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Hydration of nucleic bases in dilute aqueous solutions

Apparent molar adiabatic and isothermal compressibilities, apparent molar volumes and their temperature slopes at 25 °C

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The concentration increment of the ultrasound velocity has been measured with an accuracy of ±0.03 cm/s in dilute aqueous solutions of a variety of nucleic bases and their derivatives in the concentration range 0.5–1.5 mg/g H_2O at temperatures of 15–35 °C. A new method for the precise measurement of ultrasound velocity in small volumes of liquids has been used. The values of the apparent molar adiabatic compressibilities plus the corresponding temperature slopes, apparent molar volumes with their temperature slopes, and apparent molar isothermal compressibilities at infinite dilution have been obtained. The regularities describing the signs of these values and their dependence on the chemical structure of the solute have been revealed. It is shown that these regularities can be described as a consequence of partial 'normalization' of some of the properties of water around the bases, namely, weaker structural contribution to compressibility, less negative temperature slope of compressibility and less negative structural contribution to the coefficient of thermal expansion of water.

1. Introduction

The interaction of nucleic acids with water has aroused considerable interest since the X-ray analysis of DNA by Franklin and Gosling [1] which showed that the DNA conformation in fibers was determined by their water content.

Since the pioneering work of Jakobson [2], various experimental techniques have been used to study the hydration of nucleic acids [3–5]. Most progress was made in studying nucleic acid hydration in fibers, films and crystals. The main absorption centers of water molecules in DNA and RNA, the amount of bound water, structural characteristics, and dielectric and other properties of water absorbed on these centers have been investigated. The hydration of nucleic acids in dilute solutions has been elucidated to a much smaller extent. Previous investigations of nucleic acid hydration in aqueous solutions were aimed at determination of the total number of water molecules in the hydration shell (hydration numbers). If one wishes to obtain information about the hydration of nucleic acids at the level of separate atomic groups, it is necessary to investigate not only the nucleic acids themselves, but also a great number of their components: bases, nucleosides and nucleotides. Unfortunately, most of the traditional experimental techniques for investigating molecular hydration in aqueous solutions suffer from the low

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solubility of most nucleic acid bases and nucleosides. Only a few methods have been used to study the hydration of nucleic bases and nucleosides in solution: calorimetry, separately or in combination with measurements of heats of sublimation [6,7], and measurements of the affinity of some bases to water [8]. The hydration of nucleosides and nucleotides was investigated by using an acoustic method [9,10]. However, up to now no studies have been published with regard to the hydration of nucleic acids and their components and analogs by means of one and the same method.

The present work is the first in a series of publications on the hydration of nucleic bases, nucleosides and nucleotides, polynucleotides and DNA by using a new method of high-precision ultrasound velocity measurements in small volumes (the error in the measurements of relative ultrasonic velocity being as small as $10^{-5}\%$). The results of measurements of partial apparent molar adiabatic and isothermal compressibilities, partial apparent molar volumes and temperature slopes of these characteristics at 25 °C for nucleic acid bases and their derivatives, together with an interpretation of the results concerning the changes in the thermodynamic properties of water in the hydration shell, are presented in this paper.

The traditional characteristics of aqueous solutions obtained from density and ultrasonic velocity measurements are the apparent molar volume ϕ_V and the apparent molar adiabatic compressibility of the solute ϕ_{KS}. The apparent molar volume of the solute is given by

$$\phi_V = \frac{V - n_1 \bar{V}_1^0}{n_2}$$

where n_1 is the number of moles of solvent, n_2 the number of moles of solute, V the solution volume and $\bar{V}_1^0$ the molar volume of pure solvent. The values of ϕ_V are quoted for the change in liquid volume due to 1 mol of solute being dissolved. Consequently, ϕ_V consists of two terms: the volume of the solute molecule $\bar{V}_M$ which is inaccessible to the surrounding molecules of solvent and the volume ΔV_h (contribution of the hydration) which is determined by the structure of solvent around the solute molecule and the difference between this structure and that of pure solvent:

$$\phi_V = \bar{V}_M + \Delta V_h$$

An estimation of $\bar{V}_M$ for simple molecules may be provided by the van der Waals volume $\bar{V}_W$ [11,12].

