# Temperature dependences of the acoustic nonlinearity parameter B/A of aqueous solutions of amino acids

T. V. Chalikian
*Yerevan Medical Institute, Department of Physics, Kirova 2, Yerevan, Armenia, USSR 375025*

A. P. Sarvazyan
*Institute of Biological Physics, Academy of Science USSR, Pushchino, Moscow Region, USSR 142292*

Th. Funck
*Max-Planck-Institute of Biophysical Chemistry (Karl-Friedrich-Bonhoeffer-Institute), D34, Göttingen, West Germany*

V. N. Belonenko
*NPO "Soyuzgaztekhnologiya," Razvilka, Moscow Region, USSR 142717*

F. Dunn
*Bioacoustics Research Laboratory, University of Illinois, 1406 West Green Street, Urbana, Illinois 61801*

A differential technique for evaluating the molar increments of the nonlinearity parameter $B/A$ has been employed for investigations of aqueous solutions of the amino acids glycine, alanine, norvaline, norleucine, arginine monohydrochloride, and lysine monohydrochloride in the temperature range 18 °C–45 °C. Some regularities of temperature dependencies of the nonlinearity parameter $B/A$ of hydration shells of charged and aliphatic groups have been found. Contributions of these groups to the value of $B/A$ molar increments of solute versus temperature have been estimated.

PACS numbers: 43.25.Ba, 43.80.Cs

## INTRODUCTION

The results of investigations of the nonlinear acoustic properties of biological media in the last decade are gaining interest for solving biomedical and physicochemical problems[1-12] The acoustic nonlinearity parameter $B/A$ is an independent acoustical characteristic that can be used to describe a medium together with attenuation, ultrasonic velocity, and impedance.[9]

Previous investigations of the nonlinearity parameter of solutions of biological compounds show that $B/A$ depends on the concentration of solute,[3,4,6,12,13] on the type of solute,[5,6,12,13] and on the character of the solute–solvent interactions.[6,10,12] For the purposes of studying relationships between nonlinear acoustic properties of solutions and chemical composition, structure, and hydration of the solute, it is most appropriate to use molar or specific increment of the nonlinearity parameter $B/A$ equal to the ratio of the difference between the $B/A$ values corresponding to solution and solvent, to the molar or specific concentration of the solute.[12]

Precise evaluation of the $B/A$ concentration increments of substances with low concentrations (not more than a few percent) became possible after development of a new differential technique, described elsewhere,[12] where $B/A$ concentration increments of some amino acids and proteins at 25 °C were reported. Therein, contributions of different atomic groups to the value of the $B/A$ molar increments of amino acids were estimated. Essential differences were found in the

nonlinear behavior of the solutions containing a solute with charged, polar and nonpolar groups. These groups differ not only by their nonlinear acoustic properties, but also by their contributions to the partial volume and compressibility of the solute,[14-16] which indicates that their hydration shells have different structures.

Temperature dependences of physico-chemical characteristics can also yield information about solute hydration. For example, data on the temperature dependences of the partial molar volume of amino acids[16] show that, in the neighborhood of charged atomic groups, water molecules exposed to a strong electric field cease to display abnormal properties, exhibiting a more linear temperature dependence of the volume compared with that of free water.

This report deals with the $B/A$ molar increments of charged and aliphatic amino acids at 18 °C–45 °C. Such investigations allow estimation of the contributions of the hydrophobic and charged atomic groups to $B/A$ molar increment of solute versus temperature and can yield new information about the nonlinear acoustic properties of hydrated water near these groups.

## I. RELATIONSHIP BETWEEN B/A MOLAR INCREMENT AND PARTIAL PHYSICO-CHEMICAL CHARACTERISTICS OF SOLUTE

The following expression for the $B/A$ concentration increment has been derived previously:[12]

$$\frac{\Delta(B/A)}{C} \frac{1}{2\rho_0 U_0}$$

$$= \frac{1}{C} \Delta\left(\frac{\partial U}{\partial p}\right)_T + ([U] + [\rho])\left(\frac{\partial U}{\partial p}\right)_{T_0} + \frac{T\alpha_0}{\rho_0 C_{p_0}}$$

$$\times \left[\frac{1}{C} \Delta\left(\frac{\partial U}{\partial T}\right)_p + ([U] + [\alpha] - [C_p])\left(\frac{\partial U}{\partial T}\right)_{p_0}\right],$$
(1)

where values related to the solvent are denoted by the subscript "0," and "$\Delta$" refers to the difference between solution and solvent for the corresponding parameter; $\rho$ is the density; $U$ is the sound velocity; $\alpha$ is the volume coefficient of thermal expansion; $C_p$ is the specific heat capacity at constant pressure; $(\partial U/\partial p)_T$ and $(\partial U/\partial T)_p$ are the pressure and temperature slopes of the sound velocity, respectively; $T$ is the absolute temperature; $C$ is the molar concentration of the solute; $[U]$, $[\rho]$, $[\alpha]$, and $[C_p]$ are the relative concentration increments of, respectively, sound velocity, density, volume coefficient of thermal expansion, and specific heat at constant pressure.

