Biophysical Chemistry, 34 (1989) 211–223
Elsevier

BIOCHE 01383

Hydration of nucleosides in dilute aqueous solutions

Ultrasonic velocity and density measurements

V.A. Buckin, B.I. Kankiya and R.L. Kazaryan

Institute of Biological Physics, Academy of Sciences of the U.S.S.R., 142292 Pushchino, Moscow Region, U.S.S.R.

Received 4 July 1988
Revised manuscript received 14 June 1989
Accepted 16 June 1989

Ultrasound velocity; Partial molar compressibility; Partial molar volume; Nucleoside

The values of the concentration increments of the ultrasound velocity and their temperature slopes, apparent molar volumes, apparent molar expansibilities, apparent molar adiabatic compressibilities and their temperature gradients for 12 nucleosides and their analogs, as well as for ribose and deoxyribose, have been obtained using precision measurements of ultrasound velocity and density. The following hydration parameters for the atomic groups of the nucleosides, reflecting the state of water in the hydration shells of these groups, have been analyzed: (1) the contribution of ribose to the values of the concentration increment of ultrasound velocity A, the apparent molar volumes ϕ_v and apparent molar adiabatic compressibilities ϕ_{ks} of nucleosides; (2) contributions of the CH_3, NH_2 and O=···–H groups of nucleic bases to the A, ϕ_v and ϕ_{ks} values of nucleosides and free nucleic bases; (3) contributions of the 2′–OH group of ribose to the values of A, ϕ_v and ϕ_{ks} nucleosides; (4) changes in the A values of nucleosides and free nucleic bases upon their protonation and deprotonation. Data have been obtained on the mutual influence of the atomic groups of nucleosides on their hydration. It is shown that the GC pairs of free deoxynucleosides undergo hydration more vigorously than the AT pairs, which contrasts with the relation of the degree of hydration of the GC and AT pairs of the double helix.

1. Introduction

Hydration is one of the most important interactions which are responsible for the secondary structure of nucleic acids and other physicochemical properties of these molecules. At present a number of experimental papers are available on the hydration of nucleic acids in aqueous solutions (for reviews, see refs 1–3), the majority of which is devoted to studies of hydration of nucleic acids as a whole. Nucleic acids are heterogeneous in composition: they contain hydrophobic, polar and charged groups, which differ in their interaction with water. A systematic investigation of nucleic acids must include studies on the state of water near different atomic groups of these molecules as well as the mutual influence of atomic groups on their hydration. The most complete investigation can be carried out when the same method utilizes hydration of both the nucleic acids per se and a large number of their components and analogs. Using precision measurements of ultrasound velocity and density, in our previous studies [4] we investigated hydration of nucleic bases and their derivatives in dilute aqueous solutions. In the present paper more complicated molecules – nucleosides – are studied.

Ultrasound velocity measurements have been used for investigation of hydration of some nucleosides. Sadikhova and Braginskaya [5] reported hydration numbers for cytidine, uridine and adenosine calculated from the data on ultra-

Correspondence address: V.A. Buckin, Institute of Biological Physics, Academy of Sciences of the U.S.S.R., 142292 Pushchino, Moscow Region, U.S.S.R.

sound velocity in aqueous solutions using the method of Shiio et al. [6]. Antosiewicz et al. [7] calculated hydration numbers for cytidine, uridine, deoxyuridine and thymidine using data on ultrasound velocity in mixtures of water and ethanol and utilizing the method of Yasunaga et al. [8]. In the method of both Shiio et al. and Yasunaga et al. the calculation of hydration numbers is based on rather strict assumptions concerning the properties of water within the hydration shell. However, not all of the assumptions agree with the available experimental data. Therefore, in analyzing the results, we have not used any of the methods for calculation of hydration numbers. Our analysis is based on a comparison of the characteristics of nucleosides with those of their components: nucleic bases, ribose and deoxyribose.

The present paper reports for the first time the values determined for the concentration increments of ultrasound velocity, A, and their changes in protonation and deprotonation reactions, apparent molar volumes, ϕ_v, apparent molar expansibilities, ϕ_E, apparent molar adiabatic compressibilities ϕ_{ks} and their temperature slopes $\Delta\phi_{ks}/\Delta T$ for all ribo- and deoxyribonucleosides and a number of their analogs (a total of 14 compounds).

The physical meaning of the above characteristics was discussed in detail in our previous publication [4]. Here, it is sufficient to mention only the basic statements. At infinite dilution, the apparent molar volume ϕ_v is the sum: $\phi_v = \overline{V}_M + \Delta V_h$, where $\overline{V}_M$ is the intrinsic molar volume of a solute molecule, inaccessible to solvent molecules and determined by its stereochemical structure, ΔV_h the hydration component which is determined by the sum of the void volume between the solute molecule and the surrounding water molecules plus the change in the volume of water, surrounding the solute molecule as a result of the solute-solvent interaction.

For simple compounds having no pockets on their van der Waals surface or for their separate atomic groups, the van der Waals volume $\overline{V}_w$ calculated as a sum of volumes of separate atoms or atomic groups can be used as an estimation of $\overline{V}_M$ [9,10]. In this case, to estimate the hydration component of the apparent volume, one can utilize the ratio:

$$\Delta V_h \cong (\phi_v - \overline{V}_w)$$

In general, the values ϕ_E, ϕ_{ks} and $\Delta\phi_{ks}/\Delta T$, as well as the hydration component, contain the contribution of the intrinsic expansibility etc. However, for simple low molecular weight compounds, for which the volume of a molecule is determined mainly by the van der Waals volume, the intrinsic thermal expansibility, compressibility and temperature slope can be neglected [4]. This is especially valid for discussion of the present results, where only a comparison of ϕ_{ks}, ϕ_E and $\Delta\phi_{ks}/\Delta T$ of the molecules with similar chemical structure is made.

The values ϕ_{ks}, ϕ_E and $\Delta\phi_{ks}/\Delta T$ have one more component which is governed by the change in conformation equilibrium of the solute molecule upon alteration of the pressure and temperature. This relaxation contribution is not large and taking it into account is necessary only for the ϕ_{ks} value that can be measured with high accuracy (as compared with ϕ_E and $\phi_{ks}/\Delta T$). The relaxation contribution of ϕ_{ks} can be calculated from the data on ultrasound absorption. This can be expressed by the following equation for the apparent molar adiabatic compressibility ϕ_{ks} corrected for the relaxation contribution $\overline{K}_r$, apparent molar expansibility ϕ_E and temperature slope of apparent molar adiabatic compressibility $\Delta\phi_{ks}/\Delta T$ at infinite dilution:

$$(\phi_{ks} - \overline{K}_r) \cong \Delta K_h$$

$$\phi_E \cong \Delta E_h$$

$$\Delta\phi_{ks}/\Delta T \cong [\Delta(\Delta K_h)]/\Delta T$$

where ΔK_h and ΔE_h are determined by the compressibility and thermal expansibility of void volumes between the solute molecule and molecules surrounding the solute and also by the changes in compressibility and thermal expansibility of water surrounding the solute molecule as a result of solute-solvent interaction.