The apparent molar adiabatic and isothermal compressibilities ϕ_{KS} and ϕ_{KT} are defined in the same way as the apparent molar volume. ϕ_{KT} is related to the molecular features of solute at infinite dilution by:

$$\phi_{KT} = \bar{K}_M + \Delta K_h + \bar{K}_r$$

where $\bar{K}_M$ is the molar compressibility of the solute molecule itself. The term ΔK_h depends on the solute-solvent interaction and characterises the compressibility of the hydration shell. $\bar{K}_r$ is the relaxation compressibility, which may exist if the solute molecules are in different conformational states or tautomeric forms with a distribution depending on the pressure and temperature [13]. As shown below, for nucleic bases in dilute aqueous solutions $\bar{K}_M$, $\bar{K}_r \simeq 0$, and in this case the value of the apparent molar compressibility of these molecules depends only on their interaction with water:

$$\phi_{KT} \simeq \Delta K_h$$

In the case of apparent molar adiabatic compressibility the terms $\bar{K}_M$ and ΔK_h are not adiabatic compressibilities because in the acoustical measurements the entropy is constant only for macrovolumes (~ the ultrasound wavelength) whereas heat exchange exists between microvolumes (for example, between the hydration shell and surrounding solvent). That is why the apparent molar adiabatic compressibility is recalculated in this article to give the apparent molar isothermal compressibility, which is more suitable for molecular interpretation (see eq. 2).

2. Materials and methods

Adenine, hypoxanthine, purine, 6-methylpurine, cytosine, uracil, thymine and pyrimidine from Sigma were used without further purification. Solutions were prepared on twice distilled and degassed water of conductivity less than 10^{-6} Ω^{-1} cm^{-1}. The pH values of the solutions ranged from 6 to 7. Concentrations of the bases were determined by weighing 5–10 mg of material to a precision of ±0.01 mg and the necessary amount of water. The concentrations were further checked by spectrophotometry except for purine, 6-methylpurine and pyrimidine. The ultraviolet spectra of both neutral and acidic solutions were recorded on a Perkin Elmer spectrophotometer. The extinction coefficients were taken from ref. 14. Fisher's modified titration method [15] was used to determine the moisture content of materials. This ranged from 0 to 0.14%. The agreement between the weighed-in concentration and that determined spectrophotometrically was always within the experimental error.

Measurements of ultrasound velocity were performed with the so-called resonance method [16]. For the purposes of the present study the accuracy in the relative ultrasound velocity measurements should be about 10^{-5}% in a small volume. The equipment for such measurements has been developed in our laboratory by Sarvazyan and coworkers [16]. The velocity of ultrasound in a liquid placed in the cell (an acoustical resonator formed by two precisely parallel transducers) is determined by measuring the maximum point of the amplitude-frequency characteristic of the cell. The resonator volume is 0.8 cm^3. A high-quality factor (about 10^4) provides the necessary precision. The mechanical stability of the cell is ensured by the fact that it has no components for adjusting the parallelism of the transducers. The required parallelism, on which the quality of the resonator depends, is ensured by a special method of cell fabrication. The cells are made of the inert metal titanium. The piezotransducers consist of lithium niobate and are covered with gold. The resonance frequency of the piezotransducers is about 10 MHz. The liquid in the cell is stirred magnetically. A detailed description of the cell construction can be found in refs. 16 and 17.

In order to avoid errors in ultrasonic velocity measurements due to inaccuracy in thermostatting, a differential technique was used. The measuring and reference channels consisted of identical cells and electronic units. The temperature in both cells was maintained by means of a water thermostat with large thermal buffer vessels taken in series. The temperature was measured to an accuracy of 0.03° C. The duration of one run, in which the difference in resonance frequencies of the cell filled with water, solution and again water was measured, was about 15 min. Reproducibility of the frequency difference between measuring and reference cells was 1–2 Hz at 7 MHz after one run. All measurements were carried out within the 7.1–7.4 MHz frequency range. The concentration increment of ultrasound velocity A is calculated from eq. 1 which is valid for relative measurements of small velocity changes [18,19]:

