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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

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ABSTRACT: Using high-precision densimetric and ultrasonic measurements, we have determined, at 25 °C, the apparent molar volumes, ϕV, and the apparent molar compressibilities, ϕK_S, of five natural and three synthetic B-form DNA duplexes with varying base compositions and base sequences. We find that ϕV ranges from 152.0 to 186.6 cm^3 mol^{-1}, while ϕK_S ranges from -73.0×10^{-4} to -32.6×10^{-4} cm^3 mol^{-1} bar^{-1}. We interpret these data in terms of DNA hydration which, by the definition employed in this work, refers to those water molecules whose density and compressibility differ from those of bulk water due to interactions with the DNA solute. This definition implies that hydration depends not just on the quantity but also on the quality of the solvent molecules perturbed by the solute. In fact, we find that the number of water molecules perturbed by the DNA duplexes (the quantity of water in their hydration shells) is approximately the same for all of the B-form double helixes studied, while the quality of this water differs as measured by its density and compressibility, thereby yielding differences in the overall hydration properties. Specifically, we find a linear relationship between the density and the coefficient of adiabatic compressibility, β_{Sh}, of water in the hydration shell of the DNA duplexes, with the range of values for β_{Sh} being only 65–80% of the value of bulk water. In the aggregate, we interpret these data as reflecting the following general features: (i) water that is perturbed by interactions with B-form DNA duplexes (i.e., the hydration shell) exhibits increased density and a diminished coefficient of adiabatic compressibility relative to bulk water; (ii) the hydration of B-form duplexes primarily depends on the base composition, while it is only weakly influenced by base sequence; (iii) duplexes with lower ϕV and ϕK_S values exhibit greater hydration; (iv) duplexes with 55–60% AT base composition exhibit the weakest hydration, while increases or decreases in AT content from this range of values lead to enhanced hydration; and (v) water in the hydration shell of GC base pairs is more dense and less compressible than water in the hydration shell of AT base pairs, suggesting that GC base pairs are solvated more strongly (are more hydrated) than AT base pairs, in contrast with conventional wisdom. Using the definition of hydration employed in this work (solute-induced perturbation of solvent density and compressibility), we propose molecular interpretations for the differential hydration properties we observe.

During the last few decades, considerable attention has been focused on characterizing the hydration of DNA (Tunis & Hearst, 1968a,b; Falk et al., 1970; Texter, 1978; Drew & Dickerson, 1981; Chevrier et al., 1986; Kennard et al., 1986; Buckin, 1987; Westhof & Beveridge, 1989; Schneider et al., 1992; Beveridge et al., 1993), as well as the changes in DNA hydration which accompany various processes, such as conformational transitions and ligand binding (Clement et al., 1973; Wolf & Hanlon, 1975; Chapman & Sturtevant, 1969, 1970; Rentzeperis et al., 1993). In the aggregate, these and other studies provide a picture in which hydration exerts a significant influence on DNA properties, such as conformation (Saenger et al., 1986; Saenger, 1987; Westhof, 1988), bending (Westhof, 1988; Park & Breslauer, 1991; Breslauer, 1991), and ligand binding (Marky & Breslauer, 1987; Breslauer et al., 1987; Remeta et al., 1993).

However, despite general acceptance that hydration can influence DNA properties, the macroscopic and microscopic character of DNA hydration remains obscure. This circumstance, in part, reflects the fact that hydration almost always is defined operationally in terms of a specific observable. Microscopic characterizations of hydration generally rely on X-ray and/or NMR studies. However, single-crystal X-ray diffraction studies on DNA fragments "see" only those waters that are crystallographically ordered, while the same waters might not be seen in the corresponding solution NMR study if they exchange too rapidly with bulk water. Thus, the two techniques that generally are used to define DNA hydration microscopically may differentially sample the DNA-perturbed solvent, that is, the so-called hydration shell. A similar circumstance plagues macroscopic characterizations of DNA hydration. For example, the differential hydration of AT and GC base pairs has been characterized by both buoyant density (Tunis & Hearst, 1968b) and microcalorimetric techniques (Mrevlishvili, 1981), with grossly different values being reported. It is quite likely that both reports are correct since the observables associated with the two techniques probably sample different subpopulations of the DNA-perturbed solvent. Thus, when discussing DNA hydration, it is important to emphasize and to discuss the nature of the observable used to define the hydration shell, since the solute-altered solvent that is sampled quite likely will depend on the observable(s) used to investigate the system.

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We have begun a program in which the hydration properties of DNA are being studied using densimetric and ultrasonic velocimetric techniques. We have three reasons for selecting these methods to investigate DNA hydration. First, the measurements can be performed in dilute aqueous solutions and therefore are not complicated by crystal-packing forces or solute–solute interactions. Second, the observables associated with these macroscopic methods are highly nonselective and therefore sense a very broad population of the solvent that is perturbed by the solute. Third, these measurements yield data that allow one to obtain a thermodynamic characterization of the solute-perturbed solvent. Such additional thermodynamic data expand the data base that we and others have derived from calorimetric studies of DNA molecules.

Both density and sound velocity in solutions are known to be sensitive to the state and the amount of water in the hydration shell of a solute (Sarvazyan, 1991). In this work, we have determined, at 25 °C, the apparent molar volume and the apparent molar adiabatic compressibility of five natural and three synthetic DNAs with different base compositions and base sequences. The resulting experimental data are interpreted in terms of DNA hydration, in particular, its dependence on the base composition and the base sequence. Independent of our microscopic interpretations of our macroscopic observables, the data reported here vastly expand the current sparse data base on the volumetric properties of DNA.

