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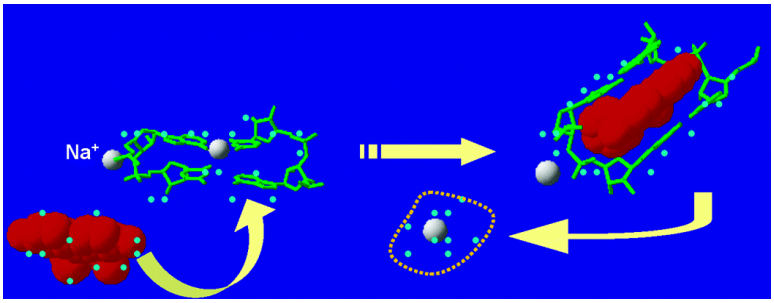

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Hydration Changes Accompanying Nucleic Acid Intercalation Reactions:Volumetric Characterizations

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Abstract: We use high precision ultrasonic velocimetric and densimetric techniques to determine at 25 °C the changes in volume, ΔV, and adiabatic compressibility, ΔK_S, that accompany the binding of ethidium to the poly(rA)poly(rU), poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC) duplexes, as well as to the poly(rU)poly(rA)poly(rU) triplex. The binding of ethidium to each of the duplexes and the triplex is accompanied by negative changes in volume, ΔV, and adiabatic compressibility, ΔK_S. We discuss the basis for relating macroscopic and microscopic properties, particularly, emphasizing how measured changes in volume and compressibility can be quantitatively interpreted in terms of the differential hydration properties of DNA and RNA structures in their ligand-free and ligand-bound states. We also estimate the entropic cost of intercalation-induced changes in hydration of each of the nucleic acid structures and the drug. In general, our results emphasize the vital role of hydration in modulating the energetics of drug−DNA binding, while also underscoring the fact that hydration must be carefully taken into account in analysis and prediction of the energetics of nucleic acid recognition.

Introduction

Intercalators represent a major class of DNA-binding agents. A typical intercalator is a planar aromatic molecule comprising at least two heteroatomic rings. The binding mode of an intercalating agent is based on intercalation in which the planar aromatic rings of the drug are inserted between adjacent base pairs of the DNA or RNA helix.[1−2] The intercalator-DNA complex is stabilized by $\pi-\pi$ stacking interactions between the drug molecule and the contacting base pairs. Intercalation induces substantial local rearrangements and major distortion of DNA helix. Specifically, as intercalation requires separation of the two base pairs, the DNA helix somewhat unwinds and elongates by ∼1 base pair spacing (∼3.4 Å) per bound drug molecule. Intercalators are capable of binding to both double helices and higher order structures including triplexes and tetraplexes.[3−8] In this connection, it should be mentioned that some recently designed intercalating agents are capable of binding more strongly to triplexes and tetraplexes compared to their duplex analogues.[4−8] The latter capability is of potential biomedical significance since it can be used to selectively stabilize and even induce formation of higher order nucleic acid structures under physiological conditions.[9,10]

Thermodynamic investigations of drug−DNA recognition are complementary to structural studies and aimed at identifying and quantifying the energetic contributions of various intra- and intermolecular interactions which stabilize/destabilize drug−DNA complexes. The thermodynamics of drug−DNA interactions, including DNA intercalation reactions, has been extensively reviewed.[11−17] These studies have revealed that hydration plays a major role in determining the binding affinity and specificity of drug−DNA interactions. However, the exact role of water in ligand-biopolymer interactions in general, and drug−DNA recognition, in particular, is still poorly understood. This deficiency is unfortunate since it prevents one from resolving the contributions of different interactions, including hydration, to the net energetics of drug−DNA binding. From a practical point of view, an understanding of hydration is important for rational design of improved DNA-binding drugs targeted with predictable affinity and specificity to selected sequences of nucleic acid structures.

