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Volumetric effects of ionization of amino and carboxyl termini of α,ω-aminocarboxylic acids

Nicolas Taulier, Tigran V. Chalikian*

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ont., Canada M5S 2S2

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Abstract

We have determined the partial molar volumes and adiabatic compressibilities of a homologous series of six α,ω-aminocarboxylic acids over a broad pH range at 25 °C. We interpret the resulting data in terms of the changes in hydration associated with neutralization of amino and carboxyl termini. By combining our volumetric results with pH-dependent data on 1-anilinonaphthalene-8-sulfonic acid fluorescence we propose the following explanation to the long-standing observation that changes in volume and compressibility accompanying neutralization of a carboxyl group depend on the type of the solute in contrast to solute-independent changes in these parameters accompanying neutralization of an amino group. Unlike amino groups, neutralized carboxyl groups are capable of forming hydrogen-bonded structures stabilized by hydrogen bonds between the carbonyl oxygen of one solute molecule and the hydroxyl group of another molecule. Formation of such hydrogen-bonded structures causes an additional decrease in solute hydration with concomitant increases in volume and compressibility. Furthermore, solutes with large aliphatic moieties may form larger associates stabilized, in addition to intermolecular hydrogen bonds, by hydrophobic interactions which will result in further increases in volume and compressibility. In the aggregate, our results emphasize the need for further studies focused on developing an understanding of the role of electrostatic interactions in stabilizing/destabilizing proteins and protein complexes.

Keywords: α,ω-Aminocarboxylic acids; Amino and carboxyl termini; State of ionization; Hydration; Compressibility; Volume

1. Introduction

Understanding the magnitude and relative importance of a variety of interactions governing the stability of proteins and protein complexes is a central topic of molecular biophysics [1–5]. In this respect, the role of electrostatic interactions is quite remarkable. On the one hand, the magnitude of free energy contribution (ΔG^0) of electrostatic interactions to protein stability is relatively modest constituting only a small fraction of the absolute value of other, more dominant contributions (e.g. hydrophobic interactions or configurational entropy) [1]. On the other hand, the ΔG^0 contribution of electrostatic interactions is comparable to the net stability of the protein system. Therefore,

*Corresponding author. Tel.: +1-416-946-3715; fax: +1-416-978-8511.

E-mail address: chalikan@phm.utoronto.ca (T.V. Chalikian).

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moderate changes in solution pH and/or ionic strength often result in drastic changes in the conformational state of the protein and/or dissociation of the protein complex.

There have been a large number of theoretical and experimental endeavors devoted to exploration of the role of charged groups in protein stability [6–12]. One aspect of these endeavors is related to studying pH-dependent modifications in protein hydration. Such modifications are associated with modulation of the state of ionization of titrable groups and may have marked effect on the energetics of protein stability and recognition [13]. Volumetric observables such as volume and compressibility have proven uniquely sensitive to the hydration properties of solutes and, more importantly, to changes in solute hydration upon various events including a change in the state of ionization of a titrable group [14–23]. Consequently, volumetric measurements have been widely applied to investigating hydration changes accompanying neutralization of positively charged amino ($-NH_3^+$) and negatively charged carboxyl ($-COO^-$) groups [24–37].

Neutralization of amino and carboxyl groups in aqueous solutions are given by the following reactions:

$$-NH_3^+ + OH^- \rightleftharpoons -NH_2 + H_2O \quad (1)$$

$$-COO^- + H^+ \rightleftharpoons -COOH \quad (2)$$

On the qualitative level, changes in volume, ΔV, and adiabatic compressibility, ΔK_S, associated with Eqs. (1) and (2) are positive indicating a net decrease in solute hydration upon neutralization of amino and carboxyl groups [24–37]. However, on the quantitative level, there are a number of unanswered questions regarding the fact that the same ionizable group within different chemical structures may exhibit significantly different values of ΔV and ΔK_S. Such disparities complicate identification of model compounds that can be used for mimicking pH-dependent ionization/neutralization equilibria of titrable protein groups. The biophysical importance of such studies is underscored by an everincreasing number of volumetric characterizations of pH-induced conformational transitions of proteins [23,38–47]. In such studies, it is often required to consider the volumetric contributions of neutralization of titrable protein groups, which can only be done, based on low molecular weight model compound-based data.

Another intriguing observation yet to be rationalized is related to neutralization of carboxyl groups. Specifically, for this process, the values of ΔV and ΔK_S tend to increase when the solute's aliphatic moiety becomes bulkier. For example, at 25 °C, the values of ΔV accompanying neutralization of the carboxyl terminus of formic, acetic, propionic, butiric, valeric, and hexanoic acids are 8.5, 11.3, 13.0, 13.9, 14.2 and 14.2 $cm^3\,mol^{-1}$, respectively [27]. For the same compounds at 25 °C, the values of ΔK_S are 12.0×10^{-4}, 18.0×10^{-4}, 25.0×10^{-4}, 28.0×10^{-4}, 30.0×10^{-4} and 30.0×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$, respectively [27]. Similar correlations have been found for neutralization of carboxyl groups in homologous series of other organic compounds that exhibit an enlargement of aliphatic portions [24,28,30,31,33,37]. Significantly, no such observations have been made for neutralization of an amino group, $-NH_3^+$. Referring to the ionization volume of carboxyl groups, Asano and le Noble [48] have pointed out in their monumental review that 'the nature of these deviations is not known; any theory to account for it should explain why the effect of small alkyl groups on the ionization volume does not apply to amines'.