As the values ϕ_{ks} (after correction for the relaxation contribution), $(\phi_v - \overline{V}_w)$, ϕ_E and $\Delta\phi_{ks}/\Delta T$ are mainly determined by solute-solvent interaction, we shall take them in discussing the results

as representing the hydration characteristics of solute molecules and their atomic groups. We will also use the hydration contribution to the ultrasound velocity increment A_h, that is a combination of ϕ_{ks} and $(\phi_v - \overline{V}_w)$ parameters, as hydration characteristics. Equality of each of these values for different molecules or atomic groups will be interpreted as equality of the four different characteristics of the hydration shells of these molecules: volume, expansibility, compressibility and temperature slope of compressibility. We term this situation equality of hydration. A difference in one of these values will be interpreted as a difference in hydration.

2. Materials and methods

Preparations of nucleic bases, nucleosides, ribose and deoxyribose from Sigma were used in the experiments. The solutions were prepared using fresh bidistilled water, the conductivity of which was less than 10^{-6} Ω^{-1} cm^{-1}. The respective pH values of aqueous solutions were pH 6.9, 6.7, and 6.8–6.5 for cytosine, cytidine, and other nucleobases and nucleosides. The concentrations of solutions were determined by weighing the dry samples and the water. The amount of water in the dry samples was determined using a modification of the method of Fischer [11] with an accuracy of 0.1%. Extinction coefficients of the ultraviolet absorption maximum were determined for all preparations with the exception of purine riboside at neutral pH values. The values of the wavelengths and extinction coefficients of the absorption maxima coincided within experimental error with tabulated data [12].

The density of solutions was measured using a DMA-602 instrument (Anton Paar) having a measuring cell of 1 cm^3 volume. Relative ultrasound velocity measurements were made on a differential interferometer of constant length with titanium cells of 1 cm^3 volume with a built-in magnetic stirrer. A more detailed description of the instruments, their adjustment, the measuring technique as well as the calculation method for the apparent molar volume ϕ_v and the concentration increment of ultrasonic velocity, $A = (U - U_0)/(U_0\rho_0 c)$ (where U and U_0 denote ultrasound velocities in the solution and solvent, respectively, ρ_0 the density of pure water, and c the molal concentration of the solute) can be found in our previous publication [4].

To determine the values of $\phi_E = (\Delta\phi_v/\Delta T)$ and $\Delta A/\Delta T$, a series of two or three solutions were measured at 18 and 32°C. First, the values of ϕ_E and $\Delta A/\Delta T$ were determined by measurements in the same solution at 18 and 32°C. The values obtained were then averaged. The values of A and ϕ_v at 25°C were determined from the series of two or three solutions at 25°C.

Titration of nucleic base and nucleoside solutions was performed differentially. Using a special syringe, the measuring and standard cells were filled with equal volumes of solution and water, respectively, with an error not exceeding 0.1%. Thereafter, an amount of aqueous solution of HCl (Reakhim, C.P.) or NaOH (Fluka, C.P.) was added to both cells via a syringe with a special adaptor. The volume of additions varied from 4 to 10 μl, the reproducibility being not less than 0.25%. After each addition, the A value was determined taking into account the dilution of the solution. In parallel, a potentiometric titration was carried out in the same volume using the same syringe.

Concentration dependences of A were obtained for adenosine, 2′-deoxyadenosine, 2′-deoxyguanosine, inosine, thymidine, cytidine and uridine. Measurements of A for the other compounds as well as of $\Delta A/\Delta T$ for all test substances were made at 0.5–1.5 mg/cm^3 for adenosine and 1.5 mg/cm^3 for other nucleosides, while determinations of ϕ_v were at 1.5 to 2 mg/cm^3. Titration was carried out at 0.6 mg/cm^3 for adenine and hypoxanthine, and at 1–1.5 mg/cm^3 for the other compounds.

The exact concentrations of alkali and acid on preparing the titration solutions were determined from the measured values of the ultrasound velocities using literature data on ultrasound velocity increments of these compounds [13].

The values of the apparent molar adiabatic compressibility were calculated from the following equation which is valid for dilute solutions:

$$\phi_{ks} = (\phi_v - A - M/2\rho_0)2\beta_0 \qquad (1)$$

where ρ_0 and β_0 denote, respectively, the density and coefficient of adiabatic compressibility of water, and M the molecular mass of the solute. The β_0 values were calculated from the data reported by Del Grosso [14] for the ultrasound velocity in pure water and the tabulated data for the density of water ρ_0.

3. Results

Fig. 1 shows the concentration dependences A of a set of nucleosides obtained in the present work. We have previously obtained the concentration dependences A for adenosine and 2′-adenosine [15]. The error in measuring the ultrasound velocity in the present paper is several times less than that reported earlier, which enabled us to perform measurements at lower concentrations. This also allowed us to repeat determinations of the concentration dependences A for adenosine and 2′-deoxyadenosine. The concentration dependences were used for estimating the A values for nucleosides pertaining to infinite dilution (extrapolation to zero concentration).

In the case of nucleosides, for which the concentration dependences of A were not measured, the A values at infinite dilution were calculated as the sum of the measured A value at a given concentration and the correction for the concentration dependence. For nucleosides with pyrimidine bases (2′-deoxyuridine and 2′-deoxycytidine), the correction was estimated from the slopes of the concentration dependences of nucleosides with a pyrimidine base (cytidine, thymidine, uridine) which are close to each other. For nucleosides with a purine base (purine riboside, guanosine, 3′-deoxyadenosine), the mean value of the slope of the concentration dependences of nucleosides with a purine base (adenosine, 2′-deoxyadenosine, inosine, 2′-deoxyguanosine) was used. The corrections were 0.1 cm^3/mol for 2′-deoxycytidine, 2′-deoxyuridine and guanosine and 0.3 cm^3/mol for purine riboside and 3′-deoxyadenosine. It should be noted that the correction values are lower or compatible with the error in determining the absolute A value for nucleosides (0.3–0.5 cm^3/mol) and, even if they were not taken into account, this would not influence the interpretation of the results.