MATERIALS AND METHODS

Materials. The five natural DNA polymers (salmon testes, *Micrococcus lysodeikticus*, human placenta, herring testes, and *Escherichia coli*) were obtained from Sigma Chemical Co. (St. Louis, MO), while the three synthetic DNA polymers [poly(dA)poly(dT), poly(dA–dT)poly(dA–dT), and poly(dG–dC)poly(dG–dC)] were purchased from Pharmacia-LKB Biochemicals (Piscataway, NJ). These eight DNAs were of the highest grade commercially available and were used without further purification. Solutions were prepared using triply distilled water with a specific conductivity of less than $10^{-6}\ \Omega^{-1}$. All measurements were performed in a pH 6.7 buffer solution consisting of 10 mM cacodylic acid–sodium cacodylate, 0.1 mM Na_2EDTA, and 8 mM NaCl, yielding a total Na^+ ion concentration of 16 mM. DNA samples were dissolved in the buffer and sonicated to reduce solution viscosities. All solutions were dialyzed at 4 °C against the same buffer using dialysis tubing with a 1000 molecular weight cutoff (Spectrum, Houston, TX). Two additional changes of buffer solution were made, with at least 48 h allowed for equilibration. The concentration of each DNA was determined spectrophotometrically using the following DNA molar extinction coefficients per nucleotide: salmon testes, ϵ_{260} = 6550 M^{-1} cm^{-1} (Breslauer et al., 1987); *Micrococcus lysodeikticus*, ϵ_{260} = 6970 M^{-1} cm^{-1} (Felsenfeld & Hirschman, 1965); human placenta, ϵ_{260} = 6410 M^{-1} cm^{-1}; herring testes, ϵ_{260} = 6460 M^{-1} cm^{-1}; *E. coli*, ϵ_{260} = 6540 M^{-1} cm^{-1} (Felsenfeld & Hirschman, 1965); poly(dA)poly(dT), ϵ_{260} = 6000 M^{-1} cm^{-1}; poly(dA–dT)poly(dA–dT), ϵ_{260} = 6650 M^{-1} cm^{-1}; poly(dG–dC)poly(dG–dC), ϵ_{254} = 8400 M^{-1} cm^{-1}. The extinction coefficients for the latter three synthetic DNA polymers were provided by the manufacturer and are consistent with previously reported values (Remeta et al., 1993). The herring testes and human placenta extinction coefficients were derived by extrapolating the data on extinction coefficients for different natural DNAs with various base-pair compositions (Felsenfeld & Hirschman, 1965) to the 57 and 60% AT contents, which correspond to these two DNAs.

In all of the experiments, the DNA concentrations fell within the range of 2–3 mM per nucleotide, which represents the lowest DNA concentrations one can study with the existing state-of-the-art acoustic and densimetric instrumentation. Throughout this article, all DNA concentrations are expressed per mole of nucleotide, unless otherwise indicated.

Densimetry. All densities were measured at 25 °C with a precision of $\pm 1.5 \times 10^{-6}$ g cm^{-3} using a vibrating tube densimeter (DMA-60, Anton Paar, Graz, Austria). The apparent molar volumes, ϕV, of DNA were calculated from the following well-known relationship (Millero, 1972):

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

where M is the molecular weight of DNA (average per nucleotide), C is the molar concentration of nucleotides, and ρ and ρ_0 are the densities of the solution and the solvent (buffer solution), respectively.

Ultrasonic Velocimetry. Solution sound velocity values, U, were measured at a frequency of 7.5 MHz using the resonator method (Eggers & Funck, 1973; Sarvazyan, 1982; Eggers, 1992) and a previously described differential technique (Sarvazyan, 1982). Ultrasonic resonator cells with sample volumes of 0.8 cm^3 were thermostated at 25 °C with an accuracy of ±0.01 °C. For the type of ultrasonic resonators used in this work, the accuracy of the sound velocity measurements was about $\pm 10^{-4}$% (Sarvazyan & Chalikian, 1991; Chalikian & Sarvazyan, 1991).

The resulting values of U were used in conjunction with the ϕV values derived from eq 1 to calculate the apparent molar adiabatic compressibility, ϕK_S, using the relationship (Barnatt, 1952; Owen & Simons, 1957):

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

where β_{S0} is the coefficient of adiabatic compressibility of the solvent, and $[U]$ is the relative molar increment of the sound velocity, which is equal to $(U - U_0)/(U_0 C)$, where U and U_0 are the sound velocities in the solution and the solvent, respectively.

For each evaluation of ϕV or ϕK_S, 3–5 independent measurements were carried out on each DNA sample within a concentration range of 2–3 mM.

RESULTS AND DISCUSSION

Table 1 shows the relative molar increments of sound velocity, $[U]$, apparent molar volume, ϕV, and apparent molar adiabatic compressibility, ϕK_S, at 25 °C of the five natural and three synthetic DNA duplexes studied in this work. Inspection of these data reveals that the values of the apparent molar volume, ϕV, and the relative molar sound velocity increment, $[U]$, cover a considerable range, from 152.0 to 186.6 cm^3 mol^{-1} for ϕV and from 57 to 68 cm^3 mol^{-1} for $[U]$. Naturally, it would be interesting to compare our data with previously reported values of ϕV and ϕK_S. Unfortunately, despite the fundamental importance of these parameters for understanding DNA hydration, such measurements have been sparse due to a lack of appropriate instrumentation. Some of the few related previous studies (Tennent & Vilbrandt, 1943; Chapman & Sturtevant, 1969; Zipper & Bünemann, 1975) report values that range from 183 to 186 cm^3 mol^{-1} for the apparent molar volumes, ϕV, of the sodium salt of calf thymus DNA. Significantly, these data fall within the range of ϕV values that we report here.

