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Partial molar volumes and adiabatic compressibilities of unfolded protein states

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Abstract

We determined the partial molar volumes, $V°$, and adiabatic compressibilities, $K°_S$, of *N*-acetyl amino acids with neutralized carboxyl termini, *N*-acetyl amino acid amides, and *N*-acetyl amino acid methylamides between 18 and 55 °C. The individual compounds in the three classes have been selected so as to collectively cover the 20 naturally occurring amino acid side chains. We interpret our experimental results in terms of the volumetric contributions and hydration properties of individual amino acid side chains and their constituent atomic groups. We also conducted pH-dependent densimetric and acoustic measurements to determine changes in volume and compressibility accompanying protonation of the aspartic acid, glutamic acid, histidine, lysine, and arginine side chains. We use our resulting data to develop an additive scheme for calculating the partial molar (specific) volume and adiabatic compressibility of fully extended polypeptide chains as a function of pH and temperature. We discuss the differences and similarities between our proposed scheme and the reported additive approaches. We compare our calculated volumetric characteristics of the fully extended conformations of apocytochrome *c* and apomyoglobin with the experimental values measured in water (for apocytochrome *c*) or acidic pH (for apomyoglobin). At these respective experimental conditions, the two proteins are unfolded. However, the comparison between the calculated and experimental volumetric characteristics suggests that neither apocytochrome *c* nor apomyoglobin are fully unfolded and retain a sizeable core of solvent-inaccessible groups.

Keywords: *N*-acetyl amino acid amides; Amino acid side chains; Proteins; Hydration; Volume; Compressibility

1. Introduction

Volume and compressibility are fundamental thermodynamic observables that have been proven sensitive to solute hydration [1–6]. Volumetric measurements have been applied to characterizing conformational states of proteins, including the native, compact intermediate, and fully and partially unfolded states, and, more recently, amyloidal fibrils [7–18]. In these studies, the fully extended conformation is a useful concept which refers to the extreme case of protein unfolding in which all atomic groups are exposed to solvent. The fully extended conformation is characterized by the lack of nonsteric interactions between the amino acid residues constituting the polypeptide chain. Therefore, to a good approximation, thermodynamic properties of a fully unfolded protein state can be evaluated from additive calculations based on the primary amino acid sequence and the group contributions of the 20 naturally occurring amino acid residues. The reliability of the additive approach critically depends on the choice of model compounds mimicking hydration and, ultimately, the volumetric properties of amino acid residues in polypeptides.

Survey of the literature reveals several additive schemes that have been proposed for calculating the partial molar volumes [19–21] and adiabatic compressibilities [22,23] of extended polypeptides. Iqbal and Verrall [22] and Kharakoz [24] have used in their models the group contributions of side chains derived from data on zwitterionic amino acids. In contrast, Makhatadze et al. [19] and Häckel et al. [20] have based their additive schemes on GlyXGly tripeptides. While these works represent an important step forward toward developing a more comprehensive understanding of the volumetric properties of

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unfolded polypeptide chains, close inspection reveals significant numerical discrepancies between the models. For example, the additive scheme proposed by Iqbal and Verrall [22] yields unrealistically negative partial compressibilities of their analyzed polypeptides. In general, zwitterionic amino acids make poor models for mimicking the hydration properties of amino acid residues in polypeptides. In zwitterionic amino acids, the side chain is not independently hydrated, since it interacts *via* overlapping solvation shells with the charged skeleton of the molecule. GlyXGly tripeptides represent a more attractive alternative. In these compounds, the amino acid side chains are flanked in both sides by peptide bonds, while their interactions with the charged but remotely located termini should be greatly reduced. However, given the bulk size of tripeptides, one cannot exclude the possibility that these molecules may have some preferred conformations (which would render the constituent atomic groups not fully solvent-exposed) depending on the size and chemical nature of the amino acid side chain. In fact, a combination of recent optical spectroscopic measurements has provided evidence for the ability of peptides (even as short as tripeptides) to form stable structures in aqueous solutions [25,26]. Such conformational preferences may have a modulating effect on hydration of the side chain in question.

The underlying hypothesis of the present study is that *N*-acetyl amino acid amides and *N*-acetyl amino acid methylamides are arguably the best (close to ideal) system for modeling the hydration properties of amino acid side chains in unfolded polypeptides. These amino acid derivatives are small which minimizes the possibility of their adoption of preferential conformations that may affect the side chain hydration. Another important feature of this class of molecules is that the side chains are flanked in both directions by peptide groups as in peptides. In addition, the termini are uncharged which reduces their interactions with the side chain. The volumetric data on *N*-acetyl amino acid amides reported to date are incomplete and mostly limited to a single temperature of 25 °C and neutral pH [27–32]. These deficiencies prevent one from using the existing data on *N*-acetyl amino acid amides in additive calculations of the volumetric properties of polypeptides, in particular, as a function of temperature and pH.

In this work, we employ high precision acoustic and densimetric techniques to evaluate, as a function of temperature, the partial molar volumes and adiabatic compressibilities of 14 *N*-acetyl amino acid amides ($CH_3CONH–CHR–CONH_2$), two *N*-acetyl amino acid methylamides ($CH_3CONH–CHR–CONHCH_3$), and eight *N*-acetyl amino acids ($CH_3CONH–CHR–COOH$). The volumetric properties of the latter group of compounds were measured at acidic pH (~pH 2) where the terminal carboxyl group can be considered uncharged. The chemical diversity of the compounds studied in this work is related to the fact that commercially available *N*-acetyl amino acid amides do not cover the entire range of naturally occurring side chains.

Furthermore, we carried out pH-dependent measurements in the solutions of the amino acid derivatives containing ionizable side chains to determine changes in volume and compressibility accompanying their neutralization. Our combined results enable us to determine the group contributions of the complete set of naturally existing amino acid side chains to volume and compressibility as a function of temperature and pH. We interpret these volumetric results in terms of the hydration properties of the amino acid side chains and describe an additive scheme for evaluating the partial molar volumes and adiabatic compressibilities of fully extended polypeptides. We use our results to calculate the temperature and pH-dependences of the partial specific volumes and adiabatic compressibilities of six globular proteins in their fully unfolded, extended conformation. By comparing our calculated volumetric parameters with the experimental data on apocytochrome *c* and apomyoglobin, we discuss the thermodynamic implications of our results for protein transitions and conformational equilibrium. In particular, our determined contributions of amino acid side chains to partial molar volume, $V°$, expansibility, $E° = (\partial V°/\partial T)_P$, and adiabatic compressibility, $K°_S$, can be used for developing more reliable quantitative approaches to interpretation of pressure perturbation calorimetric and high pressure data on temperature- and pressure-induced conformational transitions of proteins.

2. Materials and methods

2.1. Materials

All amino acid derivatives used in the studies reported here were of the highest purity commercially available and used without further purification. *N*-acetyl alanine amide, *N*-acetyl valine amide, *N*-acetyl leucine amide, *N*-acetyl isoleucine amide, *N*-acetyl proline amide, *N*-acetyl phenylalanine amide, *N*-acetyl tryptophan amide, *N*-acetyl methionine amide, *N*-acetyl glutamine amide, *N*-acetyl aspartic acid amide, *N*-acetyl glutamic acid amide, *N*-acetyl lysine amide hydrochloride, *N*-acetyl arginine amide acetate, *N*-acetyl glycine methylamide, and *N*-acetyl histidine methylamide were purchased from Bachem Bioscience, Inc (King of Prussia, PA, USA). *N*-acetyl glycine amide, *N*-acetyl tyrosine amide, *N*-acetyl glycine, *N*-acetyl alanine, *N*-acetyl phenylalanine, *N*-acetyl tryptophan, *N*-acetyl cysteine, *N*-acetyl serine, and *N*-acetyl threonine as well as sodium acetate were purchased from Sigma-Aldrich Canada, Ltd. (Oakville, Ontario, Canada). *N*-acetyl asparagine was obtained from Fluka (Buchs, Switzerland). All amino acid derivatives except *N*-acetyl serine and *N*-acetyl threonine were in L-stereoisomeric form. *N*-acetyl serine and *N*-acetyl threonine were a mixture of D- and L-stereoisomeric forms.

Solutions of the amino acid derivatives were prepared using doubly distilled water which had been degassed by boiling. The concentrations of the samples were determined by weighing 10–20 mg of a solute material with a precision of ±0.02 mg and dissolving the sample in a known amount of water. All amino 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 to 5 °C above the experimental temperature before placing them into the acoustic or densimetric cell. The preheating was performed in hermetically sealed plastic tubes to prevent evaporation.

2.2. Preparation of apocytochrome c

Horse heart cytochrome *c* was purchased from Sigma-Aldrich Canada (Oakville, Ontario, Canada). Apocytochrome *c* was obtained and purified from the holoprotein following the previously described protocol [33]. The heme was removed by acid acetone extraction after the thioether bridges connecting the heme with the polypeptide chain of the protein had been cleaved by silver sulfate in the presence of acetic acid. Following the removal of the unreacted silver sulfate and the cleaved heme by centrifugation, the apoprotein was reacted with 2-mercaptoethanol to get rid of the bound silver. The resulting apoprotein was extensively dialyzed against water at 4 °C and used without lyophilization. The content of the holocytochrome *c* was assessed spectrophotometrically by measuring light absorption in the Soret region at 25 °C and using an extinction coefficient, ε_{408}, of 106,500 $M^{-1}cm^{-1}$ [9]. Within the limit of the accuracy of our spectrophotometric measurements (<0.5%), we could not detect any contamination of the apocytochrome *c* solution by the holoprotein. The concentration of apocytochrome *c* was determined spectrophotometrically at 25 °C using the extinction coefficient ε_{277}=10,500±300 $M^{-1}cm^{-1}$, which was determined by dry weight analysis. This result is in good agreement with the previously reported ε_{277} of 10,800 $M^{-1}cm^{-1}$ [33].

2.3. Methods

All densities were measured using a vibrating tube densimeter (DMA 5000, Anton Paar, Austria) with a precision of $\pm 1.5\times10^{-4}$% at 18, 25, 40 and 55 °C. The apparent molar volume, ϕV, was calculated from the equation:

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

where M is the molecular weight of the solute; m is its molal concentration; ρ and ρ_0 are the densities of the solution and solvent, respectively. Values for the density of water were taken from the work of Kell [34].

