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Volumetric characterization of the hydration properties of heterocyclic bases and nucleosides

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Abstract

We have determined the partial molar volumes, expansibilities and adiabatic compressibilities of six heterocyclic nucleic acid bases, five ribonucleosides and six 2′-deoxyribonucleosides within the temperature range 18–55°C. We interpret the resulting data in terms of the hydration of the component hydrophobic and polar atomic groups. From our temperature-dependent volumetric studies, we found that the total contraction of water caused by polar groups of each individual heterocyclic base and nucleoside depends on the proximity and chemical nature of other functional groups of the solute. In addition, the compressibility contributions of polar groups vary greatly in sign and magnitude depending on the surrounding functional groups. In agreement with previous studies, our results are suggestive of little or no interaction between the sugar and base moieties of a nucleoside. In general, our data shed light into the hydration properties of individual heterocyclic bases and nucleosides, which may have significant implications for the sequence-dependent hydration of nucleic acids. We discuss the potential importance of our results for developing an understanding of the role that solvent plays in the stabilization/destabilization of nucleic acid structures.

Keywords: Heterocyclic bases; Nucleosides; Hydration; Volume; Expansibility; Compressibility

1. Introduction

Hydration is a term commonly used for denoting solute–water interactions in the vicinity of solute molecules. Hydration is widely acknowledged to represent a major determinant of the thermodynamics of stability and recognition of nucleic acid structures [1–12]. In recognition of this fact, the hydration properties of DNA and RNA have been the subject of intensive scrutiny in which a variety of structural, spectroscopic, thermodynamic and computer simulation techniques have been widely employed [13–25]. In this respect, volumetric techniques, including ultrasonic velocimetry and high precision densimetry, have already proven useful and highly informative in hydration related investigations [26–32]. The volumetric parameters of a solute, such as

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the partial molar volume, expansibility and adiabatic compressibility, being sensitive to the total amount of solute hydration, can also be used for discriminating between water molecules solvating charged, polar and non-polar atomic groups [29–32].

Volumetric measurements have been successfully applied to studying hydration of nucleic acids and their complexes (for recent reviews, see [31,32]). Just recently, we have used densimetric and ultrasonic velocimetric measurements to characterize the hydration properties of double stranded DNA and RNA duplexes as a function of structure, base composition, and base sequence [33,34] as well as the changes in DNA hydration associated with drug binding [35]. A survey of the literature reveals that the majority of volumetric studies of nucleic acids have been conducted only at around room temperature [31,32]. This limitation is unfortunate since solute hydration, in particular, DNA hydration, is temperature-dependent. Consequently, volumetric investigations of nucleic acids over a wider temperature range are required for a more complete characterization of DNA hydration.

As a first step toward this goal, one needs to determine, as a function of temperature, the volumetric properties of simple analogs of nucleic acids, including heterocyclic nucleic acid bases and ribo- and deoxyribonucleosides. Previously reported volumetric data on nucleic acid bases and ribonucleosides all have been obtained at a single temperature of 25°C which limits the applicability of the reported data to DNA studies [32,36–39]. It should be noted that thermodynamic (e.g. volumetric) data on low molecular weight model compounds cannot always be directly used for microscopic interpretation of macroscopic results on biopolymers (e.g. nucleic acids). However, knowledge provided by thermodynamic studies on small molecules often enables one to gain important insights that ultimately can be used for understanding the thermodynamics of biopolymer hydration. In this respect, temperature-dependent data on the partial molar volume, expansibility, and adiabatic compressibility of heterocyclic nucleic acid bases and nucleosides can be useful for microscopic interpretation of volumetric results on polymeric and oligomeric nucleic acids in terms of hydration and other intra- and intermolecular interactions. In addition, volumetric investigation of heterocyclic bases and nucleosides is of independent physico-chemical interest since these molecules consist of only polar and hydrophobic moieties. Consequently, heterocyclic bases and nucleosides make good model systems for exploring the hydration properties of polar and hydrophobic groups which are ubiquitously present in virtually all biologically significant macromolecules.

In this paper, we report our experimental results on the partial molar volume, expansibility, and adiabatic compressibility of some nucleic acid bases and ribo- and deoxyribonucleosides at 18, 25, 40 and 55°C. We interpret our data in terms of hydration of polar and non-polar groups and discuss implications of our results for the stability of nucleic acids and nucleic acid complexes. In particular, our results suggest that, in nucleic acid bases and nucleosides, the hydration properties of polar groups are strongly modulated by the proximity and relative position of other functional groups of the molecule. This finding may have important implications for the sequence- and structure-dependent patterns of hydration of DNA and RNA and, consequently, for the stability and recognition thermodynamics of these structures.

2. Materials and methods

All heterocyclic bases (uracil, thymine, cytosine, purine, adenine, hypoxanthine), ribonucleosides (uridine, cytidine, adenosine, guanosine, and inosine), and deoxyribonucleosides (2′-deoxyuridine, thymidine, 2′-deoxycytidine, 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine) used in this study were purchased from Sigma-Aldrich Canada (Mississauga, Ontario, Canada). These chemicals were of the highest purity commercially available and, therefore, used without further purification. Solutions of heterocyclic bases and nucleosides were prepared with doubly distilled water which was degassed by boiling. The concentration of each sample was de-

termined by weighing 10–20 mg of solute with a precision of ± 0.03 mg and dissolving in a known amount of water. All heterocyclic bases and nucleosides were dried under vacuum in the presence of phosphorus pentoxide for at least 3 days prior to weighing. To prevent formation of air bubbles, all solutions were preheated to 5°C above the experimental temperature before placing them into the ultrasonic or densimetric cells.

All densities were measured using a vibrating tube densimeter (DMA-60, Anton Paar, Austria) with a precision of $\pm 1.5 \times 10^{-6}$ g cm^{-3} at 18, 25, 40 and 55°C. The apparent molar volume, ϕV, was calculated from the standard equation [40]:

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

where M is the molecular weight of a solute; m is the molal concentration; and ρ and ρ_0 are the densities of the solution and solvent, respectively. Values for the density of water were taken from the work of Kell [41].

The solution sound velocities values, required to calculate the apparent molar adiabatic compressibility, ϕK_S, of each solute were measured with a precision of $\pm 2 \times 10^{-4}$% at 18, 25, 40 and 55°C using the resonator method [42–47] at a frequency of approximately 7 MHz. Ultrasonic resonator cells with sample volumes of 0.8 cm^3 were thermostated with an accuracy of ± 0.01°C, and a previously described differential technique was employed for all measurements [43].

Apparent molar adiabatic compressibility values for the solutes were calculated from the densimetric and ultrasonic data using the expression [48,49]:

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

where β_{S0} is the coefficient of adiabatic compressibility of water; $[U]$ is the relative molar sound velocity increment of a solute and is equal to $(U - U_0)/(U_0 C)$; U and U_0 are the sound velocities in the solution and solvent, respectively; and C is the molar concentration. The coefficient of adiabatic compressibility of water, β_{S0}, required to evaluate ϕK_S from Eq. (2) was calculated from the data on density [41] and sound velocity [50], since $\beta_{S0} = (\rho_0 U_0^2)^{-1}$.

For each evaluation of ϕV or ϕK_S, three to four independent measurements were carried out at a concentration of approximately 1 mg ml^{-1} for all of the heterocyclic bases and nucleosides.

3. Results

Tables 1–3 show the relative molar increments of sound velocity, $[U]$, apparent molar volumes, ϕV, and apparent molar adiabatic compressibilities, ϕK_S, of the heterocyclic nucleic acid bases and nucleosides at 18, 25, 40 and 55°C, respectively. Errors were estimated by taking into account uncertainties due to the determination of the concentrations, temperature drifts, and apparatus limitations. The concentration dependences of the apparent molar volumes and compressibilities of the heterocyclic bases and nucleosides are not strong in the range of concentrations used in the present work [36,39]. Within the limits of experimental error, the apparent molar volumes, ϕV, and adiabatic compressibilities, ϕK_S, we have determined at concentrations of approximately 1 mg ml^{-1} coincide with the values of the partial molar volume, V°, and adiabatic compressibility, K°_S, obtained by extrapolation to infinite dilution.

The temperature dependences of the partial molar volumes, V°, that we had measured were approximated by second order polynomial functions. The temperature derivatives of V° were then determined by analytical differentiation of the approximating functions at the required temperatures. Table 4 presents the resulting data as the partial molar expansibility [equal to the temperature slope of the partial molar volume, since $E^\circ = (\partial V^\circ/\partial T)_P$] at 18, 25, 40 and 55°C.

Table 5 compares our data on V°, K°_S and E° at 25°C with the previous literature reports that exist. In general, there is reasonable agreement between our data and published data for the volumetric characteristics of the heterocyclic bases and nucleosides.