(1) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18−30.
(2) Lerman, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *49*, 94−102.
(3) Lehrman, E. A.; Crothers, D. M. *Nucleic Acid Res.* **1977**, *4*, 1381−1392.
(4) Scaria, P. V.; Shafer, R. H. *J. Biol. Chem.* **1991**, *266*, 5417−5423.
(5) Escude, C.; Nguyen, C. H.; Kukreti, S.; Janin, Y.; Sun, J.-S.; Bisagni, E.; Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3591−3596.
(6) Haq, I.; Trent, J. O.; Chowdhry, B. Z.; Jenkins, T. C. *J. Am. Chem. Sci.* **1999**, *121*, 1768−1779.
(7) Mergny, J.-L.; Lacroix, L.; Teulade-Fichou, M.-P.; Hounsou, C.; Guittat, L.; Hoarau, M.; Arimondo, P. B.; Vigneron, J.-P.; Lehn, J.-M.; Riou, J.-F.; Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3062−3067.
(8) Koeppel, F.; Riou, J.-F.; Laoui, A.; Mailliet, P.; Arimondo, P. B.; Labit, D.; Petitgenet, O.; Helene, C.; Mergny, J.-L. *Nucl. Acid. Res.* **2001**, *29*, 1087−1096.
(9) Mergny, J.-L.; Mailliet, P.; Lavelle, F.; Riou, J.-F.; Laoui, A.; Helene, C. *Anti-Cancer Drug Des.* **1999**, *14*, 327−339.
(10) Praseuth, D.; Guieysse, A. L.; Helene, C. *Biochim. Biophys. Acta* **1999**, *1489*, 181−206.
(11) Chaires, J. B. *Anti-Cancer Drug Des.* **1996**, *11*, 569−580.
(12) Chaires, J. B. *Biopolymers.* **1997**, *44*, 201−215.
(13) Lane, A. N.; Jenkins, T. C. *Q. Rev. Biophys.* **2000**, *33*, 255−306.
(14) Ren, J.; Jenkins, T. C.; Chaires, J. B. *Biochemistry* **2000**, *39*, 8439−8447.
(15) Haq, I.; Jenkins, T. C.; Chowdhry, B. Z.; Ren, J.; Chaires, J. B. *Methods Enzymol.* **2000**, *323*, 373−405.
(16) Haq, I.; Ladbury, J. *J. Mol. Recognit.* **2000**, *13*, 188−197.
(17) Haq, I. *Arch. Biochem. Biophys.* **2002**, *403*, 1−15.

Densimetric and acoustic techniques have proven useful in studying nucleic acid hydration.[18−22] Volumetric parameters of a solute, such as the partial molar volume and adiabatic compressibility, are sensitive to the amount of solute hydration (the total number of water molecules incorporated within the hydration shell of a solute). In addition, an important feature of the partial molar volume and adiabatic compressibility observables is that they can be used for discriminating between water molecules solvating charged, polar, and nonpolar atomic groups of a solute. In this work, we employ densimetric and ultrasonic velocimetric techniques to characterize changes in hydration associated with the binding of ethidium bromide (3,8-diamino 6-phenyl-5-ethylphenanthridinium), a prototypical intercalator, to various DNA and RNA structures. We report the changes in volume and adiabatic compressibility that accompany ethidium association with the poly(rA)poly(rU), poly(dAdT)-poly(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC) duplexes as well as the poly(rU)poly(rA)poly(rU) triplex. The major goal of this work is to characterize the differential hydration properties of each DNA and RNA structure in their ligand-free and ligand-bound states. On the basis of our volumetric data, we find that the binding of ethidium to DNA or RNA may cause either release or uptake of water molecules from the hydration shell of the host structure depending on its structure and composition. We discuss the impact of hydration on the energetics of drug−DNA binding events.

Results

To confirm independently that ethidium bromide, in fact, binds to the host duplexes and the poly(rU)poly(rA)poly(rU) triplex under the conditions of our study, we measured CD spectra of each DNA and RNA host structure in the absence and presence of the intercalator at various drug-to-DNA binding ratios and observed the induced signal at ~304 nm characteristic of bound ethidium. Figure 1, parts a and b, shows two representative binding profiles for the complexation of ethidium with the poly(rA)poly(rU) (panel A) and poly(dAdT)poly(dAdT) (panel B) host duplexes at 20 mM NaCl.

Figures 2 depicts changes in the relative molar sound velocity increment, Δ[U] (panel A), volume, ΔV (panel B), and adiabatic compressibility, ΔK_S (panel C), for the poly(rA)poly(rU) RNA duplex in the absence and presence of ethidium bromide at various drug-to-RNA binding ratios, r (where $r = [EB]/[RNA]$), at 20 mM NaCl. We have measured similar dependences for poly(rA)poly(rU) at 80 and 300 mM NaCl (are not shown). Figures 3, 4, and 5 show changes in the relative molar sound velocity increment, Δ[U] (panels A), volume, ΔV (panels B), and adiabatic compressibility, ΔK_S (panels C), for the poly-(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)-poly(dIdC) duplexes, respectively, in the absence and presence of ethidium bromide at various drug-to-DNA binding ratios, r, at 20 mM NaCl. Figures 6 presents the r dependences of the relative molar sound velocity increment, Δ[U] (panel A), volume, ΔV (panel B), and adiabatic compressibility, ΔK_S (panel C), for the poly(rU)poly(rA)poly(rU) RNA triplex at 300 mM NaCl.