To answer these questions, we have conducted pH-dependent measurements of density and sound velocity aimed at determining the changes in volume and adiabatic compressibility associated with neutralization of the amino and carboxyl termini in a homologous series of α,ω-aminocarboxylic acids. At neutral pH, these zwitterionic molecules consist of oppositely charged amino and carboxyl termini separated by a nonbranched chain of methylene, $-CH_2-$, groups. This is the third work from our group in which we use α,ω-aminocarboxylic acids as a model system for studying the hydration properties of protein groups. In our previous investigations, we have studied the hydration of aliphatic and charged atomic groups in H_2O ad D_2O [49,50]. In this work, we expand these explorations to investigate changes in hydra-

tion of α,ω-aminocarboxylic acids associated with neutralization of their amino and carboxyl termini.

2. Materials and methods

All the α,ω-aminocarboxylic acids used were of the highest purity commercially available and were used without further purification. Specifically, β-alanine, 4-aminobutanoic acid, 5-aminopentanoic acid, 6-aminohexanoic acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid were purchased from Sigma-Aldrich Canada (Mississauga, Ont., Canada). Solutions of HCl and NaOH were purchased from BDH Inc. (Toronto, Ont., Canada) and Fisher Scientific Canada (Nepean, Ont., Canada), respectively, while 1-anilinonaphthalene-8-sulfonic acid (ANS) was obtained from Sigma-Aldrich Canada. Solutions of α,ω-aminocarboxylic acids were prepared using doubly distilled degassed water. 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 α,ω-aminocarboxylic acids were dried for several days under vacuum in the presence of phosphorus pentoxide.

All the densimetric and ultrasonic measurements reported here were conducted at a temperature of 25 °C. Solution sound velocities and absorptions were measured using a differential technique and a previously described resonator method at a frequency of 7.2 MHz [51–54]. 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. The accuracy of all the sound velocity relative measurements achieved with this procedure is approximately $\pm 10^{-4}$%, while the accuracy of the relative sound absorption measurements is ± 2% [55,56].

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. Acoustic titration experiments were performed by adding equal aliquots of 0.1 or 1 M HCl or NaOH solutions to both the sample and the reference cells filled with the same volume of 0.80 cm^3 of the α,ω-aminocarboxylic acid solution and water, respectively. Additions were made using microliter 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.

All densities were measured with a precision of $\pm 1.5\times 10^{-6}$ g cm^{-3} using a vibrating tube densimeter (DMA-60, Anton Paar, Graz, Austria). The apparent molar volumes, ϕV, of α,ω-aminocarboxylic acids were then calculated from the following relationship [57]:

$$\phi V = M/\rho - (\rho-\rho_0)/(\rho\rho_0 m) \quad (3)$$

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 molal concentration of the solute.

The volume change, ΔV, accompanying neutralization of the carboxyl or amino terminus of the α,ω-aminocarboxylic acids was calculated using the following equation:

$$\Delta V = \left[(\rho-\rho_0) - (\rho_{\text{solution}} - \rho_{\text{solvent}}) \times (1+V'/V_0)\right]/(\rho_0 C_0) \quad (4)$$

where V_0 is the initial volume of the α,ω-aminocarboxylic acid 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 α,ω-aminocarboxylic acid solution and water,

respectively; $\rho_{solution}$ and $\rho_{solvent}$ are, respectively, the densities of the α,ω-aminocarboxylic acid solution and the solvent to which the same volume of the HCl or NaOH solution has been added.

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 α,ω-aminocarboxylic acids using the relationship [58,59]:

$$\phi K_S = \beta_{S0}(2\phi V - 2[U] - M/\rho_0) \quad (5)$$

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

Differentiating Eq. (5) yields the expression

$$\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U]) \quad (6)$$

where ΔV and $\Delta[U]$ are, respectively, the changes in the volume and in the relative molar sound velocity increment of an α,ω-aminocarboxylic acid upon neutralization of its carboxyl or amino group. This relationship allows one to calculate the adiabatic compressibility change, ΔK_S, accompanying neutralization of the carboxyl or amino group of an α,ω-aminocarboxylic acid.

The pH of the α,ω-aminocarboxylic 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 1–3 mg ml^{-1} for each of the solutes studied. The concentration dependences of the apparent molar volumes and adiabatic compressibilities of α,ω-aminocarboxylic acids are negligible in the range of concentrations used in the present work [32,34]. Within the limits of experimental error, the apparent molar volumes, ϕV, and adiabatic compressibilities, ϕK_S, we have determined in the concentration range of 1–3 mg ml^{-1} coincide with the values of the partial molar volume, V^0, and the adiabatic compressibility, K_S^0, obtained by extrapolation to infinite dilution. Therefore, below, we do not discriminate between the apparent and molar volumetric characteristics of the α,ω-aminocarboxylic acids.

ANS emission fluorescence measurements were performed as a function of pH in a 1 cm pathlength cuvette at 25 °C using an AVIV model ATF 105 spectrofluorometer. The ANS samples were excited at 370 nm with the emission light intensity recorded at 515 nm. ANS at a concentration of 0.01 mM was dissolved in a 15-mM solution of 8-aminooctanoic acid, and HCl titration was carried out in the fluorescence cuvette. To take into account any possible pH-dependent change in ANS fluorescence that is not related to its interaction with the α,ω-aminocarboxylic acid, we also performed an identical HCl titration of 0.01 mM solution ANS in water. The resulting pH-dependent emission spectrum of free ANS was subsequently subtracted from that of the ANS–8-aminooctanoic acid mixture. When calculating the final titration profile of the mixture, changes in ANS concentration resulting from the addition of HCl aliquots have been taken into account.