Table 1: Relative Molar Increments of Sound Velocity, $[U]$, Apparent Molar Volume, ϕV, and Apparent Molar Adiabatic Compressibility, ϕK_S, for Various Natural and Synthetic DNA Duplexes at 25 °C[a]

DNA	M	% AT	$[U]$ ($cm^3\ mol^{-1}$)	ϕV ($cm^3\ mol^{-1}$)	$\phi K_S \times 10^4$ ($cm^3\ mol^{-1}\ bar^{-1}$)
poly(dA)poly(dT)	331.7	100	67.0 ± 0.8	169.0 ± 1.5	−57.5 ± 2.0
poly(dA–dT)poly(dA–dT)	331.7	100	68.0 ± 0.8	170.0 ± 1.5	−57.5 ± 2.0
human placenta	331.9	60	59.2 ± 1.0	181.6 ± 1.5	−39.1 ± 2.0
herring testes	331.9	57	56.9 ± 0.8	186.6 ± 1.0	−32.6 ± 1.5
salmon testes	331.9	57	59.3 ± 1.0	185.0 ± 1.5	−36.3 ± 2.0
E. coli	332.0	50	61.5 ± 1.0	182.7 ± 1.5	−40.4 ± 2.0
M. lysodeikticus	332.1	28	61.7 ± 0.9	167.5 ± 1.5	−54.4 ± 2.0
poly(dG–dC)poly(dG–dC)	332.2	0	67.0 ± 1.0	152.0 ± 1.5	−73.0 ± 2.0

[a] The indicated errors include contributions from the solute concentration, as well as any temperature variability, in the measuring cells.

With regard to the apparent molar adiabatic compressibility, ϕK_S, inspection of Table 1 reveals values that range from -73.0×10^{-4} to $-32.6 \times 10^{-4}\ cm^3\ mol^{-1}\ bar^{-1}$. Unfortunately, the only two previous studies that report ϕK_S values for DNA (Buckin et al., 1989a,b) measured ϕK_S at much lower temperature (1 °C) and therefore their data cannot be compared directly with our 25 °C data, particularly since ϕK_S is strongly temperature-dependent. In fact, the ϕK_S data listed in Table 1 represent the first reported values for the apparent molar adiabatic compressibility of DNA duplexes at 25 °C.

It should be emphasized once again that we observe a wide range of values for both the apparent molar volume and the apparent molar adiabatic compressibility of DNA duplexes that possess different base-pair compositions and different sequences. This variation can be interpreted as suggesting that the types and strengths of DNA–solvent interactions depend on the specific nature of the polymer. We will elaborate on this interpretation later in this article. However, we will first describe the types of molecular interactions that are thought to contribute to the ϕV and ϕK_S values reported here.

Interpretation of ϕV and ϕK_S Data in Terms of Hydration. The standard interpretation of the apparent molar volume, ϕV, and the apparent molar adiabatic compressibility, ϕK_S, of a solute in terms of hydration effects is based on the following two simple relationships (Shiio et al., 1955):

$$\phi V = V_M + \Delta V_h = V_M + n_h(V_h - V_0) \quad (3)$$

$$\phi K_S = K_M + \Delta K_h = K_M + n_h(K_{Sh} - K_{S0}) \quad (4)$$

where V_M and K_M are the intrinsic molar volume and the intrinsic molar adiabatic compressibility of a solute molecule, respectively; ΔV_h and ΔK_h represent, respectively, the volume and compressibility effects of hydration; V_h and V_0 are the partial molar volumes of water in the hydration shell of a solute and in the bulk state, respectively; K_{Sh} and K_{S0} are the partial molar adiabatic compressibilities of water in the hydration shell of a solute and in the bulk state, respectively; and n_h is the hydration number, which corresponds to the number of water molecules in the hydration shell of a solute molecule. Some notations of partial molar properties include a superscript zero or bar over the parameter, which for convenience has been excluded in the notation used here. The ΔV_h term (the volume effect of hydration) in eq 3 originates from two sources: (i) volume changes in the solvent induced by interactions with solute molecules, and (ii) the void volume around solute molecules due to the mutual thermal motion of solute and solvent molecules (Kharakoz, 1989, 1992; Chalikian et al., 1993). Similarly, the compressibility changes in the solvent and the compressibility of the void space around solute molecules contribute to the ΔK_h term in eq 4. Thus, the terms V_h and K_{Sh} in eqs 3 and 4 are determined, not only by the intrinsic partial molar volume and the partial molar compressibility of water in the hydration shell of a solute but also by positive contributions from the volume and compressibility of the void space around solute molecules.

Equations 3 and 4 and the foregoing discussion suggest that the values of the apparent molar volume and the apparent molar adiabatic compressibility of a solute are sensitive to both the quantity and the quality of hydration. The quantity of hydration corresponds to the amount of solvating water molecules and is represented in eqs 3 and 4 as the hydration number, n_h. The quality of hydration is reflected in the values of the partial molar volume, V_h, and the partial molar adiabatic compressibility, K_{Sh}, of the water in the hydration shell of a solute, since the volume and compressibility characteristics of water surrounding an atomic group are sensitive to its chemical nature (Sarvazyan & Kharakoz, 1977; Buckin et al., 1989c; Kharakoz, 1989, 1991; Sarvazyan, 1991; Chalikian et al., 1993, 1994). Electrostatic solute–solvent interactions cause strong decreases in both the partial molar volume, V_h, and the partial molar compressibility, K_{Sh}, of water surrounding charged atomic groups or ions (Buckin et al., 1989c; Kharakoz, 1989, 1991) at 25 °C. By contrast, hydrophobic groups cause almost no change in V_h and small negative changes in K_{Sh} at 25 °C (Sarvazyan & Kharakoz, 1977; Kharakoz, 1989, 1991; Chalikian et al., 1993). Polar groups, which can form hydrogen bonds with water molecules, decrease V_h while their influence on K_{Sh} strongly depends on their proximity to other polar or charged groups (Kharakoz, 1989, 1991, 1992; Sarvazyan, 1991). The contributions of specific atomic groups to the apparent molar characteristics of solutes have been determined experimentally through studies on a homologous series of different low molecular weight model compounds (Sarvazyan & Kharakoz, 1977; Millero et al., 1978; Mishra & Ahluwalia, 1984; Buckin, 1988; Buckin et al., 1989c; Kharakoz, 1989, 1991; Chalikian et al., 1993, 1994). Such data can be used to assist us in interpreting the molecular origins of the macroscopic data reported here (see Table 1) or, in other words, in interpreting our experimental data in terms of DNA hydration.