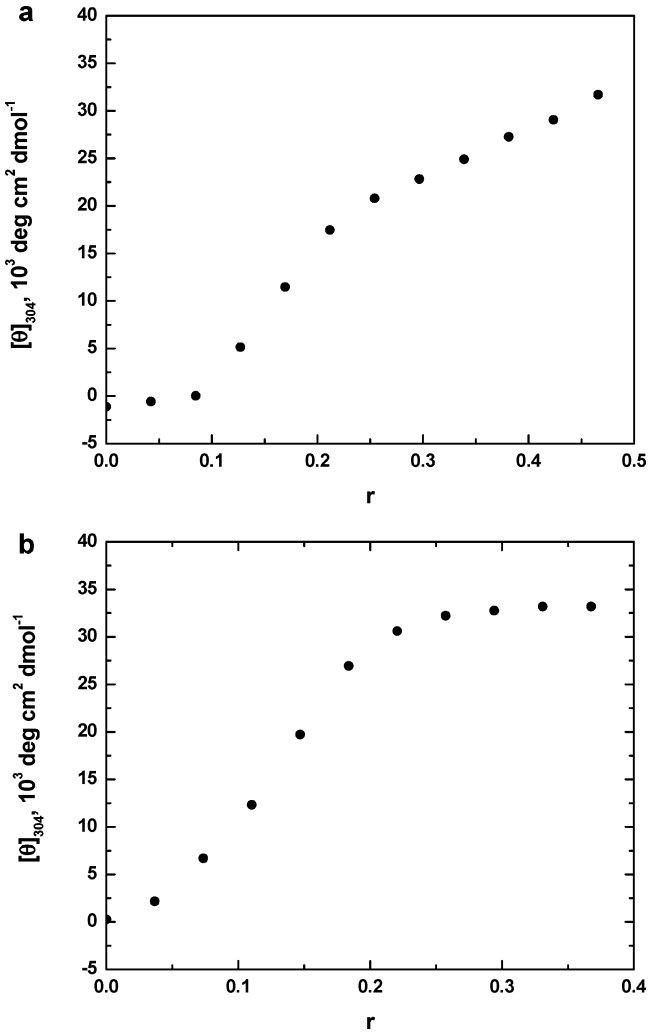

Figure 1. Molar ellipticity (per mole of nucleotides) of the solution containing the poly(rA)poly(rU) (panel A) and poly(dAdT)poly(dAdT) (panel B) duplexes in the absence and presence of ethidium bromide versus the ethidium-to-nucleotide ratio, r, at 20 mM NaCl.

One point of concern in interpreting our volumetric data is how would stacking of ethidium in solution affect our results. In this connection, it should be noted that, since we perform differential solution-versus-solvent acoustic and densimetric measurements, only the differential stacking of the drug in the DNA solution and pure buffer would affect our data. This notion may be of importance since the concentration of free drug in the DNA solution is smaller than that in the pure buffer. To clarify this point, we have carried out concentration-dependent measurements of the partial molar volume, $V°$, and adiabatic compressibility, $K_S°$, of ethidium bromide (not shown). Our data reveal that, within the range of our experimental concentrations (0.5 to 3 mg/mL), the values of $V°$ and $K_S°$ are very slightly dependent on concentration. Consequently, we conclude that ethidium stacking does not strongly influence our results.

Discussion

Primary and Secondary Binding of Ethidium to Nucleic Acid Structures. Inspection of Figure 1a reveals two distinctive features of ethidium association with poly(rA)poly(rU). First, the sigmoidal shape of the binding profile is suggestive of cooperative binding, an observation consistent with previous

(18) Sarvazyan, A. P. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 321−342.
(19) Chalikian, T. V.; Sarvazyan, A. P.; Breslauer, K. J. *Biophys. Chem.* **1994**, *51*, 89−109.
(20) Chalikian, T. V.; Breslauer, K. J. *Biopolymers* **1998**, *48*, 264−280.
(21) Chalikian T. V. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 207−235.
(22) Buckin, V. A. *Mol. Biol.* **1987**, *21*, 512−525.