The values of $[\rho]$, $[\alpha]$, and $[C_p]$ are related to the apparent molar volume $\phi V$, thermal expansibility $\phi E$, and heat capacity $\phi C_p$ as follows:

$$[\rho] = \Delta\rho/\rho_0 C = M/\rho_0 - \phi V,$$
(2)

$$[\alpha] = \Delta\alpha/\alpha_0 C = \phi E/\alpha_0 - \phi V,$$
(3)

$$[C_p] = \Delta C_p/C_{p_0} C \simeq (\phi C_p/C_{p_0} - M)/\rho_0,$$
(4)

where $M$ is the molecular weight of the solute.

Expressions (2–4) can be substituted in Eq. (1) and, taking into account that the apparent molar adiabatic compressibility $\phi K_s = (\phi V - [U] - M/2\rho_0) 2\beta_{so}$, where $\beta_{so}$ is the coefficient of adiabatic compressibility of the solvent,[17] yields

$$\frac{\Delta(B/A)}{C} \frac{1}{2\rho_0 U_0}$$

$$= \frac{1}{C} \Delta\left(\frac{\partial U}{\partial p}\right)_T + \left(\frac{M}{2\rho_0} - \frac{\phi K_s}{2\beta_{so}}\right)\left(\frac{\partial U}{\partial p}\right)_{T_0}$$

$$+ \frac{T\alpha_0}{\rho_0 C_{p_0}}\left[\frac{1}{C} \Delta\left(\frac{\partial U}{\partial T}\right)_p$$

$$+ \left(\frac{\phi E}{\alpha_0} - \frac{\phi K_s}{2\beta_{so}} - \frac{\phi C_p}{C_{p_0}\rho_0} + \frac{M}{2\rho_0}\right)\left(\frac{\partial U}{\partial T}\right)_{p_0}\right].$$
(5)

The concentration increments of the pressure and temperature slopes of the sound velocity can be calculated from the pressure and temperature slopes of $[U]$ as

$$\frac{1}{C} \Delta\left(\frac{\partial U}{\partial p}\right)_T = U_0\left(\frac{\partial [U]}{\partial p}\right)_T + [U]\left[\left(\frac{\partial U}{\partial p}\right)_{T_0} + \beta_{T_0} U_0\right],$$
(6)

$$\frac{1}{C} \Delta\left(\frac{\partial U}{\partial T}\right)_p = U_0\left(\frac{\partial [U]}{\partial T}\right)_p + [U]\left[\left(\frac{\partial U}{\partial T}\right)_{p_0} - \alpha_0 U_0\right],$$
(7)

where $\beta_{T_0}$ is the coefficient of isothermal compressibility of the solvent.

Apparent molar volume, adiabatic compressibility,

thermal expansion, and heat capacity are known as characteristics of the solute that are sensitive to solute hydration structure. Equation 5, showing the relation between the $B/A$ molar increment and $\phi V$, $\phi K_s$, $\phi E$, and $\phi C_p$, exhibits the reason for the sensitivity of $\Delta(B/A)/C$ to solute hydration. Data on $\Delta(B/A)/C$ can be used in hydration studies as a new independent characteristic.

Another advantage of Eq. (5) is the possibility of direct use of values of $\phi V$, $\phi K_s$, $\phi E$, and $\phi C_p$ available from the literature, without having to recalculate them into the corresponding relative molar increments $[\rho]$, $[\alpha]$, and $[C_p]$.

## II. MATERIALS AND METHODS

All preparations of amino acids, except norvaline, were obtained from Merck. Norvaline was purchased from Sigma Chemical. All amino acids were in 1-stereoisomeric form and used without further purification.

Solutions of amino acids were prepared with twice-distilled water. The concentrations of the amino acids in solution were determined by weighing 30 to 40 mg of the solute material, with precision $\pm 0.05$ mg, and the necessary amount of water. The amino acids were dried at 110 °C for 6 h before weighing. Molar concentrations were reca'culated from the molal values using literature data of the apparent molar volumes of the amino acids (14–16) by means of the expression

$$C = (\phi V/1000 + 1/m\rho_0)^{-1},$$
(8)

where $m$ is the molal concentrations of solute.

In order to evaluate the $B/A$ molar increments of amino acids, the ultrasonic velocity molar increments $[U]$ were measured, as well as their pressure and temperature slopes using Eqs. (5)–(7). Measurements of $[U]$ were carried out in the pressure range 1–300 bar at 18 °C, 25 °C, 35 °C, and 45 °C. Other data necessary for the calculation of $\Delta(B/A)/C$ were obtained from the literature. Data on the pressure and temperature dependences of the sound velocity in water have been reported by Chen and Millero.[18] Data on density, specific heat capacity at constant pressure, coefficient of isothermal compressibility, and volume coefficient of thermal expansion of water have been reported by Pitzer et al.[19] Data on $\phi V$ and $\phi E$ of the amino acids reported by Kharakoz,[15,16] and on $\phi C_p$ by Jolicoeur et al.,[20] were used.

The concentrations of the amino acids in the solution studied are as follows: glycine, 0.347 mol/kg; alanine, 0.286 mol/kg; norvaline, 0.173 mol/kg; norleucine, 0.126 mol/kg; arginine monohydrochloride, 0.120 mol/kg; and lysine monohydrochloride, 0.128 mol/kg.