3. Results

3.1. pH-induced changes in volume

Fig. 1 presents the pH-dependences of the partial molar volume, V^0, of the α,ω-aminocarboxylic acids. We fit these dependences by the following analytical functions:

$$V^0 = V^0_{neut} + \Delta V_{OH}/(1 + 10^{pK_a - pH}) \quad (7a)$$

$$V^0 = V^0_{neut} + \Delta V_{H}/(1 + 10^{pH - pK_a}) \quad (7b)$$

where V^0_{neut} is the partial molar volume of an α,ω-aminocarboxylic acid at neutral pH; ΔV_{OH} is the change in volume upon deprotonation of the amino terminus at alkaline pH; ΔV_H is the change in volume upon protonation of the carboxyl terminus at acidic pH; and pK_a is the dissociation constant of the amino (Eq. (7a)) or carboxyl (Eq. (7b)) terminus. Eq. (7a) is used for treating the data on neutralization of the amino terminus at alkaline pH, whereas Eq. (7b) is used for treating the data on neutralization of the carboxyl terminus at acidic pH. From the fit of the pH-dependences shown in Fig. 1 by Eqs. (7a) and (7b), we determine the values of ΔV_{OH}, ΔV_H and pK_a for each protonation and deprotonation reaction studied here.

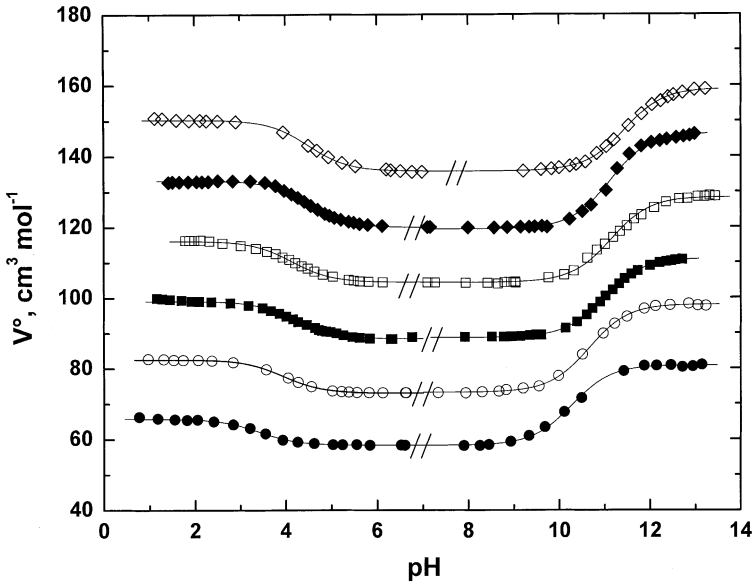

Fig. 1. The pH dependences of the partial molar volumes of β-alanine (●), 4-aminobutanoic acid (○), 5-aminopentanoic acid (■), 6-aminohexanoic acid (□), 7-aminoheptanoic acid (♦), and 8-aminooctanoic acid (◇). The fitting of the experimental data (solid lines) was accomplished using Eqs. (7a) and (7b), as explained in text.

The results of the analysis for ΔV_{OH} and ΔV_H are given in the second columns of Tables 1 and 2. Fig. 2 shows the dependence of ΔV_H on the number of $-CH_2-$ groups in α,ω-aminocarboxylic acids from this work (●) along with literature data on similar dependences for homologous series of α,ω-aminocarboxylic acids determined by Lepori and Mollica [60] (■) and Shahidi [61] (♦), α-carboxylic acids determined by Høiland [27] (□) and Srivastava et al. [62] (○), and dicarboxylic acids determined by Høiland [28] (◇).

3.2. pH-induced changes in sound velocity and absorption

Figs. 3 and 4 present, respectively, the pH-dependences of the relative molar sound velocity increment, $[U]$, and molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$, of the α,ω-

Table 1
The changes in the relative molar sound velocity increment, $\Delta[U]_{OH}$, volume, ΔV_{OH}, and adiabatic compressibility, ΔK_{SOH}, accompanying neutralization of the amino terminus in α,ω-aminocarboxylic acids at 25 °C

	ΔV_{OH} $cm^3\ mol^{-1}$	$\Delta[U]_{OH}$ $cm^3\ mol^{-1}$	ΔK_{SOH} $10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$
Glycine[a]	23±1	−39±1	56±2
β-Alanine	22±1	−31±1	48±2
4-Aminobutanoic acid	24±1	−34±1	54±2
5-Aminopentanoic acid	22±1	−37±1	55±2
6-Aminohexanoic acid	24±1	−38±1	56±2
7-Aminopentanoic acid	25±1	−37±1	56±2
8-Aminooctanoic acid	23±1	−37±1	54±2

[a] From Ref. [36].