$$A \equiv \frac{u - u_0}{u_0 c} = \frac{f - f_0}{f_0 c \rho_0} \cdot (1 + \gamma) \qquad (1)$$

where c is molal concentration of solute, u and u_0 ultrasound velocities of the solution and solvent, respectively, and f and f_0 frequencies in the maxima of the resonance peaks for the cell filled with solution and solvent, respectively. γ is a constant depending on the geometry and material of the cell. The value of γ is about 10^{-2} or less [19]. The precise value of γ was obtained by calibration of the cell with NaCl solution for which there are many data obtained by different methods. The most precise data were obtained by Owen and Kronick [20], Garnsen et al. [21] and Millero et al. [22]. The values of A for NaCl obtained in these works by extrapolation to infinite dilution are equal to 43.1, 44.1 and 43.3 cm^3/mol, respectively. According to our result, for NaCl the extrapolated value, in the concentration range 0.2–2 mg/ml to infinite dilution, of $(f - f_0)/f_0 c \rho_0$ is equal to 43.1 cm^3/mol. Thus, the γ value in eq. 1 was assumed to be zero.

The A value and its temperature slope were determined from two to three runs at 18 and 32° C. The same solution was used at both temperatures in order to reduce the additional uncertainty in the temperature slope of A resulting from errors in the concentration determination. The A values at 25° C were calculated as the averaged values at the upper and lower temperatures. Additional measurements of A at 20, 25 and 30° C were performed for purine and pyrimidine and at 25° C for cytosine. The values of A at

25 °C and $\Delta A/\Delta T$ measured at 20–30 and at 18–32 °C agreed within experimental error.

The solution density was measured using an Anton Paar DMA 602 densitometer. This technique employs the difference in vibrational frequencies of a quartz U-tube filled with water and the solution. It was calibrated by measuring the vibrational frequency of the tube filled with water and air. Measurements of the temperature dependence of the density were taken in duplicate runs at 18 and 32 °C similarly to the procedure used for velocity measurements. For each preparation two to three density measurements at 25 °C were also made. The apparent molar volume ϕ_V was calculated from the well-known equation:

$$\phi_V = \frac{M}{\rho} - \frac{\rho - \rho_0}{\rho_0 \rho c}$$

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

Measurements of A were performed at the following concentrations of the solutions: 0.6–0.7 mg/g H_2O for adenine and hypoxanthine, 1–1.5 mg/g H_2O for pyridine, and 1 mg/g H_2O for cytosine, uracil, thymine, purine and 6-methylpurine. When measuring ϕ_V the following concentrations were used: 0.5–0.6 mg/g H_2O for adenine and hypoxanthine, 1.5–2 mg/g H_2O for uracil, thymine, cytosine and pyrimidine, and 1–1.5 mg/g H_2O for purine and 6-methylpurine. Moreover, the concentration dependence of A at 20 °C was obtained for purine over the concentration range 0.01–0.24 M.

3. Results

Table 1 presents the values obtained for the concentration increment of ultrasound velocity (A), apparent molar volume (ϕ_V), apparent molar adiabatic compressibility (ϕ_{KS}), apparent molar isothermal compressibility (ϕ_{KT}), and the temperature slopes of these parameters ($\Delta A/\Delta T$, $\Delta\phi_{KS}/\Delta T$, $\Delta\phi_V/\Delta T$) for nucleic bases and their derivatives. The values of the van der Waals volumes $\bar{V}_w$ and the differences ($\phi_V - \bar{V}_w$) are also listed in table 1. The van der Waals volumes were calculated from data on the contribution of atoms to van der Waals volumes of organic molecules [11,12].

For some of the nucleic bases the values of A and ϕ_V depend on the concentration due to stacking association of the molecules in aqueous solutions [23]. Thus, the problem of the concentration dependences of A and ϕ_V requires additional discussion. The A value at 25 °C for 6-methylpurine (table 1) is obtained by extrapolation of the concentration dependence of A [24] to infinite dilution. The value of ϕ_{KS} for 6-methylpurine at 25 °C is calculated according to this A value. The A value for purine in table 1 is obtained by extrapolation to infinite dilution of the concentration dependence of A (measurements in the range 0.005–0.2 M). The difference of 0.2 cm^3/mol between the values of A at infinite dilution and at a concentration of 1.0 mg/g H_2O (measurements of A at 20–30 and 18–32 °C were made at this concentration) was supposed to be the same at 20 and 25 °C. This assumption is correct because this difference is less than the error in the determination of the A value.