Measurements of the concentration dependence of ultrasound velocity in solutions of thymidine, cytidine and uridine were reported in ref. 16. Our data are in agreement with those results at concentrations above 0.05 M but differ below 0.05 M. At lower solution concentrations, the error in estimating A is determined only by the error in ultrasound velocity measurements, since the increase in ultrasound velocity in solution, as compared to that in water, is very low being $\Delta u/u \approx 2 \times 10^{-2}\%$ at 5 mM. To estimate the A value with an accuracy of 1% and to analyze the concentra-

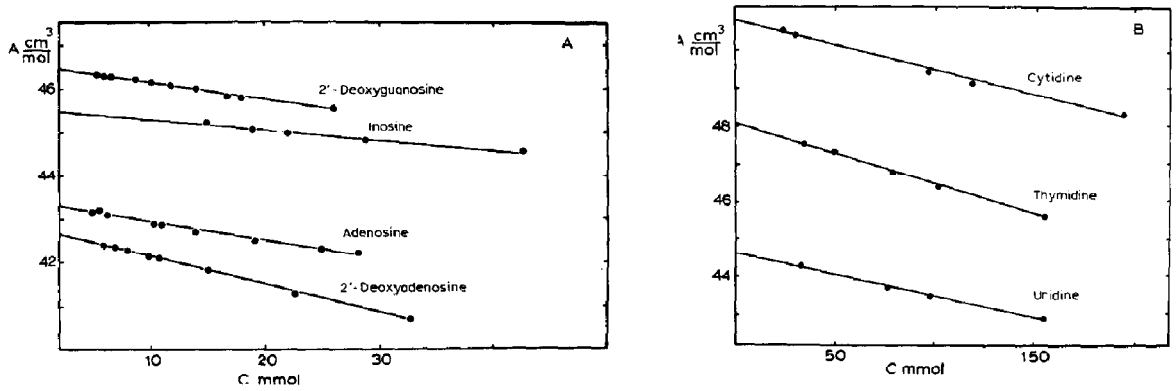


Fig. 1. Dependence of the concentration increment of ultrasound velocity, A, on solution concentration, c, for: (A) purine nucleosides and (B) pyrimidine nucleosides in aqueous solution at 25 °C.

tion dependence A at the above concentrations, the error in determining the $\Delta u/u$ value should not exceed $\pm 1 \times 10^{-4}$% [17]. The error $\Delta u/u$ in the method for measuring ultrasound velocity that we used was $\pm 0.3 \times 10^{-4}$% (see ref. 4), which is more than an order of magnitude better than that reported in ref. 16.

The concentration dependence of A of the ribose and deoxyribose is small and can be neglected [19,20]. The concentration dependences ϕ_v of the studied compounds are also small [16,18]. Therefore, the values of ϕ_v reported here can be attributed to infinite dilution.

Table 1 lists the values of A and ϕ_v and calculated from them ϕ_{ks} values for the compounds studied which are attributed to infinite dilution. The table also gives the values of $\Delta A/\Delta T$, ϕ_E and $\Delta\phi_{ks}/\Delta T$ obtained at the concentrations mentioned in section 2.

Fig. 2 depicts the curves for acidic and alkaline titration of nucleic bases and nucleosides. The changes in concentration increment of the ultrasound velocity upon protonation and deprotonation of nucleic bases A, calculated from these data, are given in table 5.

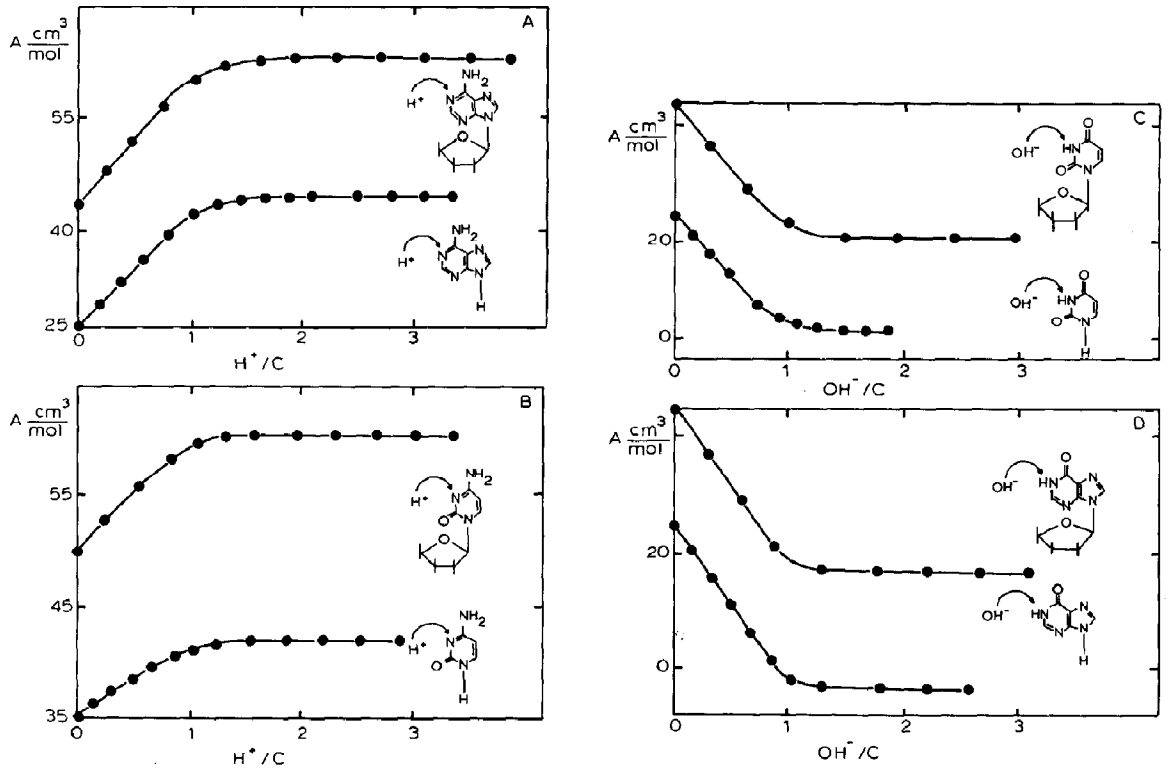


Fig. 2. Alteration of the concentration increment of ultrasound velocity A for nucleic bases and nucleosides in aqueous solution upon acidic titration for: (A) adenine ($c = 5.9$ mM) and adenosine ($c = 4.6$ mM); (B) cytidine ($c = 4.5$ mM) and cytosine ($c = 6.7$ mM), and upon alkaline titration: (C) uridine ($c = 3.0$ mM) and uracil ($c = 4.6$ mM), (D) inosine ($c = 4.0$ mM) and hypoxanthine ($c = 5.9$ mM). H^+ and OH^- denote the respective concentrations of HCl and NaOH added in solution upon titration; c, concentration of base or nucleoside. Temperature, 25 °C; the pH values in aqueous solutions ($H^+/c = 0$; $OH^-/c \approx 0$) varied from pH 6.4 to 6.9.