reports.[17,23−27] For example, Bresloff and Crothers[23] found that the binding of ethidium bromide to poly(rA)poly(rU), poly(dAdT)poly(dAdT), and poly(dIdC)poly(dIdC) is cooperative with the cooperativity parameter ranging between 1.9 and 2.3. The structural basis for the observed cooperativity is not very well understood. In one model, the cooperativity of drug−DNA binding is assumed to originate from a drug-induced allosteric transition of DNA between two conformers of which one has a stronger affinity for the drug.[17,24,25] Although this model was originally put forward to account for the cooperativity of complexation of minor groove binders with DNA, its main concepts can probably be extended to intercalation reactions.[24,25]

Another observation is that, after the primary binding of ethidium to poly(rA)poly(rU) (roughly at r of ∼0.25, which corresponds to one drug per two base pairs), secondary binding takes place as suggested by a linear increase in the post-binding baseline. This observation, which is consistent with previous reports, appears to reflect the electrostatic binding of the cationic drug to negatively charged phosphate groups.[28−30] Secondary binding was observed also for the poly(rA)poly(rU) duplex at 80 and 300 mM NaCl and for the poly(rU)poly(rA)poly(rU) triplex at 300 mM NaCl.

Inspection of Figure 1b reveals that, analogous to poly(rA)poly(rU), the primary binding of ethidium to poly(dAdT)poly(dAdT) is cooperative. In fact, cooperative binding was observed for all the duplexes and the triplex studied in this work. Further inspection of Figure 1b reveals that, in contrast to poly(rA)poly(rU), there is no significant secondary binding of ethidium to the poly(dAdT)poly(dAdT) duplex as can be judged by the horizontal post-binding baseline. Secondary binding was not observed for the poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) DNA duplexes either.

In the aggregate, our CD data are suggestive of cooperative binding of ethidium to all the DNA or RNA structures investigated in this study. Our CD data further suggest that ethidium binds to secondary binding sites of the poly(rA)poly(rU) duplex and poly(rU)poly(rA)poly(rU) triplex but not the poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC) DNA duplexes.

Changes in Volume and Adiabatic Compressibility Accompanying the Primary Binding. Inspection of Figures 2 and 6 reveals that ethidium association with the poly(rA)poly(rU) host duplex and the poly(rU)poly(rA)poly(rU) host triplex causes initial decreases in volume (panels B) and adiabatic compressibility (panels C) followed by subsequent increases in both observables. To account for this observation, recall that ethidium association with the two RNA structures occurs through the binding of the drug to the primary and secondary sites. In line with this notion, we propose that the primary binding of the drug to poly(rA)poly(rU) and poly(rU)poly(rA)poly(rU) is accompanied by decreases in volume, V, and compressibility, K_S. By contrast, the secondary binding is accompanied by increases in V and K_S. Hence, as a reasonable approximation, we propose that the minima of the r dependences of volume (panels B of Figures 2 and 6) and compressibility (panels C of Figures 2 and 6) roughly correspond to the volume, ΔV, and compressibility, ΔK_S, changes (normalized per mole of nucle-