Table 1
Relative molar sound velocity increments, $[U]$ (cm^3 mol^{-1}), as a function of temperature, T, for the nucleic bases and nucleosides

Compounds	18°C	25°C	40°C	55°C
Uracil	28.7 ± 0.4	23.8 ± 0.4	17.7 ± 0.4	10.0 ± 0.5
Thymine	37.6 ± 0.4	32.8 ± 0.4	23.4 ± 0.4	16.4 ± 0.5
Cytosine	38.1 ± 0.4	33.9 ± 0.4	26.8 ± 0.4	21.8 ± 0.5
Purine	26.8 ± 0.4	21.3 ± 0.4	13.3 ± 0.4	7.2 ± 0.5
Adenine	29.4 ± 0.4	23.9 ± 0.4	15.1 ± 0.4	10.5 ± 0.5
Hypoxanthine	29.8 ± 0.4	25.6 ± 0.4	16.2 ± 0.4	12.4 ± 0.5
Uridine	49.1 ± 0.5	42.2 ± 0.5	32.8 ± 0.6	26.9 ± 0.6
Cytidine	55.4 ± 0.5	48.2 ± 0.5	39.4 ± 0.6	32.4 ± 0.6
Adenosine	50.3 ± 0.5	43.1 ± 0.5	30.6 ± 0.6	23.0 ± 0.6
Guanosine	52.0 ± 0.5	46.1 ± 0.5	35.6 ± 0.6	28.5 ± 0.6
Inosine	51.6 ± 0.5	43.4 ± 0.5	34.7 ± 0.6	25.9 ± 0.6
2′-Deoxyuridine	47.1 ± 0.5	40.5 ± 0.5	31.5 ± 0.6	22.6 ± 0.6
Thymidine	54.6 ± 0.5	47.0 ± 0.5	35.4 ± 0.6	25.5 ± 0.6
2′-Deoxycytidine	51.1 ± 0.5	45.4 ± 0.5	35.9 ± 0.6	27.9 ± 0.6
2′-Deoxyadenosine	48.0 ± 0.5	42.0 ± 0.5	30.9 ± 0.6	21.7 ± 0.6
2′-Deoxyguanosine	52.2 ± 0.5	45.0 ± 0.5	36.3 ± 0.6	26.2 ± 0.6
2′-Deoxyinosine	50.8 ± 0.5	44.4 ± 0.5	35.6 ± 0.6	26.3 ± 0.6

4. Discussion

4.1. Partial molar volume

The partial molar volume of a solute, $V°$, can be interpreted in terms of intrinsic and hydration contributions based on concepts of scaled particle theory (SPT) [51–54]:

$$V^{\cdot} = V_C + V_I + \beta_{T0} RT \quad (3)$$

Table 2
Partial molar volumes, $V°$ (cm^3 mol^{-1}), as a function of temperature, T, for the nucleic bases and nucleosides

Compounds	18°C	25°C	40°C	55°C
Uracil	70.2 ± 0.4	71.8 ± 0.4	73.6 ± 0.4	74.6 ± 0.5
Thymine	86.3 ± 0.4	88.7 ± 0.4	90.1 ± 0.4	91.6 ± 0.5
Cytosine	72.5 ± 0.4	73.5 ± 0.4	74.6 ± 0.4	75.8 ± 0.5
Purine	83.3 ± 0.4	84.1 ± 0.4	85.8 ± 0.4	87.8 ± 0.5
Adenine	88.0 ± 0.4	89.6 ± 0.4	92.4 ± 0.4	93.5 ± 0.5
Hypoxanthine	80.9 ± 0.4	82.9 ± 0.4	86.3 ± 0.4	88.8 ± 0.5
Uridine	150.7 ± 0.6	152.2 ± 0.6	153.4 ± 0.7	154.8 ± 0.7
Cytidine	152.2 ± 0.6	153.4 ± 0.6	155.0 ± 0.7	156.4 ± 0.7
Adenosine	169.2 ± 0.6	170.8 ± 0.6	173.0 ± 0.7	175.3 ± 0.7
Guanosine	172.0 ± 0.6	175.4 ± 0.6	177.0 ± 0.7	177.8 ± 0.7
Inosine	162.2 ± 0.6	165.5 ± 0.6	167.3 ± 0.7	168.9 ± 0.7
2′-Deoxyuridine	149.4 ± 0.6	151.9 ± 0.6	152.7 ± 0.7	154.6 ± 0.7
Thymidine	166.4 ± 0.6	167.6 ± 0.6	169.0 ± 0.7	170.4 ± 0.7
2′-Deoxycytidine	153.0 ± 0.6	154.0 ± 0.6	155.8 ± 0.7	157.1 ± 0.7
2′-Deoxyadenosine	168.9 ± 0.6	170.3 ± 0.6	172.0 ± 0.7	175.3 ± 0.7
2′-Deoxyguanosine	172.0 ± 0.6	173.4 ± 0.6	175.1 ± 0.7	178.3 ± 0.7
2′-Deoxyinosine	161.7 ± 0.6	162.8 ± 0.6	163.9 ± 0.7	166.2 ± 0.7

Table 3
Partial molar adiabatic compressibilities, K°_S (10^{-4} cm^3 mol^{-1} bar^{-1}), as a function of temperature, T, for the nucleic bases and nucleosides

Compounds	18°C	25°C	40°C	55°C
Uracil	−13.4 ± 0.7	−7.4 ± 0.7	−0.5 ± 0.7	6.6 ± 0.9
Thymine	−13.4 ± 0.7	−6.7 ± 0.7	2.7 ± 0.7	9.5 ± 0.9
Cytosine	−19.5 ± 0.7	−14.4 ± 0.7	−7.1 ± 0.7	−2.1 ± 0.9
Purine	−3.3 ± 0.7	2.3 ± 0.7	10.3 ± 0.7	16.7 ± 0.9
Adenine	−8.4 ± 0.7	−1.9 ± 0.7	7.9 ± 0.7	12.2 ± 0.9
Hypoxanthine	−15.2 ± 0.7	−9.8 ± 0.7	1.3 ± 0.7	2.1 ± 0.9
Uridine	−19.0 ± 1.0	−11.4 ± 1.0	−2.1 ± 1.2	3.4 ± 1.2
Cytidine	−23.0 ± 1.0	−15.0 ± 1.0	−6.0 ± 1.2	0.5 ± 1.2
Adenosine	−13.8 ± 1.0	−5.6 ± 1.0	6.6 ± 1.2	14.2 ± 1.2
Guanosine	−20.0 ± 1.0	−11.4 ± 1.0	−1.1 ± 1.2	4.8 ± 1.2
Inosine	−21.8 ± 1.0	−11.1 ± 1.0	−2.2 ± 1.2	5.9 ± 1.2
2′-Deoxyuridine	−11.0 ± 1.0	−2.7 ± 1.0	5.4 ± 1.2	13.7 ± 1.2
Thymidine	−8.8 ± 1.0	−0.7 ± 1.0	10.0 ± 1.2	18.7 ± 1.2
2′-Deoxycytidine	−11.0 ± 1.0	−4.8 ± 1.0	4.7 ± 1.2	11.8 ± 1.2
2′-Deoxyadenosine	−4.5 ± 1.0	2.1 ± 1.0	12.5 ± 1.2	22.2 ± 1.2
2′-Deoxyguanosine	−12.9 ± 1.0	−5.0 ± 1.0	3.6 ± 1.2	14.0 ± 1.2
2′-Deoxyinosine	−14.2 ± 1.0	−7.2 ± 1.0	1.0 ± 1.2	10.2 ± 1.2

where $V_C = V_M + V_T$ is the cavity volume, that is the volume of a cavity in a solvent enclosing the solute molecule; V_M is the intrinsic volume of a solute molecule (for small molecules, V_M can be reasonably approximated by the van der Waals volume, V_W); V_T is the 'thermal' volume (the volume of the void space surrounding the solute molecule) which is due to the thermally induced mutual molecular vibrations of the solute and the solvent; V_I is the 'interaction volume' which represents the change in the solvent volume under the influence of solute–solvent interactions; β_{T0} is the coefficient of isothermal compressibility of the solvent; R is the universal gas constant; and T

Table 4
Partial molar expansibilities, E° (cm^3 mol^{-1} K^{-1}), as a function of temperature, T, for the nucleic bases and nucleosides

Compounds	18°C	25°C	40°C	55°C
Uracil	0.21 ± 0.05	0.18 ± 0.04	0.10 ± 0.04	0.02 ± 0.06
Thymine	0.24 ± 0.05	0.20 ± 0.04	0.11 ± 0.04	0.03 ± 0.06
Cytosine	0.11 ± 0.05	0.10 ± 0.04	0.08 ± 0.04	0.06 ± 0.06
Purine	0.10 ± 0.05	0.11 ± 0.04	0.12 ± 0.04	0.14 ± 0.06
Adenine	0.27 ± 0.05	0.23 ± 0.04	0.13 ± 0.04	0.06 ± 0.06
Hypoxanthine	0.29 ± 0.05	0.26 ± 0.04	0.20 ± 0.04	0.13 ± 0.06
Uridine	0.15 ± 0.08	0.13 ± 0.07	0.01 ± 0.07	0.06 ± 0.09
Cytidine	0.15 ± 0.08	0.14 ± 0.07	0.10 ± 0.07	0.07 ± 0.09
Adenosine	0.19 ± 0.08	0.18 ± 0.07	0.16 ± 0.07	0.13 ± 0.09
Guanosine	0.36 ± 0.08	0.28 ± 0.07	0.10 ± 0.07	−0.08 ± 0.09
Inosine	0.34 ± 0.08	0.27 ± 0.07	0.13 ± 0.07	0 ± 0.09
2′-Deoxyuridine	0.20 ± 0.08	0.17 ± 0.07	0.11 ± 0.07	0.06 ± 0.09
Thymidine	0.14 ± 0.08	0.13 ± 0.07	0.01 ± 0.07	0.07 ± 0.09
2′-Deoxycytidine	0.15 ± 0.08	0.14 ± 0.07	0.10 ± 0.07	0.07 ± 0.09
2′-Deoxyadenosine	0.11 ± 0.08	0.13 ± 0.07	0.18 ± 0.07	0.22 ± 0.09
2′-Deoxyguanosine	0.11 ± 0.08	0.13 ± 0.07	0.17 ± 0.07	0.21 ± 0.09
2′-Deoxyinosine	0.08 ± 0.08	0.09 ± 0.07	0.12 ± 0.07	0.15 ± 0.09

Table 5
Comparison with literature values of the partial molar volume, V° ($cm^3\ mol^{-1}$), adiabatic compressibility, K°_S ($10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$) and expansibility, E° ($cm^3\ mol^{-1}\ K^{-1}$), at 25°C for the nucleic bases and nucleosides