Table 1

Concentration increment of ultrasound velocity A and its temperature slope $\Delta A/\Delta T$, apparent molar volume ϕ_v, apparent molar expansibility ϕ_E, apparent molar adiabatic compressibility ϕ_{ks} and its temperature slope $\Delta\phi_{ks}/\Delta T$ for nucleosides and their analogs at 25 °C

Substance	Molecular mass (Da)	A (cm^3/mol)	$-\Delta A/\Delta T$ (cm^3/mol per K)	ϕ_v (cm^3/mol)	ϕ_E (cm^3/mol per K)	ϕ_{ks} ($\times 10^4$) (cm^3/mol per bar)	$\Delta\phi_{ks}/\Delta T$ ($\times 10^4$) [b] (cm^3/mol per bar per K)
Purine riboside	252.2	40.9 ± 0.3	0.74 ± 0.06	–	–	–	–
Adenosine	267.2	43.3 ± 0.3	0.78 ± 0.06	170.8 ± 0.7	0.26 ± 0.02	−5.8 ± 0.9	0.98 ± 0.08
2′-Deoxy-adenosine	251.2	42.3 ± 0.3	0.85 ± 0.006	169.8 ± 0.7	0.25 ± 0.02	1.3 ± 0.9	1.01 ± 0.08
3′-Deoxy-adenosine	251.2	43.3 ± 0.5	0.84 ± 0.06	–	–	0.4 [a]	1.01
Inosine	268.2	45.5 ± 0.3	–	164.6 ± 0.6	–	−13.8 ± 0.8	–
Guanosine	283.2	48.7 ± 0.6	0.99 ± 0.07	178.2 ± 0.6	–	−10.9 ± 1.1	1.15
2′-Deoxy-guanosine	267.2	46.5 ± 0.4	0.90 ± 0.07	173.7 ± 0.6	–	−5.8 ± 0.9	1.05
Cytidine	243.2	50.7 ± 0.4	0.76 ± 0.03	153.7 ± 0.5	–	−17.0 ± 0.8	0.99
2′-Deoxy-cytidine	227.2	46.6 ± 0.3	0.73 ± 0.06	153.4 ± 0.5	–	−6.2 ± 1.0	0.90
Uridine	244.2	44.6 ± 0.3	0.87 ± 0.06	151.7 ± 0.5	–	−13.8 ± 0.7	1.08
2′-Deoxy-uridine	228.2	40.4 ± 0.4	0.86 ± 0.04	152.2 ± 0.4	–	−2.6 ± 0.7	1.03
Thymidine	242.2	48.1 ± 0.6	0.96 ± 0.03	167.6 ± 0.6	–	−1.8 ± 0.7	1.12
Ribose	150.1	33.4 ± 0.2	0.48 ± 0.02	95.1 ± 0.3	0.12 ± 0.01	−12.1 ± 0.4	0.59 ± 0.03
2′-Deoxy-ribose	134.1	32.2 ± 0.2	0.47 ± 0.02	94.7 ± 0.4	0.09 ± 0.01	−4.8 ± 0.5	0.53 ± 0.03

[a] In calculations of the ϕ_{ks} values of 3′-deoxyadenosine, the ϕ_v value of 2′-deoxyadenosine was used as ϕ_v.

[b] In calculations of $\Delta\phi_{ks}/\Delta T$ for nucleosides whose ϕ_E value was not measured, 0.255 cm^3/mol per K, the average for adenosine and 2′-deoxyadenosine, was used as ϕ_E. To justify such an approach, the result reported in our previous paper can be given: for all nucleic bases for which the ϕ_E values were measured (total of five compounds), these values were the same within experimental error.

4. Discussion

4.1. Relaxation compressibility

The relaxation contribution to the apparent molar adiabatic compressibility and the concentration increment of the ultrasound velocity of nucleosides can be calculated from the data on the frequency dependence of ultrasound absorption in aqueous solutions of these molecules. Such measurements were performed by Rhodes and Shimmel [21] and Hemmes et al. [22,23]. The excessive absorption in nucleoside solutions and, correspondingly, the relaxation compressibility are explained by the *syn-anti* isomerization, i.e., by the

Table 2

Relaxation contributions to the values of concentration increment of ultrasound velocity A_r and apparent molar adiabatic compressibility $\bar{K}_r$ of nucleosides

Nucleoside	$-A_r$ (cm^3/mol)	$\bar{K}_r$ ($\times 10^4$) (cm^3/mol per bar)
Adenosine [a]	2.5	2.2
2′-Deoxyadenosine [a]	2.0	1.8
Guanosine [b]	1.8	1.6
Inosine [b]	0.1	0.1
Uridine [b]	0	0
Cytidine [b]	0	0

[a] Calculated from the data of Hemmes et al. [22].

[b] Calculated from the data of Rhodes and Shimmel [21].

rotation of the nucleic base relative to the ribose around the glycosidic bond, and are described by a process with one relaxation time. Table 2 lists the values of relaxation contributions to the concentration increment of ultrasound velocity A_{rel} and the apparent adiabatic compressibility calculated from the data of Rhodes and Shimmel [21] and Hemmes et al. [22,23] using the equation:

$$A_{rel} = [(\alpha\lambda)_{max}]/\pi c\rho \cdot 1/\left[1 + (f/f_{rel})^2\right] = 2\beta_0 \bar{K}_{rel}$$

where $(\alpha\lambda)_{max}$ is the maximum value of ultrasound absorption per wavelength, f_{rel} the relaxation frequency, and f the frequency at which ultrasound velocity is measured [24]. The values $(\alpha\lambda)_{max}/c$ and f_{rel} in practice do not depend on concentration [21–23]. The relaxation contribution is far from zero only in the case of the purine nucleosides, adenosine and guanosine. For inosine, the relaxation contribution is small which is in good agreement with NMR data indicative of the prevailing *anti* conformation in aqueous solution [25], in contrast to other purine nucleosides having a significant proportion of the *syn* conformation. For purine riboside, no experimental data are available on ultrasound absorption. However, it can be supposed that the $\bar{K}_{rel}$ value for purine riboside is approximately the same as that for adenosine, since the conformations of these molecules, having similar chemical compositions in aqueous solution, are closely related [25]. In the case of the pyrimidine nucleosides, uridine and cytidine, the calculated A value is negligible. Let us assume that the contribution of $\bar{K}_{rel}$ to ϕ_{ks} for deoxyuridine, deoxycytidine and thymidine is also very small and therefore negligible.

The temperature dependence A_{rel} calculated from the data of Rhodes and Shimmel [21] on ultrasound absorption at different temperatures is insignificant and can be neglected.