## III. ANALYSIS OF THE ACCURACY OF B/A MEASUREMENTS

Descriptions of the experimental setup for precise measurement of ultrasonic velocity as a function of temperature and pressure have been published elsewhere.[12,21] Measurements of the sound velocity were carried out by the so-called resonator method.[22,23] Analysis of the ultrasonic resonator cell method, which is the basis of the velocimeter, shows that

accuracy of relative measurements of sound velocity is the order of $\pm 1.5 \times 10^{-4}\%$ within the pressure range 1 to 1000 bar.[24] That is, changes in sound velocity $\Delta U$ of the order of 1% of the sound velocity in pure water $U_0$, which correspond to the measurements of the present work, can be determined with the relative accuracy of $\pm 0.015\%$.

However, the accuracy of determination of the sound velocity relative molar increments $[U]$ are limited mostly by the precision of the determination of solute concentration and the instability of the temperature in the ultrasonic cell. Taking into account these sources of error, the accuracy of determination of $[U]$ becomes $\pm 0.3\%$. The relative accuracy of determination of the pressure and temperature slopes of $[U]$ are of the order of 5% to 7%, which is related to limitations of numerical differentiation. Obviously, values of $1/C(\partial U/\partial P)_T$ and $1/C(\partial U/\partial T)_P$ in Eq. (1) also can be evaluated with the same precision.

The values of $[\rho]$ [see Eq. (1)] for most substances can be calculated from literature data with precision $\pm 1\%$, and the values of $[C_p]$ and $[\alpha]$ with precision $\pm 5\%$ to 10%.

The thermodynamical characteristics of water necessary for calculation of $\Delta(B/A)/C$ are known with much greater accuracy than those of the solute, and therefore do not limit the accuracy of the measurements.

The contributions of the first, second, and third terms of Eq. (1) to the total value of $\Delta(B/A)/C$ for most of the biological compounds studied are about 30% to 50%, 40% to 60%, and 1% to 10%, respectively. Therefore, the maximum error of the $\Delta(B/A)/C$ evaluation should be equal to $\pm$ (0.5 of 7% + 0.6 of 1% + 0.1 of 10%) = $\pm 5\%$. This value is much greater than the error determination of the solute concentration ($\pm 0.3\%$), and thus the increment of the nonlinearity parameter $\Delta(B/A)$ also can be determined with the accuracy $+5\%$. Within the concentration range 1%–5%, the value of $B/A$ for most biological compounds does not exceed 0.2 to 0.3,[3–6,12,21] which is 4% to 6% of the total value of $B/A$ of dilute aqueous solutions; roughly equal to 5.0 at room temperatures.[10] Therefore, the error of evaluation of small changes of $B/A$ is approximately equal to $\pm 0.2\%$ to 0.3% of the value of $B/A$ of the aqueous solution.

## IV. RESULTS AND DISCUSSIONS

Figures 1 to 6 show the pressure dependences of the relative molar increments of sound velocity for glycine, alanine, norvaline, norleucine, lysine monohydrochloride, and arginine monohydrochloride in the temperature range 18 °C to 45 °C.

The value of $[U]$, at each applied pressure, was determined from

$$[U]_p = (\Delta U_p/U_{0p} C_1)(\rho_{01}/\rho_{0p}),$$

where subscripts 1 and $p$ refer to atmospheric and applied pressure, respectively.

Increasing the pressure increases the density of a solution. As a result, the molar concentration of solute, equal to number of moles per unit volume, increases. For dilute solutions, the ratio of densities at atmospheric and elevated pressures is approximately equal to that for the pure solvent, and

the term $\rho_{01}/\rho_{0p}$ is used for considering the change occurring in the molar concentration of solute with increasing pressure.

As is seen from Figs. 1–6, a linear decrease of $[U]$ with pressure is observed for all the amino acids, in the temperature range studied. The pressure slope of $[U]$ is specific for the particular type of amino acid, and this value can be used for the characterization of the solute. For example, comparison of aliphatic amino acids shows that increase of number of aliphatic-$CH_2$-radicals in the side chains leads to a decrease of the $[U]$ pressure slope. Thus the amino acids with long aliphatic side chains, viz., norvaline and norleucine, have the most negative values of $\Delta[U]/\Delta p$ at all temperatures. Using the measured values of $[U]$, $\Delta[U]/\Delta p$, and $\Delta[U]/\Delta T$, the molar increments of $B/A$ of the studied amino acids in the temperature range 18 °C–45 °C were calculated using Eqs. (5)–(7), and these are shown in Table I. Figure 7 is the data of Table I exhibiting the contributions of the various atomic groups to the $\Delta(B/A)/C$ value. This provides for speculation about the nonlinear acoustic behavior of the charged and aliphatic groups.

For analysis of the nonlinear properties of the charged groups, one can use the $\Delta(B/A)/C$ data of glycine and the amino acids lysine and arginine having charged side chain radicals. Glycine does not have a side chain and, therefore, only two charged $HN_3^+$ and $COO^-$ groups mainly contribute to its $\Delta(B/A)/C$ value.

The $\Delta(B/A)/C$ values of glycine are positive throughout the temperature range considered. This means that the contributions of the charged groups are positive and that their presence in the solute molecules increases the total acoustical nonlinearity of the solution.

Lysine monohydrochloride and arginine monohydrochloride, which have additional charged groups relative to glycine, are characterized by significantly greater values of $\Delta(B/A)/C$. However, not all the differences between the values of $\Delta(B/A)/C$ corresponding to glycine and lysine–HCl, or arginine–HCl, are due to the additional side chains. Some contributions to these differences are a result of the presence at $Cl^-$ ions in the respective solutions.