Compounds	V°		K°_S		E°	
Uracil	71.8[a]	71.7[b]	−7.4[a]	−9.5[b]	0.18[a]	0.15[b]
		72.3[c]				
Thymine	88.7[a]	88.2[b]	−6.7[a]	−8.4[b]	0.20[a]	0.14[b]
		88.7[c]				
Cytosine	73.5[a]	73.3[b]	−14.4[a]	−15.3[b]	0.10[a]	0.13[b]
		73.6[c]				
Purine	84.1[a]	84.5[b]	2.3[a]	1.3[b]	0.11[a]	0.15[b]
		84.40[d]		1.5[d]		
Adenine	89.6[a]	89.3[b]	−1.9[a]	−3.5[b]	0.23[a]	0.14[b]
Hypoxanthine	82.9[a]	84.1[b]	−9.8[a]	−8.7[b]	0.26[a]	
Uridine	152.2[a]	151.7[e]	−11.4[a]	−13.8[e]	0.13[a]	
		152.3[c]				
		151.45[d]		−17.0[d]		
Cytidine	153.4[a]	153.7[e]	−15.0[a]	−17.0[e]	0.14[a]	
		154.2[c]				
		153.50[d]		−18.2[d]		
Adenosine	170.8[a]	170.8[e]	−5.6[a]	−5.8[e]	0.18[a]	0.26[d]
		171.4[c]				
Guanosine	175.4[a]	178.2[e]	−11.4[a]	−10.9[e]	0.28[a]	
Inosine	165.5[a]	164.6[e]	−11.1[a]	−13.8[e]	0.27[a]	
2′-Deoxyuridine	151.9[a]	152.2[e]	−2.7[a]	−2.6[e]	0.17[a]	
Thymidine	167.6[a]	167.6[e]	−0.7[a]	−1.8[e]	0.13[a]	
2′-Deoxycytidine	154.0[a]	153.4[e]	−4.8[a]	−6.2[e]	0.14[a]	
2′-Deoxyadenosine	170.3[a]	169.8[e]	2.1[a]	1.3[e]	0.13[a]	0.25[e]
2′-Deoxyguanosine	173.4[a]	173.7[e]	−5.0[a]	−5.8[e]	0.13[a]	
2′-Deoxyinosine	162.8[a]		−7.2[a]		0.09[a]	

[a] This study.
[b] [37].
[c] [39].
[d] [36].
[e] [38].

is the absolute temperature. It is customarily assumed that V_I mostly reflects a decrease in the solvent volume (solvent contraction) resulting from hydration of polar and charged atomic groups of a solute [54]. The ideal term, $\beta_{T0}RT$, describes the volume effect related to the kinetic contribution to the pressure of a solute molecule due to its translational degrees of freedom. The $\beta_{T0}RT$ term is small and weakly depends on temperature (it increases from 1.1 $cm^3\ mol^{-1}$ at 10°C to 1.2 $cm^3\ mol^{-1}$ at 60°C).

Determination of the thermal volume, V_T, in Eq. (3) is not straightforward especially for non-spherical molecules. There are different approaches for evaluating V_T. These approaches are all model-dependent and may often yield substantially different values of V_T for the same solute [54]. One approach to calculating V_T is based on the assumption that the 'thickness', Δ, of the thermal volume is constant at a given temperature and does not depend on the chemical nature of solvent-exposed atomic groups of a solute [54]. With this simplification, determination of V_T becomes a geometric task, with which the main difficulty is to find the most appropriate geometric approximation of the shape of a solute molecule. The 'barrel', as proposed in Kharakoz [54], is a reasonable approximation for heterocyclic bases. It can also be used as a model for describing the shape of nucleosides even though a

'bent barrel' (since the plane of sugar is tilted with respect to the plane of nucleic base) should be a better approximation. The cavity volume, V_C, in Eq. (3) for each of the compounds studied in this work has been calculated using the equation [54]:

$$V_C = \pi N_A \left[(4/3)(r+\Delta)^3 + \pi R(r+\Delta)^2 + 2R^2(r+\Delta) \right] \quad (4)$$

where N_A is Avogadro's number; r is the half thickness of the barrel; and R is the radius of the barrel (for more details, see [54]).

The thickness of the thermal volume, Δ, for calculations using Eq. (4) is chosen to be 0.50 Å at 18 and 25°C [54]. At 40 and 55°C, Δ is taken to be equal to 0.51 and 0.52 Å, respectively, to account for an increase in the thermal volume with increasing temperature as suggested by our calculations based on scaled particle theory (SPT) [51–53]. The value of r in Eq. (4) is taken equal to 1.77 Å (half thickness of an aromatic ring [54]). The values of R for each compound studied in this work have been evaluated from Eq. (4) based on the following assumption: if Δ in Eq. (4) is set equal to zero, then the cavity volume, V_C, of a solute should become equal to its van der Waals volume, V_W. The van der Waals volumes, V_W, of the heterocyclic nucleic acid bases and nucleosides have been calculated using the additive scheme and group contributions presented by Bondi [55], and the values of R have been evaluated accordingly. The van der Waals volumes of uracil, thymine, cytosine, purine, adenine, hypoxanthine, uridine, cytidine, adenosine, guanosine, inosine, 2′-deoxyuridine, thymidine, 2′-deoxycytidine, 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine are equal to 55.8, 66.0, 55.5, 54.7, 62.8, 62.1, 118.7, 118.4, 125.7, 133.1, 125.0, 114.1, 124.4, 113.8, 121.1, 128.5 and 120.5 $cm^3\ mol^{-1}$, respectively. For the same compounds, our calculated values of R are equal to 1.47, 1.73, 1.46, 1.44, 1.65, 1.63, 2.80, 2.80, 2.92, 3.05, 2.91, 2.72, 2.90, 2.71, 2.85, 2.97 and 2.83 Å, respectively.

Equipped with these estimates, we use Eq. (4) in conjunction with Eq. (3) to calculate the interaction volumes, V_I, for the nucleic acid bases and nucleosides as a function of temperature. Table 6 presents the results of these calculations. As noted above, the interaction volume, V_I, represents a decrease in the solvent volume due to solute–solvent interactions in the vicinity of

Table 6
Interaction volumes, V_I ($cm^3\ mol^{-1}$), as a function of temperature, T, for the nucleic bases and nucleosides

Compounds	18°C	25°C	40°C	55°C
Uracil	−23.9 ± 0.4	−22.3 ± 0.4	−21.5 ± 0.4	−21.4 ± 0.5
Thymine	−22.9 ± 0.4	−20.5 ± 0.4	−20.2 ± 0.4	−19.7 ± 0.5
Cytosine	−21.1 ± 0.4	−20.1 ± 0.4	−19.9 ± 0.4	−19.6 ± 0.5
Purine	−9.1 ± 0.4	−8.3 ± 0.4	−7.6 ± 0.4	−6.5 ± 0.5
Adenine	−16.4 ± 0.4	−14.8 ± 0.4	−13.1 ± 0.4	−12.9 ± 0.5
Hypoxanthine	−22.4 ± 0.4	−20.4 ± 0.4	−18.0 ± 0.4	−16.4 ± 0.5
Uridine	−32.9 ± 0.6	−31.4 ± 0.6	−31.7 ± 0.7	−31.8 ± 0.7
Cytidine	−31.4 ± 0.6	−30.2 ± 0.6	−30.1 ± 0.7	−30.2 ± 0.7
Adenosine	−23.9 ± 0.6	−22.2 ± 0.6	−21.7 ± 0.7	−20.9 ± 0.7
Guanosine	−31.8 ± 0.6	−28.4 ± 0.6	−28.4 ± 0.7	−29.2 ± 0.7
Inosine	−30.1 ± 0.6	−26.8 ± 0.6	−26.6 ± 0.7	−26.5 ± 0.7
2′-Deoxyuridine	−27.9 ± 0.6	−25.4 ± 0.6	−26.1 ± 0.7	−25.6 ± 0.7
Thymidine	−25.1 ± 0.6	−23.9 ± 0.6	−24.1 ± 0.7	−24.2 ± 0.7
2′-Deoxycytidine	−23.6 ± 0.6	−22.6 ± 0.6	−22.3 ± 0.7	−22.4 ± 0.7
2′-Deoxyadenosine	−18.6 ± 0.6	−17.2 ± 0.6	−17.1 ± 0.7	−15.3 ± 0.7
2′-Deoxyguanosine	−25.2 ± 0.6	−23.8 ± 0.6	−23.7 ± 0.7	−22.0 ± 0.7
2′-Deoxyinosine	−24.2 ± 0.6	−23.1 ± 0.6	−23.6 ± 0.7	−22.8 ± 0.7

charged and polar groups. Nucleic acid bases and nucleosides do not contain any charged groups. Consequently, for these substances, V_I represents a quantitative volumetric measure of the hydration properties of polar groups: a more negative value of V_I of a solute generally correlates with its stronger hydration. Inspection of the data in Table 6 reveals a number of significant observations with regard to the hydration properties of the heterocyclic bases, ribonucleosides, and deoxyribonucleosides.

4.1.1. Heterocyclic bases

The heterocyclic nucleic acid bases (rows 2–7 in Table 6) exhibit significantly different values of the interaction volume, V_I, which do not always correlate with the number of polar groups in the molecule. For example, at 25°C, the value of V_I of uracil with four polar atomic groups is -22.3 cm^3 mol^{-1} while that of adenine with five polar groups is only -14.8 cm^3 mol^{-1}. This disparity is consistent with the net hydration of a solute being determined not only by the total number of polar groups in the solute molecule but also by the relative location of these groups and, possibly, the presence of other functional groups [30,32,54,56].

At all temperatures, the pyrimidine-based heterocyclic bases studied here (including uracil, thymine, and cytosine each having four polar groups) are not much different (within $\pm 10\%$) with respect to their interaction volumes, V_I. For example, at 25°C, the values of V_I of uracil, thymine, and cytosine are equal to -22.3, -20.5 and -20.1 cm^3 mol^{-1}, respectively. Thus, judging by the polar group-induced contraction of water, the hydration properties of the pyrimidine-based heterocyclic bases are quite similar. In contrast, the interaction volumes, V_I, of the purine-based nucleic acid bases (including purine, adenine, and hypoxanthine, with purine having four polar groups and adenine and hypoxanthine both having five polar groups) are significantly different. For example, at 25°C, the values of V_I of purine, adenine, and hypoxanthine are equal to -8.3, -14.8 and -20.4 cm^3 mol^{-1}, respectively. Hence, judging by polar group-induced contraction of water, hypoxanthine is hydrated most extensively followed by adenine and purine.