4.2. Hydration of ribonucleosides

4.2.1. Qualitative analysis

The reported $(\phi_v - \bar{V}_w)$, ϕ_{ks}, ϕ_E and $\Delta\phi_{ks}/\Delta T$ values for nucleosides are characterized by the same regularities as those corresponding to nucleic bases: (1) high sensitivity of the $(\phi_v - \bar{V}_w)$ and ϕ_{ks} values to the chemical composition of a nucleoside

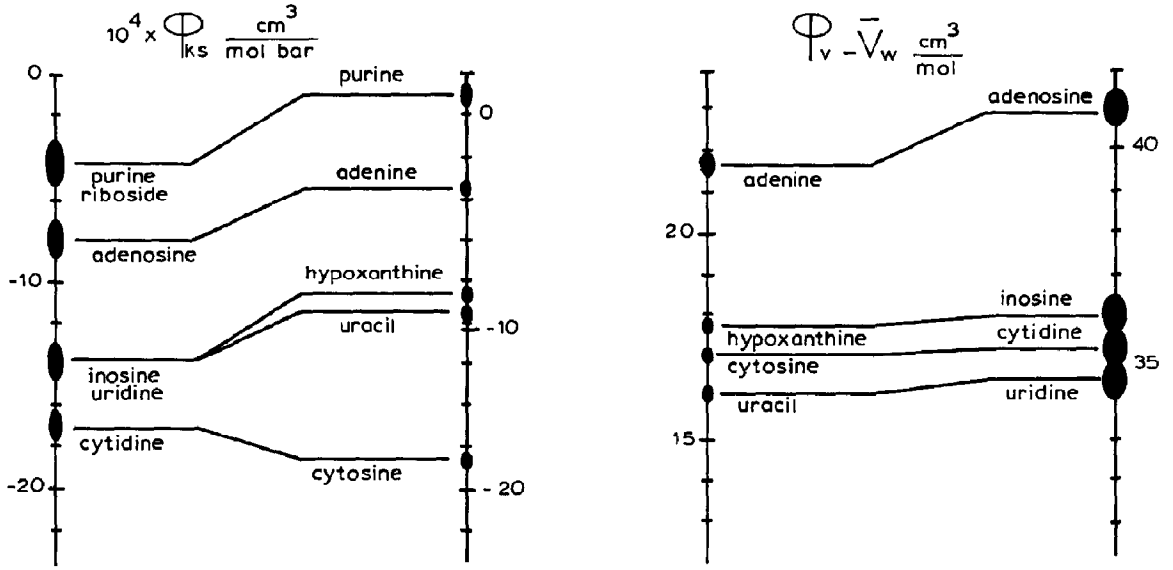


Fig. 3. Scale of apparent compressibility ϕ_{ks} corrected for the relaxation contribution and difference between the apparent volume and van der Waals volume of the molecule $(\phi_v - \bar{V}_w)$ for nucleic bases and nucleosides. In calculating ϕ_{ks} of purine riboside, the sum of the values ϕ_v of purine [4] and $\delta\phi_v$ of the ribose contribution (81 cm^3/mol, see section 4.2.2.1) was used as ϕ_v. The joining of ribose to the base results in a similar shift on the ϕ_{ks} and $(\phi_v - V_w)$ scales with the exception of cytidine. This means that all peculiarities in the hydration of free bases persist for the bases of nucleosides as well (with the exception of cytidine).

and low sensitivity of the ϕ_E and $\Delta\phi_{ks}/\Delta T$ values to the chemical composition; (2) negative ϕ_{ks} values and positive $(\phi_v - \bar{V}_w)$, ϕ_E and $\Delta\phi_{ks}/\Delta T$ values [4]. This supports the contention that the physical parameters of water, surrounding the nucleosides in solution, are comparable to those of water around the nucleic bases. In particular, as in the case of bases [4], one can speak about a partial 'normalization' (decrease in abnormality) of water surrounding nucleosides in aqueous solution.

Fig. 3 shows scales of ϕ_{ks} corrected for $\bar{K}_r$ and $(\phi_v - \bar{V}_w)$ values for nucleic bases and nucleosides. The relative arrangement of the bases and the corresponding nucleosides is the same on both the ϕ_{ks} and $(\phi_v - \bar{V}_w)$ scales. The association of ribose to a base leads to a shift in both scales which is the same within the experimental error for all of the nucleosides studied (with the exception of cytosine). This means that all characteristic properties of hydration for free bases are also retained for the bases within nucleosides. An exception is cytosine: its association with ribose is accompanied by a shift in the ϕ_{ks} scale which is significantly smaller than that for other bases. It was shown earlier [4] that the hydration parameters of cytosine differ greatly from those of other bases. The reason for the difference of shifts in the ϕ_{ks} scale between cytidine and other nucleosides is discussed below.

4.2.2. Quantitative analysis

In a quantitative analysis of the hydration of nucleosides, it is more convenient to use the contributions of individual atomic groups to the hydration parameters, and not the absolute values of the hydration parameters of molecules. The difference in the values X for molecules with and without a given atomic group is henceforth designated as the contribution of this group to the X parameter. Contributions will be denoted δX. According to the definition, δX of the R group within the R'-R molecule represents the change in X value in the conventional reaction: $R'\text{-}H \xrightarrow{\delta X} R'\text{-}R$. As mentioned above, among all of our hydration parameters obtained for nucleosides, only $(\phi_v - \bar{V}_w)$ and ϕ_{ks} are sensitive to chemical composition, and therefore will be analyzed. The most important factor in a quantitative comparison of nucleoside parameters is the problem of the experimental error in their determination. The ϕ_v value is determined directly in the experiment. The ϕ_{ks} value is calculated from the ϕ_v and A values using eq. 1. Correspondingly, the error in determining ϕ_{ks} is the sum of ϕ_v and A errors, the ϕ_v error being significantly greater than that of A. Thus, in a quantitative analysis of nucleoside hydration it is more convenient to use the value of δA as the hydration parameter of the atomic groups instead of $\delta\phi_{ks}$.

4.2.2.1. Influence of the base on ribose hydration in a ribonucleoside molecule. Table 3 lists the contributions of ribose to the values of the apparent molar volume, concentration increment of ultrasound velocity and temperature slopes of these values. The contributions to the concentration increment of the ultrasound velocity are corrected with the relaxation component being taken into account, i.e., they do not contain the relaxation contribution. The values of contributions have

Table 3

Ribose contributions to the values of concentration increment of ultrasound velocity δA, apparent molar volume $\delta\phi_v$ and hydration components of these contributions δA_h and $\delta(\phi_v - \bar{V}_w)$ for ribonucleosides

Nucleoside	δA [a] (cm³/mol)	δA_h [a] (cm³/mol)	$\delta\phi_v$ (cm³/mol)	$\delta(\phi_v - \bar{V}_w)$ (cm³/mol)	$\delta\phi_{ks}$ ($\times 10^4$) [a] (cm³/mol per bar)
Purine riboside	20.3 ± 0.5	24.5	–	–	–
Adenosine	20.1 ± 0.5	24.3	81.5 ± 0.9	19.4	−4.5 ± 1.2
Inosine	20.1 ± 0.5	24.3	80.5 ± 0.8	18.4	−5.2 ± 1.1
Cytidine	16.1 ± 0.7	20.3	80.4 ± 0.7	18.3	−1.7 ± 1.2
Uridine	19.7 ± 0.5	23.9	80.6 ± 0.7	18.5	−4.3 ± 1.1

[a] δA, δA_h and $\delta\phi_{ks}$ values have been corrected for the relaxation contribution.

been calculated from the data of tables 1 and 2, as well as on the basis of the A and ϕ_v values and the temperature slopes of these values for the nucleic bases reported in ref. 4.