The contribution of one charged group, which in a first approximation can be assumed equal to half of the $\Delta(B/A)/C$ value of glycine, decreases from $240\ \mathrm{cm}^3/\mathrm{mol}$ at
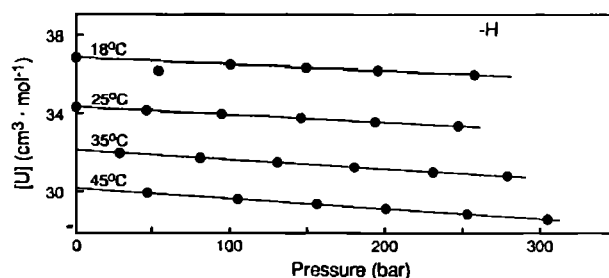


FIG. 1. Pressure dependences of sound velocity relative molar increments for glycine at 18 °C–45 °C. The side chain radical chemical composition of the amino acid is shown on the figure.
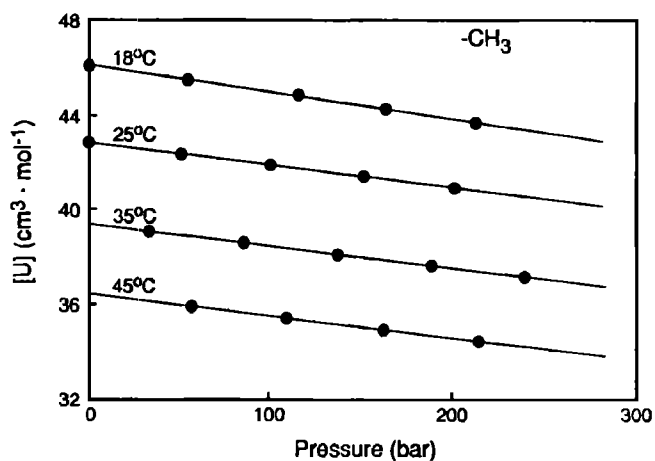
FIG. 2. Pressure dependences of sound velocity relative molar increments for alanine at 18 °C–45 °C. The side chain radical chemical composition of the amino acid is shown on the figure.
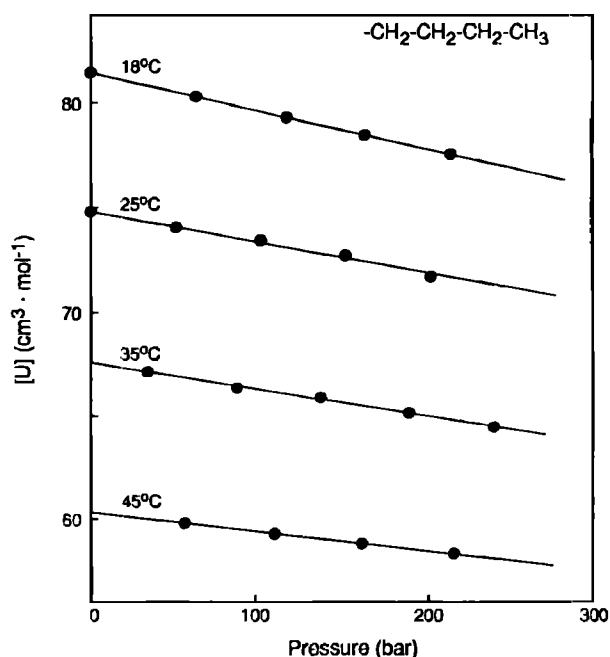


FIG. 4. Pressure dependences of sound velocity relative molar increments for norleucine at 18 °C–45 °C. The side chain radical chemical composition of the amino acid is shown on the figure.

18 °C to 150 cm³/mol at 45 °C, as seen in Fig. 7. Lysine–HCl and arginine–HCl have negative temperature slopes of $\Delta(B/A)/C$ that are steeper than those of glycine, as seen from Table II. This is due both to the additional charged groups in the side chain radicals of lysine and arginine and to the presence of Cl⁻ ions in their solutions.

One can assume that, in the temperature range 18 °C–45 °C, the larger the number of charged groups in the solute molecules, the greater the value of $\Delta(B/A)/C$ and the more negative its temperature slope.

Data on the acoustic nonlinearity parameter $B/A$ of the solutions of amino acids with the aliphatic side chains show higher sensitivity of $B/A$ to the structure of solute and its

hydration shell compared with other acoustic parameters, for example, with sound velocity. Figure 8 shows dependences of $\Delta(B/A)/C$ and $[U]$ of the aliphatic amino acids on the number of carbon atoms in the side chain radicals at 25 °C. One can see that the differences between sound velocity relative molar increments $[U]$ corresponding to the isomers valine and norvaline, as well as for leucine, isoleucine, and norleucine, are considerably small compared with the data on the nonlinearity parameter $B/A$ molar incre-
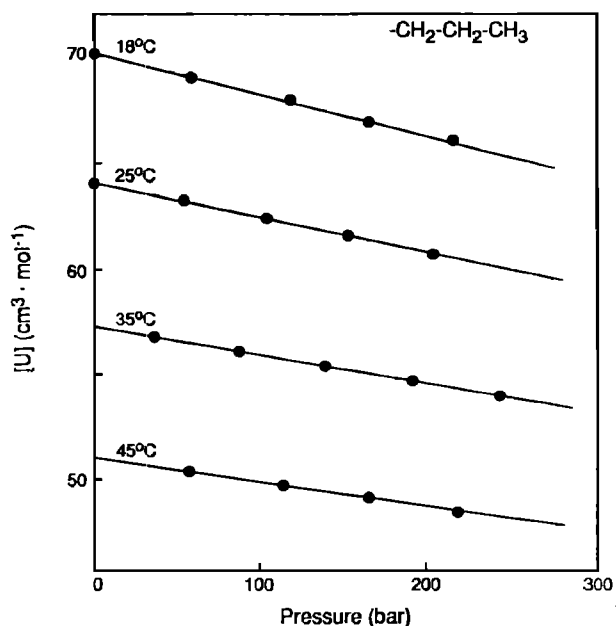


