7.7 (■) and pH 3.1 (□) at 25°C. In addition, Figure 1 shows the CD spectrum of poly(L-lysine) that has been preincubated for 5 h at pH 11.1 and 51°C (♦). Inspection of Figure 1 reveals that, at neutral pH, poly(L-lysine) and poly(L-glutamic acid) exhibit CD spectra typical for the random-coil conformation. By contrast, poly(L-lysine) at pH 11.9 and poly(L-glutamic acid) at pH 3.1 are in the α-helical conformation as can be judged by the two characteristic minima at 208 and 222 nm. Poly(L-lysine) that has been preincubated at pH 11.1 and 51°C exhibits a CD spectrum characteristic of β-sheet conformation. Figure 2(A) and (B) show the CD profiles of the pH-induced coil-to-helix transitions of poly(L-lysine) (A) and poly(L-glutamic acid) (B) measured at 222 nm. Inspection of Figure 2 reveals that poly(L-lysine) and poly(L-glutamic acid) undergo cooperative (sigmoidal) pH-induced coil-to-helix transitions with midpoints at pH 9.8 and 5.5, respectively. These values coincide with literature data.[25–27]

Figures 3 and 4 depict the pH-dependencies of the relative molar sound velocity increment, $[U]$, and partial molar volume, V°, of poly(L-lysine) at 25°C. Inspection of Figures 3 and 4 reveals that a change in the solution pH from ≈5 to ≈12 (before and after the coil-to-helix transition) causes a decrease in $[U]$ of poly(L-lysine) of 46.2 ± 0.8 cm^3mol^{-1} and an increase in V° of 25.6 ± 0.8 cm^3mol^{-1}. Using Eq. (4), we calculate a change in adiabatic compressibility, ΔK°_S, accompanying alkalinization of the poly(L-lysine) solution from ≈pH 5 to ≈pH 12 of (64.3 ± 1.4) × 10^{-4} $cm^3mol^{-1}bar^{-1}$. Our value of ΔV is in good agreement with 22.8 cm^3mol^{-1}, the volume change reported for the base-titration of poly(L-lysine) in 0.2 *M* NaBr by Noguchi.[48]

Figures 5 and 6 depict the pH-dependencies of the relative molar sound velocity increment, $[U]$, and

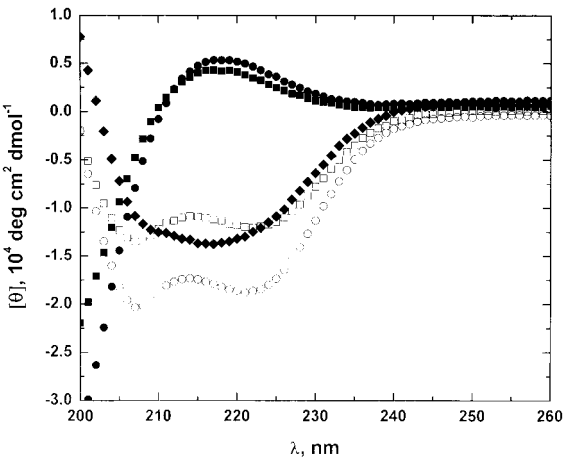
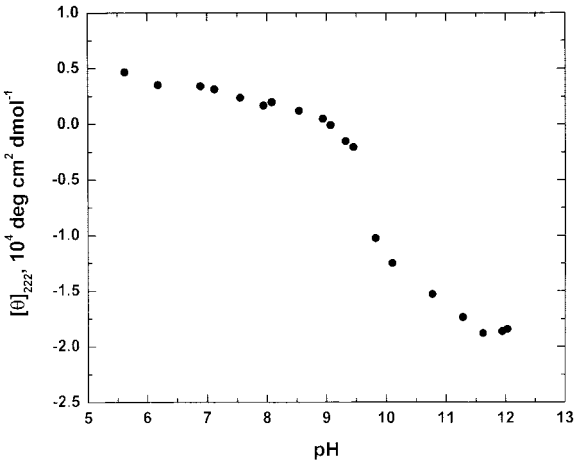
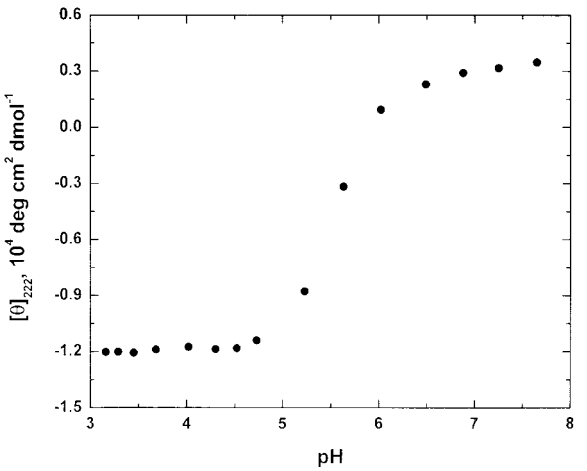