Table 2
The changes in the relative molar sound velocity increment, $\Delta[U]_H$, volume, ΔV_H, and adiabatic compressibility, ΔK_{SH}, accompanying neutralization of the carboxyl terminus in α,ω-aminocarboxylic acids at 25 °C

	ΔV_H $cm^3\ mol^{-1}$	$\Delta[U]_H$ $cm^3\ mol^{-1}$	ΔK_{SH} $10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$
Glycine[a]	6±1	−2±1	7±2
β-Alanine	7.5±1	−1.5±1	8±2
4-Aminobutanoic acid	9.5±1	−9±1	17±2
5-Aminopentanoic acid	11±1	−11.5±1	20.5±2
6-Aminohexanoic acid	12±1	−18±1	27±2
7-Aminopentanoic acid	13±1	−20±1	32.5±2
8-Aminooctanoic acid	14.5±1	−24±1	34.5±2

[a] From Ref. [36].

aminocarboxylic acids. The pH-dependent change in $[U]$ associated with neutralization of an ionizable group can be presented as a sum of three terms:

$$[U] = [U]_{neut} + \Delta[U]_{hyd} + \Delta[U]_{rel} \quad (8)$$

where $[U]_{neut}$ is the relative molar sound velocity increment of an α,ω-aminocarboxylic acid at neutral pH; $\Delta[U]_{hyd}$ is the hydrational component of a change in $[U]$ which is due to a pH-induced change in solute hydration accompanying deprotonation of the amino terminus or protonation of the carboxyl terminus; and $\Delta[U]_{rel}$ is the relaxation contribution to $[U]$ caused by proton transfer reactions which accompany protonation/deprotonation of amino and carboxylic groups [36,63–74].

The pH-dependence of $\Delta[U]_{hyd}$ can be approximated by the following functions:

$$\Delta[U]_{hyd} = \Delta[U]_{OH}/(1 + 10^{pK_a - pH}) \quad (9a)$$

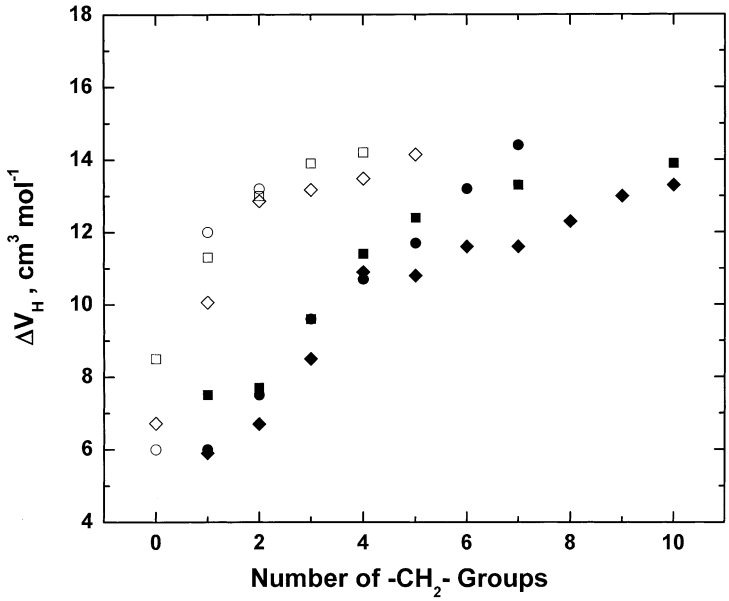

Fig. 2. The dependences on the number of –CH_2– groups of changes in volume accompanying neutralization of carboxyl termini in α,ω-aminocarboxylic acids (this work (●); Ref. [60] (■); and Ref. [61] (♦)), α-carboxylic acids (Ref. [27] (□); and Ref. [62] (○)), and dicarboxylic acids (Ref. [28] (◇)).

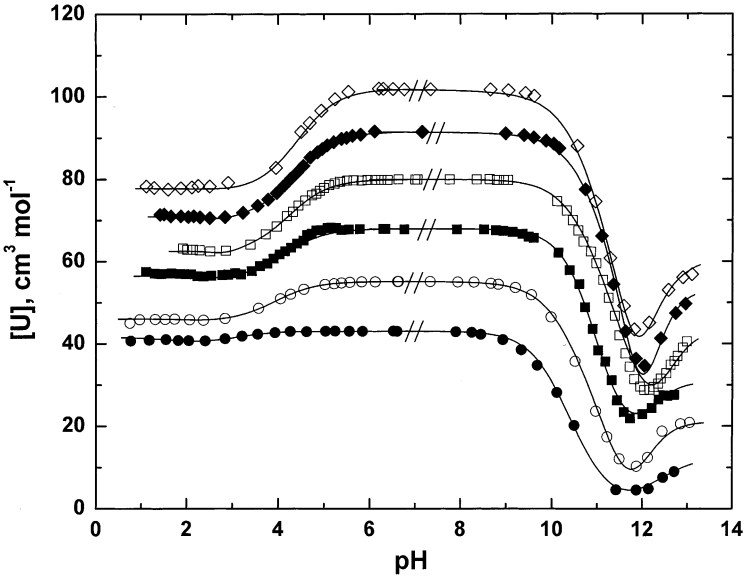

Fig. 3. The pH dependences of the relative molar sound velocity increments of β-alanine (●), 4-aminobutanoic acid (○), 5-aminopentanoic acid (■), 6-aminohexanoic acid (□), 7-aminoheptanoic acid (♦), and 8-aminooctanoic acid (◇). The fitting of the experimental data (solid lines) was accomplished using Eqs. (8), (9a), (9b), (10) and (11), as explained in text.