FIG. 3. Pressure dependences of sound velocity relative molar increments for norvaline at 18 °C–45 °C. The side chain radical chemical composition of the amino acid is shown on the figure.
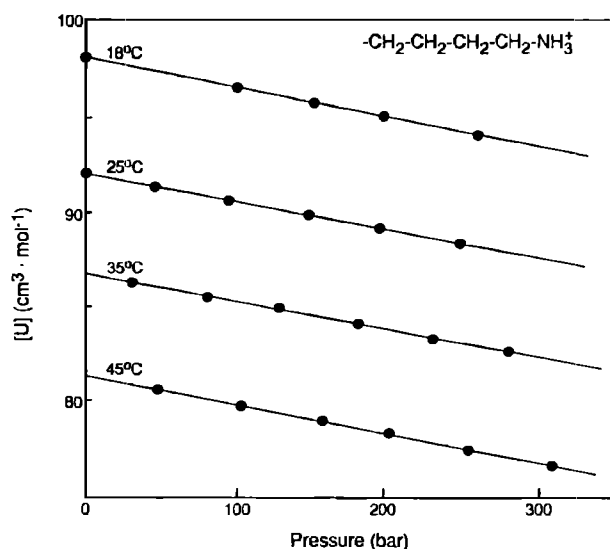


FIG. 5. Pressure dependences of sound velocity relative molar increments for lysine monohydrochloride at 18 °C–45 °C. The side chain radical chemical composition of the amino acid is shown on the figure.
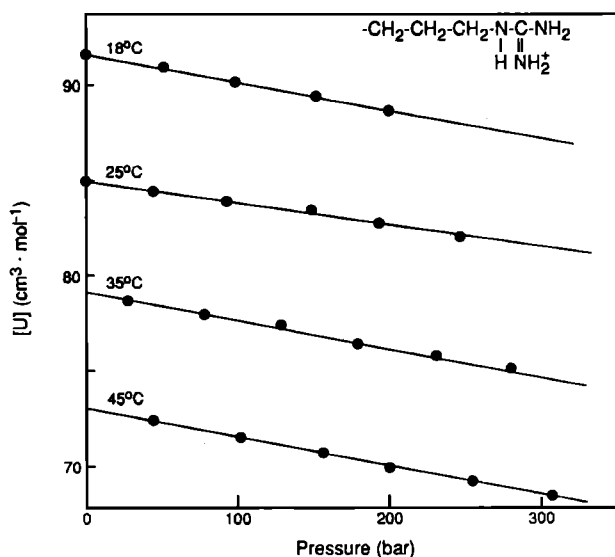
FIG. 6. Pressure dependences of sound velocity relative molar increments for arginine monohydrochloride at 18 °C–45 °C. The side chain radical chemical composition of the amino acid is shown on the figure.



FIG. 7. Temperature dependences of the molar increments of the nonlinearity parameter $B/A$ of amino acids: O: glycine; □: alanine; ●: norvaline; △: norleucine; ■: lysine monohydrochloride; ▲: argine monohydrochloride.

ments. While data on $[U]$ as a function of the number of carbon atoms in the aliphatic amino acids can be approximated beyond alanine by a straight line, the $\Delta(B/A)/C$ data occupy large areas. This emphasizes the high sensitivity of the nonlinearity parameter $B/A$ of these solutions to features of the hydration structure of the solute.

Figure 9 shows the dependences of $[U]$ and $\Delta(B/A)/C$ of aliphatic amino acids, with nonbranched side chains, on the number of carbon atoms in the temperature range 18 °C–45 °C. It is seen that the curves for $[U]$ have breaks at the point corresponding to alanine, beyond which these dependences form straight lines. This phenomenon is related to the following. Glycine, in contrast to all the other amino acids, does not have a side chain. Therefore, its hydration shell is formed only by water molecules attracted and oriented in the electrostatic field of $-NH_3^+$ and $-COO^-$ charged atomic groups. In the case of alanine, the hydrophobic $-CH_3$ group changes the structure of the hydration shell and reduces accessibility of the charged groups of solute to the water molecules. Therefore, the observed difference between $\Delta(B/A)/C$ values corresponding to alanine and glycine is

due not only to the appearance of the aliphatic group, but also to the changes in the $[U]$ value, which means that interaction between this group and the zwitterionic skeleton of amino acid does not affect the relative molar increment of sound velocity.

The situation is more complex in the case of $\Delta(B/A)/C$. At the lower temperatures, the observed minimum in the temperature dependences of $\Delta(B/A)/C$ is shifted toward higher numbers of the carbon atoms. At lower temperatures, breaks exist at every point corresponding to one of the amino acids with the nonbranched side chains. The possible nature of this phenomenon, related to the change of water structure with temperature, is described as follows.