FIGURE 1 The far UV CD spectra of the polypeptides at 25°C: poly(L-lysine) at pH 5.6 (●); poly(L-lysine) at pH 11.9 (○); poly(L-lysine) at pH 11.1 that had been preincubated for 5 h at 51°C (♦); poly(L-glutamic acid) at pH 7.7 (■); and poly(L-glutamic acid) at pH 3.1 (□).



(A)



(B)

FIGURE 2 CD profiles of the pH-induced coil-to-helix transitions of poly(L-lysine) (A) and poly(L-glutamic acid) (B) measured at 222 nm.

partial molar volume, $V°$, of poly(L-glutamic acid) at 25°C. Inspection of Figures 5 and 6 reveals that acidification of the polypeptide solution from ≈pH 8.5 to ≈pH 3 (before and after the coil-to-helix transition) causes a decrease in $[U]$ of 12.0 ± 0.8 cm^{3}mol^{-1} and an increase in $V°$ of 10.2 ± 0.8 cm^{3}mol^{-1}. From Eq. (4) we calculate a change in adiabatic compressibility, $\Delta K°_S$, accompanying acidification of the poly(L-glutamic acid) solution from ≈pH 8.5 to ≈pH 3 of (19.9 ± 1.4) × 10^{-4} cm^{3}mol^{-1}bar^{-1}. Our values of $\Delta[U]$, ΔV, and ΔK_S are in qualitative agreement with −14.5 cm^{3}mol^{-1}, 11.4 cm^{3}mol^{-1}, and 23.2 × 10^{-4} cm^{3}mol^{-1}bar^{-1}, the values of $\Delta[U]$, ΔV, and ΔK_S that can be calculated for the acid-titration of poly(L-glutamic acid) in 0.01 *M* NaCl at 25°C from the pH-dependent density and sound velocity data reported by Noguchi and Yang.[49,50]

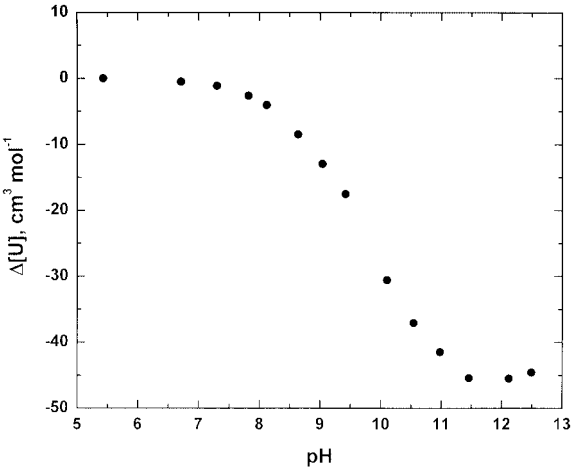


FIGURE 3 The pH-dependence of the relative molar sound velocity increment, $[U]$, of poly(L-lysine) at 25°C.

It should be noted that the pH-induced changes in $[U]$ presented in Figures 3 and 5 can be rationalized if one takes into account two effects: structural and hydration changes of the polypeptides resulting from neutralization of their amino or carboxyl groups and the coil-to-helix transitions; and the relaxation effect caused by periodical shifts in equilibrium of proton-transfer reactions due to changes in temperature and pressure in the field of ultrasonic waves.[10,51–53] In this article, we have considered only the structural and hydration contribution by subtracting the relaxation contribution from the measured values of $[U]$. The relaxation contributions have been determined from our measured data on ultrasonic absorption, $[\alpha\lambda]$ (not shown), according to a standard procedure described previously.[52]

Table V presents the differential values of $\Delta[U]$, ΔV, and ΔK_S of poly(L-lysine) at pH 11.1 and 25°C in