4.2.2.2. Purine ribonucleosides. The contributions of ribose to each value investigated are equal for all of the ribonucleosides studied with a purine base: purine riboside, adenosine and inosine. This signifies that, within experimental error, the degree of hydration of ribose is identical in the three nucleosides. This result can be made quantitative by comparing the error in determining the ribose contributions to the A and ϕ_v values with the value of the hydration component A and ϕ_v. The hydration component of the ribose contribution to the apparent molar volume, i.e., the $\delta(\Delta V_h)$ value, is determined by the difference $\delta(\phi_v - \bar{V}_w)$, as stated in section 1. The equation for the hydration component of ultrasound velocity (δA_h) can be derived from eq. 1 by excluding all non-hydration terms from A:

$$\delta A_h = \delta A - \delta \bar{V}_w + \delta M/2\rho_0$$
$$= \delta(\Delta \bar{V}_h) - [\delta(\Delta K_h)]/2\beta_0$$

The δA_h and $\delta(\phi_v - \bar{V}_w)$ values are given in table 3. From these data, it can be assumed that the close agreement in the δA value for ribose among purine riboside, adenosine and inosine is indicative of the difference in hydration of the ribose, if any, between these compounds being no greater than 6%. From the data on $\delta\phi_v$ it follows that the difference in hydration of ribose between adenosine and inosine does not exceed 14%. The following data can be given as a quantitative illustration of possible changes in the A and ϕ_v values for small variations in the degree of ribose hydration: for ribose, xylose and arabinose, which have the same chemical composition and differ only in the direction of the C–OH bond, the ϕ_v values vary by 3 cm^3/mol [26], while the A values vary by 4–5 cm^3/mol (calculated from the data reported in refs. 19 and 20); this exceeds the difference in $\delta\phi_v$ and δA values of ribose for the ribonucleosides studied with a purine base which is no greater than 2.7 and 1.2 cm^3/mol, respectively.

The investigated nucleosides differ in both chemical composition of the nucleic base and conformation. The difference in composition is the consequence of the C_6 position of the nucleic base in purine riboside, adenosine and hypoxanthine containing H, NH_2 and O groups, respectively. Moreover, in the case of inosine, the N_1 atom possesses a proton. The most essential difference in their conformations concerns variations in the angle of rotation of the plane of the nucleic base relative to the ribose around the glycosidic bond [25,27–30]. The molecules of inosine in aqueous solution adopt an *anti* conformation in which the nucleic base is rotated around the glycosidic bond to the opposite side from the five-membered ring of ribose. In the case of adenosine, judging from NMR data [27] and ultrasound absorption measurements [22], 25% of the molecules in aqueous solution are in the *syn* conformation in which the nucleic base faces the ribose. Comparable distributions of *syn* and *anti* conformations exist for purine riboside molecules [25]. All mentioned above means that the hydration of ribose in the ribonucleosides being investigated with a purine base does not depend on alterations in conformation. This conclusion is consistent with the results reported by Hemmes et al. [22] on ultrasound absorption in adenosine solutions which show that the volume effect of the *syn-anti* isomerization reaction does not exceed 1 cm^3.

4.2.2.3. Pyrimidine nucleosides. Two ribonucleosides with a base of the pyrimidine type, uridine and cytidine, have been studied in the present paper. For uridine, the contribution of ribose to the A and ϕ_v values is the same (within experimental error) as that for nucleosides with a base of the purine type. For cytidine, the contribution of ribose to ϕ_v is equal to that for uridine, and the contribution to A is 3.6 ± 1.2 cm^3/mol lower. The difference between the contributions of ribose to A for cytidine as compared to those for the other nucleosides could be the result of: (i) the hydration behavior of the cytidine base and the influence of atomic groups of the base on the hydration of ribose, or of (ii) the conformational properties of cytidine. The first possibility is in agreement with the conclusion drawn in the previous paper that the hydration parameters of cytosine differ from those of other nucleic bases and their analogs [4]. The conformational properties of

cytidine have been studied by use of NMR spectroscopy [31]. In solution, about 20% of cytidine molecules are in the *syn* conformation [31], whereas molecules of uridine adopt only the *anti* conformation [32]. Differences in conformation of the five-membered ribose ring between cytidine and uridine are small [28,31]. When cytidine is in the *syn* conformation, the C=O group can be located near the ribose, which should lead to the mutual dehydration of this group and the atomic groups of ribose. The contribution of these groups to the A_h value is positive [4], which can lead to a decrease in the δA of ribose observed experimentally. The question then arises as to whether it is possible to explain the observed differences in the contributions of ribose within cytidine and other nucleosides as being the result of 20% of the molecules existing in the *syn* conformation. If this is indeed possible, then, taking into account the error in the NMR method for determining the proportion of the *syn* conformation which is about 10% [31], the dehydration of cytidine upon transformation of 1 mol cytidine from the *anti* to the *syn* conformation should lead to a decrease in the A and A_h values by 9–40 cm^3/mol, which is too large. For comparison, it may be noted that 9 cm^3/mol corresponds to a 40% decrease in δA_h of ribose, viz., 3-fold greater than for δA_h of the NH_2 group. Thus, either the NMR method underestimates the value for the fraction of cytidine molecules adopting the *syn* conformation in solution, or the observed difference in the contribution of ribose as compared to other nucleosides is due, at least in part, to the uniqueness of the hydration shell of cytidine or its base, cytosine.

4.2.2.4. Influence of ribose on the hydration parameters of atomic groups of a nucleic base. Table 4 summarizes the contributions of the three atomic groups, NH_2, H–···=O and CH_3, of nucleic bases and nucleosides to the A and ϕ_v values. The contributions by the atomic groups of the free bases have been calculated from the data reported in ref. 4. As shown in table 4, there is reasonable agreement between the contributions to all the investigated values (within experimental error) for each atomic group of a free nucleic base and nucleoside. This assertion can be quantified via a comparison of the experimental error in determining the contributions with the hydration components of the corresponding contributions (Table 4). By comparing the δA_h values, it can be shown that if there is any influence exerted by ribose on the hydration of the NH_2 group within the adenosine molecule, then it does not exceed 45%; in the case of the H–···=O groups of inosine this effect does not exceed 25% and for the CH_3 group of thymidine, the influence of deoxyribose does not exceed 50%. From the data on the apparent molar volume, it follows that the influence of deoxyribose on the hydration of the CH_3 group of thymidine, if any, does not exceed 50%.