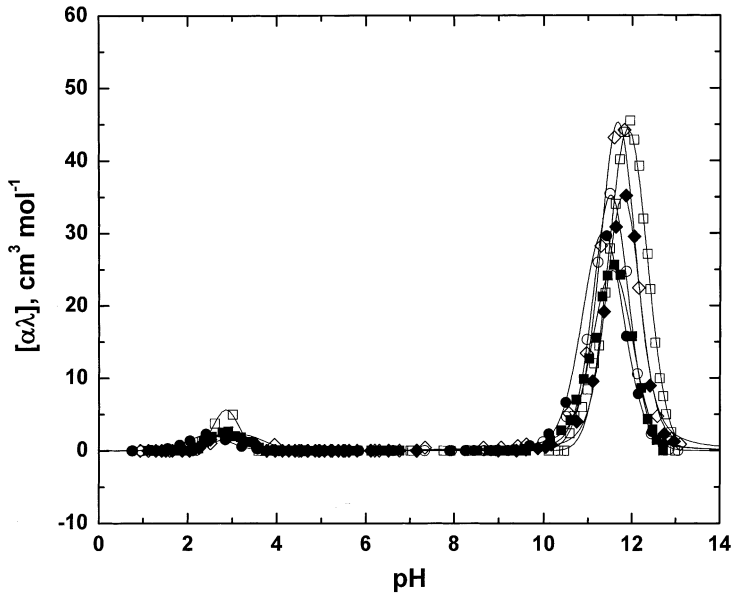

Fig. 4. The pH dependences of the molar increments of ultrasonic absorption per wavelength of β-alanine (●), 4-aminobutanoic acid (○), 5-aminopentanoic acid (■), 6-aminohexanoic acid (□), 7-aminoheptanoic acid (♦), and 8-aminooctanoic acid (◇). The fitting of the experimental data (solid lines) was accomplished using Eqs. (10), (10a), (10b), (10c) and (10d), as explained in text.

$$\Delta[U]_{hyd} = \Delta[U]_H/(1+10^{pH-pK_a}) \quad (9b)$$

$\Delta[U]_{OH}$ and $\Delta[U]_H$ are the changes in the relative molar sound velocity increment upon deprotonation of the amino terminus and protonation of the carboxyl terminus, respectively.

Recall that proton transfer reactions which accompany protonation/deprotonation of titrable groups result in a relaxation increase in ultrasonic absorption which, if expressed per wavelength, $\Delta(\alpha\lambda)$, can be calculated using the relationship [36,63–74]:

$$\Delta(\alpha\lambda) \approx (\pi\Delta V^2\Gamma)/(\beta_{S0}RT)\left[\omega\tau/(1+\omega^2\tau^2)\right] \quad (10)$$

where ω is the angular frequency of ultrasound; ΔV is the reaction volume; τ is the relaxation time of the proton transfer reaction; and Γ is a concentration dependent coefficient. For Eq. (1), Γ and τ are given by the equations:

$$\Gamma^{-1} = \left[(2+10^{pH-pK_a}+10^{pK_a-pH})/C\right] + 10^{14-pH} \quad (10a)$$

$$\tau^{-1} = k_f\left[C/(1+10^{pH-pK_a}) + 10^{pH-14} + 10^{pK_a-14}\right] \quad (10b)$$

where k_f is the rate constant for the forward reaction.

For Eq. (2), Γ and τ can be calculated from the relationships:

$$\Gamma^{-1} = \left[(2+10^{pH-pK_a}+10^{pK_a-pH})/C_k\right] + 10^{pH} \quad (10c)$$

$$\tau^{-1} = k_f\left[C/(1+10^{pK_a-pH}) + 10^{-pH} + 10^{-pK_a}\right] \quad (10d)$$

The relaxation term, $\Delta[U]_{rel}$, in Eq. (8) can be calculated from the molar increment of ultrasonic absorption per wavelength, $[\alpha\lambda]$, using the expression [64]:

$$\Delta[U]_{rel} = -[\alpha\lambda]/(2\pi\omega\tau) \quad (11)$$

We analyse these acoustic data using a previously described method, in which the relaxation characteristics of a proton transfer reaction were determined from ultrasonic velocity and absorption measured as a function of pH at a single frequency [36,75]. We use this method in conjunction with our measured pH-dependences of $[U]$ (Fig. 3) and $[\alpha\lambda]$ (Fig. 4) to calculate the relaxation contribution of $\Delta[U]_{rel}$ as a function of pH. Subtracting $\Delta[U]_{rel}$ from $([U]-[U]_{neut})$ (Eq. (8)), we evaluate $\Delta[U]_{hyd}$ that we further approximate using Eqs. (9a) and (9b). Based on this approximation, we calculate the values of $\Delta[U]_{OH}$ and $\Delta[U]_H$ that are presented in the third columns of Tables 1 and 2, respectively. In these calculations, we use the values of pK_a determined from analysing the partial molar volume data.

3.3. pH-induced changes in adiabatic compressibility

The values of ΔV_{OH}, ΔV_H, $\Delta[U]_{OH}$ and $\Delta[U]_H$ have been used in conjunction with Eq. (6) to calculate the changes in adiabatic compressibility accompanying neutralization of the amino, ΔK_{SOH}, and carboxyl, ΔK_{SH}, termini of the α,ω-aminocarboxylic acids. The results of these calculations are given in the fourth columns of Table 1 (ΔK_{SOH}) and Table 2 (ΔK_{SH}). Fig. 5 shows the dependence of ΔK_{SH} on the number of $-CH_2-$ groups in α,ω-aminocarboxylic acids from this work (●), along with literature data for a similar dependence for a homologous series of α-carboxylic acids determined by Høiland [27] (○).