The solution sound velocities and absorptions were measured at 18, 25, 40 and 55 °C using the resonator method at a frequency of about 7.2 MHz [35–38]. Ultrasonic resonator cells with sample 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 [38]. In this technique, two identical resonator cells (sample and reference) are placed in a common thermostated environment, with the difference in the ultrasound velocities in the two cells being measured. The accuracy of all the sound velocity relative measurements achieved with this design is about $\pm10^{-4}$%, while the accuracy of the relative sound absorption measurements is ±2% [38,39].

The acoustic characteristics of a solute which 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.

Apparent molar adiabatic compressibility values for the solutes were calculated from the density and ultrasonic velocity data using the expression [40]:

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

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

The coefficient of the adiabatic compressibility of water, β_{S0}, required to evaluate ϕK_S from Eq. (2) was calculated from the data on density [34] and sound velocity [41], since $\beta_{S0}=(\rho_0 U_0^2)^{-1}$. For each evaluation of ϕV or ϕK_S, three to five independent measurements were carried out within a concentration range of 2–3 mg/ml. Our reported values of ϕV or ϕK_S represent the averages of these measurements, while the errors were calculated as standard deviations.

2.4. Measurements in the solutions of N-acetyl amino acids

To minimize the influence of the ionizable carboxyl terminus of the *N*-acetyl amino acids (*N*-acetyl glycine, *N*-acetyl alanine, *N*-acetyl phenylalanine, *N*-acetyl tryptophan, *N*-acetyl cysteine, *N*-acetyl serine, *N*-acetyl threonine, and *N*-acetyl asparagine) on the side chains, their partial molar volume and adiabatic compressibilities were determined at low ~pH 2 where the terminus is uncharged. The initial values of pH of the *N*-acetyl amino acid solutions were within the range of 2.3 to 2.8. The pH of the *N*-acetyl amino acid solutions was further lowered by HCl; equal aliquots of HCl were incrementally added to both the solution and the solvent (water). The relative molar sound velocity increment, $[U]$, and apparent molar volume, ϕV, of the solute were determined from the differential solution–*versus*–solvent measurements at each pH point. To ensure full neutralization of the carboxyl terminus, the pH-dependent measurements of $[U]$ and $V°$ were performed until these volumetric parameters level off (at ~pH 2). The plateau values of $[U]$ and $V°$ were used to calculate apparent molar adiabatic compressibilities, ϕK_S, at each experimental temperature.

2.5. Volume and compressibility changes accompanying neutralization of ionizable side chains

To determine changes in relative molar sound velocity increment, volume, and compressibility accompanying neutralization of the aspartic acid, glutamic acid, histidine, lysine, and arginine side chains, we performed pH-dependent densimetric and acoustic measurements. The pH-dependent density and ultrasonic velocity and absorption measurements were performed as previously described [42,43].

Acoustic titration experiments were performed by adding equal aliquots of 0.1 or 1 M HCl (aspartic acid, glutamic acid,

Table 1
Relative molar sound velocity increments, $[U]$ (cm^3 mol^{-1}), as a function of temperature

Compounds	18 °C	25 °C	40 °C	55 °C
N-Ac-Gly-NH_2	38.5±0.5	34.2±0.4	27.1±0.1	21.4±0.2
N-Ac-Gly-NH-Me	48.8±0.1	42.0±0.1	31.7±0.1	25.0±0.2
N-Ac-Gly	31.7±0.1	27.6±0.5	18.6±0.4	12.1±0.3
N-Ac-Ala-NH_2	48.3±0.1	42.7±0.2	33.4±0.4	25.5±0.2
N-Ac-Ala	40.6±0.4	34.5±0.4	24.5±0.4	15.9±0.5
N-Ac-Val-NH_2	67.9±0.1	60.1±0.1	47.0±0.7	33.5±0.9
N-Ac-Leu-NH_2	81.1±0.3	71.0±0.4	54.2±0.3	40.9±0.6
N-Ac-Ile-NH_2	80.2±0.7	70.2±0.1	55.0±0.6	40.9±0.1
N-Ac-Pro-NH_2	61.4±0.5	54.1±0.5	43.0±0.4	33.7±0.2
N-Ac-Phe-NH_2	77.2±0.1	67.6±0.1	51.0±0.3	39.7±0.3
N-Ac-Phe	68.0±0.2	58.2±0.4	41.6±0.1	28.1±0.2
N-Ac-Trp-NH_2	75.2±0.3	67.0±0.5	49.5±0.8	38.7±0.4
N-Ac-Trp	69.3±0.6	59.3±0.3	43.4±0.1	29.6±0.1
N-Ac-Met-NH_2	68.7±0.1	61.3±0.2	45.7±0.4	33.7±0.3
N-Ac-Cys	46.2±0.1	39.5±0.6	27.2±0.1	17.9±0.1
N-Ac-Tyr-NH_2	63.1±0.6	54.4±0.3	41.3±0.2	29.9±0.3
N-Ac-Ser	38.1±0.5	32.1±0.3	22.9±0.3	17.7±0.6
N-Ac-Thr	47.1±0.2	40.9±0.2	30.3±0.1	22.2±0.2
N-Ac-Asn	40.5±0.1	36.5±0.2	24.3±0.2	19.6±0.2
N-Ac-Gln-NH_2	56.6±0.5	50.5±0.1	41.0±0.3	33.4±0.1
N-Ac-Asp-NH_2 (pH 2.9 [a])	43.7±0.4	37.9±0.2	27.9±0.9	21.2±0.6
N-Ac-Glu-NH_2 (pH 3.2 [a])	51.4±0.2	44.5±0.4	34.0±0.1	25.7±0.4
N-Ac-His-NH-Me (pH 8.7 [a])	64.4±0.1	56.8±0.5	43.5±0.5	33.5±0.1
N-Ac-Lys-NH_2·HCl (pH 4.4 [a])	96.6±0.4	89.1±0.1	77.0±0.1	68.4±0.7
N-Ac-Arg-NH_2·CH_3COOH (pH 6.3 [a])	114.3±0.2	103.6±0.3	87.6±0.5	73.9±0.2

[a] Experimental pH at 25 °C.

and histidine) or NaOH (lysine and arginine) solutions to both the sample and the reference cells filled with the same volume of 0.80 cm^3 of the respective amino acid derivative solution and water. Additions were made using Hamilton syringes equipped with a Chaney adapter (Hamilton Co., Reno, NV). In calculating the relative molar sound velocity increment, $[U]$, and molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$, we took into account the changes in the sound velocity in the solvent, U_0, and in the molar concentration of the solute, C, that result from addition of acid or base solution.

Densimetric pH-titrations were performed in a specially designed vial equipped with a magnetic stirring bar. The total volume of the vial is 4 ml. The inlet and outlet of the vial were connected to the two ends of a *U*-shaped densimetric cell *via* tygon tubing, with one of the tubes passing through a peristaltic pump. The latter is used to remove the solution from the cell into the vial to add an aliquot of the titrant and to drive the solution back to cell after a thorough mixing in the vial. Two consecutive titrations with HCl or NaOH were conducted for the *N*-acetyl amino acid amide solution and the solvent, using the same initial volume of 3.00 ml and the same added aliquots of the titrant. The partial molar volumes of the amino acid derivatives at each pH point were calculated using Eq. (1) in which ρ and ρ_0 correspond to the densities of water and amino acid solutions containing the same concentration of HCl or NaOH.

The pH of the *N*-acetyl amino acid amide solutions was measured separately, using the same amounts and concentrations of solutions and titrant, as for the ultrasonic and densimetric measurements. The absolute error of the pH measurements was ±0.01 pH units.

3. Results

3.1. Relative molar sound velocity increments, partial molar volumes, and partial molar adiabatic compressibilities of amino acid derivatives

Tables 1–3 present the relative molar sound velocity increments, $[U]$, apparent molar volumes, ϕV, and apparent molar adiabatic compressibilities, ϕK_S, of the amino acid derivatives studied here at 18, 25, 40, and 55 °C. Our results are in good agreement with the available literature data.

Previous studies have revealed that the apparent molar volumes [28,30,44] and the apparent molar adiabatic compressibilities

Table 2
Apparent molar volumes, ϕV (cm^3 mol^{-1}), as a function of temperature

Compounds	18 °C	25 °C	40 °C	55 °C
N-Ac-Gly-NH_2	90.1±0.1	90.5±0.1 91.02 [a] 90.56 [b]	91.9±0.2 92.36 [a]	93.3±0.2 93.53 [a]
N-Ac-Gly-NH-Me	108.0±0.3	108.8±0.4 108.93 [d]	110.1±0.2	111.5±0.1
N-Ac-Gly	87.2±0.1	88.3±0.2 88.39 [c]	89.8±0.1	90.8±0.2
N-Ac-Ala-NH_2	107.1±0.1	107.3±0.3 108.06 [a] 108.06 [b]	109.1±0.2 109.31 [a]	111.2±0.2 110.75 [a]
N-Ac-Ala	105.0±0.2	105.4±0.1 105.78 [c]	107.1±0.2	108.7±0.2
N-Ac-Val-NH_2	137.6±0.1	138.2±0.1 138.92 [a] 139.00 [d]	139.6±0.6 140.71 [a]	142.3±0.1 142.50 [a]
N-Ac-Leu-NH_2	154.9±0.2	155.9±0.2 156.09 [a]	157.9±0.2 158.15 [a]	160.7±0.6 160.31 [a]
N-Ac-Ile-NH_2	153.6±0.6	154.3±0.1 153.9 [a]	155.7±0.6 156.0 [a]	158.4±0.4 158.3 [a]
N-Ac-Pro-NH_2	125.2±0.2	126.1±0.1 126.51 [d]	128.2±0.3	130.0±0.2
N-Ac-Phe-NH_2	168.8±0.1	170.4±0.3	172.8±0.1	175.7±0.1
N-Ac-Phe	166.1±0.1	168.2±0.2	171.2±0.1	173.5±0.1
N-Ac-Trp-NH_2	191.2±0.1	192.6±0.1	195.5±0.1	198.0±0.1
N-Ac-Trp	186.8±0.1	188.8±0.1	192.4±0.2	194.6±0.5
N-Ac-Met-NH_2	152.2±0.2	153.3±0.1	154.9±0.3	157.6±0.2
N-Ac-Cys	116.4±0.3	117.9±0.5	120.1±0.1	122.4±0.2
N-Ac-Tyr-NH_2	171.7±0.3	172.7±0.2	175.4±1.0	178.4±0.5
N-Ac-Ser	104.5±0.1	105.3±0.1	107.2±0.1	108.6±0.2
N-Ac-Thr	120.5±0.3	121.3±0.1	123.3±0.2	125.5±0.2
N-Ac-Asn	121.2±0.3	122.2±0.1	124.3±0.6	126.2±0.2
N-Ac-Gln-NH_2	140.0±0.1	141.3±0.1	143.1±0.1	144.8±0.1
N-Ac-Asp-NH_2	119.8±0.1	121.1±0.1	123.2±0.3	124.7±0.5
N-Ac-Glu-NH_2	136.2±0.5	137.3±0.7	139.2±0.1	141.1±0.3
N-Ac-His-NH-Me	164.9±0.3	165.8±0.4	168.0±0.1	170.0±0.4
N-Ac-Lys-NH_2·HCl	171.7±0.2	172.5±0.4	174.8±0.2	176.7±0.2
N-Ac-Arg-NH_2·CH_3COOH	209.9±0.5	211.6±0.3	214.1±0.1	215.8±0.7