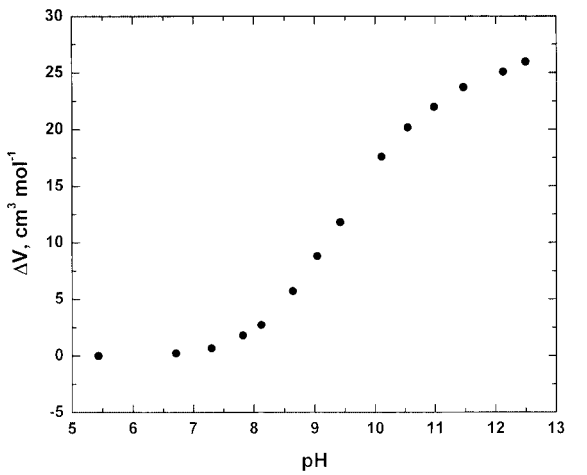


FIGURE 4 The pH-dependence of the partial molar volume, $V°$, of poly(L-lysine) at 25°C.

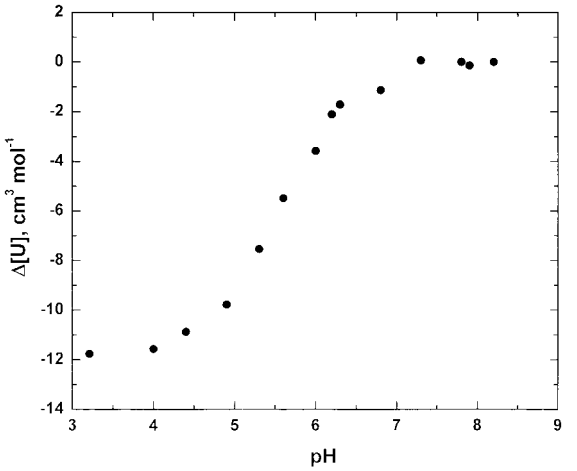


FIGURE 5 The pH-dependence of the relative molar sound velocity increment, $[U]$, of poly(L-glutamic acid) at 25°C.

its β-sheet (preincubated at 51°C) and α-helical conformations. Inspection of data in Table V reveals that the values of $[U]$, $V°$, and $K°_S$ exhibited by poly(L-lysine) in its α-helical and β-sheet conformations are distinct. Specifically, the relative molar sound velocity increment, $[U]$, of the β-sheet conformation is smaller than that of the α-helical conformation ($\Delta[U] = -3.9 \pm 0.8$ cm^3mol^{-1}), while the partial molar volume, $V°$, and adiabatic compressibility, $K°_S$, of the β-sheet conformation are larger than that of the α-helical conformation [$\Delta V = 2.6 \pm 0.8$ cm^3mol^{-1} and $\Delta K_S = (5.8 \pm 1.4) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$]. These values are in good agreement with -1.7 cm^3mol^{-1}, 4.2 cm^3mol^{-1}, and 5.3×10^{-4} $cm^3mol^{-1}bar^{-1}$, the values of $\Delta[U]$, ΔV, and ΔK_S, respectively, for the α-to-β transition of poly(L-lysine) in 0.2 *M* NaBr at 26°C that can be calculated from the density and sound velocity data reported by Noguchi and Yang.[49]

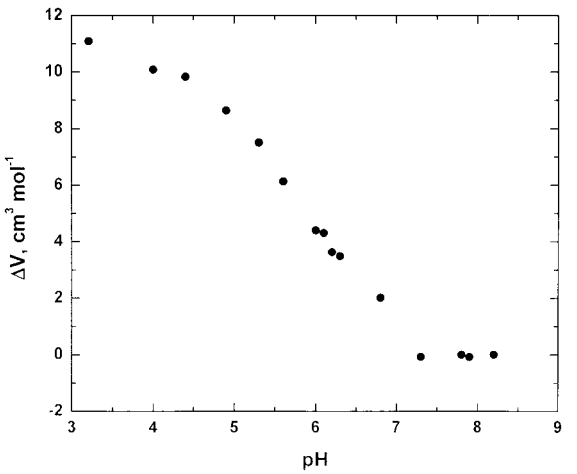


FIGURE 6 The pH-dependence of the partial molar volume, $V°$, of poly(L-glutamic acid) at 25°C.