The term hydration shell, as used here and as reflected in eqs 3 and 4, refers to those water molecules around the solute whose physicochemical properties (particularly, density and compressibility) differ from those of bulk water. In other words, the hydration shell comprises those water molecules which, under the influence of the solute, are physicochemically distinguishable from bulk water. This operational definition of a hydration shell may not exactly coincide with other operational definitions based on different observables derived from other experimental methods. For example, as noted earlier, in crystallography, one primarily detects the highly ordered water molecules around a solute and ascribes these

solvent molecules to the hydration shell. However, some water molecules in the vicinity of a solute that exhibit altered physicochemical characteristics may not be ordered sufficiently to diffract X-rays. On the other hand, such crystallographically detected, ordered water molecules may be indistinguishable from bulk water by some particular physicochemical parameter. In low-temperature scanning calorimetry, the term hydration shell corresponds only to those water molecules which, upon lowering the temperature below the freezing point of bulk water, do not undergo a water to ice transition (Mrevlishvili, 1981). However, water molecules that fail to freeze may be only a subpopulation of those molecules that have altered physicochemical properties.

Clearly, the determination of the number of water molecules associated with and/or influenced by a solute will depend on the method used to investigate the system. Different observables may yield different estimates of the degree of hydration of a solute. Thus, in general, the frequently encountered terms more hydrated or less hydrated should be used with caution and always discussed in terms of the methods used to evaluate the degree of hydration. With such qualifications in mind, and in the context of a single physicochemical property of water characterizing a common observable, it can at times be instructive to use the terms more hydrated or less hydrated to reflect the strengths and the types of solute–solvent interactions (the quality of hydration), rather than just defining hydration in terms of the number of water molecules in the hydration shell of a solute (the quantity of hydration).

Acoustic and densimetric studies on a large variety of low molecular weight model compounds reveal that solute-induced changes in the properties of water in the vicinity of an atomic group of a solute extend to a distance of 3–4 Å, which corresponds to 1–1.5 layers of water molecules (Buckin, 1987; Buckin et al., 1989c; Sarvazyan, 1991; Chalikian et al., 1993, 1994). Thus, at least from the point of view of acoustodensimetric measurements, the quantity of the hydration shell consists primarily of water molecules that contact a solute directly. In the case of DNA, this interpretation is consistent with the infrared studies of Falk et al. (1963) who, on the basis of the OH stretching frequencies of water around DNA, concluded that "all but the first layer of adsorbed water molecules have properties identical with those of liquid water".

The number (quantity) of water molecules in the first monolayer around DNA can be considered as a lower limit for the hydration number, n_h, in eqs 3 and 4. This number can be defined as the ratio of the accessible surface area of DNA (S_M) to the surface area of a water molecule (S_W). Thus, n_h is equal to S_M/S_W, where the surface area of a water molecule, S_W, is 9 Å^2. According to the calculations of Alden and Kim (1979), the accessible surface area, S_M, of AT-rich polymers of B-DNA is equal to 175.2 Å^2 per nucleotide, while the corresponding S_M value for GC-rich B-DNA is equal to 171.5 Å^2 per nucleotide. Dividing these values by 9 Å^2, we calculate the hydration number, n_h, in eqs 3 and 4 (the number of water molecules in the first monolayer around DNA) to be 19.5 per "average" nucleotide for an AT base pair and 19.1 per "average" nucleotide for a GC base pair in B-form duplexes [an assumption that may not be correct for the poly(dA)-poly(dT) duplex, which is believed to assume an altered non-B conformation (Leslie et al., 1980; Alexeev et al., 1987)]. Thus, the hydration number, n_h, defined exclusively from geometrical considerations, is essentially the same for AT and GC base pairs in B-form duplexes, that is, 19.3 ± 0.2 water molecules per nucleotide. However, it should be emphasized that the number estimated above must be considered as only the lower limit of the hydration number, n_h. The true value of n_h is expected to be higher because, in the simple geometric treatment just described, the contribution of the counterions to the total accessible surface area is not taken into account. More realistic estimations of n_h for the sodium salt of DNA, calculated from the accessible surfaces of both the DNA itself and the sodium counterions, yield numbers that range from 22 to 26 (Buckin, 1987). We will use the average n_h value of 24 ± 2 in the calculations described below, keeping in mind that this number may not be correct for the poly(dA)poly(dT) duplex, since it may assume a non-B-form conformation (Leslie et al., 1980; Alexeev et al., 1987).

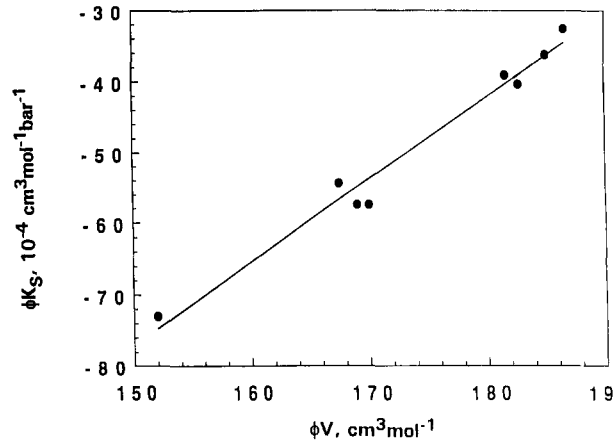

FIGURE 1: Plot of the apparent molar adiabatic compressibilities, ϕK_S, versus the apparent molar volumes, ϕV, of DNA duplexes.