One can adduce the following arguments for the other nucleic bases, for which the extrapolation to infinite dilution has not been made. For hypoxanthine and adenine the A values were obtained at concentrations 50% lower than that for 6-methylpurine. These substances stack more weakly than 6-methylpurine [25]. At any rate, the difference between the measured value and that at infinite dilution is too small to affect the interpretation. The pyrimidine bases are known to stack much less than the purine bases [25], so the difference in A between the measured value and the values for infinite dilution is negligibly small in this case. The concentration dependence of ϕ_V is less than that of A and can be neglected [26,27]. The concentration dependences of $\Delta A/\Delta T$ and $\Delta\phi_V/\Delta T$ can also be neglected within the experimental error because the relative errors in the values of $\Delta A/\Delta T$ and $\Delta\phi_V/\Delta T$ are considerably larger than in the values of A and ϕ_V. The arguments mentioned above enable one to assume the data given in table 1 as relating to infinite dilution. The ϕ_{KS} values in table 1 are calculated from the data on ϕ_V and A from the relation for

Table 1

Concentration increments of sound velocity (A), apparent molar volumes (ϕ_V), apparent molar isothermal compressibilities (ϕ_{KS}), apparent molar isothermal compressibilities (ϕ_{KT}), van der Waals volumes ($\bar{V}_w$) and temperature slopes ($\Delta A/\Delta T$, $\Delta\phi_V/\Delta T$, $\Delta\phi_{KS}/\Delta T$) of nucleic bases and their derivatives in aqueous solution at 25°C

Molecule	M	A (cm^3/mol) [a]	ϕ_V (cm^3/mol) [b]	$\bar{V}_w$ (cm^3/mol) [c]	($\phi_V - \bar{V}_w$) (cm^3/mol)	ϕ_{KS} ($\times 10^4$) (cm^3/mol per bar) [d]	ϕ_{KT} ($\times 10^4$) (cm^3/mol per bar) [e]	$\Delta A/\Delta T$ (cm^3/mol per K)	$\Delta\phi_V/\Delta T$ (cm^3 mol^{-1} K^{-1} bar^{-1})	$\Delta\phi_{KS}/\Delta T$ ($\times 10^5$) (cm^3 mol^{-1} K^{-1} bar^{-1}) [f]
Purine	120.12	22.8±0.2	84.5±0.2	60.2	24.3	1.3±0.3	5.6	−0.71±0.02	0.15±0.01	0.75±0.02
6-Methylpurine	134.15	29.1±0.2	101.8±0.2	70.7	31.1	4.9±0.3	8.9	−0.77±0.02	–	0.89±0.02
Adenine	135.14	25.4±0.2	89.3±0.2	67.6	21.7	−3.5±0.3	0.4	−0.71±0.02	0.14±0.01	0.76±0.02
Hypoxanthine	136.12	25.5±0.2	84.1±0.2	66.3	17.8	−8.7±0.3	−4.7	−0.64±0.02	–	0.72±0.02
Pyrimidine	80.09	23.7±0.5	70.0±0.3	42.7	27.3	5.5±0.6	9.5	−0.71±0.02	–	0.74±0.02
Cytosine	111.11	34.6±0.3	73.3±0.2	56.3	17.0	−15.3±0.4	−11.6	−0.63±0.02	0.13±0.01	0.73±0.02
Uracil	112.09	24.9±0.2	71.7±0.2	54.9	16.1	−9.5±0.4	−5.3	−0.59±0.02	0.15±0.01	0.68±0.02
Thymine	126.12	33.0±0.2	88.2±0.2	65.3	22.9	−8.4±0.3	−4.5	−0.78±0.02	0.14±0.01	0.84±0.02

[a] For purine and 6-methylpurine the A values were obtained by extrapolation to infinite dilution, for adenine and hypoxanthine the A values were obtained at concentrations of 0.6–0.7 mg/cm^3, and for other molecules at 1 mg/cm^3.