3.4. pH-dependence of ANS fluorescence in the solution of 8-aminooctanoic acid

Fig. 6 depicts the pH-dependence of the differential fluorescence emission intensity of ANS in a solution of 8-aminooctanoic acid and water. Inspection of Fig. 6 reveals that the fluorescence intensity of ANS sigmoidally increases with a decrease in pH. The observed increase in fluorescence intensity suggests that the microenvironment of ANS becomes increasingly nonpolar when the solution pH decreases from neutral to acidic.

4. Discussion

The partial molar volume of a solute, V^0, can be interpreted in terms of the intrinsic and hydra-

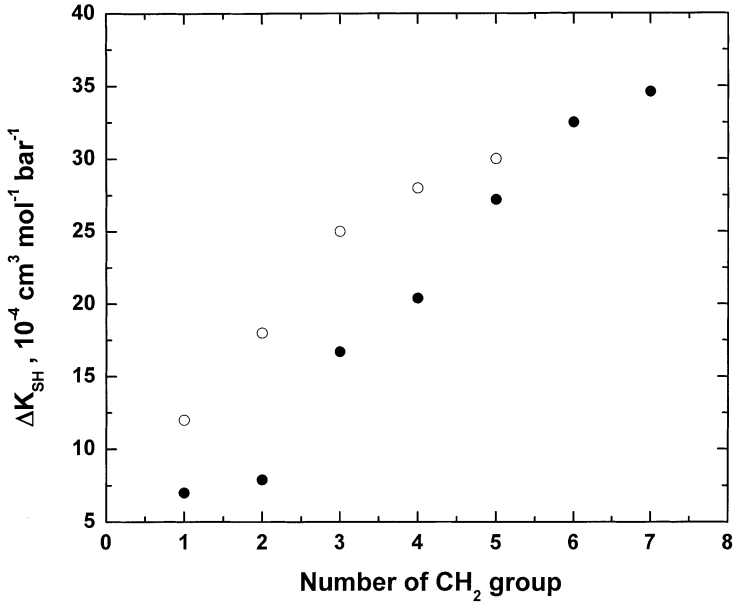

Fig. 5. The dependences on the number of $-CH_2-$ groups of changes in adiabatic compressibility accompanying neutralization of carboxyl termini in α,ω-aminocarboxylic acids (this work (●)) and α-carboxylic acids (from Ref. [27] (○)).

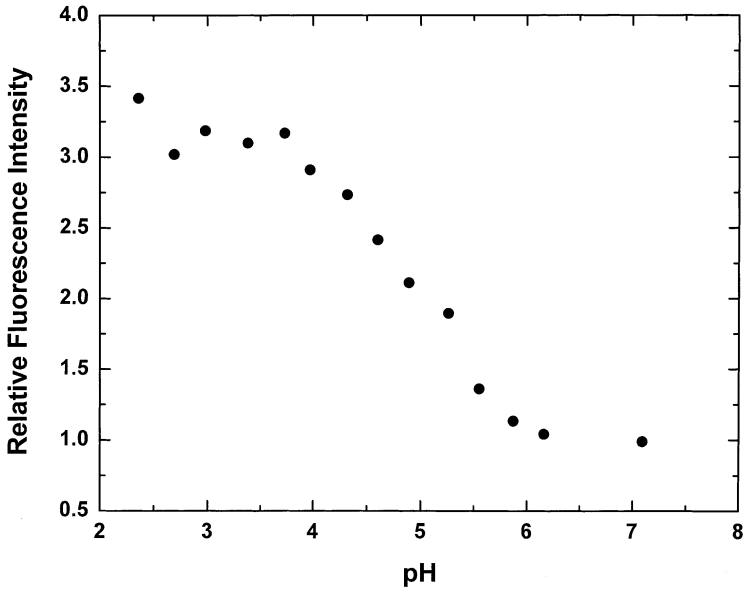

Fig. 6. The pH dependence of the differential fluorescence intensity of ANS in a solution of 8-aminooctanoic acid and water at 515 nm. The excitation wavelength is 370 nm.

tion contributions based on concepts of scaled particle theory [76–79]:

$$V^0 = V_M + V_T + V_I + \beta_{T0}RT \quad (12)$$

where V_M is the intrinsic volume of a solute molecule; V_T is the 'thermal' volume, which is the volume of the void space surrounding the solute molecule as a result of thermally induced mutual molecular vibrations of the solute and the solvent; V_I is the 'interaction volume' which represents the change in the solvent volume under the influence of solute–solvent interactions; β_{T0} is the coefficient of isothermal compressibility of the solvent; R is the universal gas constant; and T is the absolute temperature. It is customarily assumed that V_I predominantly reflects a decrease in the solvent volume resulting from hydration of polar and charged solute groups. The ideal term, $\beta_{T0}RT$, describes the volume effect related to the kinetic contribution to the pressure of a solute molecule due to its translational degrees of freedom.

The intrinsic volume, V_M, of a small solute is roughly equal to its van der Waals volume, V_W. The thermal volume can be conceptually presented as a layer of 'empty' space surrounding the solute. The thickness of the layer, δ, as a first approximation, should not depend on the chemical nature of the solvent-exposed atomic groups of a solute [79,80]. At 25 °C, various estimates have yielded the values of δ ranging from 0.50 to 0.56 Å [79–81]. Thus, as a first approximation, the thermal volume is determined by the geometric properties of the solute.

Eq. (1) or Eq. (2) should not result in any significant alterations in V_M and V_T, since changes in the state of ionization of the termini do not cause any appreciable alterations in the shape or the van der Waals volume of α,ω-aminocarboxylic acid. Therefore, ΔV_{OH} and ΔV_H mainly reflect the pH-dependent changes in the interaction volume, V_I, that is determined by the nature of solute–solvent interactions. However, the situation may dramatically change if neutralization of the carboxyl terminus of a solute results in its aggregation (see below). In this event, in addition to V_I, the values of V_M and V_T can also be affected.