The difference between the intrinsic volumes, V_M, of AT and GC base pairs is not large: 162.0 cm^3 mol^{-1} for AT base pairs and 159.3 cm^3 mol^{-1} for GC base pairs (Pavlov & Fedorov, 1983). The intrinsic compressibility, K_M, of DNA is on the order of 5×10^{-4} cm^3 mol^{-1} bar^{-1} (7–15% of the total values of ϕK_S) and depends only weakly on the type of DNA (Buckin et al., 1989b). Thus, on the basis of the relationships described in eqs 3 and 4, the differences we have measured in the apparent molar volumes, ϕV, and the apparent molar adiabatic compressibilities, ϕK_S, for different DNA duplexes (see Table 1) will primarily reflect differences in duplex hydration, as manifested in ΔV_h and ΔK_h. In the discussions that follow, we describe empirical correlations evident from our data and interpret these correlations in terms of DNA hydration.

Water in the Hydration Shell of B-Form DNA Exhibits an Increase in Density and a Decrease in the Coefficient of Adiabatic Compressibility Relative to Bulk Water. Figure 1 shows that the apparent molar adiabatic compressibility, ϕK_S, of DNA depends linearly on its apparent molar volume, ϕV, so that we can write:

$$\phi K_S = A(\phi V) + B \quad (5)$$

where A and B are universal constants for all of the DNA duplexes studied here. From a least-squares fit of our data, we estimate the value of A (the slope) to be equal to 1.15×10^{-4} bar^{-1}, while we estimate the value of B (the intercept) to be equal to −0.025 cm^3 mol^{-1} bar^{-1}. Since $K_{Sh} = \beta_{Sh} V_h$ (where β_{Sh} is the coefficient of adiabatic compressibility of water in the hydration shell of DNA) and $V_h = M_W/\rho_h$ (where M_W is the molecular weight of water and ρ_h is the density of water in the hydration layer), eq 5 can be rewritten in conjunction with these relationships and eqs 3 and 4 to yield:

$$\beta_{Sh} = A + B_1\rho_h \quad (6a)$$

where $B_1 = [A(V_M/n_h - V_0) + (B - K_M)/n_h + K_{S0}]/M_W$. Using the previously reported K_M value of 5×10^{-4} cm^3 mol^{-1} bar^{-1} (Buckin et al., 1989b) and the average values $n_h = 24$ and $V_M = 160.1$ cm^3 mol^{-1}, we estimate B_1 to be equal to -0.85×10^{-4} cm^3 g^{-1} bar^{-1}.

Thus, for the eight duplexes investigated here, the specific form of eq 6a is

$$\beta_{Sh}\ (\text{bar}^{-1}) = 1.15 \times 10^{-4} - 0.85 \times 10^{-4}\rho_h\ (\text{g cm}^{-3}) \quad (6b)$$

Equation 6b shows that there is a linear relationship between the density and the coefficient of adiabatic compressibility of water in the hydration shell of a DNA duplex. This relationship seems to be universal for double-stranded B-form DNA. An important conclusion implicit in eq 6 is that solute–solvent interactions increase the density of water in the hydration shell of B-form duplex DNA relative to bulk water, while decreasing the coefficient of adiabatic compressibility of this perturbed water.

From eqs 3–6, the density, ρ_h, and the coefficient of adiabatic compressibility, β_{Sh}, of water in the hydration shell of the DNA duplexes studied here can be estimated. This calculation yields the following range of values: $\rho_h = 0.94$–1.01 g cm^{-3} (very close to the value for bulk water) and $\beta_{Sh} = (30$–$35) \times 10^{-6}$ bar^{-1} (well below the value for bulk water). However, this calculation of ρ_h includes contributions from the volume of the void space around DNA. After correction for this excess volume (as explained below), the actual intrinsic density of the water in the hydration shell of DNA will well exceed 1.0 g cm^{-3}, the density of bulk water.

As just noted, one must consider the contribution of the volume of the void space around DNA to convert our calculated ρ_h values into true estimates of the actual (intrinsic) density of water in the hydration shell of DNA. In a previous study, we estimated the volume of the void volume around α,ω-aminocarboxylic acids to be as much as 60–65% of their intrinsic volumes (Chalikian et al., 1993). If one assumes a qualitatively similar proportion for DNA duplexes (a reasonable assumption since the hydration shells of short α,ω-aminocarboxylic acids are formed predominantly under the influence of electrostatic solute–solvent interactions, as is the case for DNA duplexes), then the real density of water in the DNA hydration shell is about 25% higher than the 0.94–1.01 g cm^{-3} range of ρ_h values noted above, yielding intrinsic densities in the range of 1.17–1.26 g cm^{-3}. This qualitative analysis, therefore, suggests that the actual density of water in the hydration shell of DNA duplexes is significantly higher than that of bulk water.

With regard to the coefficient of adiabatic compressibility of water in the hydration shell of DNA, β_{Sh}, we have calculated a range of values of $(30$–$35) \times 10^{-6}$ bar^{-1}. This range corresponds to 65–80% of the value for bulk water, which is equal to 45×10^{-6} bar^{-1} at 25 °C (Fine & Millero, 1973). In this connection, it should be noted that the compressibility of bulk water approaches the compressibility of water in the hydration shell of DNA only under hydrostatic pressures greater than 1000 bar (Fine & Millero, 1973). Such low compressibility values previously have been observed only in the hydration shells of charged atomic groups or ions, where water molecule dipoles experience strong electrostatic contraction (Desnoyers et al., 1965; Sarvazyan, 1991). Thus, our data suggest that electrostatic DNA–solvent interactions play the predominant role in the formation of the primary hydration shell of duplex DNA, a conclusion consistent with conventional wisdom. Consequently, in the absence of fortuitous compensations, we can conclude that nonelectrostatic interactions of water with the accessible polar and nonpolar atomic groups of duplex DNA contribute only secondarily to the individual features of hydration of a particular DNA duplex.