[b] The ϕ_V values were obtained for adenine and hypoxanthine at concentrations of 0.5–0.6 mg/cm^3, for uracil, thymine, cytosine and pyrimidine at 1.5–2 mg/cm^3, and for purine and 6-methylpurine at 1–1.5 mg/cm^3.

[c] Calculated from the data of Bond and Edward on the contribution of atoms to the van der Waals volumes of organic molecules [11,12].

[d] Calculated from the A and ϕ_V values.

[e] Calculated from the values of ϕ_{KS}, $\Delta\phi_V/\Delta T$ and literature data on ϕ_{cp}; see the text.

[f] Calculated from the average values of the temperature slopes for A and ϕ_V in the range 18–32°C.

infinite dilution [28]:

$$\phi_{KS} = 2\beta_0(\phi_V - A - M/(2\rho_0))$$

where β_0 is the adiabatic compressibility coefficient for pure water.

The value of $\Delta\phi_V/\Delta T$ for hypoxanthine and pyrimidine molecules was not measured. In this case an average value of $\Delta\phi_V/\Delta T$ for all other bases equal to 0.14 cm^3 mol^{-1} K^{-1} was used for calculations of $\Delta\phi_{KS}/\Delta T$. This can be done because the $\Delta\phi_V/\Delta T$ value for nucleic bases differing in chemical structure is equal to 0.13–0.15 cm^3 mol^{-1} degree^{-1}, i.e., the same within experimental error. Moreover, the major part of the $\Delta\phi_{KS}/\Delta T$ value is due to $\Delta A/\Delta T$, and the contribution of $\Delta\phi_V/\Delta T$ does not exceed 20%.

The values of ϕ_{KT} are calculated from the relation:

$$\phi_{KT} = \phi_{KS} + \frac{\bar{E}_1^{0^2} T}{\bar{C}_{p1}^0}\left(2\frac{\phi_E}{\bar{E}_1^0} - \frac{\phi_{cp}}{\bar{C}_{p1}^0}\right) \tag{2}$$

where $\bar{E}_1^0$ and $\bar{C}_{p1}^0$ are the molar expansibility and heat capacity of pure water, ϕ_E and ϕ_{cp} the apparent molar expansibility and heat capacity of the solute at infinite dilution and T the absolute temperature [28]. For calculations, the values of $\Delta\phi_V/\Delta T$ obtained in this work were used instead of ϕ_E. The apparent molar heat capacities of adenine, cytosine, thymine and uracil were obtained from ref. 29 and for thymine and uracil from ref. 16. The value of ϕ_{cp} averaged with respect to adenine, cytosine, thymine and uracil was used as ϕ_{cp} for other bases. One can do so because the contribution of the second term within parentheses in eq. 2 to ϕ_{KT} does not exceed 0.18×10^{-4} cm^3 mol^{-1} bar^{-1} for nucleic bases with a known ϕ_{cp}, which is less than the experimental error in ϕ_{KT}. To estimate the value of $(\Delta\phi_{KT}/\Delta T - \Delta\phi_{KS}/\Delta T)$ the measurements of ϕ_V at 15, 25 and 35 ° C were performed for purine and cytosine for the calculation of $\Delta\phi_E/\Delta T$. The values of $\Delta\phi_E/\Delta T$ are equal to $(0.17 \pm 0.09) \times 10^{-4}$ cm^3 mol^{-1} K^{-2}) for both substances. The estimated difference between $\Delta\phi_{KT}/\Delta T$ and $\Delta\phi_{KS}/\Delta T$ is insignificant for the interpretation given in section 4.