Aliphatic groups in the $\zeta$, $\gamma$, or $\varepsilon$ positions and charged groups of the zwitterionic skeletons of amino acids are far from each other, and therefore interactions between them are realized through a few layers of water molecules, in the hydration shell of the solute molecules. At low temperatures, when there are strong hydrogen bonds between water molecules, these interactions strongly influence the structure of the solute hydration shell. The result of these interactions is the appearance of the numerous breaks on the $\Delta(B/A)/C$ dependences. Temperature increase leads to in-

TABLE I. Molar increments of the nonlinearity parameter $B/A$ of amino acids (cm³/mol) versus temperature (°C).

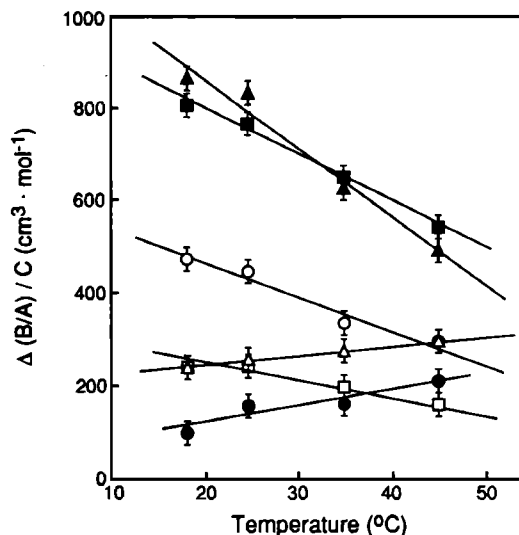| Amino acids | ($T$°C) 18 | 25 | 35 | 45 |
|---|---|---|---|---|
| Glycine | 480 ± 30 | 450 ± 30 | 340 ± 30 | 300 ± 30 |
| Alanine | 250 ± 30 | 250 ± 30 | 200 ± 30 | 160 ± 30 |
| Norvaline | 100 ± 30 | 160 ± 30 | 170 ± 30 | 210 ± 30 |
| Norleucine | 240 ± 30 | 260 ± 30 | 270 ± 30 | 300 ± 30 |
| Lysine–HCl | 800 ± 30 | 760 ± 30 | 650 ± 30 | 540 ± 30 |
| Arginine–HCl | 860 ± 30 | 830 ± 30 | 630 ± 30 | 490 ± 30 |

TABLE II. Temperature slopes of the molar increments of the nonlinearity parameter $B/A$.

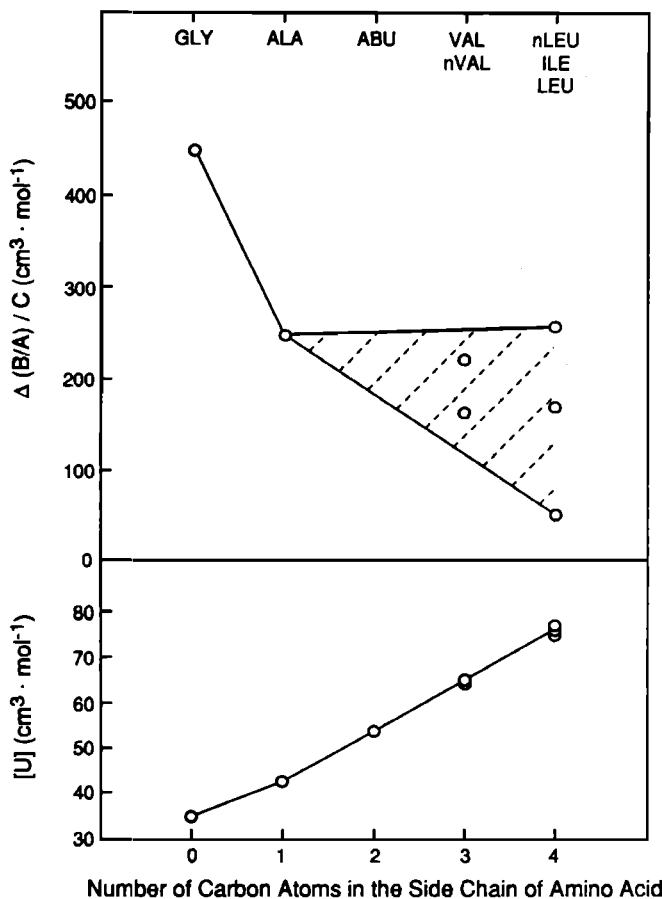| Amino acids | $\Delta[\Delta(B/A)/C]/\Delta T$, cm³/mol/K |
|---|---|
| Glycine | $-7.5 \pm 1.0$ |
| Alanine | $-4.0 \pm 1.0$ |
| Norvaline | $4.0 \pm 1.0$ |
| Norleucine | $1.5 \pm 1.0$ |
| Lysine–HCl | $-10.0 \pm 1.0$ |
| Arginine–HCl | $-14.5 \pm 1.0$ |