4.1.2. Ribonucleosides

Structurally, a ribonucleoside represents a heterocyclic base covalently linked to the ribose residue. Inspection of the data in Table 6 (rows 8–12) reveals that, analogously to nucleic acid bases, the pyrimidine-based ribonucleosides (including uridine and cytidine) are quite similar while the purine-based ribonucleosides (including adenosine, guanosine, and inosine) are somewhat distinct with respect to their interaction volumes, V_I. For example, at 25°C, the values of V_I for uridine and cytidine are equal to -31.4 and -30.2 cm^3 mol^{-1}, respectively, while those for adenosine, guanosine, and inosine are equal to -22.2, -28.4 and -26.8 cm^3 mol^{-1}, respectively. Hence, judging by the values of V_I, guanosine is hydrated most extensively amongst the purine-based ribonucleosides, followed by inosine and adenosine.

The apparent V_I contribution of the ribose moiety can be calculated as the difference in the interaction volume, V_I, between a given ribonucleoside and its corresponding heterocyclic base. We have performed such calculations for the pairs uridine/uracil, cytidine/cytosine, adenosine/adenine, and inosine/hypoxanthine. The average V_I contribution of the ribose residue in the pyrimidine-based ribonucleosides (uridine and cytidine) equals -10.0 ± 0.4 cm^3 mol^{-1} and, within error, does not depend on temperature. The average V_I contribution of the sugar residue in the purine-based ribonucleosides (adenosine and inosine) is somewhat larger (less negative) and equal to -8.1 ± 0.8 cm^3 mol^{-1}. Both values are significantly larger (less negative) than the interaction volume, V_I, of free ribose. For free ribose, V_I is equal to -21.9 ± 0.3, -22.8 ± 0.3, -22.3 ± 0.4 and -25.0 ± 0.5 at 18, 25, 40 and 55°C, respectively [57]. There are three possible reasons for the observed disparity. The first possibility is related to the fact that the ribose residue in a ribonucleoside contains one less hydroxyl residue (in the 1-position) than free ribose. The V_I contribution of a hydroxyl group in ribose can be approximately estimated by comparing the V_I values of free ribose and free 2′-deoxyribose [57]. For example, at 25°C, the differential value of V_I of free 2′-deoxyribose and free ribose is -6.6 cm^3 mol^{-1} [57]. The second possibility is that the

ribose and base moieties of a ribonucleoside may interact with each other via their hydration shells. The fact that the apparent interaction volume of the ribose residue, ΔV_I, of the pyrimidine-based ribonucleosides is slightly more negative (-10.0 ± 0.4 cm^3 mol^{-1}) than that for the purine-based ribonucleosides (-8.1 ± 0.8 cm^3 mol^{-1}) may suggest that in the latter, the base–ribose interactions are slightly stronger. Finally, the third possibility may originate from the fact that the thermal volumes, V_T, (required for V_I calculations) of the ribonucleosides and free ribose [57] have been calculated using different approaches. Therefore, a direct comparison of the interaction volume, V_I, of the ribose residue of the ribonucleosides from this work and that of free ribose from our previous work [57] may be misleading. It is difficult to separate the relative contributions of each of the three possible factors just outlined to the apparent difference in the interaction volume of the ribose residue of ribonucleosides and free ribose, but, most probably, each factor plays some role. However, it should be noted that, based on our compressibility results (see below), sugar–base interactions (if any) in nucleosides are rather weak.

4.1.3. Deoxyribonucleosides

A deoxyribonucleoside consists of the covalently linked heterocyclic base and deoxyribose residue. Inspection of the data in Table 6 (rows 13–18) reveals that, analogous to nucleic acid bases and ribonucleosides, the pyrimidine-based deoxyribonucleosides (including 2′-deoxyuridine, thymidine, and 2′-deoxycytidine) are not significantly different (within $\pm 10\%$) with respect to their interaction volumes, V_I, while the purine-based deoxyribonucleosides (including 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine) exhibit a broader range of V_I values. For example, at 25°C, the values of V_I for 2′-deoxyuridine, thymidine, and 2′-deoxycytidine are equal to -25.4, -23.9 and -22.6 cm^3 mol^{-1}, respectively, while those for 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine are -17.2, -23.8 and -23.1 cm^3 mol^{-1}, respectively. Thus, judging by the values of V_I, thymidine and 2′-deoxycytidine are similarly hydrated, while 2′-deoxyguanosine and 2′-deoxyinosine are hydrated more extensively than 2′-deoxyadenosine. This observation may have important implications for understanding the hydration properties of double stranded DNA molecules.

The apparent V_I contribution of the 2′-deoxyribose moiety can be calculated by subtracting the interaction volume, V_I, of a heterocyclic base from that of its corresponding 2′-deoxyribonucleoside. We have performed such calculations for the pairs 2′-deoxyuridine/uracil, thymidine/thymine, 2′-deoxycytidine/cytosine, 2′-deoxyadenosine/adenine, and 2′-deoxyinosine/hypoxanthine. The average V_I contribution of the 2′-deoxribose in both pyrimidine- and purine-based 2′-deoxyribonucleosides equals -3.4 ± 1.0 cm^3 mol^{-1} and, within error, does not depend on temperature. This value is significantly larger (less negative) than the interaction volume, V_I, of free 2′-deoxyribose. For free 2′-deoxyribose, V_I is equal to -14.8 ± 0.3, -16.2 ± 0.3, -16.6 ± 0.4 and -18.4 ± 0.5 at 18, 25, 40 and 55°C, respectively [57]. Analogous to the ribonucleosides, the observed disparity in V_I can be related to the lack of the hydroxyl residue in the 1-position of the deoxyribose residue in a deoxyribonucleoside, possible interactions between the sugar and base moieties of a deoxyribonucleoside, and the fact that the thermal volumes, V_T, of the deoxyribonucleosides and free 2′-deoxyribose [57] have been calculated using different approaches. It is our opinion that all factors may contribute to the apparent difference in the interaction volume of the deoxyribose residue of deoxyribonucleosides and free 2′-deoxyribose, but it is difficult to evaluate the relative importance of each of the three factors.

4.2. Partial molar expansibility

The partial molar expansibility, $E°$, of a solute is a fundamental thermodynamic characteristics which is sensitive to solute–solvent interactions. However, expansibility measurements still find only a limited use in hydration studies as compared to volume and compressibility measurements. One reason is that the partial molar expansibility data on biological compounds are relatively scarce. Another reason is that the relative

precision of expansibility measurements on biological compounds is generally low as compared to volume and compressibility measurements. Nevertheless, expansibility data are potentially useful and may provide valuable information on the thermodynamics of solute hydration, for example, on the differential enthalpy of water of solute hydration and bulk water [58].

The partial molar expansibility, E°, of a solute is the first temperature derivative of its partial molar volume, V°. Consequently, the relationship for E° can be obtained by differentiating Eq. (3):

$$E^\circ = E_M + E_T + E_I + [\partial(\beta_{T0}RT)/\partial T]_P \quad (5)$$

where

$$E_M = (\partial V_M/\partial T)_P;\ E_T = (\partial V_T/\partial T)_P;\ \text{and}\ E_I = (\partial V_I/\partial T)_P.$$

The contribution of the ideal term, $[\partial(\beta_{T0}RT)/\partial T]_P$, is relatively small and can be safely neglected: depending on temperature, the ideal term in Eq. (5) is between 0 and 0.005 $cm^3\ mol^{-1}\ K^{-1}$. Consequently, the partial molar expansibility, E°, of a solute is mainly determined by its intrinsic expansibility, E_M, the expansibility of the thermal volume, E_T, and the solute-induced change in the solvent expansibility, E_I, the 'interaction expansibility'. It should be noted that slight solute-induced structural changes in the solvent in the vicinity of non-polar groups which contribute little to V_I, may, nevertheless, have a significant impact on E_I (since expansibility is a derivative of volume). Currently, there is no reliable way to discriminate between the E_T and E_I terms in Eq. (5). Therefore, on a practical level, it is convenient not to separate these two terms but treat them together as a single hydration contribution, ΔE_h. Thus, Eq. (5) can be reduced to the form

$$E^\circ = E_M + \Delta E_h = E_M + n_h(E_h - E_0) \quad (6)$$

where E_h and E_0 are the partial molar expansibilities of water of hydration and bulk water; and n_h is the hydration number, that is the number of water molecules within the solute hydration shell.

For small molecules, such as heterocyclic nucleic acid bases and nucleosides, the intrinsic expansibility, E_M, in Eq. (6) is small and can be neglected in our analysis. Furthermore, for nucleic acid bases and nucleosides, ΔE_h can be presented as a sum of the expansibility contributions of polar, ΔE_{pol}, and hydrophobic, ΔE_{hyd}, groups. Consequently, one obtains the following simple relationship:

$$E^\circ = \Delta E_{pol} + \Delta E_{hyd} \quad (7)$$

In our analysis, we assume that the hydrophobic contribution, ΔE_{hyd}, for each of the solutes studied is proportional to its hydrophobic van der Waals surface area, S_{Whyd}:

$$\Delta E_{hyd} = E(CH_2)S_{Whyd}/S_W(CH_2) \quad (8)$$

where $E(CH_2)$ and $S_W(CH_2)$ are the expansibility contribution and van der Waals area (22.4 $Å^2$) of a $-CH_2-$ group in an extended chain (e.g. in α,ω-aminocarboxylic acids).

In α,ω-aminocarboxylic acids, $E(CH_2)$ is positive over the entire temperature range studied and equal to 0.018, 0.020, 0.025 and 0.030 $cm^3\ mol^{-1}\ K^{-1}$ at 18, 25, 40 and 55°C, respectively [59]. The hydrophobic van der Waals surface areas, S_{Whyd}, of uracil, thymine, cytosine, purine, adenine, hypoxanthine, uridine, cytidine, adenosine, guanosine, inosine, 2′-deoxyuridine, thymidine, 2′-deoxycytidine, 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine are equal to 43.2, 66.8, 43.2, 48.2, 48.2, 48.2, 103.5, 103.5, 108.4, 96.8, 108.4, 116.4, 140.0, 116.4, 109.8, 109.8 and 121.4 $Å^2$, respectively. These surface areas have been calculated using the additive procedure and group contributions presented by Bondi [55]. Armed with these values, we now use Eqs. (7) and (8) to calculate the polar contributions ΔE_{pol} for each compound studied here. Table 7 presents results of these calculations. Inspection of the data in Table 7 reveals a number of interesting observations.