4. Discussion

4.1. Relaxational compressibility

The relaxation contribution $\bar{K}_r$ to the apparent compressibility of nucleic bases at infinite dilution is determined by two processes. The first is protonation and deprotonation of the nucleic bases. The pK values of these molecules are in the acidic or alkaline pH range [14]. Therefore, the contribution of this process to ϕ_{KS} at neutral pH can be neglected. The second is interconversion of different tautomeric forms. The purines may exist in water mainly in two forms differing in the position of a hydrogen atom (N_7HN_9 and N_7N_9H) [30,31]. The dynamics of interconversion of these forms was investigated for adenine by Dreyfus et al. [31]. The estimation of $\bar{K}_r$ from the data of these authors shows that its value is negligibly small. This is due to two causes. The interconversion of tautomeric forms occurs with participation of protons and hydroxyls or the anionic form of the base [31] but their concentrations at neutral pH are very small and the amplitude of the relaxation process is insignificant. Furthermore, the interconversion of purine isomers is a slow process [31] with a relaxational frequency much lower than 7 MHz which is our operating frequency. Therefore, we can assume that $\bar{K}_r = 0$. For pyrimidines the equilibrium between the tautomeric forms is shifted strongly toward one of the forms [30,32]. Consequently, the value of $\bar{K}_r$ for these molecules can be neglected. Moreover, all the above-stated arguments for the absence of a relaxational term in ϕ_{KS} for purines are also valid for pyrimidines.

The relaxational components of ϕ_E and ϕ_{cp} can provide an additional contribution to ϕ_{KT}. However, as mentioned above, the differences in $\Delta\phi_V/\Delta T$ values for various purines and pyrimidines are within the experimental error. The contribution of ϕ_{cp} to ϕ_{KT} is insignificant. Therefore, the relaxational components in ϕ_E and ϕ_{cp} can be neglected.

4.2. The intrinsic compressibility of bases

The intrinsic compressibility of bases ($\bar{K}_M$) is determined by two processes: the pressure dependence of the distance between the atomic nuclei (compressibility of covalent bonds) and the change in van der Waals radii of atoms with pressure (the compressibility of atoms). The compressibility of covalent bonds can be estimated from the compressibility of diamonds. The estimation shows that the contribution of compressibility of covalent bonds to ϕ_{KT} can be neglected. The compressibility of nucleic base atoms can be estimated from that of solid graphite, with the π-electron system in the planes of carbon atoms similar to that of the nucleic bases. Using the data on graphite compressibility [33] in a direction perpendicular to the planes, we find for purines that compressibility of atoms is a small fraction of ϕ_{KT} and does not exceed 0.1×10^{-4} cm^3 mol^{-1} bar^{-1}, which is within the experimental error. The previous statement shows that the contribution of the values of intrinsic compressibility of the bases to the value of ϕ_{KT} is negligibly small. Therefore, the value of the apparent compressibility at infinite dilution depends only on the interaction of these molecules with solvent:

$$\phi_{KT} \cong \Delta K_h$$

4.3. *Correlation between apparent molar characteristics: Relation to the structure of nucleic bases*

A correlation between ϕ_{KT} and $(\phi_V - \bar{V}_w)$ is depicted in fig. 1. One can see that increasing the number of polar groups in nucleic bases results in a decrease of ϕ_{KT} and $(\phi_V - \bar{V}_w)$. This may be interpreted as follows. Water molecules in the hydration shell of a base can form hydrogen bridge-like bonds between various polar groups of the base [34,35]. This decreases the mobility of water molecules which in turn would decrease the molar volume of water and the structural component of water compressibility. For pure water the value of this structural component is 60% at 25°C [36,37].

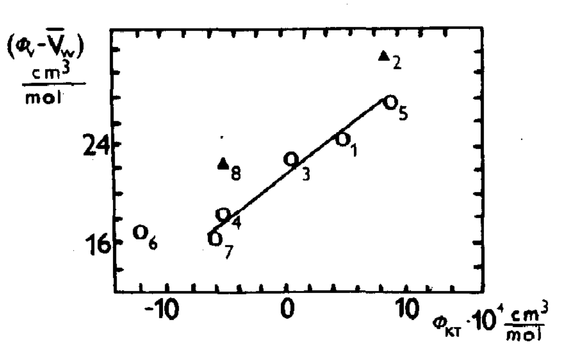

Fig. 1. Plot of $(\phi_V - \bar{V}_w)$ vs. ϕ_{KT} for nucleic bases and their derivatives at 25°C. (▲) Indicates molecules with methyl groups. (1) Purine, (2) 6-methylpurine, (3) adenine, (4) hypoxanthine, (5) pyrimidine, (6) cytosine, (7) uracil, (8) thymine.