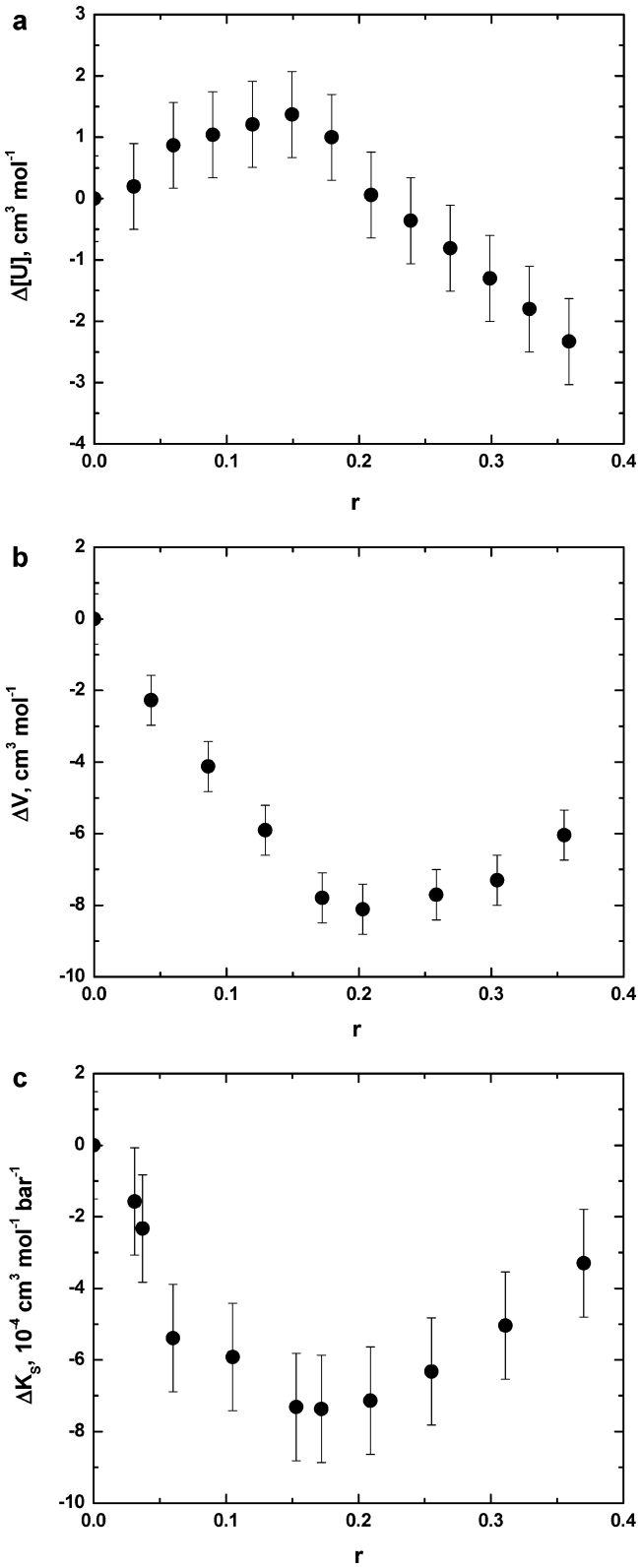

Figure 2. Changes in the relative molar sound velocity increment, Δ[U] (panel A), volume, ΔV (panel B), and adiabatic compressibility, ΔK_S (panel C), of the poly(rA)poly(rU) duplex versus the ethidium-to-RNA ratio, r, at 20 mM NaCl.

(23) Bresloff, J. L.; Crothers, D. M. *Biochemistry* **1981**, *20*, 3547−3553.
(24) Hogan, M.; Dattagupta, N.; Crothers, D. M. *Nature* **1979**, *278*, 521−524.
(25) Dattagupta, N.; Hogan, M.; Crothers, D. M. *Biochemistry* **1980**, *19*, 5998−6005.
(26) Dattagupta, N.; Hogan, M.; Crothers, D. M. *Biochemistry* **1981**, *20*, 1438−1445.
(27) Wilson, W. D. *Prog. Drug Res.* **1987**, *31*, 193−221.
(28) Waring, M. J. *J. Mol. Biol.* **1965**, *13*, 269−282.
(29) Le Pecq, J.-B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87−106.
(30) Le Pecq, J.-B. *Methods Biochem. Anal.* **1971**, *20*, 41−86.