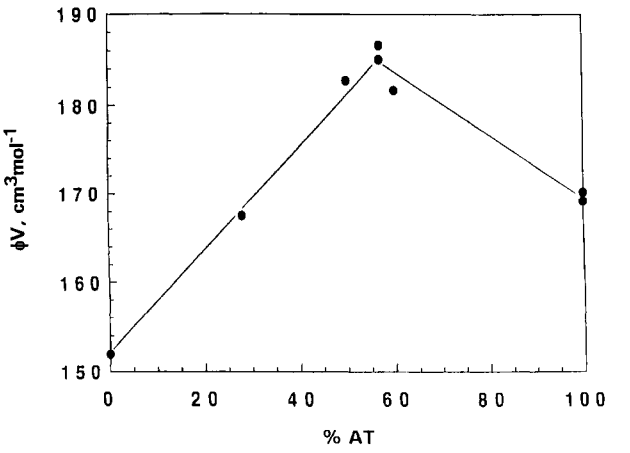

FIGURE 2: Dependence of the apparent molar volume, ϕV, of DNA duplexes on the AT content.

As mentioned above, the number of solvating water molecules, n_h, is probably the same for all of the B-form DNA duplexes studied (perhaps with the exception of the poly(dA)poly(dT) duplex, which may assume an altered conformation). Thus, lower values of ϕV and ϕK_S for one duplex versus another may be interpreted as indicative of a higher density and a lower compressibility for the water in its hydration shell, a feature that suggests stronger DNA–solvent interactions due to a higher degree of contraction of the water molecule dipoles in the DNA hydration shell. Using the more widely employed verbiage, this higher degree of contraction of water molecules (stronger solute–solvent interactions) in the hydration shell of a DNA duplex may be referred to as reflective of greater hydration. Although this description represents an oversimplification of the complex processes associated with all solute–solvent interactions, it helps to describe the hydration features of a solute in commonly accepted, intuitive terms. Thus, for the sake of our discussions, we will refer to lower values of the apparent molar volume and the apparent molar adiabatic compressibility of DNA duplexes as reflecting greater hydration.

Influence of Base Composition and Sequence on DNA Hydration. Figures 2 and 3 show, respectively, the dependence on AT base-pair content of the apparent molar volumes, ϕV, and the apparent molar adiabatic compressibilities, ϕK_S, of DNA duplexes. Note that the two plots appear similar to each other. Specifically, an increase in AT content from 0 to about 60% leads to significant increases in both ϕV and ϕK_S, while further increases in AT content from 60 to 100% lead to decreases in the values of both ϕV and ϕK_S. Significantly, both the apparent molar volume, ϕV, and the apparent molar adiabatic compressibility, ϕK_S, of poly(dG–dC)poly(dG–dC) are substantially lower than those of the two all-AT polymers, which have nearly identical values of ϕV and ϕK_S. We describe and will discuss here a number of generalizations concerning the state of water in the hydration shell of DNA duplexes that are suggested by the data and trends illustrated in Figures 2 and 3.

Inspection of the trends in Figures 2 and 3 suggests that base composition appears to be the primary factor in determining the hydration of DNA, while base sequence is of

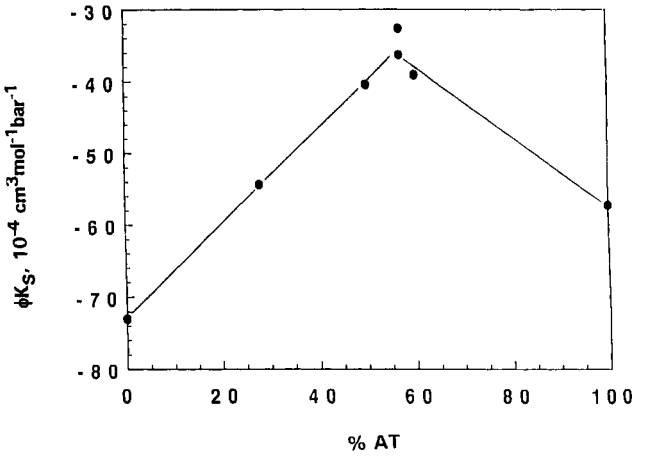

FIGURE 3: Dependence of the apparent molar adiabatic compressibility, ϕK_S, of DNA duplexes on the AT content.

secondary importance. This conclusion derives from the observation that duplexes with the same base compositions but different base sequences (e.g., salmon testes and herring testes DNA duplexes with 57% AT content) exhibit nearly equal values of ϕV and ϕK_S. Furthermore, the data listed in Table 1 and illustrated in Figures 2 and 3 suggest that DNA duplexes with 100% AT or GC contents are more hydrated than random sequence duplexes, with the minimum hydration being observed for duplexes with AT contents around 55–60%. Thus, the hydration-dependent characteristics of a DNA duplex cannot be estimated as a simple weighted sum of the contributions from AT and GC base pairs. Perhaps the regularity of some sequences produces periodic structural scaffolds that promote resonance extension of hydration networks, thereby causing duplex hydration properties to be influenced by more than global base composition or local sequence effects. In other words, long-range solute–solvent interactions may be facilitated by a regular matrix of solvent binding sites produced by a periodic DNA structure. For this reason, DNA duplexes with patterned, repeating sequences (e.g., 100% AT or GC base pairs) rather than random sequences may have hydration shells that include more solvent molecules, particularly involving waters from the second and even third layers of the surrounding solvent. In fact, the periodicity of regular DNA structures may correlate with the second and/or third peaks in the radial distribution function of bulk water, thus causing some additional coordination of water at distances which exceed that associated with local hydration near isolated atomic groups. Clearly, further studies are required to understand the origin of the intriguing maxima in Figures 2 and 3.