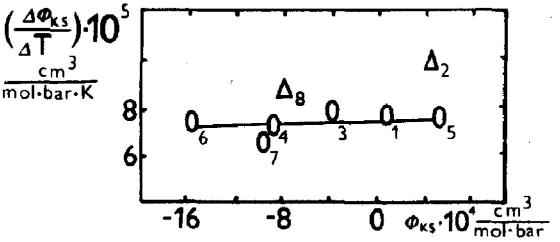

Fig. 2. Plot of apparent molar adiabatic compressibility ϕ_{KS} vs. its temperature slope for nucleic bases and their derivatives at 25°C. (Δ) Indicates molecules with methyl groups. (1) Purine, (2) 6-methylpurine, (3) adenine, (4) hypoxanthine, (5) pyrimidine, (6) cytosine, (7) uracil, (8) thymine.

The $(\phi_V - \bar{V}_w)$ dependence on ϕ_{KT} for bases without methyl groups can be approximated by a straight line with the only exception being cytosine. According to other methods, the properties of cytosine also differ from those of other bases [29]. The bases with methyl groups, thymine and 6-methylpurine, do not follow this dependence. This is a result of essential differences in the hydration of hydrophobic (methyl) and polar groups. This difference will be discussed in the following paper. The slope of the $(\phi_V - \bar{V}_w)$ dependence on ϕ_{KT} is 0.75×10^4 bar. This is much greater than the analogous slope for electrolytes (0.45×10^4 bar) [38,39], which is indicative of the different hydration of hydrophobic and charged atomic groups and molecules. $\Delta\phi_{KS}/\Delta T$ vs. ϕ_{KS} dependencies are plotted in fig. 2. As in the case examined above, 6-methylpurine and thymine do not follow the general dependence. Another interesting conclusion can be drawn from fig. 2: large changes in ϕ_{KS} (and therefore in ϕ_{KT}) correspond to insignificant changes in $\Delta\phi_{KS}/\Delta T$.

4.4. Regularities in apparent molar characteristics: Relation to the anomalies of water

Two types of qualitative regularities can be revealed from the data given in figs. 1 and 2, and table 1. The first concerns the signs of the values for the partial apparent molar characteristics. For all investigated molecules ($\phi_V - \bar{V}_w$), $\Delta\phi_V/\Delta T$ and $\Delta\phi_{KS}/\Delta T$ are positive at 25 °C while ϕ_{KS} and ϕ_{KT} may be either positive or negative. The second deals with the sensitivity of different characteristics of the chemical structure of a base. There exist so-called 'sensitive' and 'insensitive' characteristics. The sensitive type are ϕ_{KS}, ϕ_{KT} and ($\phi_V - \bar{V}_w$). ϕ_{KS} and ϕ_{KT} change sign due to the structure of the base and ($\phi_V - \bar{V}_w$) undergoes a 2-fold variation (see fig. 1). The insensitive characteristics $\Delta\phi_V/\Delta T$ and $\Delta\phi_{KS}/\Delta T$ depend weakly on the chemical structure of nucleic bases (see figs. 1 and 2 and table 1).

The above-stated regularities can be explained by anomalies in the properties of water. As is known water has some anomalous properties at room temperature which distinguish it form any other liquid. The extremum in the temperature dependences of density, compressibility and heat capacity may be cited as examples of such anomalies. The anomalies of water are due to its unique structure, more exactly to the dependence of structure on pressure and temperature [36,37,40]. One can assume that when water molecules are in contact with nucleic bases their behaviour becomes more 'normal' and the properties of water should approach those of normal liquids. The assumption of partial 'normalization' of the properties of water in the hydration shell of a solute helps to explain the regularities mentioned above. One should take into account that ΔK_h is represented by the sum:

$$\Delta K_h = K_{1,2} + \Delta K_1$$

where $K_{1,2}$ is the compressibility of the contacts and the space between the molecules of solute and the surrounding water molecules which depends on the thermal motion of the solute molecule and its stereochemical structure; ΔK_1 is defined by the changes in compressibility of water around the molecules of solute. The values of ϕ_E ($\cong \Delta\phi_V/\Delta T$) and $\Delta\phi_{KS}/\Delta T$ are presented as the sum of two analogous terms. Details of the division of ΔK_h into two terms are given in refs. 41–43. The contribution of the first term in eq. 4 to the measured characteristics is always positive. The sign of the second term can be estimated using the assumption of normalization of the properties of water near the nucleic base.