FIG. 8. Dependences of the $B/A$ molar increments and sound velocity relative molar increments of the aliphatic amino acids on the number of carbon atoms in the side chains at 25 °C. The $\Delta(B/A)/C$ values of valine, leucine, and isoleucine are from Sarvazyan et al.[12] The values of the sound velocity relative molar increments of amino acids are from Kharakoz.[15]
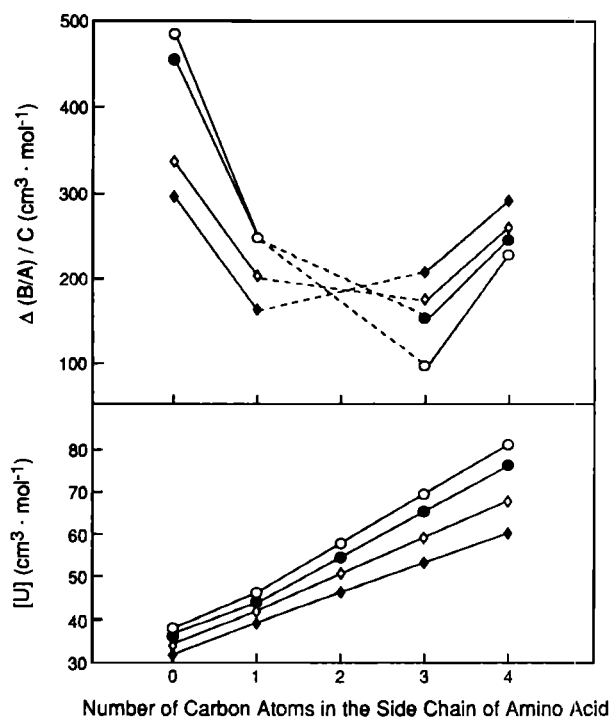


FIG. 9. Dependences of the $B/A$ molar increments and sound velocity relative molar increments of amino acids with nonbranched aliphatic side chains, on the number of carbon atoms in the side chains at $\bigcirc$: 18 °C; $\bullet$: 25 °C; $\Diamond$: 35 °C; $\blacklozenge$: 45 °C. The sound velocity relative molar increment data of aminobutiric acid are from Kharakoz.[15]

crease of the portion of the water molecules with the broken hydrogen bonds. Influence of the interatomic interactions on the solute hydration becomes weak, and contributions of added aliphatic groups become approximately the same.

Data, as exhibited in Figs. 8 and 9, indicate that $B/A$ is very sensitive to the hydration features of the amino acids, compared with the sound velocity, especially if there are interactions between atomic groups far from each other.

In order to estimate the nonlinear contribution of a single aliphatic group far from the zwitterionic skeleton of the amino acid, it is reasonable to use the difference between $\Delta(B/A)/C$ values corresponding to norleucine and norvaline.

The temperature dependences of the contributions of the charged [half value of the $\Delta(B/A)/C$ of glycine] and aliphatic groups are depicted in Fig. 10. The temperature slopes of the nonlinear contributions of the charged and nonpolar aliphatic groups are, respectively, approximately $-4$ and $-1$ cm$^3$/mol/K. It is pertinent to inquire into the cause of negative temperature slopes of these contributions.

Data on the nonlinearity parameter $B/A$ of water[10,25] show that the temperature slope of $B/A$ is considerably greater than that of alcohols,[10,26] which are also liquids with

hydrogen bonds between molecules. The high-temperature slope of $B/A$ of water (approximately 0.02 K$^{-1}$ at 25 °C) can be explained using common assumptions of the two-state structure of water.[10] This implies that the high-temperature slope of $B/A$ is one of many other abnormal characteristics of water.

The high negative slope of the contribution of the charged groups to the value of $\Delta(B/A)/C$ of the solute means that $B/A$ of hydrated water near these groups increases with temperature less rapidly than that of free water. This can be explained assuming that water molecules in the strong electric field of charged atomic groups exhibit more "normal" characteristics and, as a result, are characterized
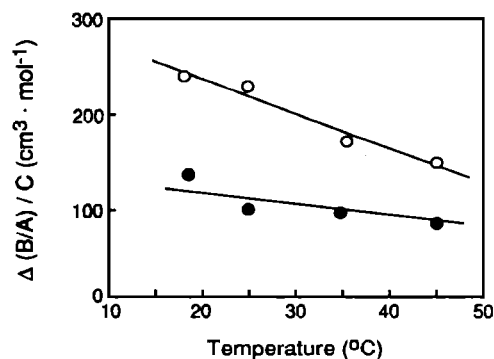


FIG. 10. Temperature dependence of the contributions of charged ($\bigcirc$) and aliphatic ($\bullet$) atomic groups to the value of the $B/A$ molar increment of solute.

by a smaller $B/A$ temperature slope, compared with free water. The small and negative temperature slope of the contributions of the aliphatic groups to the $\Delta(B/A)/C$ value of the solute (comparable with the errors of measurement) (Fig. 10) indicates that in the hydration shell of these groups, the temperature dependence of $B/A$ differs slightly from that of bulk water.

## V. SUMMARY

Experimental data on the molar increments of the non-linearity parameter of six amino acids, in the temperature range 18 °C–45 °C, have been reported. The contributions of charged and aliphatic groups to the $B/A$ molar increment of solute versus temperature have been estimated.

In the hydration shell of the charged groups, $B/A$ increases with temperature less rapidly than that of free water, while the temperature dependence of $B/A$ of the hydration shell of the aliphatic group differs slightly from that of free water.

It was shown that the nonlinearity parameter $B/A$, relative to sound velocity, which is one of the most widely used characteristics in hydration studies, could be very sensitive to the features of the solute hydration shell structure.

## ACKNOWLEDGMENT

[1] T. G. Muir and E. L. Carstensen, "Prediction of nonlinear acoustic effects at biomedical frequencies and intensities," Ultrasound Med. Biol. 6, 345–357 (1980).