Moving from the global duplex to the component base pairs, the trends illustrated in Figures 2 and 3 also can be interpreted as suggesting that GC base pairs are more hydrated than AT base pairs. This suggestion contrasts with earlier studies (Tunis & Hearst, 1968b; Mrevlishvili, 1981), which conclude that AT base pairs are more hydrated. Significantly, however, the researchers of these previous studies based their conclusions on observables (e.g., buoyant density and heat capacity) that are different from those measured in this work. In this study, our operational definition of more hydrated is based on the density and compressibility of the water perturbed by the presence of the solute. Thus, when we say that GC base pairs are more hydrated than AT base pairs, strictly speaking we are saying that water in the hydration shell of GC base pairs is more dense and less compressible (reflective of stronger solute–solvent interactions) compared with water in the hydration shell of AT base pairs. Clearly, caution must be exercised when proclaiming a solute more or less hydrated, since this property may depend on the observable used to define it.

Hydration of the Homopolymeric Duplex Poly(dA)poly(dT) versus the Alternating Copolymer Duplex Poly(dA–dT)poly(dA–dT). At 25 °C, we find no difference between the values of ϕV and ϕK_S for the poly(dA)poly(dT) and poly(dA–dT)poly(dA–dT) duplexes. It is tempting to interpret this result as suggesting similar overall hydrations for these two all-AT polymer duplexes, at least in so far as the density and compressibility of the primary hydration shells are concerned. However, such a conclusion is predicated on the assumption that the accessible surface area and, consequently, the number of solvating water molecules are the same for the poly(dA)poly(dT) and poly(dA–dT)poly(dA–dT) duplexes. This assumption may not be correct since the homopolymeric duplex is believed to adopt a non-B-form conformation (Pilet et al., 1975; Leslie et al., 1980; Chuprina, 1985) with a spine of hydration in the minor groove (Drew & Dickerson, 1981; Kopka et al., 1983), a feature that has been proposed to be one of the factors that stabilize its noncanonical structure (Alexeev et al., 1987). In fact, according to Alexeev et al. (1987), the minor groove of the poly(dA)poly(dT) duplex is narrowed by 2.8 Å compared to a classic B-form duplex.

These features may, in the aggregate, result in a lower total accessible surface area for the noncanonical homopolymeric duplex, thereby resulting in a lower hydration number, n_h, for the poly(dA)poly(dT) duplex relative to classic B-form DNA duplexes. According to eqs 3 and 4, such a reduction in n_h would result in a lower volume (higher density) and lower compressibility for water in the hydration shell of the poly(dA)poly(dT) duplex compared with the poly(dA–dT)poly(dA–dT) duplex and compared with the values listed in Table 1. In such a case, the conventional picture that the homopolymer is more hydrated than the alternating copolymer (Pilet et al., 1975; Leslie et al., 1980; Chuprina, 1985; Breslauer et al., 1987; Buckin et al., 1989a,b; Marky & Kupke, 1989; Marky & Macgregor, 1990) would reflect stronger DNA–solvent interactions in the hydration shell (the quality of hydration), despite the involvement of fewer water molecules, n_h (the quantity of hydration). In discussing such potential interpretations of the data, one also must consider the possibility that the ordered water molecules detected crystallographically in the minor groove of AT tracts (Drew & Dickerson, 1981; Kopka et al., 1983) may be "invisible" to our acoustodensimetric measurements. Another consideration is that the non-B conformation of the poly(dA)poly(dT) homopolymeric duplex can be disrupted thermally to produce a more B-like conformation at temperatures near the 25 °C value used in this work (Chan et al., 1990). Thus, the temperature dependence of the observables reported here also needs to be evaluated. Clearly, additional studies are required to distinguish between these multiple interpretations of the data in terms of the hydration properties of the homopolymeric poly(dA)poly(dT) duplex and the alternating copolymeric poly(dA–dT)poly(dA–dT) duplex.

Hydration and DNA Structure. Inspection of the data in Table 1 reveals that poly(dG–dC)poly(dG–dC) has an apparent molar volume, ϕV, that is 17 ± 3 cm^3 mol^{-1} lower than the ϕV for poly(dA–dT)poly(dA–dT). If we assume similar global B-form conformations for these two duplexes, it may be possible to rationalize this result in terms of the structural differences between GC and AT base pairs using the following logic. In addition to intrinsic volume differences

(the intrinsic volume of a GC base pair is about 3 cm^3 mol^{-1} lower than that of an AT base pair), differential contributions to the partial molar volume can arise from the chemical nature of the base-pair functional groups exposed to solvent. Specifically, the polar N-2 amino group of guanine may decrease the partial molar volume of a DNA molecule with G residues (compared to the corresponding DNA molecule without G residues) by forming additional hydrogen bonds with water molecules. Such a proposition is consistent with the results of studies on low molecular weight compounds, which reveal that the interaction between an amino group and water molecules can cause a volume reduction as large as 7 cm^3 mol^{-1} (Kharakoz, 1992).