[a] Ref. [28].
[b] Ref. [30].
[c] Ref. [31].
[d] Ref. [32].

Table 3
Apparent molar adiabatic compressibilities, ϕK_S (10^{-4} cm^3 mol^{-1} bar^{-1}), as a function of temperature

Compounds	18 °C	25 °C	40 °C	55 °C
N-Ac-Gly-NH_2	−6.0±0.5	−1.7±0.4 −1.58 [a] −1.99 [b]	5.7±0.2	11.0±0.2
N-Ac-Gly-NH-Me	−5.5±0.3	1.4±0.4 1.28 [a]	11.1±0.2	17.9±0.2
N-Ac-Gly	−2.9±0.1	1.8±0.4	10.5±0.1	16.4±0.4
N-Ac-Ala-NH_2	−5.9±0.1	−0.6±0.3 −0.68 [a] −0.72 [b]	8.7±0.4	16.7±0.2
N-Ac-Ala	−1.1±0.3	4.6±0.5	14.3±0.4	22.3±0.5
N-Ac-Val-NH_2	−8.7±0.1	−1.1±0.1 −0.68 [a]	11.1±0.8	24.2±0.8
N-Ac-Leu-NH_2	−11.5±0.3	−1.3±0.4 −1.92 [a]	14.6±0.3	27.5±0.7
N-Ac-Ile-NH_2	−12.3±0.8	−2.5±0.1 −2.9 [a]	11.6±0.7	26.5±0.3
N-Ac-Pro-NH_2	−13.5±0.5	−5.7±0.4	6.0±0.4	14.5±0.2
N-Ac-Phe-NH_2	−10.7±0.1	−0.6±0.3	15.4±0.3	26.6±0.3
N-Ac-Phe	−5.2±0.1	5.4±0.4	21.7±0.3	34.1±0.3
N-Ac-Trp-NH_2	−6.3±0.3	2.3±0.5	19.3±0.7	29.6±0.3
N-Ac-Trp	−5.4±0.6	5.4±0.5	21.5±0.5	33.9±0.6
N-Ac-Met-NH_2	−10.8±0.2	−3.1±0.2	11.5±0.4	23.2±0.3
N-Ac-Cys	−10.6±0.1	−3.1±0.4	9.2±0.2	18.4±0.3
N-Ac-Tyr-NH_2	−2.5±0.6	6.1±0.3 5.7 [a]	19.0±0.9	30.0±0.5
N-Ac-Ser	−6.7±0.4	−0.5±0.4	8.8±0.2	13.8±0.2
N-Ac-Thr	−6.8±0.3	−0.4±0.5	10.1±0.2	18.2±0.5
N-Ac-Asn	−6.0±0.3	−1.5±0.4	10.6±0.6	15.5±0.2
N-Ac-Gln-NH_2	−9.5±0.4	−2.7±0.1 −2.56 [a]	6.7±0.3	13.9±0.1
N-Ac-Asp-NH_2	−10.5±0.4	−3.7±0.2	6.5±0.8	12.8±0.7
N-Ac-Glu-NH_2	−8.7±0.5	−1.4±0.7	8.5±0.1	16.9±0.4
N-Ac-His-NH-Me	−4.4±0.3	3.2±0.6	16.0±0.4	26.2±0.3
N-Ac-Lys-NH_2·HCl	−34±0.4	−25.8±0.4	−12.9±0.2	−4.4±0.6
N-Ac-Arg-NH_2·CH_3COOH	−37.9±0.5	−27.0±0.4	−10.9±0.5	2.1±0.6

[a] Ref. [29].
[b] Ref. [27].

[29] of *N*-acetyl amino acid amides do not depend significantly on concentration. We estimate that the difference between the apparent molar volumes, ϕV, measured in this study (for solute concentrations in the range of 2–3 mg/ml) and the corresponding partial molar volumes, $V°$, obtained by extrapolation to infinite dilution does not exceed 0.05 cm^3 mol^{-1}, a variance that falls well within the experimental error. Similarly, the difference between the apparent molar adiabatic compressibilities, ϕK_S, of the solutes at the concentrations used in this study and the partial molar adiabatic compressibilities, $K°_S$, is less than 0.2×10^{-4} cm^3 mol^{-1} bar^{-1}, which also falls within the experimental error. Thus, hereafter, we do not discriminate between the apparent molar and partial molar characteristics of the amino acid derivatives.

3.2. Volume and compressibility changes accompanying neutralization of ionizable amino acid side chains

Despite the four decades of studying the volumetric changes accompanying neutralization/ionization of the aspartic acid, glutamic acid, histidine, lysine, and arginine side chains, there is still no consensus about the exact values of these changes in polypeptides [5,45,46]. A common shortcoming of all previously employed model compounds is the presence of other titrable groups (as in zwitterionic amino acids) in addition to the ionizable side chain under question. The overlapping titration of more than one ionizable group leads to a complex interplay between their hydration shells which may modify the volumetric properties of neutralization of such groups in a manner which is complex and difficult to predict. *N*-acetyl amino acid amides do not have any ionizable termini except the titrable groups in question. In addition, the side chains are flanked by peptide groups. Consequently, changes in hydration and related changes in volumetric properties accompanying neutralization of ionizable side chains in *N*-acetyl amino acid amides should closely resemble those in polypeptides.

Ionization/neutralization reactions of aspartic and glutamic acids (Reaction 1), histidine (Reaction 2), and lysine and arginine (Reaction 3) are given by the following equilibria:

$$-COO^- + H^+ \leftrightarrow -COOH \quad \text{(Reaction 1)}$$

$$\equiv N + H^+ \leftrightarrow \equiv NH^+ \quad \text{(Reaction 2)}$$

$$-NH_3^+ + OH^- \leftrightarrow -NH_2 + H_2O \quad \text{(Reaction 3)}$$

Our measured pH-dependences of the relative molar sound velocity increment, $\Delta[U]$, and volume, ΔV, of the amino acid derivatives with ionizable side chains in the acidic and alkaline range (not shown) had profiles typical of such dependences [42,43]. Changes in volume accompanying HCl [Eq. (3a)] and NaOH [Eq. (3b)] titrations were fit by the following equations:

$$\Delta V = \Delta V_H/\left(1+10^{pH-pKa}\right) \quad (3a)$$

$$\Delta V = \Delta V_{OH}/\left(1+10^{pKa-pH}\right) \quad (3b)$$

where ΔV_H is the change in volume caused by protonation of the side chain at acidic pH; ΔV_{OH} is the change in volume accompanying deprotonation of the side chain at alkaline pH; and p*K*a is the dissociation constant of the side chain. From the fit of our measured pH-dependent volume changes by Eqs. (3a) and (3b), we determined the values of ΔV_{OH}, ΔV_H, and p*K*a for each protonation/deprotonation reaction studied here.

The pH-dependent change in $[U]$, the relative molar sound velocity increment, associated with ionization/neutralization of a titrable group can be presented as the sum of two terms:

$$\Delta[U] = \Delta[U]_{hyd} + \Delta[U]_{rel} \quad (4)$$

where $\Delta[U]_{hyd}$ is the hydration component which represents a change in solute hydration accompanying the protonation/deprotonation reaction; and $\Delta[U]_{rel}$ is the relaxation component caused by proton-transfer reactions [42,43,47].

The pH-dependence of $\Delta[U]_{hyd}$ can be approximated by the following functions which are similar to Eqs. (3a) and (3b):

$$\Delta[U]_{hyd} = \Delta[U]_H/\left(1+10^{pH-pKa}\right) \quad (5a)$$

$$\Delta[U]_{hyd} = \Delta[U]_{OH}/\left(1+10^{pKa-pH}\right) \quad (5b)$$

Table 4a
Dissociation constants, p*K*a, and changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying protonation of titrable side chains and terminal carboxyl and amino groups at 18 °C

Side chain	p*K*a	ΔV, cm^3 mol^{-1}	ΔK_S, 10^{-4} cm^3 mol^{-1} bar^{-1}
Asp [a]	3.9±0.1	11.2±0.7	22.8±0.8
Glu [a]	4.4±0.1	10.9±0.2	22.9±0.2
His [b]	6.3±0.1	−1.5±0.1	−11.4±0.7
Lys [c]	10.5±0.1	−5.6±0.2	−12.1±1.1
Arg [c]	12.5	13.2	1.1
–COOH [a, d]	3.3±0.1	10.2±0.5	18.3±0.9
$-NH_2$ [c, d]	8.2±0.1	−6.8±0.4	−17.3±0.7

[a] Reaction $-COO^- + H^+ \leftrightarrow -COOH$.
[b] Reaction $=N + H^+ \leftrightarrow -NH^+$.
[c] Reaction $-NH_2 + H^+ \leftrightarrow -NH_3^+$.
[d] Terminal groups in triglycine from V. S. Gindikin and T. V. Chalikian (unpublished).

where $\Delta[U]_H$ is the change in $[U]$ caused by protonation of the side chain at acidic pH; $\Delta[U]_{OH}$ is the change in $[U]$ accompanying deprotonation of the side chain at alkaline pH.