Table V Changes in Relative Molar Sound Velocity Increment, $\Delta[U]$, Volume, ΔV, and Adiabatic Compressibility, ΔK_S, Accompanying the α-to-β Transition of Poly(L-Lysine) at 25°C

$\Delta[U]$ cm^3mol^{-1}	ΔV cm^3mol^{-1}	ΔK_S 10^{-4} $cm^3mol^{-1}bar^{-1}$
-3.9 ± 1.0	2.6 ± 0.5	5.8 ± 1.4

DISCUSSION

Poly(L-Alanine), Poly(L-Proline), and Poly(L-Threonine)

There is an ongoing discussion about whether or not the thermodynamic properties of unfolded polypeptide chains can be modeled by additive calculations using group contributions of constituent atomic groups. In a good solvent, unstructured polypeptides adopt a random-coil-like conformation with the majority of its amino acid residues being solvent-exposed.[54] It is plausible to expect that, in the random coil-like conformation, the thermodynamic properties of a polypeptide chain, including its partial molar volume, expansibility, and adiabatic compressibility, are equal to the sum of the group contributions of constituent amino acids. The amino acid group contributions can be obtained from volumetric studies on strategically selected sets of short oligopeptides.

Based on the foregoing discussion, the volume and compressibility contributions of the alanine, proline, and threonine residues can be presented as the sums of the group contributions of the glycyl unit (—$COCH_2NH$—) and the alanine (—CH_3), proline (—$CH_2CH_2CH_2$—), and threonine (—$CHCH_3OH$) side chains. The volume, expansibility, and compressibility contributions of the glycyl unit have been determined based on volumetric studies of oligoglycines by several authors.[55–57] Based on our previous results, at 25°C, the volume, expansibility, and compressibility contributions of the glycyl unit are equal to 37.4 $\pm$ 0.5 cm^3mol^{-1}, 0.12 $\pm$ 0.03 $cm^3mol^{-1}K^{-1}$, and $-(1.1 \pm 0.9) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively.[55]

Poly(L-Alanine). The "additive" values of $V°$, $E°$, and $K°_S$ of the alanine residue can be derived, for example, by adding to the values of $V°$, $E°$, and $K°_S$ of the

glycyl unit the relevant differential values (ΔV, ΔE, and ΔK_S) of the GlyAlaGly and GlyGlyGly tripeptides. At 25°C, the values of ΔV and ΔE are equal to 18.3 cm^3mol^{-1} and 0.008 $cm^3mol^{-1}K^{-1}$, respectively.[58] The value of ΔK_S is 3.7×10^{-4} $cm^3mol^{-1}bar^{-1}$.[59] Hence, we calculate the volume, expansibility, and compressibility contributions of the alanine residue at 25°C to be 55.7 cm^3mol^{-1} (= 37.4 + 18.3), 0.13 $cm^3mol^{-1}K^{-1}$ (= 0.12 + 0.008), and 2.6×10^{-4} $cm^3mol^{-1}bar^{-1}$ ($= -1.1 \times 10^{-4} + 3.7 \times 10^{-4}$), respectively. Comparison of these estimates with our experimental data on $V°$, $E°$, and $K°_S$ of poly(L-alanine) (presented in Tables II, III, and IV) reveals the following discrepancies between the measured and additively calculated quantities: the experimentally measured value of $V°$ is 5.4 cm^3mol^{-1} smaller than the estimate; the experimentally measured value of $E°$ is 0.05 $cm^3mol^{-1}K^{-1}$ smaller than the estimate; and the experimentally measured value of $K°_S$ is 9.6×10^{-4} $cm^3mol^{-1}bar^{-1}$ smaller than the estimate. The observed disparities suggest that poly(L-alanine) is not in the fully solvent-exposed, random coil-like conformation.

To rationalize the fact that the measured value of $V°$ of poly(L-alanine) is smaller than the additive estimate, we propose that, due to partial burial of side chains and/or the peptide backbone, the thermal contribution to the partial molar volume of the polypeptide decreases [see Eq. (6) below].[11,16,60] In turn, this decrease may result in a net reduction in $V°$. Further, the smaller value of experimental $E°$ relative to the estimate also suggests that poly(L-alanine) is not fully solvent-exposed, because water solvating protein groups exhibit a partial molar expansibility that is ≈40% higher than that of bulk water.[11]

Finally, the observation that the measured value of $K°_S$ is significantly smaller (more negative) than the additive estimate is consistent with poly(L-alanine) adopting a conformation (or ensemble of conformations) in which the peptide groups are brought together and exert their influence on solvating water molecules as "closely located" rather than "isolated" polar groups. Recall that closely located (clustered) polar groups strongly influence solvating water molecules and exhibit highly negative compressibility contributions, while the contributions of isolated polar groups may be close to zero or even positive.[8,10,11,19,61,62]

Poly(L-Proline). The "additive" values of $V°$, $E°$, and $K°_S$ of the proline residue can be calculated by adding to the values of $V°$, $E°$, and $K°_S$ of the glycyl unit the corresponding volumetric differences ΔV, ΔE, and ΔK_S between the GlyProGly and GlyGlyGly tripeptides. At 25°C, the values of ΔV and ΔE are equal to 33.1 cm^3mol^{-1} and 0.029 $cm^3mol^{-1}K^{-1}$, respectively.[58] Unfortunately, the partial molar adiabatic compressibility, $K°_S$, of GlyProGly has not been reported.