Firstly, the values of ΔE_{pol} for the nucleic acid

Table 7
Expansibility contribution of polar groups, ΔE_{pol} ($cm^3\ mol^{-1}\ K^{-1}$), as a function of temperature, T, for the nucleic bases and nucleosides

Solutes	18°C	25°C	40°C	55°C
Uracil	0.18 ± 0.05	0.14 ± 0.04	0.05 ± 0.04	-0.04 ± 0.06
Thymine	0.18 ± 0.05	0.14 ± 0.04	0.04 ± 0.04	-0.06 ± 0.06
Cytosine	0.08 ± 0.05	0.06 ± 0.04	0.03 ± 0.04	0 ± 0.06
Purine	0.06 ± 0.05	0.07 ± 0.04	0.07 ± 0.04	0.08 ± 0.06
Adenine	0.23 ± 0.05	0.18 ± 0.04	0.07 ± 0.04	0 ± 0.06
Hypoxanthine	0.26 ± 0.05	0.18 ± 0.04	0.14 ± 0.04	0.07 ± 0.06
Uridine	0.07 ± 0.08	0.04 ± 0.07	-0.02 ± 0.07	-0.08 ± 0.09
Cytidine	0.07 ± 0.08	0.05 ± 0.07	-0.01 ± 0.07	-0.07 ± 0.09
Adenosine	0.11 ± 0.08	0.08 ± 0.07	0.03 ± 0.07	-0.02 ± 0.09
Guanosine	0.28 ± 0.08	0.19 ± 0.07	-0.01 ± 0.07	-0.21 ± 0.09
Inosine	0.25 ± 0.08	0.17 ± 0.07	0.01 ± 0.07	-0.15 ± 0.09
2′-Deoxyuridine	0.10 ± 0.08	0.07 ± 0.07	-0.02 ± 0.07	-0.10 ± 0.09
Thymidine	0.03 ± 0.08	0 ± 0.07	-0.06 ± 0.07	-0.12 ± 0.09
2′-Deoxycytidine	0.06 ± 0.08	0.03 ± 0.07	-0.03 ± 0.07	-0.09 ± 0.09
2′-Deoxyadenosine	0.02 ± 0.08	0.03 ± 0.07	0.06 ± 0.07	0.08 ± 0.09
2′-Deoxyguanosine	0.02 ± 0.08	0.03 ± 0.07	0.05 ± 0.07	0.07 ± 0.09
2′-Deoxyinosine	-0.02 ± 0.08	-0.01 ± 0.07	-0.01 ± 0.07	-0.01 ± 0.09

bases and nucleosides vary in a wide range with no apparent correlation to the number of polar groups in an individual molecule. At 25°C, ΔE_{pol} ranges from -0.01 ± 0.07 for 2′-deoxyinosine to $0.19 \pm 0.07\ cm^3\ mol^{-1}\ K^{-1}$ for guanosine.

Secondly, at 25°C and below, the values of ΔE_{pol} for the nucleic acid bases and ribonucleosides studied here are generally positive. For the deoxyribonucleosides, the values of ΔE_{pol}, although mostly positive, are generally smaller and close to zero. This observation suggests that the hydration properties of polar groups in deoxyribonucleosides are distinct from those of polar groups in nucleic acid bases and ribonucleosides. At 55°C, for all the compounds studied, ΔE_{pol} is either negative or close to zero.

Thirdly, for all the compounds studied here (perhaps, with the exception of 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine), ΔE_{pol} decreases with temperature. The negative sign of the temperature slope of the expansibility contribution of polar groups, ΔE_{pol}, coincides with that of charged groups while being opposite to the positive sign of the temperature slope of the expansibility contribution of hydrophobic groups [59,60]. This distinction reflects the differential thermodynamics of hydration of polar and nonpolar atomic groups. Because of large experimental uncertainty, it is difficult to assess if the positive sign of $\Delta E_{pol}/\Delta T$ observed for 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine (rows 16–18 of Table 3) is statistically significant.

In general, relatively large experimental uncertainties of the data presented in Table 7 prevent us from a more detailed analysis of the partial expansibility of polar groups. Further experiments, perhaps, with more concentrated solutions of heterocyclic bases and nucleosides are required to more fully exploit unique possibilities provided by this relatively untapped volumetric observable (E°) for exploring the hydration properties of low molecular weight analogs of nucleic acids.

4.3. Partial molar adiabatic compressibility

Analogous to the partial molar expansibility [see Eq. (6)], the partial molar adiabatic compressibility, K°_S, of a solute can be conveniently presented as a sum of the intrinsic, K_M, and hydration, ΔK_h, contributions [30–32]:

$$K^\circ_S = K_M + \Delta K_h = K_M + n_h(K_h - K_0) \quad (9)$$

where K_M is the intrinsic compressibility of a solute molecule; and K_h and K_0 are the partial molar adiabatic compressibilities of water of hydration and bulk water, respectively.

Note that the hydration term, ΔK_h, in Eq. (9) should have contributions from the compressibility of the thermal volume, K_T [see Eq. (3)], and the solute-induced change in solvent compressibility, K_I, the 'interaction compressibility'. However, from the practical point of view, it is difficult to reliably discriminate between these two contributions. For small molecules, such as nucleic acid bases and nucleosides, the intrinsic compressibility, K_M, is small and generally can be neglected [29–32,37,38,56]. Furthermore, the hydration contribution, ΔK_h, in Eq. (9) can be presented as the sum of the compressibility contributions of polar, ΔK_{pol}, and hydrophobic, ΔK_{hyd}, groups. Consequently, one obtains the following relationship for the partial molar adiabatic compressibility, $K°_S$, of the nucleic acid bases and nucleosides:

$$K^{\circ}_S = \Delta K_{pol} + \Delta K_{hyd} \quad (10)$$

The hydrophobic contribution, ΔK_{hyd}, for each heterocyclic base or nucleoside studied in this work can be assumed to be proportional to its hydrophobic van der Waals surface area, S_{Whyd}:

$$\Delta K_{hyd} = K(CH_2) S_{Whyd} / S_W(CH_2) \quad (11)$$

where $K(CH_2)$ is the compressibility contribution of a $-CH_2-$ group in an extended chain (e.g. in α,ω-aminocarboxylic acids).

In α,ω-aminocarboxylic acids, $K(CH_2)$ is equal to -3.3×10^{-4}, -1.6×10^{-4}, 1.2×10^{-4} and 2.9×10^{-4} cm^3 mol^{-1} bar^{-1} at 18, 25, 40 and 55°C, respectively [59]. We use Eqs. (10) and (11) to calculate the polar contributions ΔK_{pol} for each compound studied here. Table 8 presents results of these calculations. Fig. 1a–c graphically present the temperature dependences of the compressibility contribution of polar groups, ΔK_{pol}, for the nucleic acid bases, ribonucleosides, and deoxyribonucleosides, respectively. Importantly, ΔK_{pol} represents a quantitative volumetric measure of hydration of polar groups in nucleic acid bases and nucleosides. It should be noted that the magnitude and even the sign of the compressibility contribution of a polar group is thought to depend on its relative position with respect to other polar groups of the solute [30,56]. As proposed by Kharakoz, at 25°C, a 'single' polar group is characterized by a positive compressibility contribution, ΔK_{pol} [$(3.8\pm0.7)\times10^{-4}$ cm^3 mol^{-1} bar^{-1}], a negative temperature slope, $\partial\Delta K_{pol}/\partial T$ [$-(1.7\pm0.8)\times10^{-5}$ cm^3 mol^{-1} bar^{-1} K^{-1}], and a positive second temperature derivative, $\partial^2\Delta K_{pol}/\partial T^2$ [$(2\pm1)\times10^{-7}$ cm^3 mol^{-1} bar^{-1} K^{-2}] [30]. By contrast, at 25°C, a 'clustered' polar group (located in the close proximity to other polar groups) has been proposed to exhibit a negative value of ΔK_{pol} [$-(5.5\pm0.7)\times10^{-4}$ cm^3 mol^{-1} bar^{-1}], a positive temperature slope, $\partial\Delta K_{pol}/\partial T$ [$(1.1\pm0.4)\times10^{-5}$ cm^3 mol^{-1} bar^{-1} K^{-1}], and a negative second temperature derivative, $\partial^2\Delta K_{pol}/\partial T^2$ [$-(2.4\pm0.9)\times10^{-7}$ cm^3 mol^{-1} bar^{-1} K^{-2}] [30]. This differential compressibility behavior of single and clustered polar groups is related to the fact that the compressibility of liquid water has a very large positive relaxation contribution [61–63]. For example, at 25°C, the relaxation contribution to water compressibility is as high as 65% [63]. The relaxation compressibility contribution of liquid water is due to the pressure-induced shift in equilibrium between two or more structural species of water molecules differing in molecular packing and, consequently, in partial molar volumes: an increase in pressure will favor species with smaller volume and vice versa [61–63]. The positive sign of ΔK_{pol} (e.g. for single polar groups) suggests that water of polar hydration is characterized by a larger relaxation contribution to compressibility than bulk water. By contrast, the negative sign of ΔK_{pol} (e.g. for clustered polar groups) indicates a diminution in the relaxation contribution to compressibility of water solvating polar groups. The sign of the temperature slope, $\partial\Delta K_{pol}/\partial T$, is indicative of the differential temperature dependence of the relaxation contribution to compressibility of water of polar hydration and bulk water. The sign of $\partial^2\Delta K_{pol}/\partial T^2$ is also very important. One anomaly of liquid water is its abnormally high second temperature derivative of compressibility,

Table 8
Compressibility contribution of polar groups, ΔK_{pol} (10^{-4} cm^3 mol^{-1} bar^{-1}), as a function of temperature, T, for the nucleic bases and nucleosides