The relaxation term, $\Delta[U]_{rel}$, in Eq. (4) can be calculated from the pH-dependent data on molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$ (not shown), using the expression:

$$\Delta[U]_{rel} = -[\alpha\lambda]/(2\pi\omega\tau) \quad (6)$$

where τ is the pH-dependent relaxation time of the proton-transfer reaction that can be determined from pH-dependent sound absorption data as described previously [42,43,47,48].

We analyze our data using a previously described method, in which the relaxation characteristics of a proton-transfer reaction are determined from the pH-dependences of ultrasonic velocity and absorption measured at a single frequency [42,43,48]. We use this method in conjunction with our measured pH-dependences of $[U]$ and $[\alpha\lambda]$ to calculate the relaxation contribution of $\Delta[U]_{rel}$ as a function of pH. Subtracting $\Delta[U]_{rel}$ from $\Delta[U]$ [see Eq. (4)] we evaluate $\Delta[U]_{hyd}$ that is subsequently approximated using Eqs. (5a) and (5b). With this approximation, we determine $\Delta[U]_{OH}$ and $\Delta[U]_H$. In these calculations, we use the values of p*K*a determined from analyzing our partial molar volume data. With the values of ΔV_H, ΔV_{OH}, $\Delta[U]_H$, and $\Delta[U]_{OH}$, we determine the respective changes in adiabatic compressibility from $\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U])$.

Table 4b
Dissociation constants, p*K*a, and changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying protonation of titrable side chains and terminal carboxyl and amino groups at 25 °C

Side chain	p*K*a	ΔV, cm^3 mol^{-1}	ΔK_S, 10^{-4} cm^3 mol^{-1} bar^{-1}
Asp	3.9±0.1	12.1±0.4	21.6±0.5
Glu	4.3±0.1	11.8±0.4	22.4±0.4
His	6.2±0.1	−1.4±0.1	−11.1±0.3
Lys	10.5±0.1	−5.9±0.5	−14.2±1.1
Arg	12.5	13.0	−2.6
–COOH [a]	3.3±0.1	10.5±0.4	18.4±0.9
$-NH_2$ [a]	8.0±0.1	−4.8±0.5	−16.9±0.9

[a] Terminal groups in triglycine from V. S. Gindikin and T. V. Chalikian (unpublished).

Table 4c
Dissociation constants, p*K*a, and changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying protonation of titrable side chains and terminal carboxyl and amino groups at 40 °C

Side chain	p*K*a	ΔV, cm^3 mol^{-1}	ΔK_S, 10^{-4} cm^3 mol^{-1} bar^{-1}
Asp	3.9±0.1	12.1±0.3	20.7±0.4
Glu	4.4±0.1	12.1±0.3	21.6±0.7
His	6.1±0.4	−1.3±0.4	−12.4±0.7
Lys	10.0±0.1	−5.8±0.3	−15.3±1.0
Arg	12.5	13.5	−7.2
–COOH [a]	3.2±0.1	11.0±0.4	17.7±0.9
$-NH_2$ [a]	7.7±0.1	−5.1±0.5	−17.9±0.9

[a] Terminal groups in triglycine from V. S. Gindikin and T. V. Chalikian (unpublished).

In contrast to other amino acids with titrable side chains studied here, the pH-dependent acoustic and densimetric measurements in the *N*-acetyl arginine amide acetate solution were carried out at a single temperature of 25 °C. Moreover, due to an extremely alkaline dissociation constant of the arginine side chain (p*K*a ≈ 12.5), we were not able to perform pH-dependent densimetric and acoustic measurements in the solution of *N*-acetyl arginine amide acetate all the way to completion (full neutralization of the ionizable group). The highest pH of these measurements was ~13.2 at which ~20% of solute molecules are still ionized. The values of ΔV_{OH} and $\Delta[U]_{OH}$ were obtained by fitting the collected experimental points with Eqs. (3a), (3b), (4), (5a), (5b), (6).

Tables 4a–d present the values of p*K*a, ΔV, and ΔK_S for protonation of the aspartic acid, glutamic acid, histidine, lysine, and arginine side chains as well as our previous data on the terminal amino and carboxyl groups in triglycine (V. S. Gindikin and T. V. Chalikian, unpublished). Volume changes for protonation of lysine and arginine and amino terminus ($-NH_2 + H^+ \leftrightarrow -NH_3^+$) were calculated by adding to the measured values of ΔV_{OH} the volume of ionization of water [$\Delta V_{ion} = V°(H_2O) - V°(H^+) - V°(OH^-)$]. The values of ΔV_{ion} are −21.9, −21.7, −22.2, and −23.1 cm^3 mol^{-1} at 18, 25, 40, and 55 °C, respectively (the value at 55 °C was obtained by extrapolation of the data obtained between 0 and 45 °C) [49]. Similarly, the protonation compressibilities for the lysine and arginine side chains and amino terminus were calculated by adding to the measured values of ΔK_{SOH} the adiabatic compressibility of ionization of water [$\Delta K_{Sion} = K°_S(H_2O) - K°_S(H^+) - K°_S(OH^-)$].

Table 4d
Dissociation constants, p*K*a, and changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying protonation of titrable side chains and terminal carboxyl and amino groups at 55 °C

Side chain	p*K*a	ΔV, cm^3 mol^{-1}	ΔK_S, 10^{-4} cm^3 mol^{-1} bar^{-1}
Asp	4.0±0.1	12.8±0.5	21.5±0.5
Glu	4.4±0.1	13.5±0.3	23.2±0.3
His	5.9±0.1	−1.0±0.3	−13.8±0.4
Lys	9.7±0.1	−5.2±0.1	−13.2±1.2
Arg	12.5	14.4	−5.2
–COOH [a]	3.2±0.1	10.7±0.5	17.1±1.0
$-NH_2$ [a]	7.2±0.1	−5.1±0.6	−14.7±1.1

[a] Terminal groups in triglycine from V. S. Gindikin and T. V. Chalikian (unpublished).

The values of ΔK_{Sion} are -52.8×10^{-4}, -49.1×10^{-4}, -44.5×10^{-4}, and -46.5×10^{-4} cm^3 mol^{-1} at 18, 25, 40, and 55 °C, respectively (the value at 55 °C was obtained by extrapolation of the data obtained between 0 and 45 °C) [49]. For arginine, the values of ΔV_{OH} and ΔK_{SOH} obtained at a single temperature of 25 °C were used to determine the protonation volume and compressibility at all experimental temperatures. While this approximation may render the values of ΔV_{OH} and $\Delta[U]_{OH}$ for arginine less accurate compared to other data, its relative effect on the additive scheme developed in this work is insignificant. Neutralization of an arginine residue in protein studies is an extremely rare event. Therefore, in most protein studies, it is sufficient to know the group contribution of charged arginine.

4. Discussion

4.1. Volume contributions of amino acid residues do not depend on the model

The volume or compressibility contribution of a specific amino acid residue in an extended polypeptide can be obtained by adding the group contribution of the glycyl unit (–CH_2CONH–) to the group contribution of the respective side chain. The latter can be obtained as the difference in partial molar volume, $V°$, between the corresponding amino acid and glycine. Table 5 lists our evaluated volume contributions for the 20 amino acid side chains as a function of temperature. For amino acids containing titrable groups (aspartic and glutamic acids, histidine, lysine, and arginine), the data presented in Table 5 refer to the unionized state of the side chain. For aspartic and glutamic acids, the uncharged group contributions, $V(-R)$, were evaluated from $V(-R)=V°(\mathrm{pH})-V°(\mathrm{Gly})+\Delta V/(1+10^{\mathrm{p}Ka-\mathrm{pH}})$, where $V°$(pH) is the partial molar volume of *N*-acetyl aspartic or glutamic acid amide at the experimental pH; $V°$(Gly) is the partial molar volume of *N*-acetyl glycine amide; ΔV and p*K*a are, respectively, the protonation volume and dissociation constant of the side chain given in Tables 4a–d. The group contribution of the histidine side chain was calculated from $V(-R)=V°(\mathrm{pH})-V°(\mathrm{Gly})-\Delta V/(1+10^{\mathrm{pH}-\mathrm{p}Ka})$, where $V°$(pH) is the partial molar volume of *N*-acetyl histidine methylamide at the experimental pH; and $V°$(Gly) is the partial molar volume of *N*-acetyl glycine methylamide. The group contribution of lysine side chain was calculated from $V(-R)=V°(\mathrm{pH})-V°(\mathrm{Gly})-V°(\mathrm{HCl})-\Delta V/(1+10^{\mathrm{pH}-\mathrm{p}Ka})$, where $V°$(pH) is the partial molar volume of *N*-acetyl lysine amide hydrochloride at the solution pH; and $V°$(HCl) is the partial molar volume of HCl from Ref. [49]. The group contribution of arginine side chain was calculated from $V(-R)=V°(\mathrm{pH})-V°(\mathrm{Gly})-V°(\mathrm{HCl})+V°(\mathrm{NaCl})-V°(CH_3COONa)-\Delta V/(1+10^{\mathrm{pH}-\mathrm{p}Ka})$, where $V°$(pH) is the partial molar volume of *N*-acetyl arginine amide acetate at the experimental solution pH; $V°$(NaCl) is the partial molar volume of NaCl from Ref. [50]; and $V°(CH_3COONa)$ is the partial molar volume of sodium acetate. Our measured values of $V°(CH_3COONa)$ are 39.0±0.2, 39.6±0.3, 40.3±0.3, and 41.2±0.5 cm^3 mol^{-1} at 18, 25,

Table 5
Partial molar volume contributions of amino acid side chains, $V(-R)$ (cm^3 mol^{-1}), as a function of temperature