The partial molar adiabatic compressibility, K_S^0, of a solute is conventionally presented as the sum of the intrinsic, K_M, and hydration, ΔK_h, contributions:

$$K_S^0 = K_M + \Delta K_h = K_M + n_h(K_h - K_0) \quad (13)$$

where K_M is the intrinsic compressibility of a solute molecule; K_h and K_0 are the partial molar adiabatic compressibilities of water of hydration and bulk water, respectively; and n_h is the hydration number that refers to the number of water molecules incorporated into the solute hydration shell.

The intrinsic compressibility, K_M, of a low molecular weight compounds is small and, usually, can be neglected [17,19–23]. Consequently, changes in adiabatic compressibility associated with Eqs. (1) and (2) (ΔK_{SOH} and ΔK_{SH}) mainly reflect changes in the hydration contribution, ΔK_h. However, should neutralization of the carboxyl terminus cause solute aggregation, the intrinsic compressibility, K_M, may become substantial (see below). This additional increase in K_M results from the presence of highly compressible voids between loosely packed monomeric subunits within the micelle-like core of the aggregates. Clearly, the compressibility of oligomeric α,ω-aminocarboxylic acids with the neutralized carboxyl termini will be significantly greater than that of monomers.

4.1. Neutralization of the amino terminus

Inspection of data in Table 1 reveals that neutralization of the amino terminus, $-NH_3^+$, of an α,ω-aminocarboxylic acid causes an increase in volume, ΔV_{OH}, and adiabatic compressibility, ΔK_{SOH}, that do not strongly depend on the number of methylene $-CH_2-$ groups in the solute molecule. This is an intriguing observation, since the amino and carboxyl termini of the α,ω-aminocarboxylic acids shorter than 6-aminohexanoic acid interact with each other via overlap of their hydration shells [49]. Apparently, some fortuitous compensation between various hydration effects causes the values of ΔV_{OH} and ΔK_{SOH} to be independent of the solute length.

The average change in volume, ΔV_{OH}, is 23 ± 1 $cm^3\ mol^{-1}$, while the average change in adiabatic compressibility, ΔK_{SOH}, is $(54 \pm 2) \times 10^{-4}$

$cm^3\,mol^{-1}\,bar^{-1}$. The observed independence of ΔV_{OH} and ΔK_{SOH} of the number of –CH_2– groups is consistent with previous reports [25,33]. In particular, based on the investigations of a large number of small model compounds, Kauzmann et al. [25] arrived at the conclusion that the value of ΔV_{OH} for neutralization of an amino group depends insignificantly on the number and nature of alkyl groups present in the solute molecule.

In this respect, it is instructive to compare the average values of ΔV_{OH} (23 ± 1 $cm^3\,mol^{-1}$) and ΔK_{SOH} [$(54\pm2)\times10^{-4}$ $cm^3\,mol^{-1}\,bar^{-1}$] determined in this work with similar data previously obtained for neutralization of the amino terminus of triglycine [37]. The values of ΔV_{OH} and ΔK_{SOH} associated with neutralization of the amino terminus of triglycine are equal to 26.5 ± 0.5 $cm^3\,mol^{-1}$ and $(67.5\pm0.9)\times10^{-4}$ $cm^3\,mol^{-1}\,bar^{-1}$, respectively [37]. These values are ~20% larger than those observed for neutralization of the amino terminus of the α,ω-aminocarboxylic acids. This disparity cannot be explained by intertermini interactions, since the two termini in both triglycine and α,ω-aminocarboxylic acids longer than 6-aminohexanoic acid are independently hydrated [49,82]. The observed disparity probably reflects 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. In fact, we propose that the peptide group in triglycine that is closest to the amino terminus may preferentially enhance the hydration of its ionized form. Such an enhancement may occur by additional orientation of water molecules simultaneously solvating the positively charged amino terminus and the adjacent polar peptide group. Mutual enhancement of hydration of closely located hydrophilic groups has been previously reported for a large number of low molecular weight model compounds [17,79,83–86].

One objective of such simple molecule-based studies is to identify the best system for modeling changes in the state of ionization of titrable groups in proteins. Such changes may occur in response to an alteration in the solution pH or a change in the microenvironment of titrable groups. The latter may occur due to a conformational transition of the protein or binding of a ligand or other macromolecule to the protein. The observed disparity between the changes in volumetric parameters accompanying neutralization of independently hydrated amino termini in triglycine and α,ω-aminocarboxylic acids reveals complexities related to modeling changes in the state of ionization of titrable protein groups. It is not yet possible unambiguously to assign the values of ΔV_{OH} and ΔK_{SOH} to neutralization of positively charged protein residues, such as lysine, arginine, and histidine. In this respect, recall that, four decades ago, Rasper and Kauzmann [24] discovered that the addition of base to a protein solution causes a volume increase of the magnitude of 16–18 $cm^3\,mol^{-1}$ which is only approximately two thirds of the value observed for neutralization of amino and imidazole groups in simple molecules. The authors rationalized this observation by proposing that ‘the environments of these groups in proteins must be somewhat different from that in small molecules’ [24]. An alternative explanation may be related to the growing body of evidence suggesting that charged groups in proteins are not fully exposed to solvent as was previously thought [10]. Consequently, hydration of charged protein groups, as well as changes in hydration accompanying neutralization of these groups, may be reduced when compared to the same groups in small molecules.