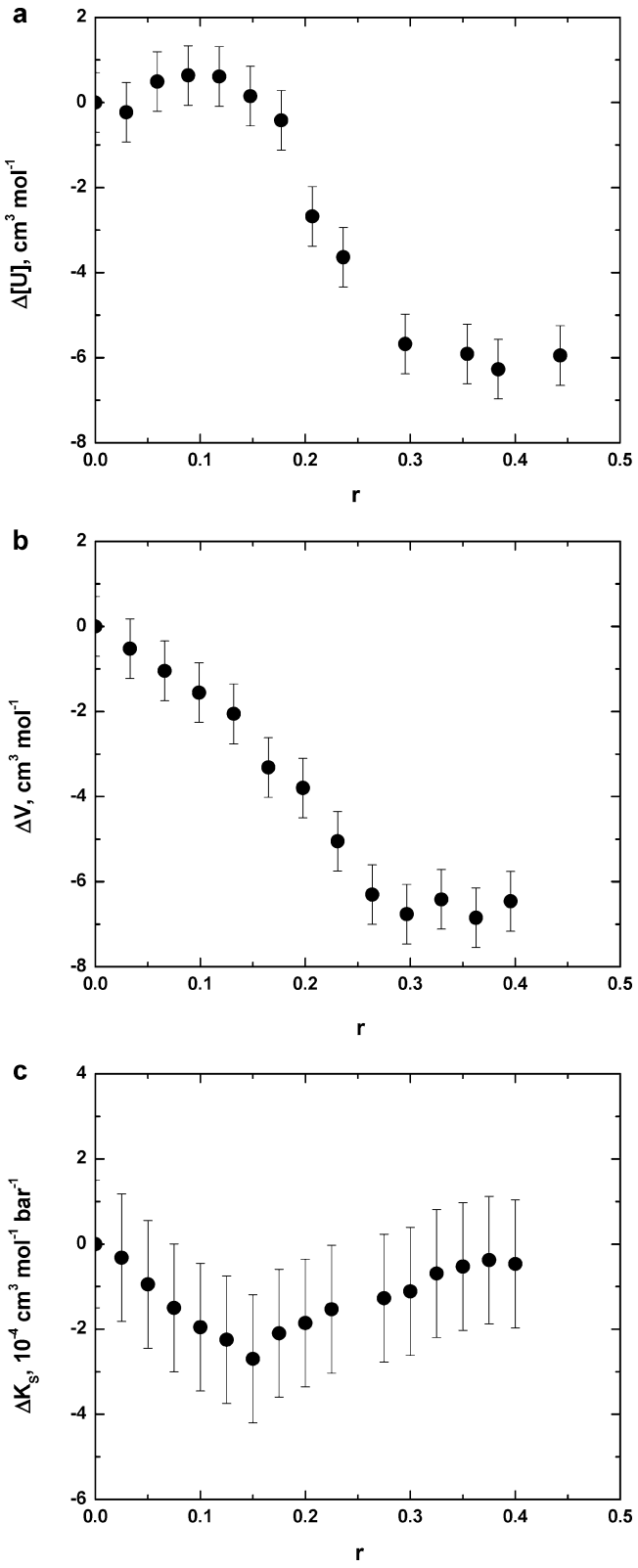

Figure 3. Changes in the relative molar sound velocity increment, Δ[U] (panel A), volume, Δ*V* (panel B), and adiabatic compressibility, Δ*K*S (panel C), of the poly(dAdT)poly(dAdT) duplex versus the ethidium-to-DNA ratio, *r*, at 20 mM NaCl.