SC	18 °C	25 °C	40 °C	55 °C
Ala	17.0±0.1	16.8±0.3	17.2±0.3	17.9±0.3
	18.2[a]	17.23[b]	18.4[a]	18.4[a]
		18.3[a]		
		16.14[c]		
		17.9[d]		
		17.5[e]		
Ala[f]	17.8±0.1	17.2±0.1	17.3±0.2	17.9±0.3
Val	47.5±0.1	47.7±0.1	47.7±0.6	49.0±0.2
	48.2[a]	47.46[b]	48.6[a]	49.0[a]
		48.3[a]		
		46.11[c]		
		49.2[d]		
		49.1[e]		
Leu	64.8±0.2	65.4±0.2	66.0±0.3	67.4±0.6
	64.3[a]	64.64[b]	65.1[a]	66.0[a]
		64.5[a]		
		63.39[c]		
		66.1[d]		
		65.7[e]		
Ile	63.5±0.6	63.8±0.1	63.8±0.5	65.1±0.4
	64.8[a]	62.61[b]	66.1[a]	67.3[a]
		65.2[a]		
		64.4[e]		
Pro	35.1±0.2	35.6±0.1	36.3±0.4	36.7±0.3
	32.9[a]	39.44[b]	33.5[a]	34.0[a]
		33.1[a]		
Phe	78.7±0.1	79.9±0.3	80.9±0.2	82.4±0.2
	80.3[a]	79.0[b]	82.2[a]	83.4[a]
		80.9[a]		
		79.22[c]		
		79.9[d]		
		79.1[e]		
Phe[f]	78.9±0.1	79.9±0.1	81.4±0.1	82.7±0.2
Trp	101.1±0.1	102.1±0.1	103.6±0.2	104.7±0.2
	98.5[a]	100.6[b]	99.9[a]	101.0[a]
		98.9[a]		
Trp[f]	99.6±0.1	101.6±0.1	102.6±0.1	103.8±0.5
Met	62.1±0.2	62.8±0.1	63.0±0.4	64.3±0.3
	64.7[a]	62.38[b]	66.4[a]	67.8[a]
		65.2[a]		
Cys[f]	29.2±0.1	29.7±0.1	30.3±0.2	31.6±0.4
	31.3[a]	30.0[g]	31.9[a]	32.3[a]
		31.5[a]		
Tyr	81.6±0.3	82.2±0.2	83.5±1.0	85.1±0.5
	82.4[a]	81.2[b]	84.0[a]	85.1[a]
		82.9[a]		
Ser[f]	17.3±0.1	17.1±0.1	17.4±0.1	17.8±0.3
	17.9[a]	17.43[b]	18.1[a]	18.2[a]
		18.0[a]		
		16.34[c]		
Thr[f]	33.3±0.3	33.1±0.1	33.5±0.2	34.7±0.3
	33.9[a]	33.64[b]	34.4[a]	34.7[a]
		34.0[a]		
		32.27[c]		
Asn[f]	34.0±0.3	34.0±0.2	34.5±0.6	35.4±0.3
	37.7[a]	33.9[f]	39.1[a]	40.2[a]
		38.1[a]		
		33.88[c]		
Gln	49.9±0.1	50.8±0.1	51.2±0.2	51.5±0.2
	50.1[a]	50.42[b]	50.9[a]	51.5[a]
		50.3[a]		

(continued on next page)

Table 5 (*continued*)

SC	18 °C	25 °C	40 °C	55 °C
Asp	30.7±0.4	31.7±0.1	32.4±0.4	32.3±0.5
	31.4 [a]	31.6 [b]	32.4 [a]	32.9 [a]
		31.7 [a]		
Glu	46.7±0.5	47.7±0.7	48.0±0.2	48.6±0.4
	47.6 [a]	46.66 [b]	48.7 [a]	49.3 [a]
		48.0 [a]		
His [h]	56.9±0.4	57.0±0.6	57.9±0.2	58.5±0.4
	59.1 [a]	55.11 [b]	60.3 [a]	61.0 [a]
		59.5 [a]		
Lys	69.6±0.2	70.1±0.4	70.7±0.3	70.6±0.3
	64.3 [a]	64.5 [a]	65.1 [a]	65.8 [a]
Arg	66.0±0.5	67.4±0.3	67.6±0.3	66.3±0.7
	71.6 [a]	72.1 [a]	73.0 [a]	73.7 [a]

[a] GlyXGly tripeptides from Ref. [20].
[b] Zwitterionic amino acids from Ref. [31].
[c] GlyX dipeptides from Ref. [52].
[d] XGlyGly tripeptides from Ref. [68].
[e] GlyGlyX tripeptides from Ref. [68].
[f] Calculated from *N*-acetyl amino acid data.
[g] Zwitterionic amino acids from Ref. [51].
[h] Calculated from *N*-acetyl amino acid methylamide data.

40, and 55 °C, respectively (measurements were conducted at a CH_3COONa concentration ~2 mg/ml).

Also shown in Table 5 are the side chain contributions calculated from the partial molar volumes of zwitterionic amino acids, glycyl dipeptides, and diglycyl tripeptides [20,31,51,52]. Comparison between the several sets of data shown in Table 5 reveals that, with the exception of Lys and Arg, the volume contribution of an amino acid side chain is essentially independent of the model compound (in most cases, within $\pm 1{-}2$ cm^3 mol^{-1}). This observation attests to the relative insensitivity of the partial molar volume observable to the microenvironment of individual atomic groups, a feature that renders additive calculations of the partial molar volumes of solutes successful.

4.2. Compressibility contributions of amino acid side chains depend on the model

The compressibility contributions of amino acid side chains were calculated by subtracting the partial molar adiabatic compressibility of glycine from that of the corresponding amino acid. Table 6 lists our calculated adiabatic compressibility contributions for the 20 amino acid side chains as a function of temperature. For amino acids with ionizable side chains, Table 6 presents the compressibility contributions of the unionized state. For aspartic acid and glutamic acid side chains, these contributions were calculated using $K_S(-R)=K°_S(\mathrm{pH})-K°_S(\mathrm{Gly})+\Delta K_S/(1+10^{pKa-pH})$, where $K°_S(\mathrm{pH})$ is the partial molar adiabatic compressibility of *N*-acetyl aspartic or glutamic acid amide at the experimental solution pH; $K°_S(\mathrm{Gly})$ is the partial molar adiabatic compressibility of *N*-acetyl glycine amide; and ΔK_S is the protonation compressibility from Tables 4a–d. For histidine, $K_S(-R)$ was calculated from $K_S(-R)=K°_S(\mathrm{pH})-K°_S(\mathrm{Gly})-\Delta K_S/(1+10^{pH-pKa})$, where $K°_S(\mathrm{pH})$ is the partial molar adiabatic compressibility of *N*-acetyl histidine methylamide at the experimental solution pH; $K°_S(\mathrm{Gly})$ is the partial molar adiabatic compressibility of *N*-acetyl glycine methyla-

Table 6
Partial molar adiabatic compressibility contributions of amino acid side chains, $K_S(-R)$ (10^{-4} cm^3 mol^{-1} bar^{-1}), as a function of temperature

SC	18 °C	25 °C	40 °C	55 °C
Ala	0.1±0.5	1.1±0.5	3.0±0.5	5.7±0.3
		3.7 [a]		
		1.5 [b]		
		−3.5 [c]		
		6.0 [d]		
		6.1 [e]		
		2.1 [f]		
Ala [g]	1.8±0.3	2.8±0.6	3.8±0.4	5.9±0.6
Val	−2.7±0.5	0.6±0.4	5.4±0.8	13.2±0.8
		1.8 [a]		
		−2.2 [b]		
		−6.7 [c]		
		5.0 [e]		
		2.5 [f]		
Leu	−5.5±0.6	0.4±0.6	8.9±0.4	16.5±0.7
		1.3 [a]		
		−3.9 [b]		
		−7.4 [c]		
		3.6 [e]		
		0.2 [f]		
Ile	−6.3±0.9	−0.8±0.4	5.9±1.3	15.5±0.4
		4.3 [a]		
		−3.7 [b]		
		1.6 [f]		
Pro	−7.5±1.4	−4.0±0.6	0.3±0.5	3.5±0.3
		−5.2 [h]		
		3.2 [b]		
Phe	−4.7±0.5	1.1±0.5	9.7±0.4	15.6±0.4
		5.8 [a]		
		−7.1 [b]		
		−1.8 [c]		
		1.2 [e]		
		0.6 [f]		
Phe [g]	−2.3±0.1	3.6±0.6	11.2±0.3	17.7±0.5
Trp	−0.3±0.6	4.0±0.6	13.6±0.7	18.6±0.4
		−4.1 [b]		
Trp [g]	−2.5±0.6	3.6±0.6	11.0±0.5	17.5±0.7
Met	−4.8±0.5	−1.4±0.4	5.8±0.4	12.2±0.4
		1.3 [a]		
		−4.0 [b]		
Cys [g]	−7.7±0.1	−4.9±0.6	−1.3±0.2	2.0±0.5
		−6.8 [b]		
Tyr	3.5±0.8	7.8±0.5	13.3±0.9	19.0±0.5
		8.9 [h]		
		−7.4 [b]		
Ser [g]	−3.8±0.4	−1.3±0.6	−1.7±0.2	−2.6±0.4
		1.3 [a]		
		−3.6 [b]		
		−4.2 [c]		
Thr [g]	−3.9±0.3	−2.2±0.6	−0.4±0.2	1.8±0.6
		−1.0 [a]		
		−4.8 [b]		
		−5.8 [c]		
Asn [g]	−3.1±0.3	−3.3±0.6	0.1±0.6	−0.9±0.4
		−2.0 [a]		
		−6.9 [b]		
		−6.3 [c]		
Gln	−3.5±0.6	−1.0±0.4	1.0±0.4	2.9±0.2
		−1.9 [h]		
		−2.3 [b]		
Asp	−6.6±0.6	−4.0±0.4	−1.1±0.8	0.2±0.7
		0 [h]		

Table 6 (*continued*)

SC	18 °C	25 °C	40 °C	55 °C
Glu	−4.1±0.7	−1.3±0.8 −0.1 [h] −2.9 [b]	1.5±0.2	4.5±0.4
His [i]	1.4±0.4	1.8±0.7 4.2 [a] −5.9 [b]	4.9±0.4	8.3±0.4
Lys	−6.0±0.6	−2.4±0.6	1.9±0.3	4.4±0.6
Arg	−14.5±0.7	−8.5±0.6	0.3±0.5	1.9±0.6