The anomalies of water can be expressed in terms of structural contributions to the thermodynamic parameters. The compressibility of water K_1 is in turn the sum of two terms:

$$K_1 = K_\infty + K_{str} \tag{3}$$

where K_{str} is the structural (relaxation) contribution caused by the changes in water structure under pressure and K_∞ the compressibility of water at frequencies higher than that of structural relaxation (~ 10^{12} Hz, see ref. 36). K_∞ is close to the compressibility of ice [36]. The large values of K_{str} is one of the anomalies of water at room temperature. Indeed, $K_{str}/K_\infty \approx 1.5$ for water at 20 °C [36] while for other liquids this ratio is in the range 0.3–0.7 [37]. The large negative structural contributions to expansibility E_{str} and to the temperature slope of compressibility $\Delta K_{str}/\Delta T$ are the other anomalies of water [37,40]. The extrema in the curves for the temperature dependences of water density and compressibility are due to the temperature dependences of E_{str} and $\Delta K_{str}/\Delta T$.

K_{str} is the most variable term of eq. 3, the same being true for the expansibility and temperature slope of compressibility. For example, the variations of K_{str}, E_{str} and $\Delta K_{str}/\Delta T$ with temperature are much greater than those of K_∞, E_∞ and $\Delta K_\infty/\Delta T$. One can expect that on changing the water structure near the surface of nucleic bases the variation of K_{str} is greater than that of K_∞ so that $\Delta K_1 \simeq \Delta K_{str}$. A similar relation should hold true for E_1 and $\Delta K_1/\Delta T$. The normalization means a decrease of the absolute values of structural contributions. Taking into account that $K_{str} > 0$ and $\Delta K_{str}/\Delta T$, $E_{str} < 0$ we have:

$$\begin{aligned} &\phi_{KT} \cong |K_{1,2}| - |\Delta K_1| \\ &\phi_E \cong |E_{1,2}| + |\Delta E_1| \\ &\frac{\Delta\phi_{KS}}{\Delta T} \cong \frac{\Delta\phi_{KT}}{\Delta T} \cong \left|\frac{\Delta K_{1,2}}{\Delta T}\right| + \left|\frac{\Delta(\Delta K_1)}{\Delta T}\right| \end{aligned} \tag{4}$$

The relation for ϕ_{KS} should have the same form as for ϕ_{KT}. According to these relations, the values of $\Delta\phi_V/\Delta T$ and $\Delta\phi_{KS}/\Delta T$ should be positive for molecules of solute for which normalization of the properties of the hydration water occurs while ϕ_{KS} and ϕ_{KT} can be either positive or negative. This result is in agreement with the first type of qualitative regularities revealed above for nucleic bases and their derivatives. Based on eq. 4 one can explain why the values of ϕ_{KS} and ϕ_{KT} are sensitive to the chemical structure of the base while $\Delta\phi_V/\Delta T$ and $\Delta\phi_{KS}/\Delta T$ are insensitive. The variations in $\Delta\phi_V/\Delta T$ and $\Delta\phi_{KS}/\Delta T$ due to the changes in hydration take place at a large constant background since both terms in eq. 4 have the same sign. In the case of ϕ_{KS} and ϕ_{KT} the two terms of eq. 4 have opposite signs and the background is either absent or very small in extent. As for the value of $(\phi_V - \bar{V}_w)$, nothing definite can be said about the sign of ΔV_1 and therefore about the sign of $(\phi_V - \bar{V}_w)$. It is expected that ΔV_1 can be either positive or negative depending on the type of the atomic groups of the solute. Consequently the value of $(\phi_V - \bar{V}_w)$ is sensitive rather than insensitive to the chemical structure of the solute.

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