We calculate the volume and expansibility contributions of the proline residue at 25°C to be 70.5 cm^3mol^{-1} (= 37.4 + 33.1) and 0.15 $cm^3mol^{-1}K^{-1}$ (= 0.12 + 0.029), respectively. The calculated volume contribution is 1.7 cm^3mol^{-1} smaller than the measured value which, at 25°C, equals 72.2 ± 0.4 cm^3mol^{-1} (see Table II). By contrast, the calculated value of $E°$ is 0.10 $cm^3mol^{-1}K^{-1}$ larger than 0.05 ± 0.02 $cm^3mol^{-1}K^{-1}$, the partial molar expansibility of poly(L-proline) at 25°C (see Table IV). It is difficult to unequivocally rationalize these observations. As a working hypothesis, one may propose that poly(L-proline) adopts a conformation in which some atomic groups are buried inside a fluctuating solvent-inaccessible core of the polypeptide. This proposition is qualitatively consistent with the observed disparities between the calculated and measured values of $V°$ and $E°$. Because of reduced hydration, the partial molar expansibility, $E°$, of the polypeptide should be smaller than that expected for the fully extended conformation. On the other hand, a loosely packed solvent-inaccessible interior of the polypeptide may cause an increase in its partial molar volume. In this scenario, the volume of the void space inside the solvent-inaccessible interior of the polypeptide prevails over any reduction in the thermal volume associated with a decrease in solvent-accessible surface area. The highly negative partial molar adiabatic compressibility [at 25°C, $K°_S = -(6.2 \pm 0.7) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$] may suggest that the peptide groups of poly(L-proline) are brought together and exhibit the hydration properties of "closely located" polar groups.

The putative nonrandom coil conformation of poly(L-proline) is consistent with the results of recent molecular dynamics simulations and vibrational CD spectroscopic measurements that suggest that a significant local ordering exists in the polypeptide.[63,64] However, additional studies are required to prove or refute the proposed conformation of poly(L-proline).

Poly(L-Threonine). The "additive" values of $V°$, $E°$, and $K°_S$ for the threonine residue can be estimated by adding to the values of $V°$, $E°$, and $K°_S$ of the glycyl unit the relevant differential values of ΔV, ΔE, and ΔK_S of the tripeptides GlyThrGly and GlyGlyGly. At 25°C, the values of ΔV, ΔE, and ΔK_S equal 34.1 cm^3mol^{-1}, 0.020 $cm^3mol^{-1}K^{-1}$, and -1.0×10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively.[58,59] Thus, the volume, expansibility, and compressibility contributions of the threonine residue at 25°C should be equal to 71.5 cm^3mol^{-1} (= 37.4 + 34.1), 0.14 $cm^3mol^{-1}K^{-1}$

(= 0.12 + 0.02), and -2.1×10^{-4} $cm^3mol^{-1}bar^{-1}$ ($= -1.1 \times 10^{-4} - 1.0 \times 10^{-4}$), respectively. Comparison of these estimates with our measured values of $V°$, $E°$, and $K°_S$ of poly(L-threonine) (presented in Tables II, III, and IV) reveals that the two sets of data are in very good agreement. At 25°C, the experimental values of $V°$, $E°$, and $K°_S$ of poly(L-threonine) are 72.1 ± 0.4 cm^3mol^{-1}, 0.11 ± 0.02 $cm^3mol^{-1}K^{-1}$, and $-(1.3 \pm 0.7) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively. Based on the agreement between the measured and additively calculated volumetric properties, we propose that, at our experimental conditions, poly(L-threonine) is in its fully unfolded, random-coil-like conformation with the majority of its atomic groups being solvent exposed.

pH-Induced Structural Transitions of Poly(L-Lysine) and Poly(L-Glutamic Acid)