[a] GlyXGly tripeptides from Ref. [69].
[b] Zwitterionic amino acids from Ref. [57].
[c] GlyX dipeptides from Ref. [70].
[d] XGly dipeptides from Ref. [70].
[e] XGlyGly tripeptides from Ref. [68].
[f] GlyGlyX tripeptides from Ref. [68].
[g] Calculated from *N*-acetyl amino acid data.
[h] GlyXGly tripeptides from Ref. [46].
[i] Calculated from *N*-acetyl amino acid methylamide data.

mide. The contribution of lysine was calculated from $K_S(-R)=K°_S(\mathrm{pH})-K°_S(\mathrm{Gly})-K°_S(\mathrm{HCl})-\Delta K_S/(1+10^{\mathrm{pH}-\mathrm{p}Ka})$, where $K°_S(\mathrm{pH})$ is the partial molar adiabatic compressibility of *N*-acetyl lysine amide hydrochloride at the experimental pH; and $K°_S(\mathrm{HCl})$ is the partial molar adiabatic compressibility of HCl from Ref. [49]. The group contribution of arginine was determined from $K_S(-R)=K°_S(\mathrm{pH})-K°_S(\mathrm{Gly})-K°_S(\mathrm{HCl})+K°_S(\mathrm{NaCl})-K°_S(\mathrm{CH_3COONa})-\Delta K_S/(1+10^{\mathrm{pH}-\mathrm{p}Ka})$, where $K°_S(\mathrm{pH})$ is the partial molar adiabatic compressibility of *N*-acetyl arginine amide acetate at the solution pH; $K°_S(\mathrm{HCl})$ is the partial molar adiabatic compressibility of HCl from Ref. [49]; $K°_S(\mathrm{NaCl})$ is the partial molar adiabatic compressibility of NaCl from Ref. [50]; and $K°_S(\mathrm{CH_3COONa})$ is the partial molar adiabatic compressibility of sodium acetate. Our measured values of $K°_S(\mathrm{CH_3COONa})$ are $-(63.6\pm0.6)\times10^{-4}$, $-(56.5\pm0.6)\times10^{-4}$, $-(47.8\pm0.6)\times10^{-4}$, and $-(42.1\pm0.9)\times10^{-4}$ $\mathrm{cm^3\ mol^{-1}\ bar^{-1}}$ at 18, 25, 40, and 55 °C, respectively.

Table 6 also shows the side chain contributions determined from the data on zwitterionic amino acids, glycyl dipeptides, diglycyl tripeptides, and GlyXGly tripeptides. Inspection of data presented in Table 6 reveals two important observations. Firstly, our determined compressibility contributions of amino acid side chains in *N*-acetyl amino acid amides are distinct from those in zwitterionic amino acids, glycyl dipeptides, and XGlyGly and GlyGlyX tripeptides. Importantly, the distinctions may involve both the magnitude and the sign of compressibility contributions. Secondly, the side chains in uncharged *N*-acetyl amino acid amides and GlyXGly tripeptides, while still being significantly different for some amino acids, share a greater degree of similarity with respect to their compressibility contributions. These observations are consistent with a picture in which hydration of a peripherally located amino acid side chain (e. g., in an XGlyGly tripeptide) is influenced by the adjacent charged amino or carboxyl terminus which causes the side chain to exhibit an altered compressibility contribution. On the other hand, the observed greater similarity between the side chain contributions in *N*-acetyl amino acid amides and GlyXGly tripeptides suggests that, in these molecules, the side chains share similarities in their microenvironment and hydration.

4.3. The hydration properties of amino acid side chains

The partial molar volume of a solute, $V°$, can be related to its hydration *via* the expression [51,53]:

$$V^\circ = V_C + V_I + \beta_{TO}RT \tag{7}$$

where V_C is the volume of the cavity in the solvent enclosing a noninteracting solute; V_I is the interaction volume that represents a change in volume accompanying the "switching on" of solute–solvent interactions; β_{T0} is the coefficient of isothermal compressibility of the solvent. The $\beta_{T0}RT$ term, which originates from the availability of the entire volume of the solution to the solute, describes the volume effect related to the kinetic contribution to the pressure of a solute molecule due to its translational degrees of freedom.

The cavity volume, V_C, consists of the van der Waals volume of the solute and the thermal volume, V_T. The latter has three components — steric, vibrational, and structural. The steric component reflects the imperfect packing of solute and solvent molecules in the solution. The vibrational component reflects thermally-induced mutual vibrational motions of solute and solvent molecules and, theoretically, should subside to zero at 0 K. The structural component reflects the open (tetrahedral) structure of water and related packing effects around solute molecules. The thermal volume has been proposed to be proportional to the van der Waals surface area, S_W, of a solute [51]. In this approximation, two molecules, which are different with respect to the chemical nature of the solvent-exposed atomic groups but exhibit the same value of S_W, should be characterized by the same thermal volume, V_T. Using this approximation and the assumption that the interaction volume, V_I, of nonpolar groups is negligibly small, Kharakoz has determined the interaction volumes of several amino acid side chains in zwitterionic amino acids [51]. We use below the same approach to evaluate the interaction volumes, V_I, of all the side chains in the amino acid derivatives investigated in this work.

Fig. 1 plots the difference between the volume contributions, $V(-R)$ (from Table 5), and the van der Waals volumes, V_W, of amino acid side chains against their van der Waals surface areas, S_W, at 18, 25, 40, and 55 °C. The values of V_W and S_W were calculated using an additive procedure and group contributions reported by Bondi [54]. For alanine, phenylalanine, and tryptophan, the average of the two evaluations of $V(-R)$ has been used in Fig. 1 (see Table 5). The straight lines passing through the points corresponding to amino acids with nonpolar side chains (Ala, Val, Leu, Ile, Pro, and Phe) represent the baselines for "noninteracting" groups with V_I of zero. Consequently, deviations of specific amino acid points from the straight lines in Fig. 1 are equal to the respective interaction volumes, V_I. Table 7 presents our evaluated interaction volumes, V_I, for all the amino acid side chains examined in this work along with the similar data evaluated by Kharakoz from zwitterionic amino acids [51]. The two sets of data are in good agreement which further attests to the additivity of the partial molar volume observable and its components.

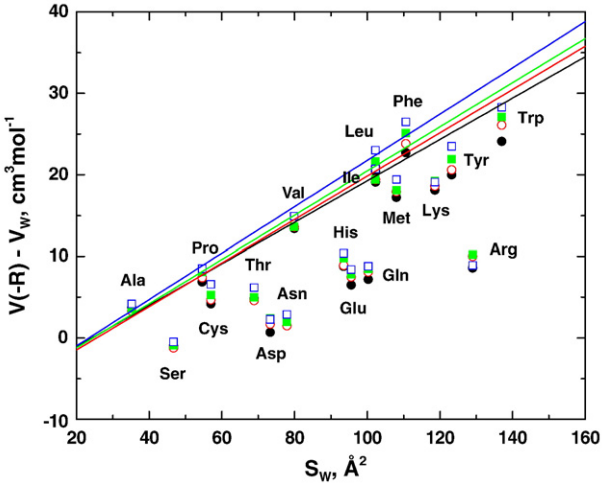

Fig. 1. Difference between the partial molar volume contributions and van der Waals, $V(-R)-V_W$, of amino acid side chains plotted against the van der Waals surface area, S_W, at 18 (black), 25 (red), 40 (green), and 55 °C (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Interaction volume can be presented as $V_I=\sum_{i=0}^{n}\alpha_i\Delta V_i$, where α_i is the fractional composition of the hydrated complex with i solvating water molecules; n is the maximum number of i for a solute or an atomic group; and ΔV_i is the volume change accompanying formation of the hydrated complex of a solute with i water molecules [55]. For the simplest case when each interacting water molecule causes the same change in volume, ΔV (hence, $\Delta V_i=i\Delta V$), one obtains $V_I=\sum_{i=0}^{n}\alpha_i i\Delta V=n_h\Delta V$, where $n_h=\sum_{i=0}^{n}\alpha_i i$ is the effective hydration number of a solute.

Since all side chains listed in Table 7 are uncharged, the interaction volumes, V_I, predominantly originate from solute–solvent hydrogen bonding. The value of ΔV for solute–solvent hydrogen bonding has been estimated to be -2.2 cm^3 mol^{-1} at 25 °C [53]. With this estimate, the effective number of solute–solvent hydrogen bonds, n_h, for each amino acid side chain can be determined by dividing its interaction volume at 25 °C by -2.2 cm^3 mol^{-1}. Our calculated values of n_h are presented in the second column of Table 7. The values of n_h range from 1.6 to 7.5 and correlate with the number of polar atomic groups in the side chain.

There have been attempts in the past to analyze the compressibility contributions of amino acid side chains in terms of the groups contributions of constituent atomic groups [56,57]. Such attempts implicitly assume that atomic groups in amino acid side chains are independently hydrated and that an atomic group exhibits the same compressibility contribution in any solute. Inspection of Table 6 suggests that neither assumption is valid for amino acid side chains. For example, the contribution of a $-CH_2-$ group can be obtained as a difference between leucine and valine, isoleucine and valine, glutamine and asparagine, and glutamic acid and aspartic acid. At 25 °C, these estimates yield the values of -0.2×10^{-4}, -1.4×10^{-4}, 2.3×10^{-4}, and 2.7×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively. However, the compressibility contribution of an independently hydrated methylene group in an unbranched aliphatic chain at 25 °C is $-(1.6\pm0.2)\times10^{-4}$ cm^3 mol^{-1} bar^{-1} [58]. The observed uncertainty in the compressibility contributions of nonpolar groups undermines the validity of determination of the group contributions of polar moieties using an additive approach rendering it virtually impossible.