Solutes	18°C	25°C	40°C	55°C
Uracil	-7.0 ± 0.7	-4.3 ± 0.7	-2.8 ± 0.7	1.0 ± 0.9
Thymine	-3.6 ± 0.7	-1.9 ± 0.7	-0.9 ± 0.7	0.9 ± 0.9
Cytosine	-13.1 ± 0.7	-11.3 ± 0.7	-9.4 ± 0.7	-7.7 ± 0.9
Purine	3.8 ± 0.7	5.7 ± 0.7	7.7 ± 0.7	10.5 ± 0.9
Adenine	-1.3 ± 0.7	1.5 ± 0.7	5.3 ± 0.7	6.0 ± 0.9
Hypoxanthine	-8.1 ± 0.7	-6.4 ± 0.7	-1.3 ± 0.7	-4.1 ± 0.9
Uridine	-3.8 ± 1.0	-4.0 ± 1.0	-7.6 ± 1.2	-10.0 ± 1.2
Cytidine	-7.8 ± 1.0	-7.6 ± 1.0	-11.5 ± 1.2	-12.9 ± 1.2
Adenosine	2.2 ± 1.0	2.1 ± 1.0	0.8 ± 1.2	0.2 ± 1.2
Guanosine	-5.7 ± 1.0	-4.5 ± 1.0	-6.3 ± 1.2	-7.7 ± 1.2
Inosine	-5.8 ± 1.0	-3.4 ± 1.0	-8.0 ± 1.2	-8.1 ± 1.2
2′-Deoxyuridine	6.1 ± 1.0	5.6 ± 1.0	-0.8 ± 1.2	-1.4 ± 1.2
Thymidine	11.8 ± 1.0	9.3 ± 1.0	2.5 ± 1.2	0.6 ± 1.2
2′-Deoxycytidine	6.1 ± 1.0	3.5 ± 1.0	-1.5 ± 1.2	-3.3 ± 1.2
2′-Deoxyadenosine	13.4 ± 1.0	10.8 ± 1.0	6.0 ± 1.2	6.5 ± 1.2
2′-Deoxyguanosine	3.3 ± 1.0	2.8 ± 1.0	-2.3 ± 1.2	-0.2 ± 1.2
2′-Deoxyinosine	3.7 ± 1.0	1.5 ± 1.0	-5.5 ± 1.2	-5.5 ± 1.2

$\partial^2 K_0/\partial T^2$ (the temperature dependence of compressibility is abnormally non-linear). Thus, as can be seen from the differentiation of Eq. (9), the negative sign of $\partial^2 \Delta K_{pol}/\partial T^2$ suggests that water of polar hydration exhibits a more linear (more normal) temperature dependence of compressibility, while the positive sign of $\partial^2 \Delta K_{pol}/\partial T^2$ is indicative of water solvating a polar group being even more abnormal than bulk water.

Based on the foregoing discussion, ΔK_{pol} as well as its temperature derivatives can be used as sensitive probes of the hydration properties of polar groups of a solute. In general, the more negative the value of ΔK_{pol} the stronger the solute–solvent interactions around the polar group and, consequently, the stronger its hydration [58]. Inspection of the data in Table 8 reveals a number of important observations with regard to the hydration properties of the heterocyclic bases, ribonucleosides, and deoxyribonucleosides.

4.3.1. Heterocyclic bases

The heterocyclic bases (rows 2–7 in Table 8) exhibit a wide range of ΔK_{pol} values which differ from each other in both magnitude and sign. For example, at 25°C, the values of ΔK_{pol} of the heterocyclic bases vary from -11.3×10^{-4} (cytosine) to 5.7×10^{-4} cm^3 mol^{-1} bar^{-1} (purine). Judging by this variation, polar groups of heterocyclic bases are characterized by significantly different hydration properties. Amongst the nucleic acid bases studied in this work, the most extensive hydration is exhibited by cytosine (with ΔK_{pol} of -11.3×10^{-4} cm^3 mol^{-1} bar^{-1} at 25°C), while purine (5.7×10^{-4} cm^3 mol^{-1} bar^{-1}) and adenine (1.5×10^{-4} cm^3 mol^{-1} bar^{-1}) are characterized by the weakest hydration (with ΔK_{pol} of 1.5×10^{-4} cm^3 mol^{-1} bar^{-1} at 25°C). Recall that our interaction volume data (see Section 4.1.1) were also suggestive of significantly weaker hydration of adenine and purine relative to other nucleic acid bases.

Inspection of Fig. 1a reveals that, for all nucleic bases, the values of ΔK_{pol} increase with temperature rise ($\partial \Delta K_{pol}/\partial T$ is positive). This observation suggests that the differential compressibility of water of polar hydration in the vicinity of nucleic bases and bulk water increases (becomes less negative) with temperature increasing. Further inspection of Fig. 1a reveals that, for all nucleic bases except adenine and hypoxanthine, the second temperature derivative of the com-

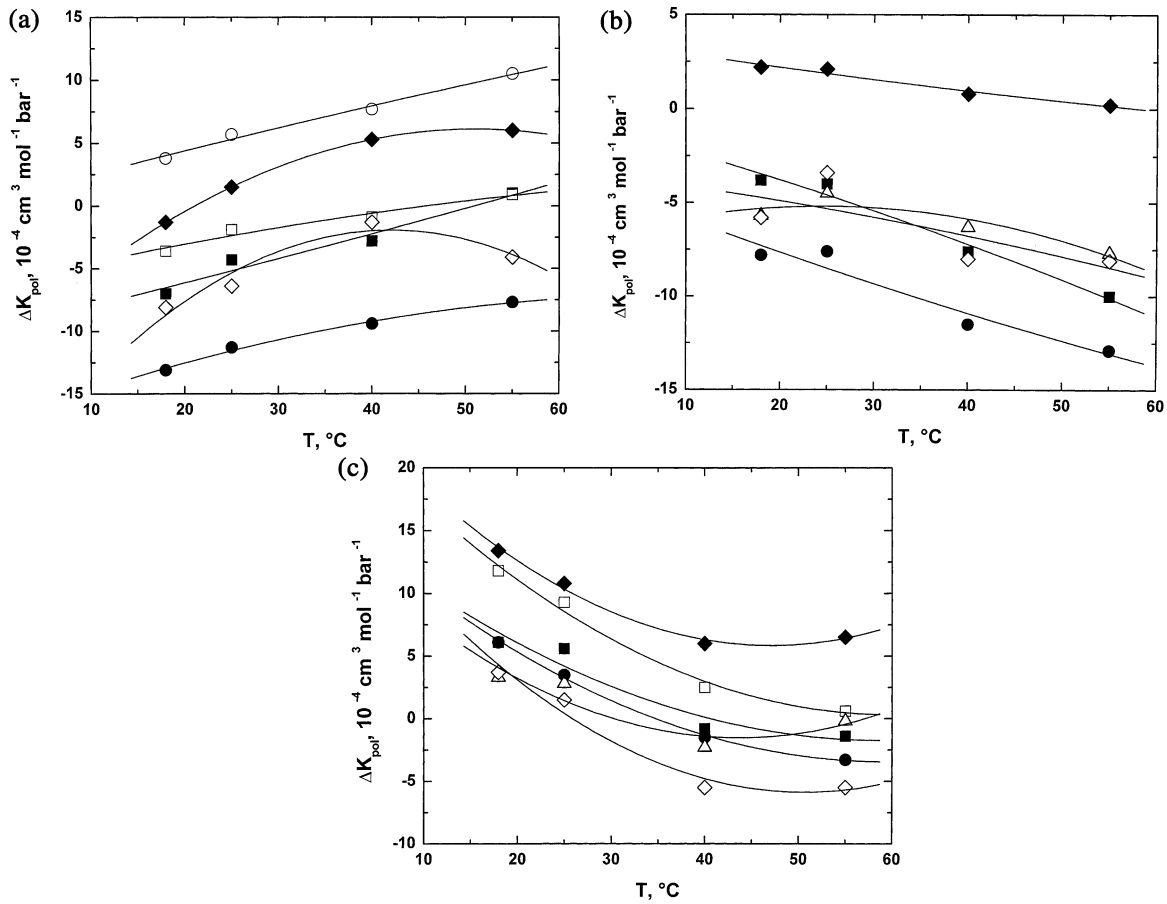

Fig. 1. (a) Temperature dependences of the compressibility contributions of polar groups of the nucleic acid bases uracil (■), thymine (□), cytosine (●), purine (○), adenine (♦), and hypoxanthine (◇); (b) temperature dependences of the compressibility contributions of polar groups of the ribonucleosides uridine (■), cytidine (●), adenosine (♦), guanosine (△), and inosine (◇); (c) temperature dependences of the compressibility contributions of polar groups of the deoxyribonucleosides 2′-deoxyuridine (■), thymidine (□), 2′-deoxycytidine (●), 2′-deoxyadenosine (♦), 2′-deoxyguanosine (△), and 2′-deoxyinosine (◇).

pressibility contribution, $\partial^2 \Delta K_{pol}/\partial T^2$, is close to zero (the temperature dependences of ΔK_{pol} are roughly linear), while, for adenine and hypoxanthine, $\partial^2 \Delta K_{pol}/\partial T^2$ is negative (the temperature dependences of ΔK_{pol} are curved). This observation suggests that the temperature dependences of the compressibility of water of polar hydration for adenine and hypoxanthine are more linear (more normal) than that of bulk water. For the rest of the heterocyclic bases, the temperature dependences of the compressibility of water of polar hydration are approximately as non-linear as that of bulk water.

4.3.2. Ribonucleosides

The ribonucleosides (rows 8–12 in Table 8), excluding adenosine, all exhibit negative values of ΔK_{pol} within the entire temperature range studied. Adenosine exhibiting positive ΔK_{pol} (2.1×10^{-4} cm^3 mol^{-1} bar^{-1} at 25°C) is characterized by the weakest hydration among the ribonucleosides. Recall that we arrived at the same conclusion based on the interaction volume data (see Section 4.1.2).

Inspection of Fig. 1b reveals that, for all the ribonucleosides studied here, ΔK_{pol} decreases with temperature rise ($\partial \Delta K_{pol}/\partial T$ is negative).

Note that this temperature-dependent behavior of the ribonucleosides is opposite to that observed for the nucleic acid bases which is reflective of the role played by the sugar ring in determining the hydration properties of ribonucleosides. Further inspection of Fig. 1b reveals that, for all ribonucleosides except inosine, the second temperature derivative of the compressibility contribution, $\partial^2\Delta K_{pol}/\partial T^2$, is close to zero. For inosine, $\partial^2\Delta K_{pol}/\partial T^2$ is negative (the temperature dependences of ΔK_{pol} are curved). However, due to the large scattering of the inosine data in Fig. 1b, it is difficult to assess if the observed non-zero value of $\partial^2\Delta K_{pol}/\partial T^2$ is statistically significant.