Coil-to-Helix Transition of Poly(L-Lysine). Recall that changes in volume, ΔV, and adiabatic compressibility, ΔK_S, upon alkalinization of the poly(L-lysine) solution from ≈pH 5 to ≈pH 12 are equal to 25.6 ± 0.8 cm^3mol^{-1} and (64.3 ± 1.4) $\times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively. The pH-induced changes in $V°$ and $K°_S$ of poly(L-lysine) can be presented as consisting of the neutralization and structural terms:

$$\Delta V = \Delta V_{neut} + \Delta V_{str} \tag{5a}$$

$$\Delta K_S = \Delta K_{Sneut} + \Delta K_{Sstr} \tag{5b}$$

The neutralization terms, ΔV_{neut} and ΔK_{Sneut}, represent changes in volume and compressibility accompanying an alteration in hydration of ionizable amino groups upon pH-induced changes in their ionization state (neutralization). The structural terms, ΔV_{neut} and ΔK_{Sneut}, represent the differences in volume and compressibility between the helix and coil states of the polypeptide at the same pH. The values of ΔV_{neut} and ΔK_{Sneut} related to base-induced neutralization of the amino group of the lysine side chain [—$(CH_2)_4NH_3^+$] can be estimated based on the data on low molecular weight model compounds. However, such an estimate is not straightforward because, depending on the model compound used, the changes in volume and compressibility accompanying neutralization of an independently hydrated amino group may be different. For example, the values of ΔV_{neut} and ΔK_{Sneut} for neutralization of the amino terminus of triglycine [NH_3^+—$CH_2(CONHCH_2)_2$—COO^-] equal 26.5 ± 0.5 cm^3mol^{-1} and (67.5 ± 0.9) $\times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively, while the same values for neutralization of the amino terminus in long α,ω-aminocarboxylic acids [NH_3^+—$(CH_2)_n$—COO^-] are 23 ± 1 cm^3mol^{-1} and (54 ± 2) $\times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively.[65,66] The observed disparity may reflect the differential interactions of nonpolar alkyl (in α,ω-aminocarboxylic acids) and polar peptide (in triglycine) groups with the adjacent amino terminus, as well as the differential response of these interactions to neutralization of the amino terminus.[66]

Given the chemical similarity, it is plausible to propose that triglycine represents a better model for neutralization of the amino group of the lysine side chain than α,ω-aminocarboxylic acids. Thus, we assume that the values of ΔV_{neut} and ΔK_{Sneut} in Eqs. (5a) and (5b) are equal to (26.5 ± 0.5) cm^3mol^{-1} and (67.5 ± 0.9) $\times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively.[66] Structural changes in volume, ΔV_{str}, and compressibility, ΔK_{Sstr}, accompanying the coil-to-helix transition of poly(L-lysine) can be calculated from Eqs. (5a) and (5b) to be -0.9 ± 1.3 cm^3mol^{-1} (25.6–26.5) and $-(3.2 \pm 2.3) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$ ($64.3 \times 10^{-4} - 67.5 \times 10^{-4}$), respectively. These estimates show that the helix-to-coil transition of poly(L-lysine) results in rather small (near zero) changes in volume and compressibility. It should be noted that Noguchi[48] also arrived at the same conclusion: the volume change associated with the helix-to-coil transition of poly(L-lysine) was found to be small, ranging from 1.0 to 1.5 cm^3mol^{-1} depending on the solution ionic strength.

α-to-β Transition of Poly(L-Lysine). The α-to-β transition of poly(L-lysine) results in increases in volume, ΔV, and compressibility, ΔK_S, of 2.6 ± 0.8 cm^3mol^{-1} and (5.8 ± 1.4) $\times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively (see Table V). These values are small but statistically significant. The β-sheet conformation of the polypeptide exhibits slightly larger values of the partial molar volume and adiabatic compressibility than the α-helical conformation.

Coil-to-Helix Transition of Poly(L-Glutamic Acid). Recall that changes in volume, ΔV, and adiabatic compressibility, ΔK_S, upon acidification of the poly(L-glutamic acid) solution from ≈pH 8.5 to ≈pH 3 are equal to 10.2 ± 0.8 cm^3mol^{-1} and (19.9 ± 1.4) $\times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively. The neutralization terms ΔV_{neut} and ΔK_{Sneut} in Eqs. (5a) and (5b) related to acid-induced neutralization of the carboxyl group of the glutamic acid side chain (—$CH_2CH_2COO^-$) can be estimated based on small molecule data. However, this is even more ambiguous than modeling neutralization of the amino group of lysine residue. It has