The observed nonadditivity of the compressibility contributions of atomic groups in amino acid side chains may reflect a complex interplay between two factors. Firstly, the hydration shells of constituent atomic groups may overlap. Secondly, interatomic interactions between adjacent groups that do not necessarily result in any significant hydration overlap may nevertheless mutually modulate the extent of solute–solvent interactions. In this respect, it should be noted that the compressibility observable, in contrast to volume, is sensitive not only to the effective number of solute–solvent interactions, n_h, but also to the distribution of the number of such interactions in the statistical assembly of solvated solute molecules [55].

Thus, compressibility contributions, while reflecting the hydration properties of individual amino acid side chains in their entirety, cannot be unequivocally rationalized in terms of individual contributions of constituent atomic groups. We propose that our measured group numbers shown in Table 6 represent the best approximation of the compressibility contributions of independently hydrated amino acid side chains in an extended polypeptide chains.

4.4. Volumetric properties of fully unfolded proteins

We use our data in conjunction with the additive approach to calculate the partial specific volumes and adiabatic compressibilities of several proteins in their fully unfolded conformations

Table 7
Hydration numbers, n_h, and interaction volumes, V_I (cm^3 mol^{-1}), of amino acid side chains as a function of temperature

SC	n_h	18 °C	25 °C	40 °C	55 °C
Trp	1.6	−4.5	−3.6	−3.4	−4.0
			−1 [a]		−1 [a]
Met	1.8	−4.1	−4.0	−4.5	−4.6
			−3 [a]		−4.5 [a]
Cys	1.7	−4.1	−3.7	−3.5	−2.9
			−4.5 [a]		−4.5 [a]
Tyr	2.5	−5.1	−5.4	−4.8	−4.8
			−6.5 [a]		
Ser	3.1	−6.7	−6.8	−6.9	−7.1
			−7 [a]		−7 [a]
Thr	3.1	−6.6	−6.9	−7.0	−6.7
			−5.5 [a]		
Asn	5.6	−12.1	−12.4	−12.5	−12.6
			−13 [a]		−13 [a]
Gln	5.4	−12.1	−11.8	−12.0	−13.0
			−12 [a]		−13.5 [a]
Asp	5.0	−11.8	−11.0	−10.8	−11.9
Glu	5.0	−11.6	−11.1	−11.5	−12.1
			−13 [a]		−13 [a]
His	4.2	−8.8	−9.2	−8.9	−9.5
			−9 [a]		−9 [a]
Lys	2.8	−5.9	−6.2	−6.3	−7.9
Arg	7.5	−17.0	−16.5	−17.1	−20.1

[a] Calculated from data on zwitterionic amino acids from Ref. [51].

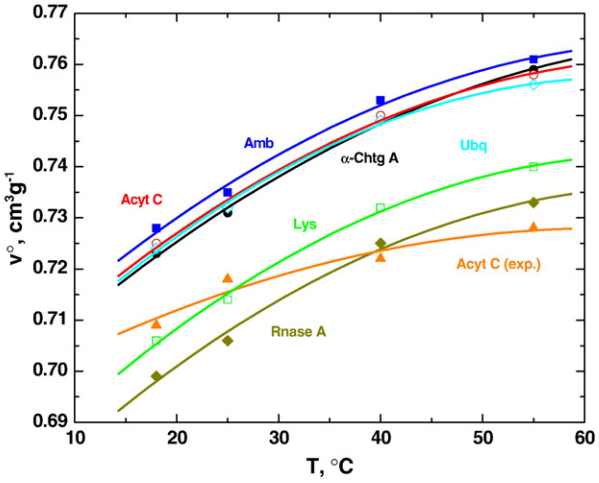

Fig. 2. Calculated temperature dependences of the partial specific volumes, $v°$, of the fully extended conformations of ubiquitine (cyan), apocytochrome (red), ribonuclease A (dark yellow), lysozyme (green), apomyoglobin (blue), and α-chymotrypsinogen A (black) at pH 7. Experimental temperature dependence of $v°$ for apocytochrome c is shown in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at 18, 25, 40, and 55 °C. The calculations were performed based on the primary amino acid structure of the polypeptide using the following additive scheme:

$$x^\circ = \Big[X^\circ(\text{trigly}) - \Delta X_{NH_2}/\left(1+10^{pH-pKa_{NH_2}}\right) + \Delta X_{COOH}/\left(1+10^{pKa_{COOH}-pH}\right) + (n-3)X(-CH_2CONH-) + \sum_{i=1}^{n} X_i(-R) + \sum_{j=1}^{l} \Delta X_j/\left(1+10^{pH-pKa_j}\right) - \sum_{k=1}^{m} \Delta X_k/\left(1+10^{pKa_k-pH}\right)\Big]/M \quad (8)$$

where $x°$ is the partial specific volume or adiabatic compressibility of the protein in question; $X°$(trigly) is the partial molar volume or adiabatic compressibility of triglycine; ΔX_{NH_2} and ΔX_{COOH} are the protonation volumes or compressibilities of the amino and carboxyl termini, respectively; pKa_{NH_2} and pKa_{COOH} are the dissociation constants of the amino and carboxyl termini, respectively; $X_i(-R)$ is the volume or compressibility contribution of the i-th amino acid side chain [for an ionizable side chain, $X_i(-R)$ corresponds to its neutral state]; n is the number of amino acid residues in the polypeptide chain; l is the number of basic side chains; pKa_j and ΔX_j are the dissociation constant and the protonation volume or compressibility of the j-th basic side chains; m is the number of acidic side chains; pKa_k and ΔX_k are the dissociation constant and the protonation volume or compressibility of the k-th acidic side chain; and M is the molecular weight of the polypeptide.

The volume, $V(-CH_2CONH-)$, and compressibility, $K_S(-CH_2CONH-)$, contributions of the glycyl unit have been determined from the volumetric data on oligopeptides containing repetitive glycyl residues. Our previous study of oligoglycines has produced the values of $V(-CH_2CONH-)$ of 36.8±0.1, 37.4±0.1, 39.0±0.1, and 39.3±0.1 $cm^3\ mol^{-1}$ and the values of $K_S(-CH_2CONH-)$ of $-(2.4\pm0.5)\times10^{-4}$, $-(1.1\pm0.5)\times10^{-4}$, $(2.0\pm0.5)\times10^{-4}$, and $(2.9\pm0.5)\times10^{-4}$ $cm^3\ mol^{-1}\ bar^{-1}$ at 18, 25, 40, and 55 °C, respectively [59]. These values are in good agreement with the oligoglycine-based data obtained by Hedwig and collaborators [60]. However, subsequent investigations from the same group of the Ala$(Gly)_n$ oligopeptides have revealed somewhat different values of $V(-CH_2CONH-)$ and $K_S(-CH_2CONH-)$ [20,60,61]. The values of $V(-CH_2CONH-)$ calculated from the Ala$(Gly)_n$ data equal 36.1, 36.3, 36.7, and 37.1 $cm^3\ mol^{-1}$ at 18, 25, 40, and 55 °C, respectively. The differences between the two sets of $V(-CH_2CONH-)$ data [from the $(Gly)_n$ and Ala$(Gly)_n$ peptides] mainly involve not the absolute values but their temperature dependence. The compressibility data on Ala$(Gly)_n$ and Ser$(Gly)_n$ oligopeptides reported by Hedwig and collaborators [61] have produced a compressibility contribution of the glycyl unit equal to $-(2.7\pm0.1)\times10^{-4}$ $cm^3\ mol^{-1}\ bar^{-1}$ at 25 °C. This value is more negative than the oligoglycine-based value of $K_S(-CH_2CONH-)$ of $-(1.1\pm0.5)\times10^{-4}$ $cm^3\ mol^{-1}\ bar^{-1}$. The volumetric disparities between the $(Gly)_n$ and Ala$(Gly)_n$ and Ser$(Gly)_n$ data are yet to be rationalized and do not permit one to unequivocally choose the group numbers for the backbone glycyl group for calculating the partial molar volume and adiabatic compressibility of a polypeptide using Eq. (8). While acknowledging this fact, we use below our data derived from studying oligoglycines [59].

Figs. 2 and 3 show, respectively, our calculated partial specific volumes, $v°$, and adiabatic compressibilities, $k°_S$, of the fully extended conformational states of ubiquitine, apocytochrome c, ribonuclease A, lysozyme, apomyoglobin, and

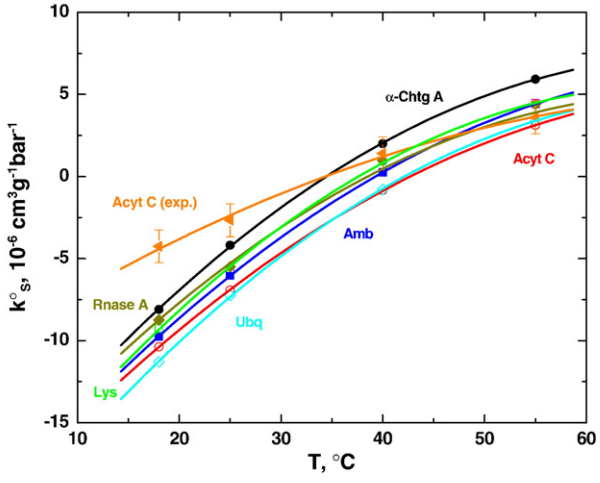

Fig. 3. Calculated temperature dependences of the partial specific adiabatic compressibilities, $k°_S$, of the fully extended conformations of ubiquitine (cyan), apocytochrome (red), ribonuclease A (dark yellow), lysozyme (green), apomyoglobin (blue), and α-chymotrypsinogen A (black) at pH 7. Experimental temperature dependence of $k°_S$ for apocytochrome c is shown in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

α-chymotrypsinogen A at pH 7 as a function of temperature. They also show the temperature dependences of our measured partial specific volume, $v°$, and partial molar adiabatic compressibility, $k°_S$, of apocytochrome *c* in water at ~pH 7. Note that apocytochrome *c* is unfolded under the physiological conditions [33,62]. Inspection of Fig. 2 reveals that the unfolded proteins studied here exhibit similar temperature-dependent changes in the partial molar specific volume. At 25 °C, the average slope $(\partial v°/\partial T)_P$ equal to the partial specific expansibility, $E°$, is $(1.27\pm0.03)\times10^{-3}$ cm^3 g^{-1} K^{-1}. This value is almost twice as high as $(0.68\pm0.03)\times10^{-3}$ cm^3 g^{-1} K^{-1}, the experimental value of the partial specific expansibility of apocytochrome *c*. To understand the origin of this disparity, recall that most atomic group are characterized by positive hydration contributions to expansibility [51,63]. Therefore, the observed disparity between the calculated and experimental partial specific expansibilities of apocytochrome *c* is consistent with the picture in which the protein is not fully extended but retains a considerable number of water-inaccessible atomic groups.