Table 5 lists the ΔA values for the protonation and deprotonation reactions of nucleic bases and nucleosides. The ΔA value is determined by alterations in the degree of hydration of a nucleic base upon transition from the neutral form to the

Table 4

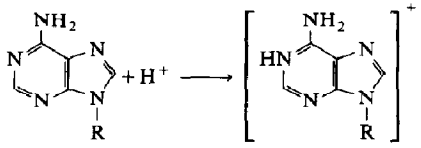
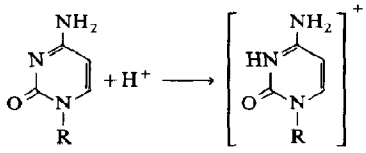
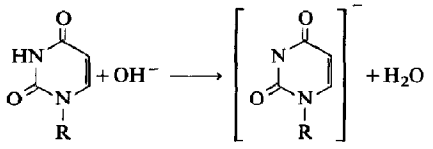
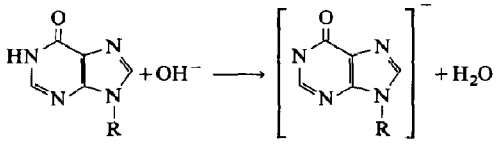
Contributions of atomic groups of nucleic bases to the values of the concentration increment of ultrasound velocity δA, of apparent molar volume $\delta\phi_v$ and their hydration components (δA_h and $\delta(\phi_v - \bar{V}_w)$)

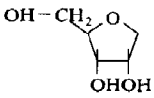
Atomic group	Substance	δA [a] (cm^3/mol)	δA_h [a] (cm^3/mol)	$\delta\phi_v$ (cm^3/mol)	$\delta(\phi_v - \bar{V}_w)$ (cm^3/mol)	$\delta\phi_{ks}(\times 10^4)$ [a] (cm^3/mol per bar)
$-NH_2$	adenine	2.6±0.4	2.7	4.8±0.4	−2.6	−4.8±0.6
	adenosine	2.4±0.6	2.5	–	–	–
H–···=O	hypoxanthine	2.7±0.4	4.8	−0.4±0.4	−6.3	−10.0±0.6
	inosine	2.5±0.6	4.6	–	–	–
$-CH_3$	thymine	7.1±0.4	3.6	16.5±0.4	6.0	1.1±0.7
	thymidine	7.7±1.0	4.2	15.4±1.0	4.9	0.8±1.4

[a] Values of δA, δA_h and $\delta\phi_{ks}$ have been corrected for the relaxation contribution.

Table 5

Changes in the concentration increment of ultrasound velocity ΔA upon protonation and deprotonation of nucleic bases (as the free species and within nucleosides)

Reaction	Substance	ΔA (cm^3/mol)	ΔA_h [a] (cm^3/mol)
	adenine adenosine	21.2 ± 0.2 21.1 ± 0.2	25.6
	cytosine cytidine	6.4 ± 0.3 10.2 ± 0.2	34.1
	uracil uridine	22.3 ± 0.2 22.2 ± 0.3	26.2
	hypoxanthine inosine	30.3 ± 0.2 31.3 ± 0.2	32.3

R = H in the case of adenine, cytosine, uracil, hypoxanthine; R =  for the other substances

[a] A_h, the hydration component of the A value of the base.

charged state as well as by hydration of H^+ (in the protonation reaction) and OH^- (in the deprotonation reaction) [33]. As follows from the data of table 5, for adenine and uridine the ΔA values of the free base and of the base within the nucleoside coincide. This signifies that the above conclusion as to the lack of effect of ribose on the hydration of the base in nucleosides is valid not only for the neutral forms but also for charged bases. In the case of hypoxanthine, the ΔA values of the base and nucleoside differ by 1 cm^3/mol, which does not greatly exceed the experimental error (0.4 cm^3/mol). A marked influence of ribose on hydration of the base within the nucleoside molecule has been observed only for cytidine, for which the ΔA values of the base and nucleoside differ by 3.8 cm^3/mol, significantly in excess of the experimental error (0.5 cm^3/mol).

4.3. Difference in hydration of ribo- and deoxyribonucleosides

It is more convenient to compare the hydration of ribo- and deoxyribonucleosides in terms of the contributions arising from the 2′-OH group of ribose. Table 6 lists the values of $\delta\phi_v$ and δA of the 2′-OH group of various nucleosides, representing the differences in ϕ_v and A values of ribo- and deoxyribonucleosides. These values are similar (within experimental error) for nucleosides with

a purine base and coincide with those for ribose. This implies that the substitution of ribose by deoxyribose in adenosine and guanosine has no effect on the hydration of the nucleic base. For nucleosides with a pyrimidine base (cytidine and uridine) this does not hold true. The δA and $\delta\phi_v$ values for 2′-OH groups of these nucleosides differ greatly from those of the free ribose and nucleosides with a purine base. This difference could arise from both the interaction between the base and deoxyribose and the conformational characteristics of deoxyribose, and thereby as a result of its hydration behavior within deoxyribonucleoside molecules with a pyrimidine base.

4.4. Hydration parameters of complementary pairs of nucleosides for DNA and RNA

The problem posed by the varying degrees of hydration of AT and GC base-pairs within the DNA double helix has become a matter arousing heated debate. The difference between the hydration properties of AT- and GC-rich DNAs in solution was studied using buoyant density and calorimetry measurements [34,35]. Both methods gave qualitatively compatible results: the influence of AT pairs of DNA on water is greater than that of GC pairs. On the whole, this agrees with X-ray scattering data on oligonucleotide crystals, according to which the AT sites of B-DNA have a regular spine of water molecules that is destroyed in the GC sites [36]. The question therefore arises as to whether this difference in hydration of the AT and GC pairs within the double helix is the result solely of the structural characteristics of the helix or if it exists in free nucleotides.

Table 6

Contributions of the -OH group of nucleosides and ribose to the values of the concentration increment of ultrasound velocity δA and apparent molar volume $\delta\phi_v$

δA and $\delta\phi_{ks}$ values have been corrected for the relaxation contributions to the A value (table 2). In the case of 3′-deoxyadenosine and 2′-deoxyguanosine for which data on A_r are unavailable, the A_r values for 2′-deoxyadenosine and guanosine, respectively, have been used. Such a substitution is justified as the A_r values per se are comparable with the error in the determination of δA and, even without the relaxation contribution being taken into account, would not affect the interpretation of the results.

Substance	Position of OH group	δA (cm^3/mol)	$\delta\phi_v$ (cm^3/mol)	$\delta\phi_{ks}$ ($\times 10^4$) (cm^3/mol per bar)
Ribose	2′	1.2±0.4	0.4±0.7	−7.3±0.9
Adenosine	2′	1.5±0.6	1.0±1.4	−6.5±1.8
Adenosine	3′	0.5±0.8	–	–
Guanosine	2′	2.2±1.0	4.5±1.2	−5.1±2.0
Uridine	2′	4.2±0.7	0.5±0.9	−11.2±1.4
Cytidine	2′	4.1±0.7	0.3±1.0	−10.8±1.8

Table 7

Values of the apparent molar adiabatic compressibility for complementary pairs of free nucleosides

The values of ϕ_{ks} corrected for the relaxation contribution $\bar{K}_{rel}$ are given in parentheses.