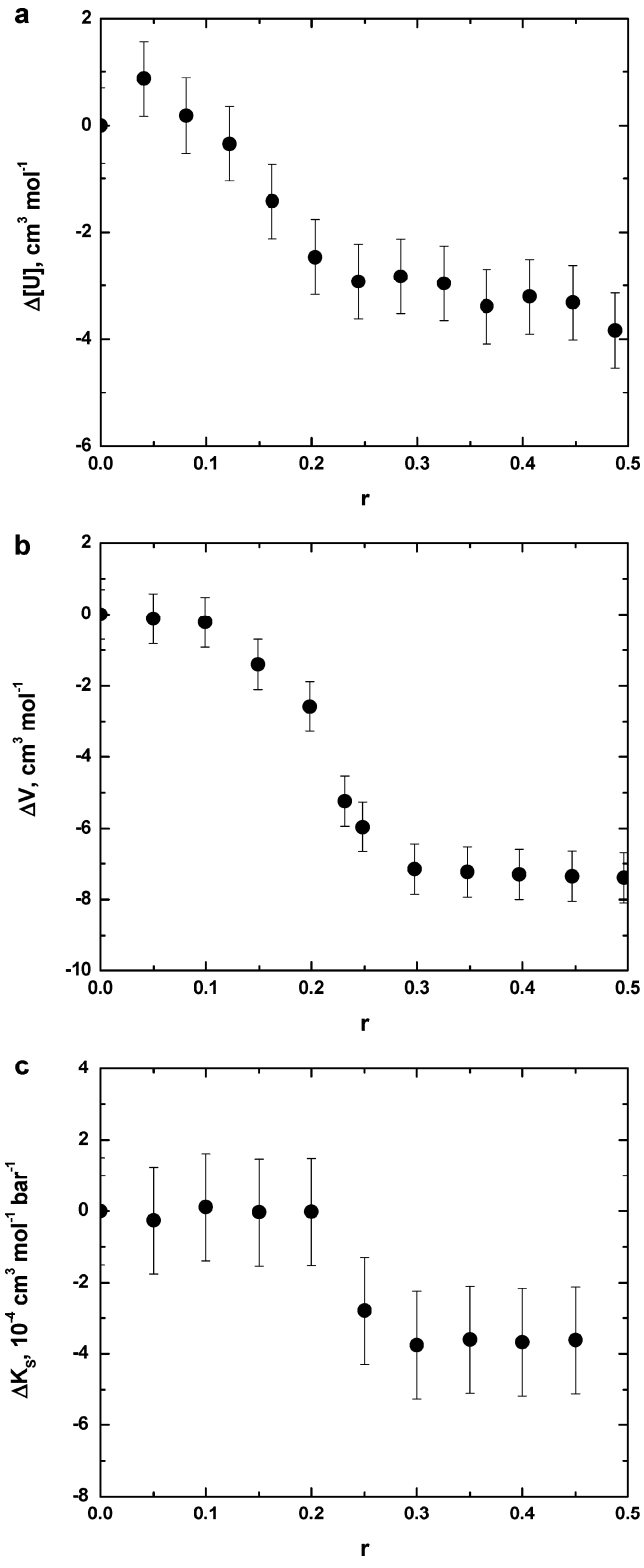

Figure 4. Changes in the relative molar sound velocity increment, Δ[U] (panel A), volume, Δ*V* (panel B), and adiabatic compressibility, Δ*K*S (panel C), of the poly(dGdC)poly(dGdC) duplex versus the ethidium-to-DNA ratio, *r*, at 20 mM NaCl.

otide) associated with saturation by ethidium of the primary binding sites of the two RNA host structures.

Inspection of Figures 3, 4, and 5 reveals that, in contrast to the RNA duplex, the binding of ethidium to the poly(dAdT)-poly(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC) DNA duplexes does not bring about any appreciable increases in either volume (panels B) or adiabatic compressibility (panels C) following the initial decreases in these variables. This observation is consistent with our CD spectroscopic data that

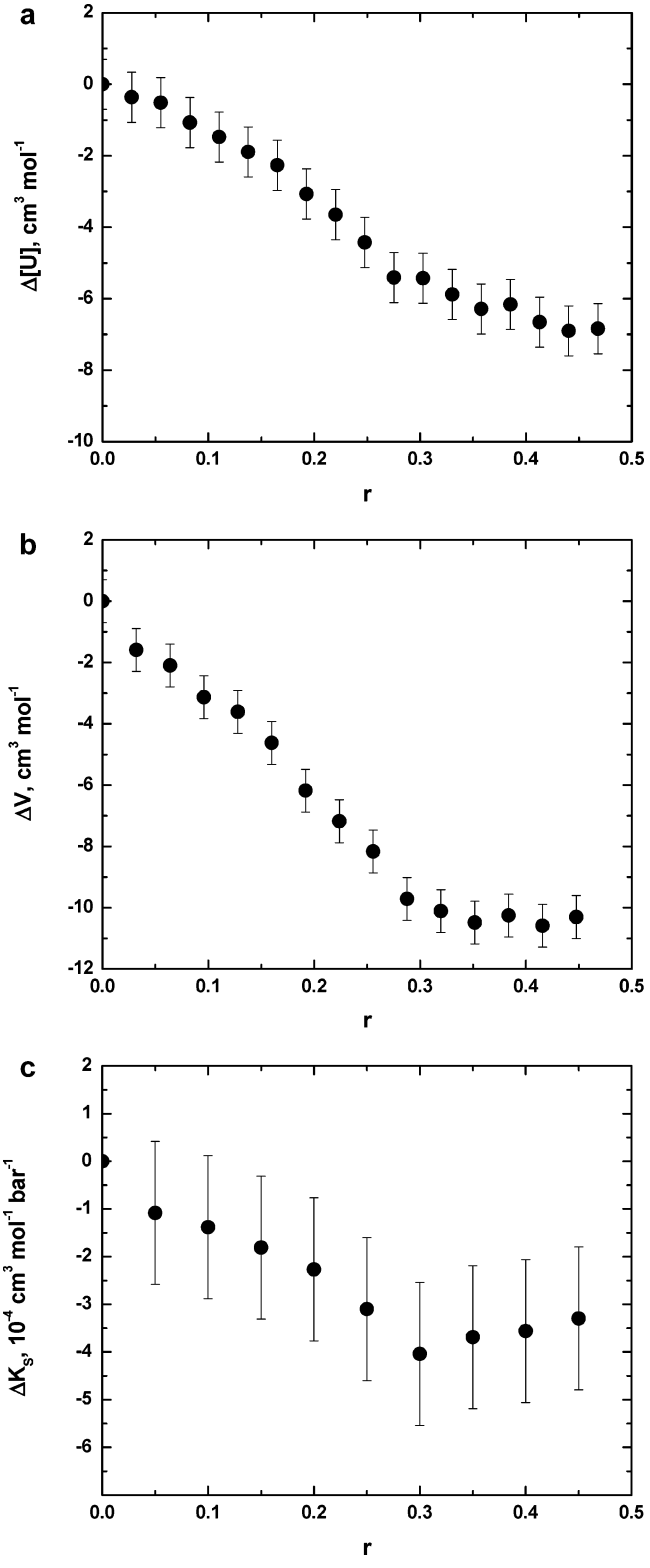

Figure 5. Changes in the relative molar sound velocity increment, Δ[U] (panel A), volume, Δ*V* (panel B), and adiabatic compressibility, ΔK_S (panel C), of the poly(dIdC)poly(dIdC) duplex versus the ethidium-to-DNA ratio, *r*, at 20 mM NaCl.