The apparent contribution of the ribose moiety to ΔK_{pol} can be calculated by subtracting the value of ΔK_{pol} of a heterocyclic base from that of its corresponding ribonucleoside. We determine the apparent ΔK_{pol} contribution of the ribose residue by comparing the pairs uracil/uridine, cytosine/cytidine, adenine/adenosine, and hypoxanthine/inosine. Fig. 2 presents the average values of the ΔK_{pol} contribution of the ribose residue (●) as a function of temperature. For comparison, Fig. 2 also presents the temperature dependence of ΔK_{pol} for free ribose (■) and free 2′-deoxyribose (□) [57]. Inspection of Fig. 2 reveals that the temperature dependence of the apparent ΔK_{pol} of the ribose residue of a ribonucleoside is significantly distinct from that of ΔK_{pol} of free ribose. There are two plausible explanations to account for this differential compressibility behavior of the ribose residue of a ribonucleoside and free ribose. The first possibility is related to the fact that the ribose residue of a ribonucleoside contains one less hydroxyl group (in the 1-position) than free ribose. The second possibility is that the ribose and base moieties of a ribonucleoside may interact with each other which would bring about a weakening of the solute hydration. To discriminate between these two possibilities we compare the apparent ΔK_{pol} of the ribose residue of a ribonucleoside with K_{pol} of free 2′-deoxyribose which contains one less hydroxyl group than free ribose (although in the 2-position) [57]. Inspection of Fig. 2 reveals that the temperature dependence of ΔK_{pol} of the ribose residue of a ribonucleoside is very similar

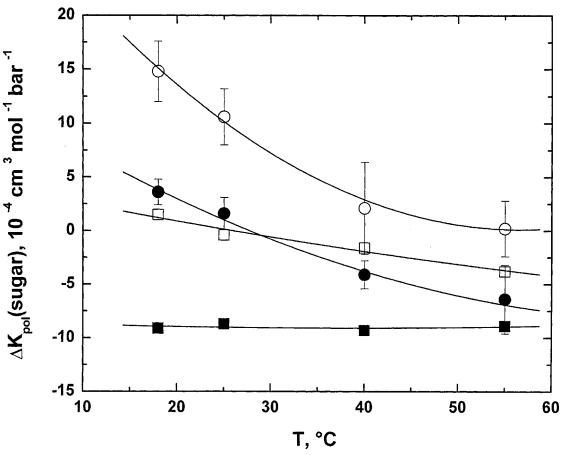

Fig. 2. Temperature dependences the compressibility contributions of polar groups of sugars: free ribose (■) [57]; free 2′-deoxyribose (□) [57]; the average value for the ribose moiety of the ribonucleosides (●); the average value for the 2′-deoxyribose moiety of the deoxyribonucleosides (○).

(although not identical) to that of K_{pol} of free 2′-deoxyribose. Judging by this similarity, we propose that the main reason for the observed disparity between the compressibility behavior of free ribose and the ribose residue of a ribonucleoside is the absence of the 1′-hydroxyl group in the latter which modifies its hydration structure in a similar manner as the absence of the 2′-hydroxyl group modifies the hydration of free 2′-deoxyribose relative to free ribose. We also propose that interactions (if any) between the base and sugar moieties of a ribonucleoside are not strong. The same observation has been made by Buckin et al. [38] based on the changes in sound velocity accompanying protonation of nucleic acid bases and ribonucleosides.

4.3.3. Deoxyribonucleosides

The deoxyribonucleosides (rows 13–18 in Table 8) are all characterized by positive values of ΔK_{pol} at 18 and 25°C, while at elevated temperatures some of them (2′-deoxycytidine, 2′-deoxyguanosine, and 2′-deoxyinosine) exhibit slightly negative values of ΔK_{pol}. In general, deoxyribonucleosides exhibit much higher (more positive) values of ΔK_{pol} than ribonucleosides. Hence, compared to ribonucleosides, deoxyribonucleosides are hy-

drated less strongly, which is consistent with the fact that, between 18 and 55°C, the value of K_{pol} of free ribose is smaller (more negative) than that of free 2′-deoxyribose [57]. This observation may have important implications for understanding the differential hydration of DNA and RNA. Inspection of the data in Table 8 reveals that, judging by the ΔK_{pol} values, at 25°C, the strongest hydration is exhibited by 2′-deoxyinosine (1.5×10^{-4} cm^3 mol^{-1} bar^{-1}) followed by 2′-deoxyguanosine (2.8×10^{-4} cm^3 mol^{-1} bar^{-1}), 2′-deoxycytidine (3.5×10^{-4} cm^3 mol^{-1} bar^{-1}), 2′-deoxyuridine (5.6×10^{-4} cm^3 mol^{-1} bar^{-1}), thymidine (9.3×10^{-4} cm^3 mol^{-1} bar^{-1}), and 2′-deoxyadenosine (10.8×10^{-4} cm^3 mol^{-1} bar^{-1}). At elevated temperatures this hydration hierarchy slightly changes. However, at all temperatures studied, 2′-deoxyadenosine exhibits the weakest hydration, an observation consistent with our conclusion which was drawn above based on the interaction volume data (see Section 4.3.3).

Inspection of Fig. 1c reveals that, for all the deoxyribonucleosides studied in this work, ΔK_{pol} is a decreasing function of temperature with the positive second temperature derivative, $\partial^2 \Delta K_{pol}/\partial T^2$. Thus, the temperature dependence of the compressibility of water solvating polar groups of the deoxyribonucleosides is more nonlinear (more abnormal) than that of bulk water. Comparison of Fig. 1b,c reveals that removal of a single hydroxyl group (in the 1-position) from the sugar moiety of a nucleoside (2′-deoxyribonucleoside vs. ribonucleoside) leads to a drastic change in the linearity of the temperature dependence of ΔK_{pol}.

To obtain the apparent ΔK_{pol} contribution of the deoxyribose moiety, we compare the deoxyribonucleosides with their corresponding bases. Specifically, we analyze the pairs uracil/2′-deoxyuridine, thymine/thimidine, cytosine/2′-deoxycytidine, adenine/2′-deoxyadenosine, and hypoxanthine/2′-deoxyinosine. Fig. 2 presents the average ΔK_{pol} contribution of the 2′-deoxyribose residue of a deoxyribonucleoside (○) as a function of temperature. Fig. 2 also presents the temperature dependence of K_{pol} for free 2′-deoxyribose (□) [57]. As is seen from Fig. 2, the temperature dependence of the apparent 2′-deoxyribose contribution to ΔK_{pol} of a deoxyribonucleoside significantly differs from that of K_{pol} of free 2′-deoxyribose. Analogous to ribonucleosides, we propose that the main reason for the observed disparity between the compressibility behavior of free 2′-deoxyribose and the deoxyribose residue of a deoxyribonucleoside is the absence of the 1′-hydroxyl group in the latter rather than the interactions between the base and sugar moieties.

5. Concluding remarks

In this work, we have determined the partial molar volumes, expansibilities, and adiabatic compressibilities of six heterocyclic nucleic acid bases (uracil, thymine, cytosine, purine, adenine, hypoxanthine), five ribonucleosides (uridine, cytidine, adenosine, guanosine, and inosine), and six deoxyribonucleosides (2′-deoxyuridine, thymidine, 2′-deoxycytidine, 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine) within the temperature range 18–55°C. For each compound studied, we have evaluated the interaction volume, that is, the contraction of water caused by solute–solvent interactions in the vicinity of polar groups. We found that the total contraction of water caused by polar groups of the heterocyclic bases and nucleosides depends on the proximity and chemical nature of other functional groups of the solute. In addition, we have calculated the compressibility contributions of polar groups for each compound studied here. The compressibility contributions of polar groups greatly vary in both sign and magnitude depending on the surrounding functional groups. In agreement with previous studies, our results are suggestive of little or no interaction between the sugar and base moieties of a nucleoside.

Based on our compressibility results, we conclude that, at room temperature, the strongest hydration among the heterocyclic bases is exhibited by cytosine, followed by hypoxanthine, uracil, thymine, adenine, and purine. Among the ribonucleosides, the strongest hydration is exhibited by cytidine, followed by guanosine, uridine, inosine, and adenosine. Among the deoxyribonucleosides, the strongest hydration is exhibited by 2′-de-

oxyinosine, followed by 2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyuridine, thymidine, and 2′-deoxyadenosine. At elevated temperatures, these hierarchies may somewhat change, but at all temperatures studied, adenosine and 2′-deoxyadenosine exhibit the weakest hydration within its corresponding class. This observation may be important for understanding the hydration properties of DNA and RNA.

Our results suggest that the hydration properties of individual heterocyclic bases and nucleosides as reflected in volume, expansibility, and adiabatic compressibility may vary greatly in range, not only in the absolute values of these volumetric characteristics, but also in their temperature dependences. In general, such volumetric characterizations should prove useful in developing an understanding of the role that solvent plays in the stabilization/destabilization of biologically important molecules.