[2] E. L. Carstensen, W. K. Law, N. D. McKay, and T. G. Muir, "Demonstration of nonlinear acoustical effects at biomedical frequencies and intensities," Ultrasound Med. Biol. 6, 359–368 (1980).

[3] W. K. Law, L. A. Frizzell, and F. Dunn, "Ultrasonic determination of the nonlinearity parameter $B/A$ for biological media," J. Acoust. Soc. Am. 69, 1210–1212 (1981).

[4] F. Dunn, W. K. Law, and L. A. Frizzell, "Nonlinear ultrasonic propagation in biological media," Br. J. Cancer 45, Suppl. V, 55–58 (1982).

[5] W. K. Law, L. A. Frizzell, and F. Dunn, "Comparison of thermodynamic and finite amplitude methods of $B/A$ measurements in biological materials," J. Acoust. Soc. Am. 74, 1295–1297 (1983).

[6] W. K. Law, L. A. Frizzell, and F. Dunn, "Determination of the nonlinearity parameter $B/A$ of biological media," Ultrasound Med. Biol. 11, 307–318 (1985).

[7] C. M. Sehgal, G. M. Brown, R. C. Baha, and J. F. Greenleaf, "Measurements and use of acoustic nonlinearity and sound speed to estimate composition of excised livers," Ultrasound Med. Biol. 12, 865–874 (1986).

[8] R. A. Apfel, "Prediction of tissue composition from ultrasonic measurements and mixture rules," J. Acoust. Soc. Am. 79, 148–152 (1986).

[9] L. Bjorno, "Characterization of biological media by means of their nonlinearity," Ultrasonics 24, 254–259 (1986).

[10] K. Yoshizumi, T. Sato, and N. Ichida, "A physicochemical evaluation of the nonlinear parameter $B/A$ for media predominantly composed of water," J. Acoust. Soc. Am. 82, 302–305 (1987).

[11] X. F. Gong, Z. M. Zhu, T. Shi, and J. H. Huang, "Determination of the acoustic nonlinearity parameter in biological media using FAIS and ITD methods," J. Acoust. Soc. Am. 86, 1–5 (1989).

[12] A. P. Sarvazyan, T. V. Chalikian, and F. Dunn, "Acoustic nonlinearity parameter $B/A$ of aqueous solutions of some amino acids and proteins," J. Acoust. Soc. Am. 88, 1555–1561 (1990).

[13] A. P. Sarvazyan, "Ultrasonic velocimetry of biological compounds," Ann. Rev. Biophys. Biophys. Chem. 20, 321–342 (1991).

[14] F. S. Millero, A. L. Surdo, and C. Shin, "The apparent molal volumes and adiabatic compressibilities of aqueous amino acids at 25 °C," J. Phys. Chem. 82, 784–792 (1978).

[15] D. P. Kharakoz, "Acoustical investigation of amino acids and protein solutions," Ph.D. dissertation, Institute of Biological Physics, Acad. Sci. USSR, Pushchino, USSR (1983).

[16] D. P. Kharakoz, "Volumetric properties of proteins and their analogs in diluted water solutions. 1. Partial volumes of amino acids at 15–55 °C," Biophys. Chem. 34, 115–125 (1989).

[17] V. A. Buckin, B. I. Kankiya, and R. L. Kazaryan, "Hydration of nucleotides in dilute aqueous solutions. Ultrasonic velocity and density measurements," Biophys. Chem. 39, 211–223 (1989).

[18] C. T. Chen and F. J. Millero, "Reevaluation of Wilson's sound-speed measurements for pure water," J. Acoust. Soc. Am. 60, 1270–1273 (1976).

[19] K. S. Pitzer, J. C. Peiper, and R. N. Busey, "Thermodynamic properties of aqueous sodium chloride solutions," J. Phys. Chem. Rev. Data 13, 1–102 (1984).

[20] C. Jolicoeur, B. Riedl, D. Desrochers, L. L. Lemelin, R. Zamoyska, and O. Enea, "Solvation of amino acid residues in water and urea–water mixtures: Volumes and heat capacities of 20 amino acids in water and in 8 molar urea at 25 °C," J. Solut. Chem. 15, 109–128 (1986).

[21] A. P. Sarvazyan, and T. V. Chalikian, "Development of a multichannel ultrasonic interferometer for the measurements under high pressures and its application to biomolecular studies," Proc. IEEE Ultrason. Symp. 2, 937–940 (1988).

[22] A. P. Sarvazyan, "Development of methods of precise ultrasonic measurements in small volumes of liquids," Ultrasonics 20, 151–154 (1982).

[23] F. Eggers and Th. Funck, "Ultrasonic measurements with milliliter liquid samples in the 0.5–100 MHz range," Rev. Sci. Instrum. 44, 969–978 (1973).

[24] A. P. Sarvazyan and T. V. Chalikian, "Theoretical analysis of an ultrasonic interferometer for precise measurements at high pressures," Ultrasonics 29, 119–124 (1991).

[25] M. P. Hagelberg, G. Holton, and S. Kao, "Calculation of $B/A$ for water from measurements of ultrasonic velocity versus temperature and pressure to 10 000 kg/cm²," J. Acoust. Soc. Am. 41, 564–567 (1967).

[26] K. L. Narayana and K. M. Swamy, "Nonlinear acoustical properties in n-amyl alcohol," Acustica 47, 51–52 (1980).