Ribonucleosides		Deoxyribonucleosides	
Pair	ϕ_{ks} ($\times 10^4$) (cm^3/mol per bar)	Pair	ϕ_{ks} ($\times 10^4$) (cm^3/mol per bar)
A+U	−19.6±1.6 (−21.8)	A+T	−0.5±1.3 (−2.3)
G+C	−27.9±1.1 (−29.5)	G+C	−12.0±1.9 (−13.6)

Table 7 lists the total hydration parameters for AT and GC pairs of ribo- and deoxyribonucleosides. The data show that AT and GC pairs of free nucleosides differ strongly with respect to hydration. However, in the case of deoxyribonucleosides, this variation contrasts with the results obtained for the DNA double helix, and the influence of the GC pairs of free deoxynucleosides on water is more pronounced vs. that of AT pairs. The opposite forms of behavior between the hydration of the AT and GC pairs of the DNA double helix and of free nucleosides can be explained as resulting from: (1) differing numbers of hydrogen bonds between the nucleic bases in the AT and GC pairs or (2) the three-dimensional structure of the double helix that leads to partial dehydration of the GC pairs or a particular type of hydration for the AT pairs. Both possibilities seem likely to result since the difference in ϕ_{ks} values between the AT and GC pairs of free nucleosides is too large. A firmer basis for justify-

ing such a conclusion can be established once the ϕ_{ks} values for AT and GC base-pairs of the DNA double helix have been measured.

5. Conclusions

(1) The properties of water surrounding nucleosides in solution do not differ significantly from those surrounding nucleic bases.

(2) Within ribonucleoside molecules, only in the case of cytidine is a noticeable influence exerted by the ribose on hydration of the atomic groups in the nucleic base.

(3) Qualitative differences in the hydration of ribo- and deoxyribonucleosides occur only for nucleosides with a pyrimidine base.

(4) Hydration of the GC pairs of free deoxyribonucleosides is more pronounced than that of the AT pair, which stands in contrast to the degree of hydration of the AT and GC pairs within the DNA double helix.

References

1 W. Saenger, Principles of nucleic acid structure (Springer, New York, 1984).
2 Y. Texter, Prog. Biophys. Mol. Biol. 33 (1978) 83.
3 V.A. Buckin, Mol. Biol. (U.S.S.R.) 21 (1987) 512.
4 V.A. Buckin, Biophys. Chem. 29 (1988) 283.
5 S.D. Sadikhova and F.I. Braginskaya, Biofizika 20 (1978) 20.
6 H. Shiio, T. Ogawa and H. Yoshihashi, J. Am. Chem. Soc. 77 (1955) 4950.
7 J. Antosiewicz, A. Yuszkiewicz and D. Shugar, J. Phys. Chem. 86 (1982) 4831.
8 T. Yasunaga, Y. Hirata, Y. Kawano and M. Miura, Bull. Chem. Soc. Jap. 37 (1964) 867.
9 A. Bondi, J. Phys. Chem. 68 (1964) 441.
10 J.T. Edward, J. Chem. Educ. 47 (1970) 261.
11 F.B. Sherman, Talanta 27 (1980) 1067.
12 G.D. Fasman, Handbook of biochemistry and molecular biology, 3rd edn. (CRC Press, Cleveland, OH, 1975) vol. 1, p. 65.
13 F.J. Millero, G.K. Ward and P.V. Chetirkin, J. Am. Chem. Soc. 61 (1977) 1492.
14 V.S. Del Grosso, J. Acoust. Soc. Am. 52 (1972) 1442.
15 P. Hemmes, A.A. Mayevsky, V.A. Buckin and A.P. Sarvazyan, J. Phys. Chem. 84 (1980) 699.
16 H. Høiland, A. Skauge and I. Stokkeland, J. Phys. Chem. 88 (1984) 6350.
17 B.I. Kankiya, S.N. Buckina, S.R. Valaeva and V.A. Buckin, in: Proc. Int. Conf. Acoustics Ultrasound '86 (Bratislava, Dom techniky CSVITS, 1986) p. 126.
18 D.D. Kasarada, Biochim. Biophys. Acta 217 (1970) 535.
19 H. Shiio, J. Am. Chem. Soc. 80 (1958) 70.
20 A. Lo Surdo, C. Shin and F.J. Millero, J. Chem. Eng. Data 23 (1978) 197.
21 L.M. Rhodes and P.S. Shimmel, Biochemistry 10 (1971) 4426.
22 P.R. Hemmes, L. Oppenheimer and F. Jordan, J. Am. Chem. Soc. 96 (1974) 6023.
23 F. Jordan, S. Nishikawa and P. Hemmes, J. Am. Chem. Soc. 102 (1980) 3913.
24 J. Stuer and E. Yeager, in: Physical acoustics, vol. 2 (Russian transl., Moscow, Mir, 1968), p. 371.
25 A.P. Zens, T.A. Bryson, R.B. Dunlap, R.R. Fisher and P.D. Ellis, J. Am. Chem. Soc. 98 (1976) 7559.
26 F. Franks, in: Biochemical thermodynamics (Elsevier, Amsterdam, 1979) p. 15.
27 R. Stolarski, L. Dudycz and D. Shugar, Eur. J. Biochem. 108 (1980) 111.
28 W. Guschlbauer, Biochim. Biophys. Acta 610 (1980) 47.
29 G. Klimke, I. Cuno, H.-D. Ludemann, R. Mengel and M.Y. Robins, Z. Naturforsch. 34c (1979) 1075.
30 F.E. Hruska, D.J. Wood and H. Singh, Biochim. Biophys. Acta 474 (1977) 129.
31 I.D. Bobruskin, M.Yu. Pokrovskaya, M.P. Kirpichnikov and V.L. Florentiev, Mol. Biol. (U.S.S.R.) 13 (1979) 1407.
32 B.J. Blackburn, A.A. Grey, C.P. Smith and F.E. Hruska, Can. J. Chem. 48 (1970) 2866.
33 V.A. Buckin, A.P. Sarvazyan, E.I. Dudchenko and P. Hemmes, J. Phys. Chem. 84 (1980) 696.
34 M.J.B. Tunis and J.E. Hearst, Biopolymers 6 (1968) 1325.
35 G.M. Mrevlishvili, Proc. Acad. Sci. U.S.S.R. 260 (1981) 761.
36 H.R. Drew and R.E. Dickerson, J. Mol. Biol. 151 (1981) 535.