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References

[1] H.R. Drew, R.E. Dickerson, Structure of a B-DNA dodecamer. III. Geometry of hydration, J. Mol. Biol. 151 (1981) 535–556.
[2] W. Saenger, W.N. Hunter, O. Kennard, DNA conformation is determined by economics in the hydration of phosphate groups, Nature 324 (1986) 385–388.
[3] W. Saenger, Structure and dynamics of water surrounding biomolecules, Annu. Rev. Biophys. Biophys. Chem. 16 (1987) 93–114.
[4] E. Westhof, Water: An integral part of nucleic acid structure, Annu. Rev. Biophys. Biophys. Chem. 17 (1988) 125–144.
[5] C.J. Morton, J.E. Jadbury, Water mediated protein–DNA interactions: The relationship of thermodynamics to structural detail, Protein Sci. 5 (1996) 2115–2118.
[6] T. Lundbäck, T. Härd, Sequence-specific DNA-binding dominated by dehydration, Proc. Natl. Acad. Sci. USA 93 (1996) 4754–4759.
[7] L. Jen-Jacobson, Protein–DNA recognition complexes: Conservation of structure and binding energy in the transition state, Biopolymers 44 (1997) 153–180.
[8] J.W.R. Schwabe, The role of water in protein–DNA interactions, Curr. Opin. Struct. Biol. 7 (1997) 126–134.
[9] J. Woda, B. Schneider, K. Patel, K. Mistry, H.M. Berman, An analysis of the relationship between hydration and protein–DNA interactions, Biophys. J. 75 (1998) 2170–2177.
[10] T. Lao, L.W. McLaughlin, Minor groove hydration is critical to the stability of DNA duplexes, J. Am. Chem. Soc. 122 (2000) 6512–6513.
[11] I. Haq, J. Ladbury, Drug–DNA recognition: Energetics and implications for design, J. Mol. Recog. 13 (2000) 188–197.
[12] X. Qu, J.B. Chaires, Hydration changes for DNA intercalation reactions, J. Am. Chem. Soc. 123 (2001) 1–7.
[13] V.A. Buckin, Experimental studies of DNA–water interaction, Mol. Biol. 21 (1987) 512–525.
[14] E. Westhof, Hydration of oligonucleotides in crystals, Int. J. Biol. Macromol. 9 (1987) 186–192.
[15] H.M. Berman, Hydration of DNA: take 2, Curr. Opin. Struct. Biol. 4 (1994) 345–350.
[16] M.A. Young, B. Jayaram, D.L. Beveridge, Intrusion of counterions into the spine of hydration in the minor groove of B-DNA: Fractional occupancy of electronegative pockets, J. Am. Chem. Soc. 119 (1997) 59–69.
[17] V.P. Denisov, G. Carlström, K. Venu, B. Halle, Kinetics of DNA hydration, J. Mol. Biol. 268 (1997) 118–136.
[18] J. Barciszewski, J. Jurczak, S. Porowski, T. Specht, V.A. Erdmann, The role of water in conformational changes of nucleic acids in ambient and high-pressure conditions, Eur. J. Biochem. 260 (1999) 293–307.
[19] M. Feig, B.M. Pettitt, Modeling high-resolution hydration patterns in correlation with DNA sequence and conformation, J. Mol. Biol. 286 (1999) 1075–1095.
[20] A.N. Lane, T.C. Jenkins, Thermodynamics of nucleic acids and their interactions with ligands, Q. Rev. Biophys. 33 (2000) 255–306.
[21] P. Auffinger, E. Westhof, Water and ion binding around RNA and DNA (C,G) oligomers, J. Mol. Biol. 300 (2000) 1113–1131.
[22] M. Feig, B.M. Pettitt, A molecular simulation picture of DNA hydration around A- and B-DNA, Biopolymers 48 (1998) 199–209.
[23] B. Halle, V.P. Denisov, Water and monovalent ions in the minor groove of B-DNA oligonucleotides as seen by NMR, Biopolymers 48 (1998) 210–233.
[24] M. Egli, V. Tereshko, M. Teplova et al., X-Ray crystallographic analysis of the hydration of A-and B-form DNA at atomic resolution, Biopolymers 48 (1998) 234–252.
[25] R.B. Macgregor, Jr., Effect of hydrostatic pressure on nucleic acids, Biopolymers 48 (1998) 253–263.
[26] H. Høiland, Partial molar volumes of biochemical model compounds in aqueous solutionsin: H.-J. Hinz (Ed.), Thermodynamic Data for Biochemistry and Biotechnology, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986, pp. 17–44.

[27] H. Durchschlag, Specific volumes of biological macromolecules and some other molecules of biological interestin: H.-J. Hinz (Ed.), Thermodynamic Data for Biochemistry and Biotechnology, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986, pp. 45–128.

[28] H. Høiland, Partial molar compressibilities of organic solutes in waterin: H.-J. Hinz (Ed.), Thermodynamic Data for Biochemistry and Biotechnology, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986, pp. 129–147.

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

[30] T.V. Chalikian, A.P. Sarvazyan, K.J. Breslauer, Hydration and partial compressibility of biological compounds, Biophys. Chem. 51 (1994) 89–109.

[31] T.V. Chalikian, K.J. Breslauer, Thermodynamic analysis of biomolecules: A volumetric approach, Curr. Opin. Struct. Biol. 8 (1998) 657–664.

[32] T.V. Chalikian, K.J. Breslauer, Volumetric properties of nucleic acids, Biopolymers 48 (1998) 264–280.

[33] T.V. Chalikian, A.P. Sarvazyan, G.E. Plum, K.J. Breslauer, The influence of base composition, base sequence, and duplex structure on DNA hydration: Apparent molar volumes and apparent molar adiabatic compressibilities of synthetic and natural DNA duplexes at 25°C, Biochemistry 33 (1994) 2394–2401.

[34] T.V. Chalikian, J. Völker, A.R. Srinivasan, W.K. Olson, K.J. Breslauer, The hydration of nucleic acid duplexes as assessed by a combination of volumetric and structural techniques, Biopolymers 50 (1999) 459–471.

[35] T.V. Chalikian, G.E. Plum, A.P. Sarvazyan, K.J. Breslauer, The influence of drug binding on DNA hydration: Acoustic and densimetric characterizations of netropsin binding to the poly(dAdT)poly(dAdT) and the poly(dA)poly(dT) duplexes and to the poly(dT)poly(dA)poly(dT) triplex at 25°C, Biochemistry 33 (1994) 8629–8640.

[36] H. Høiland, A. Skauge, I. Stokkeland, Changes in partial molar volumes and isentropic partial molar compressibilities of stacking of some nucleobases and nucleosides in water at 298.15 K, J. Phys. Chem. 88 (1984) 6350–6353.

[37] V.A. Buckin, Hydration of nucleic bases in dilute aqueous solutions. Apparent molar adiabatic and isothermal compressibilities, apparent molar volumes, and their temperature slopes at 25°C, Biophys. Chem. 29 (1988) 283–292.

[38] V.A. Buckin, B.I. Kankia, R.L. Kazaryan, Hydration of nucleosides in dilute aqueous solutions. Ultrasonic velocity and density measurements, Biophys. Chem. 34 (1989) 211–223.

[39] N. Kishore, J.C. Ahluwalia, Partial molar heat capacities and volumes of transfer of nucleic acid bases, nucleosides and nucleotides from water to aqueous solutions of sodium and calcium chloride at 25°C, J. Solution Chem. 19 (1990) 51–64.

[40] D.W. Kupke, Density and volume change measurementsin: S.J. Loch (Ed.), Physical Principles and Techniques of Protein Chemistry, Part C, Academic Press, New York, London, 1973, pp. 1–75.

[41] G.S. Kell, Thermodynamic and transport properties of fluid waterin: F. Franks (Ed.), Water. A Comprehensive Treatise, 1, Plenum Press, New York, London, 1972, pp. 363–412.

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

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

[44] A.P. Sarvazyan, E.E. Selkov, T.V. Chalikian, Constant-path acoustic interferometer with transition layers for precision measurements in small liquid volumes, Sov. Phys. Acoust. 34 (1988) 631–634.

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

[46] F. Eggers, Ultrasonic velocity and attenuation measurements in liquids with resonators, extending the MHz frequency range, Acustica 76 (1992) 231–240.

[47] F. Eggers, U. Kaatze, Broad-band ultrasonic measurement techniques for liquids, Meas. Sci. Technol. 7 (1996) 1–19.

[48] S. Barnatt, The velocity of sound in electrolytic solutions, J. Chem. Phys. 20 (1952) 278–279.

[49] B.B. Owen, H.L. Simons, Standard partial molal compressibilities by ultrasonics. 1. Sodium chloride and potassium chloride at 25°C, J. Phys. Chem. 61 (1957) 479–482.

[50] V.A. Del Grosso, C.W. Mader, Speed of sound in pure water, J. Acoust. Soc. Am. 52 (1972) 1442–1446.

[51] R.A. Pierotti, Aqueous solutions of nonpolar gases, J. Phys. Chem. 69 (1965) 281–288.

[52] F.H. Stillinger, Structure in aqueous solutions of nonpolar solutes from the standpoint of scaled-particle theory, J. Solution Chem. 2 (1973) 141–158.

[53] R.A. Pierotti, A scaled particle theory of aqueous and nonaqueous solutions, Chem. Rev. 76 (1976) 717–726.

[54] D.P. Kharakoz, Partial molar volumes of molecules of arbitrary shape and the effect of hydrogen bonding with water, J. Solution Chem. 21 (1992) 569–595.

[55] A. Bondi, van der Waals volumes and radii, J. Phys. Chem. 68 (1964) 441–451.

[56] D.P. Kharakoz, Volumetric properties of proteins and their analogs in diluted water solutions. 2. Partial adiabatic compressibilities of amino acids at 15–70°C, J. Phys. Chem. 95 (1991) 5634–5642.

[57] T.V. Chalikian, Ultrasonic and densimetric characterizations of the hydration properties of polar groups in monosaccharides, J. Phys. Chem. 102 (1998) 6921–6926.

[58] T.V. Chalikian, Structural thermodynamics of solute hydration, J. Phys. Chem. (2001) in press.

[59] T.V. Chalikian, A.P. Sarvazyan, K.J. Breslauer, Partial molar volumes, expansibilities, and compressibilities of α,ω-aminocarboxylic acids in aqueous solutions between 18 and 55°C, J. Phys. Chem. 97 (1993) 13017–13026.
[60] D.P. Kharakoz, Volumetric properties of proteins and their analogs in diluted water solutions. 1. Partial molar volumes of amino acids at 15–55°C, Biophys. Chem. 34 (1989) 115–125.
[61] C.M. Davis Jr., J. Jarzynski, Liquid water-acoustic properties: absorption and relaxationin: F. Franks (Ed.), Water. A Comprehensive Treatise, 1, Plenum Press, New York, London, 1972, pp. 443–461.
[62] A. Hvidt, A discussion of the expansivity and compressibility of liquid water, Acta Chem. Scand. A 32 (1978) 675–680.
[63] J.V. Leyendekkers, Aqueous solutions. Part 2. — The compressibility and acoustic relaxations of water, J. Chem. Soc. Faraday Trans. 1 (81) (1985) 1–10.