The remaining difference between the apparent molar volumes of the poly(dG–dC)poly(dG–dC) and the poly(dA–dT)poly(dA–dT) duplexes may be due to the influence of the hydrophobic CH_3 group of thymine on the water molecules in the major groove of the poly(dA–dT)poly(dA–dT) duplex. In the major groove, which is lined with polar functional groups, water molecules are exposed to strong electrostatic fields and, as a result, should manifest low partial molar volumes, as well as low partial molar adiabatic compressibilities compared with bulk water (similar to the hydration shells of other charged molecules or atomic groups). The presence of the thymine CH_3 group in the major groove of the poly(dA–dT)poly(dA–dT) duplex may replace some of the electrostatic solute–solvent interactions by hydrophobic interactions with the methyl group, thereby resulting in increases in both the apparent molar volume and the apparent molar adiabatic compressibility of a DNA duplex with T residues. Thus, the difference we measure for the ϕV of the poly(dG–dC)poly(dG–dC) and poly(dA–dT)poly(dA–dT) duplexes can, in large part, be rationalized in terms of differences in the solvent accessibility of the functional groups associated with these two duplexes. It is gratifying to note that this microscopic interpretation of our macroscopic data is consistent with the DNA hydration patterns discerned from crystallographic data by Schneider et al. (1992). Specifically, these authors report that, in the major groove of B-form DNA duplexes, the polar N4 atoms of cytosines are more hydrated than the polar O4 atoms of thymines. They propose that this differential hydration results from the influence of the thymine methyl group, which serves to partly shield the O4 atom of thymine, thereby reducing its solvent accessibility and, hence, its hydration relative to the unshielded N4 atoms of cytosines.

The data in Table 1 also reveal that the apparent molar adiabatic compressibility, ϕK_S, of the poly(dG–dC)poly(dG–dC) duplex is $(15 \pm 4) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} lower than that of the poly(dA–dT)poly(dA–dT) duplex. As with the ϕV data, this difference may be ascribed to the interaction with solvent of the N-2 amino group of guanine and the CH_3 group of thymine. The contribution of the polar amino group to the apparent molar compressibility of a solute can be as much as -6×10^{-4} cm^3 mol^{-1} bar^{-1} (Kharakoz, 1991). The positive influence of the CH_3 group is more difficult to estimate. However, in peptide studies, the influence of a methyl side chain on the hydration of a positively charged amino terminus is found to increase the partial molar compressibility by 6×10^{-4} cm^3 mol^{-1} bar^{-1} (unpublished data from our laboratory). Taken together, these effects may help to explain the difference in ϕK_S that we measure for the poly(dG–dC)poly(dG–dC) duplex versus the poly(dA–dT)poly(dA–dT) duplex. It should be emphasized, however, that the usefulness of our macroscopic data does not depend on the veracity of our microscopic interpretations, which are presented here only as a basis for further discussions.

CONCLUDING REMARKS

We have evaluated the partial molar volumes and the partial molar adiabatic compressibilities of five natural and three synthetic DNA duplexes at 25 °C using high-precision density and sound velocity measurements. We have interpreted the resulting data in terms of DNA hydration and have estimated the density and the coefficient of adiabatic compressibility of water in the DNA hydration shell. We have also proposed that the number of water molecules that are under the influence of the atomic groups of DNA is limited mostly to the first hydration layer and is approximately the same for all B-form DNA. We found that the physical characteristics of water in the DNA hydration shell and, consequently, the type and strength of the solute–solvent interactions depend strongly on the base content and secondarily on the base sequence. Analysis of our data revealed that water in the hydration shell of B-form DNA duplexes exhibits increased density and decreased compressibility relative to bulk water. More quantitatively, we found a linear relationship between the density, ρ_h, and the coefficient of adiabatic compressibility, β_{Sh}, of water in the hydration shell of a B-form DNA duplex. On the basis of these results, we estimated the intrinsic density of water in the hydration shell of a B-form DNA duplex to be in the range of 1.17–1.26 g cm^{-3} (after correcting for the void space volume) and the corresponding coefficient of adiabatic compressibility to be between 30×10^{-6} and 35×10^{-6} bar^{-1}, a range that is 65–80% of the value for bulk water.

We found that, in general, duplexes with lower ϕV and ϕK_S values exhibit greater hydration. We also found that DNA with a base composition of 55–60% AT base pairs exhibited the weakest hydration. Increases and/or decreases in AT content from this value resulted in enhanced DNA hydration. We did not detect any difference between the apparent molar volumes, ϕV, or apparent molar adiabatic compressibilities, ϕK_S, of the homopolymeric duplex poly(dA)poly(dT) and the alternately copolymer duplex poly(dA–dT)poly(dA–dT). If one assumes that the two duplexes have the same n_h values, this similarity in ϕV and ϕK_S may be interpreted as reflecting equivalent qualities of hydration for these two all-AT polymeric duplexes. Alternatively, if under the conditions of our study the poly(dA)poly(dT) duplex assumed a noncanonical conformation with a narrow minor groove and a distorted helical axis, this might cause the number of water molecules in its hydration shell, n_h, to be lower than that in the B-form poly(dA–dT)poly(dA–dT) duplex. Such a reduction in n_h would result in a lower volume (higher density) and lower compressibility for water in the hydration shell of the poly(dA)poly(dT) duplex compared with the poly(dA–dT)poly(dA–dT) duplex. Thus, the conventional picture that the homopolymer is more hydrated may reflect stronger DNA–solvent interactions (the quality of hydration) rather than more waters of hydration, n_h (the quantity of hydration).

Finally, we found that water in the hydration shell of GC base pairs is more dense and less compressible than water in the hydration shell of AT base pairs. On the basis of our operational definition of hydration, these properties suggest that GC base pairs are more strongly hydrated than AT base pairs. This conclusion may reflect solvent interactions with the base functional groups. Specifically, the polar N-2 amino group of guanine in the minor groove of the GC base pair may enhance hydration by forming additional hydrogen bonds with water molecules, while the nonpolar CH_3 group of thymine

in the major groove of AT base pairs may, via its hydrophobic character, reduce hydration by disrupting some of the strong electrostatic DNA–water interactions in the major groove. Independent of the veracity of such molecular interpretations, the ϕV and ϕK_S data reported here provide important new volumetric data on DNA duplexes, which ultimately are required to understand the central role that hydration plays in modulating DNA properties.

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