Inspection of Fig. 3 reveals that our calculated partial specific adiabatic compressibilities, $k°_S$, of fully extended polypeptides are significantly less negative than the previous estimates [5,14,22,23]. For example, at 25 °C, the average value of $k°_S$ is $-(5.9\pm0.4)\times10^{-6}$ cm^3 g^{-1} bar^{-1}. This value is more than twice as high as -13.5×10^{-6} cm^3 g^{-1} bar^{-1}, the average value reported by Kharakoz in what appears to be the most careful additive calculation reported to date [23]. The disparity reflects more negative contributions of the amino acid side chains (based on zwitterionic amino acid data) and the glycyl unit (based on old and rather inaccurate data on oligoglycines) used by Kharakoz [23]. All proteins studied in this work exhibit similar temperature dependences of partial specific adiabatic compressibility, $k°_S$; at 25 °C, the average value of $(\partial k°_S/\partial T)_P$ is equal to $(5.0\pm0.1)\times10^{-7}$ cm^3 g^{-1} bar^{-1} K^{-1}.

Within the entire temperature range studied, the calculated partial specific adiabatic compressibility, $k°_S$, of the fully extended state of apocytochrome *c* is lower (more negative) than the experimental value. For example, at 25 °C, the experimental value of $k°_S$ of apocytochrome *c* is -2.7×10^{-6} cm^3 g^{-1} bar^{-1}, a value typical of unfolded proteins [14,64,65]. At the same temperature, the calculated value of $k°_S$ is significantly lower and equals -6.9×10^{-6} cm^3 g^{-1} bar^{-1}. At room temperature, the majority of atomic groups exhibit negative hydration contributions to compressibility [57,66]. Consequently, judging by the differential compressibility (-2.7×10^{-6} *versus* -6.9×10^{-6} cm^3 g^{-1} bar^{-1}), apocytochrome *c* is not fully unfolded at our experimental conditions and retains a sizeable core of water-inaccessible atomic groups. This notion, which is in agreement with the expansibility data discussed above, is further supported by the differential temperature slopes of $k°_S$. Our calculated temperature dependence of $k°_S$ of apocytochrome *c* is steeper than the measured one. At 25 °C, the experimental value of $(\partial k°_S/\partial T)_P$ is 2.8×10^{-7} cm^3 g^{-1} bar^{-1} K^{-1} against the calculated value of 4.8×10^{-7} cm^3 g^{-1} bar^{-1} K^{-1}. Since most atomic group are characterized by positive temperature slopes of partial compressibility [57], the observed disparity is consistent with the protein being not fully extended and retaining a significant number of water-inaccessible atomic groups.

Our experimental data in conjunction with Eq. (8) can be used for studying pH-dependent protein behavior. As an example, Fig. 4 depicts our calculated pH-dependences of the partial specific volume (panel A) and adiabatic compressibility (panel B) of apomyoglobin at 25 °C. Fig. 4 also shows the experimental pH-dependences of $v°$ and $k°_S$ of apomyoglobin in 20 mM NaCl from our previous work [67]. At these conditions, the protein is native (N) at pH 6, while adopting a molten globule (MG) conformation at pH 4 and an unfolded (*U*) conformation at pH 2 [67]. As is seen from Fig. 4a, the partial specific volume of apomyoglobin in the fully extended conformation is 0.014 cm^3 g^{-1} smaller than that of the native state, while being close to that of the molten globule state (see the inset). The unfolded conformation exhibits a volume which is

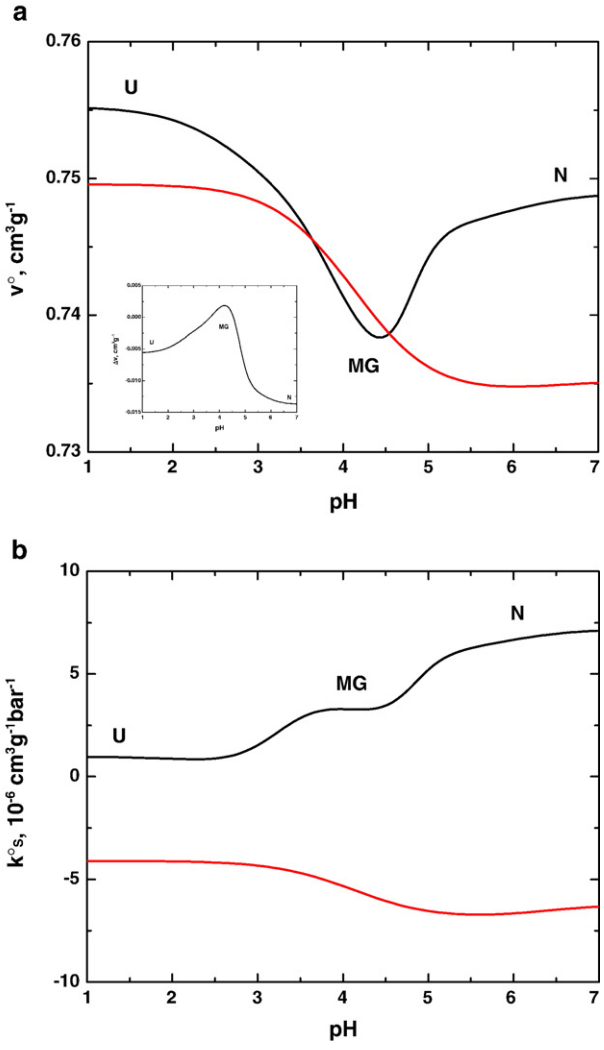

Fig. 4. Calculated (red) and experimental (black) pH-dependences of the partial specific volumes, $v°$(panel A), and adiabatic compressibilities, $k°_S$ (panel B), of apomyoglobin at 25 °C. Inset in panel A shows the difference between the calculated and experimental partial specific volumes of apomyoglobin as a function of pH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.006 $cm^3\ g^{-1}$ higher relative to the fully extended conformation, however the differential volume is less negative than for the native state. Inspection of Fig. 4b reveals that, as the protein denatures from the native to molten globule and further to unfolded conformation, the difference between its partial specific adiabatic compressibility and that of the fully extended conformation becomes smaller. However, even at pH 2, the unfolded state of apomyoglobin exhibits a higher partial specific adiabatic compressibility than its fully extended conformation. This observation is consistent with the picture in which the acid-induced unfolded state of apomyoglobin is not fully unfolded and retains a sizeable core of solvent-inaccessible atomic groups.

5. Conclusion

We determined the volumetric properties of *N*-acetyl amino acid amides, *N*-acetyl amino acid methylamides, and *N*-acetyl amino acids with neutralized carboxyl termini between 18 and 55 °C. The individual compounds in the three classes were selected so as to collectively cover the 20 naturally occurring amino acid side chains. We also determined changes in volume and compressibility accompanying the protonation/deprotonation reactions of amino acids with ionizable side chains. We rationalized our data in terms of the volumetric contributions and hydration properties of each individual amino acid side chain and its constituent atomic groups. In particular, we evaluated the contributions to interaction volumes, V_I, of all amino acid side chains. Based on the assumption that the interaction volumes, V_I, of uncharged side chains predominantly originate from solute–solvent hydrogen bonding, we estimated the effective number of solute–solvent hydrogen bonds, n_h, for each amino acid side chain. Our calculated values of n_h range from 1.6 to 7.5 and correlate with the number of polar atomic groups in the side chain. The compressibility contributions of the amino acid side chains cannot be unequivocally rationalized in an additive way as the sum of contributions of constituent atomic groups. The observed nonadditivity of the compressibility contributions of atomic groups in amino acid side chains may reflect a complex interplay between two factors. Firstly, the hydration shells of constituent atomic groups may overlap. Secondly, interatomic interactions between adjacent groups that do not necessarily result in significant hydration overlap may nevertheless mutually modulate the extent of solute–solvent interactions. The compressibility observable, in contrast to volume, is sensitive not only to the effective number of solute–solvent interactions, n_h, but also to the distribution of solute–solvent interactions in the statistical assembly of solvated solute species.

We used our data to develop an additive scheme that is further used to calculate the partial specific volumes, $v°$, and adiabatic compressibilities, $k°_S$, of the fully extended conformational states of ubiquitine, apocytochrome *c*, ribonuclease A, lysozyme, apomyoglobin, and α-chymotrypsinogen A as a function of temperature at pH 7. The unfolded proteins studied here exhibit similar partial specific adiabatic compressibilities, $k°_S$, and the temperature slopes of $v°$ and $k°_S$. At 25 °C, the average value of $k°_S$ is $-(5.9\pm0.4)\times10^{-6}$ $cm^3\ g^{-1}\ bar^{-1}$; the average slope $(\partial v°/\partial T)_P$ is $(1.27\pm0.003)\times10^{-3}$ $cm^3\ g^{-1}\ K^{-1}$; while the average value of $(\partial k°_S/\partial T)_P$ is $(5.0\pm0.1)\times10^{-7}$ $cm^3\ g^{-1}\ bar^{-1}\ K^{-1}$.

We calculated the values of $v°$ and $k°_S$ for the fully extended conformation of apomyoglobin as a function of pH at 25 °C. We compared the calculated values of $v°$ and $k°_S$ of the fully extended states of apocytochrome *c* and apomyoglobin with the experimental values of $v°$ and $k°_S$ of their unfolded states. Apocytochrome c is unfolded at native conditions (water at neutral pH), while apomyoglobin denatures to the molten globule state and further to the unfolded state upon a decrease in pH from 7 to 2. The comparison between the calculated and experimental values of $k°_S$, $(\partial v°/\partial T)_P$, and $(\partial k°_S/\partial T)_P$ suggests that neither apocytochrome *c* nor apomyoglobin are fully unfolded under the experimental conditions of our study and retain a sizeable core of solvent-inaccessible groups.

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