been known for some time that changes in volume and compressibility accompanying neutralization of an independently hydrated carboxyl group increase with increasing the number of aliphatic groups in the solute molecule.[66] For example, the values of ΔV_{neut} and ΔK_{Sneut} for neutralization of the carboxyl terminus of glycine (with a single -CH_2- group in the molecule) are 6 ± 1 cm^3mol^{-1} and (7 ± 2) × 10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively, while increasing to 14.5 ± 1 cm^3mol^{-1} and (34.5 ± 2) × 10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively, for 8-aminooctanoic acid (with seven -CH_2- groups).[66] Similarly, ΔV_{neut} and ΔK_{Sneut} for neutralization of the carboxyl terminus of triglycine are 10.5 ± 0.5 cm^3mol^{-1} and (18.4 ± 0.9) × 10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively, while increasing to 13.5 ± 0.5 cm^3mol^{-1} and (25.1 ± 0.9) × 10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively, for GlyGlyIle.[65] To rationalize this experimental reality, we have recently proposed that neutralized carboxyl groups are capable of forming aggregates stabilized by hydrogen bonds between the carbonyl oxygen of one solute molecule and the hydroxyl group of another molecule.[66] Formation of such hydrogen-bonded structures causes an additional decrease in solute hydration with concomitant increases in volume and compressibility. Significantly, solutes with bulky aliphatic moieties may form even larger aggregates in which the sizeable solvent-inaccessible cores are stabilized, in addition to intermolecular hydrogen bonds, by hydrophobic interactions. Formation of such aggregates facilitates further increases in volume and compressibility.

In this work, we modeled neutralization of the glutamic acid side chain by the carboxyl terminus of triglycine. Triglycine is an appropriate model. Firstly, it does not have a sizeable aliphatic moiety. And secondly, its carboxyl terminus is independently hydrated.[55] Hence, the values of ΔV_{neut} and ΔK_{Sneut} in Eqs. (5a) and (5b) are assumed to be equal to (10.5 ± 0.5) cm^3mol^{-1} and (18.4 ± 0.9) × 10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively.[65] Structural changes in volume, ΔV_{str}, and compressibility, ΔK_{Sstr}, accompanying coil-to-helix transition of poly(L-glutamic acid) can be calculated from Eqs. (5a) and (5b) to be equal to −0.3 ± 1.3 cm^3mol^{-1} (10.2–10.5) and (1.5 ± 2.3) × 10^{-4} $cm^3mol^{-1}bar^{-1}$ (19.9 × 10^{-4} – 18.4 × 10^{-4}), respectively. Thus, analogous to poly(L-lysine), the coil-to-helix transition of poly(L-glutamic acid) brings about near zero changes in volume and compressibility. This conclusion coincides with the results of Noguchi and Yang,[50] who concluded that the volume change associated with the coil-to-helix transition of poly(L-glutamic acid) is on the order of 1.0 cm^3mol^{-1}.

Hydration Changes Accompanying the Conformational Transitions of Poly(L-Lysine) and Poly(L-Glutamic Acid). Our previous volumetric data suggest that ordered structures, such as native globular proteins and double stranded nucleic acids with repetitive nucleotide sequences, influence solvating water molecules beyond the first coordination layer.[10,11,19,67] In other words, the hydration shell of an ordered macromolecular structure consists of at least two hydration layers, while the hydration shell of a small molecule and an unordered peptide comprises only a single layer of water molecules.[10,11,19,67] It is of interest to examine if the formation of α-helical or β-sheet structures leads to an enhancement of polypeptide hydration.

The partial molar volume of a solute, $V°$, can be interpreted in terms of hydration based on the following expression[11,60,68,69]:

$$V° = V_M + V_T + V_I + \beta_{T0}RT \qquad (6)$$

where V_M is the intrinsic volume of a solute; V_T is the thermal volume that represents the volume of the void space around the solute molecule resulting from thermally induced mutual vibrations of the solute and solvent molecules; V_I is the interaction volume that represents solvent contraction due to hydrogen bonding or electrostriction; β_{T0} is the coefficient of isothermal compressibility of the solvent; R is the universal gas constant; and T is the absolute temperature. The interaction volume, V_I, correlates to the number of solvent-exposed polar and charged groups, while the thermal volume, V_T, correlates to the solvent-accessible surface area of a solute.[11,60]

The partial molar adiabatic compressibility, $K°_S$, of a solute can be interpreted in terms of hydration based on the following expression[7–11]:

$$K°_S = K_M + \Delta K_h \qquad (7)$$

where K_M is the intrinsic compressibility of a solute; and ΔK_h represents the hydration-induced change in solvent compressibility.