4.2. Neutralization of the carboxyl terminus

Inspection of Table 2 reveals that, in contrast to ΔV_{OH} and ΔK_{SOH}, increases in volume, ΔV_H, and adiabatic compressibility, ΔK_{SH}, accompanying neutralization of the carboxyl terminus of α,ω-aminocarboxylic acids depend on the number of –CH_2– groups. Specifically, the values of ΔV_H and ΔK_{SH} increase with increasing the number of –CH_2– groups. These trends are graphically illustrated in Figs. 2 and 5. The value of ΔV_H increases from 6 $cm^3\,mol^{-1}$ for glycine to 14.5 $cm^3\,mol^{-1}$ for 8-aminooctanoic acid, while the value of ΔK_{SH} increases from 7×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ for glycine to 34.5×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ for 8-aminooctanoic acid. As mentioned in Section 1,

similar observations have been made for neutralization of carboxyl groups in other solutes with sizeable aliphatic moieties [24,27,28,30,31,33,37]. For example, changes in volume, ΔV_H, and adiabatic compressibility, ΔK_{SH}, accompanying neutralization of the carboxyl terminus of triglycine are 10.5 $cm^3\ mol^{-1}$ and 18.4×10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$, respectively, while increasing to 13.5 $cm^3\ mol^{-1}$ and 25.1×10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$, respectively, for GlyGlyIle in which a bulky aliphatic side chain is adjacent to the carboxyl terminus [37].

Further inspection of the data in Table 2 reveals that the values of ΔV_H and ΔK_{SH} continue to increase even beyond 6-aminohexanoic acid when the two termini become independently hydrated and cease interacting with one another [49]. To rationalize these intriguing observations, we propose that α,ω-aminocarboxylic acids with neutralized carboxyl termini form hydrogen-bonded structures that are stabilized by intermolecular hydrogen bonds between the carbonyl oxygen, =O, of one solute molecule and the hydroxyl group, –OH, of another molecule. This notion is in line with the well-known fact that neutral forms of carboxylic acids, R–COOH, are capable of forming hydrogen-bonded dimers [87–90]. We further propose that the longer homologues with larger aliphatic moieties form micelle-like oligomeric aggregates. These aggregates are stabilized by hydrophobic interactions in addition to the hydrogen bonds between neutralized carboxyl groups. Formation of such aggregates influences the values of ΔV_H and ΔK_{SH} in two ways. Firstly, aggregation causes a decrease in solute hydration due to the burial from the solvent of previously solvent-exposed atomic groups. A decrease in solute hydration, especially, burial of polar groups, brings about increases in the partial molar volume and adiabatic compressibility of α,ω-aminocarboxylic acid species with neutralized carboxyl termini with concomitant increases in ΔV_H and ΔK_{SH}. Secondly, micelle-like aggregates possess bulky solute-inaccessible intrinsic cores of loosely packed monomeric subunits which are highly compressible. Thus, the intrinsic compressibility, K_M, of the solute increases which, in turn, causes an increase in the overall partial molar adiabatic compressibility of α,ω-aminocarboxylic acid species with neutralized carboxyl termini (Eq. (13)). As a result, the value of ΔK_{SH} increases even to a greater extent.

These rationalizations are in qualitative agreement with our pH-dependent data on ANS fluorescence intensity in the solution of 8-aminooctanoic acid presented in Fig. 6. Recall that ANS is a fluorescence dye that exhibits a significant increase in its fluorescence intensity upon a change in its microenvironment from polar to nonpolar. Inspection of Fig. 6 reveals that the ANS fluorescence intensity in the solution of 8-aminooctanoic acid sigmoidally increases upon a decrease in pH. This observation is consistent with the binding of ANS to the hydrophobic core of micelle-like oligomeric aggregates formed by the molecules of 8-aminooctanoic acid with neutralized carboxyl termini.

In the aggregate, independent of the veracity of our ‘explanations’, experimental data presented in this work suggest that the longer the α,ω-aminocarboxylic acid homologue the stronger the volumetric effects of neutralization of its carboxyl terminus. The proposal that the observed anomalies of neutralization of the carboxyl terminus may be related to solute aggregation, while qualitatively rationalizing our volumetric and spectroscopic data, is somewhat speculative in nature. Clearly, additional explorations are required to further unveil the origins of the observed anomalies. One viable strategy is to carry out concentration-dependent and pH-dependent studies of neutralization of carboxyl termini of α,ω-aminocarboxylic acids, including a determination of the dissociation constants (pK_a) and neutralization-induced changes in volume and compressibility. Such concentration-dependent studies may allow evaluation of many aggregation-related characteristics including the critical micelle concentrations of the longer α,ω-aminocarboxylic acid homologues.

4.3. Modeling neutralization of ionizable groups of proteins

Results of this work in conjunction with literature data suggest that amino, $-NH_3^+$, and carboxyl,

–COO^-, groups in different compounds may produce significantly different changes in volume and compressibility upon their neutralization. These discrepancies may be due to the proximity of other functional groups or be related to aggregation of neutralized species. Given these complexities, it is clear that we still lack the understanding required for modeling changes in volumetric parameters associated with neutralization/ionization of titrable amino acid residues in proteins. This deficiency emphasizes the need for further investigations. One strategy is to expand pH-dependent volumetric studies to proteins that do not undergo conformational transitions at extremes of pH. Such work is in progress using the densimetric and ultrasonic velocimetric measurements employed here.

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