Based on Eqs. (6) and (7), changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying the coil-to-helix and α-to-β transitions of poly(L-lysine) and poly(L-glutamic acid) can be presented as follows:

$$\Delta V = \Delta V_M + \Delta V_T + \Delta V_I \qquad (8)$$

$$\Delta K_S = \Delta K_M + \Delta\Delta K_h \qquad (9)$$

The fact that the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) are accompanied by insignificant changes in volume, ΔV, and compress-

ibility, ΔK_S, may suggest that these transitions cause only slight alterations of polypeptide hydration or fortuitous compensation between different contributions to ΔV and ΔK_S. A coil-to-helix transition causes formation of two interpeptide hydrogen bonds [between the *n*th and *(n + 4)*-th amino acid residues] with concomitant disruption of two solute-solvent hydrogen bonds per amino acid residue. This should not bring about any appreciable changes in the intrinsic volume, V_M, and compressibility, K_M. However, formation of a solute-solvent hydrogen bond is accompanied by an average decrease in the solvent volume of 2.2 cm^3mol^{-1}.[60] Consequently, the coil-to-helix transition of a polypeptide should result in a change in the interaction term in Eq. (8), ΔV_I, of about 4.4 cm^3mol^{-1} (2×2.2). Apparently, this increase in V_I is offset by a decrease in the thermal volume, V_T, which should accompany burial of previously solvent-accessible surface upon helix formation.

The compressibility effect of disruption of solute-solvent hydrogen bonds between water molecules and peptide groups should not be large, because the entire compressibility contribution of a peptide group at 25°C is only $(0.5 \pm 0.8) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$.[55] This notion is consistent with the near zero value of ΔK_S for the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid). Hence, our volume and compressibility data suggest that the coil-to-helix transitions of the two polypeptides do not result in any significant enhancement of solute hydration.

The α-to-β transition of poly(L-lysine) should not lead to additional disruption of solute-solvent hydrogen bonds. Notwithstanding, this transition is accompanied by slight but statistically significant increases in volume and compressibility that are equal to 2.5 ± 0.8 cm^3mol^{-1} and $(5.8 \pm 1.4) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively. This observation may suggest that the β-sheet conformation of poly(L-lysine) is slightly less hydrated than its α-helical conformation, which may be merely a consequence of burying some additional surface in the β-sheet relative to the α-helical conformation. Clearly, further investigations are required for confirming or refuting this notion or testing its generality for other peptides with different amino acid sequences.

CONCLUDING REMARKS

We have determined the partial molar volumes, expansibilities, and adiabatic compressibilities for poly(L-alanine), poly(L-proline), and poly(L-threonine) are within the temperature range of 18–55°C. We have compared the measured volumetric properties of the polypeptides with results of additive calculations performed under the assumption of independent hydration and full solvent exposure of constituent atomic groups. Based on this comparison, poly(L-alanine) and poly(L-proline) are not fully unfolded and, probably, retain some solvent-inaccessible core. By contrast, poly(L-threonine) is fully unfolded with the majority of its atomic groups being solvent-exposed.

In addition, we have determined at 25°C changes in volume, ΔV, and adiabatic compressibility, ΔK_S, associated with the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid) and the α-to-β transition of poly(L-lysine). We have measured near zero changes in volume and compressibility accompanying the coil-to-helix transitions of poly(L-lysine) and poly(L-glutamic acid). Based on this observation, we have proposed that, in the absence of fortuitous compensations, the coil-to-helix transitions of the polypeptides do not result in any significant enhancement of hydration. By contrast, the α-to-β transition of poly(L-lysine) causes slight but statistically significant increases in volume and compressibility equal to 2.36 ± 0.8 cm^3mol^{-1} and $(5.8 \pm 1.4) \times 10^{-4}$ $cm^3mol^{-1}bar^{-1}$, respectively. This observation is consistent with the β-sheet conformation of poly(L-lysine) being slightly less hydrated than its α-helical conformation. The differential hydration of the α-helix and β-sheet conformations of poly(L-lysine) may reflect burial of some additional surface in the β-sheet relative to α-helical conformation.

In the aggregate, our results provide a quantitative volumetric description of the hydration properties of the polypeptides. Independent of the veracity of the proposed interpretations, the experimental data presented in this work represent an objective empirical basis for volumetric characterizations of denatured protein states. Such characterizations should prove useful in developing an understanding of the role that the solvent plays in the stabilization/destabilization of folded protein structures.

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