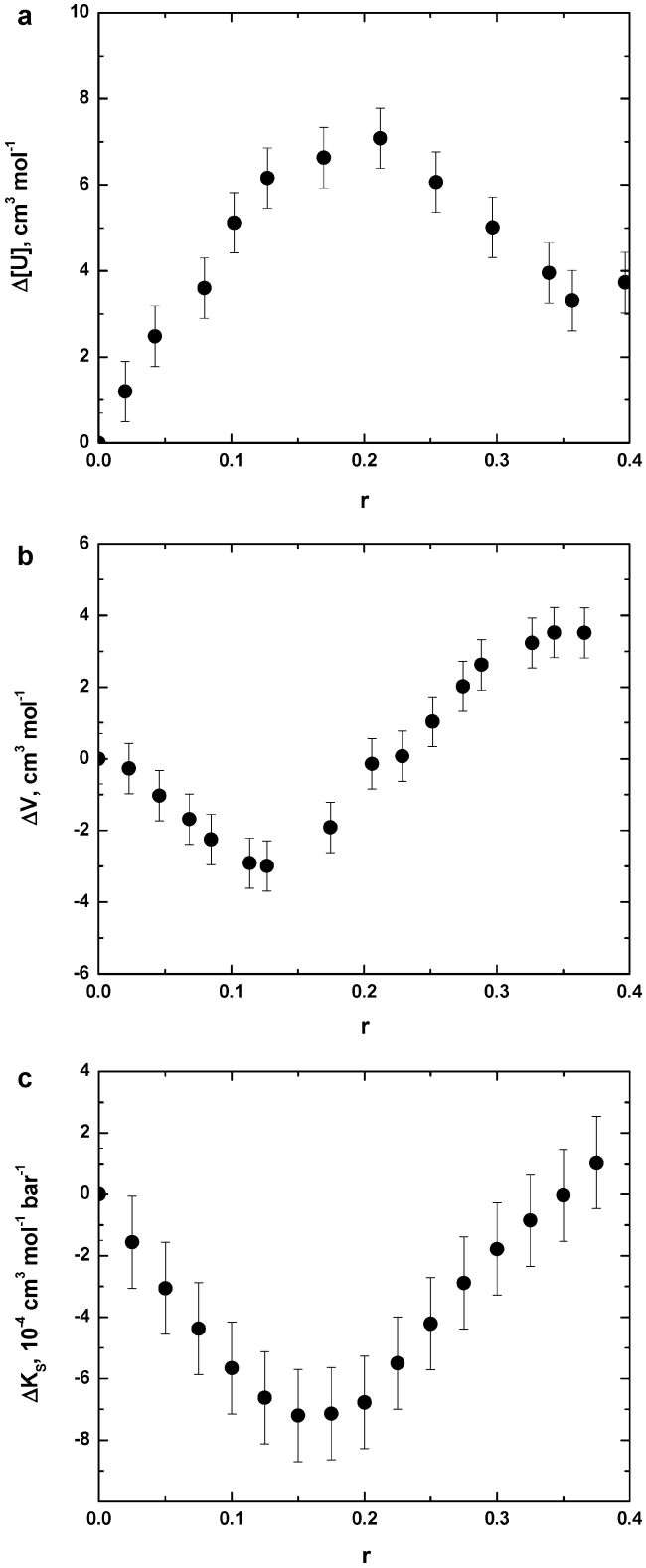

Figure 6. Changes in the relative molar sound velocity increment, Δ[U] (panel A), volume, Δ*V* (panel B), and adiabatic compressibility, ΔK_S (panel C), of the poly(rU)poly(rA)poly(rU) triplex versus the ethidium-to-RNA ratio, *r*, at 300 mM NaCl.

reveal no secondary binding of the intercalator to the three DNA duplexes. Thus, the values of Δ*V* (panels B) and ΔK_S (panels C) corresponding to the plateaus in Figures 3, 4, and 5 represent the changes in volume, Δ*V*, and adiabatic compressibility, ΔK_S, associated with saturation by ethidium of the primary binding sites of the poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC) duplexes (normalized per mole of nucleotide).

Ionic Strength Dependence of Δ*V* and ΔK_S. Figures 7a and b show the salt dependences of the changes in volume, Δ*V*

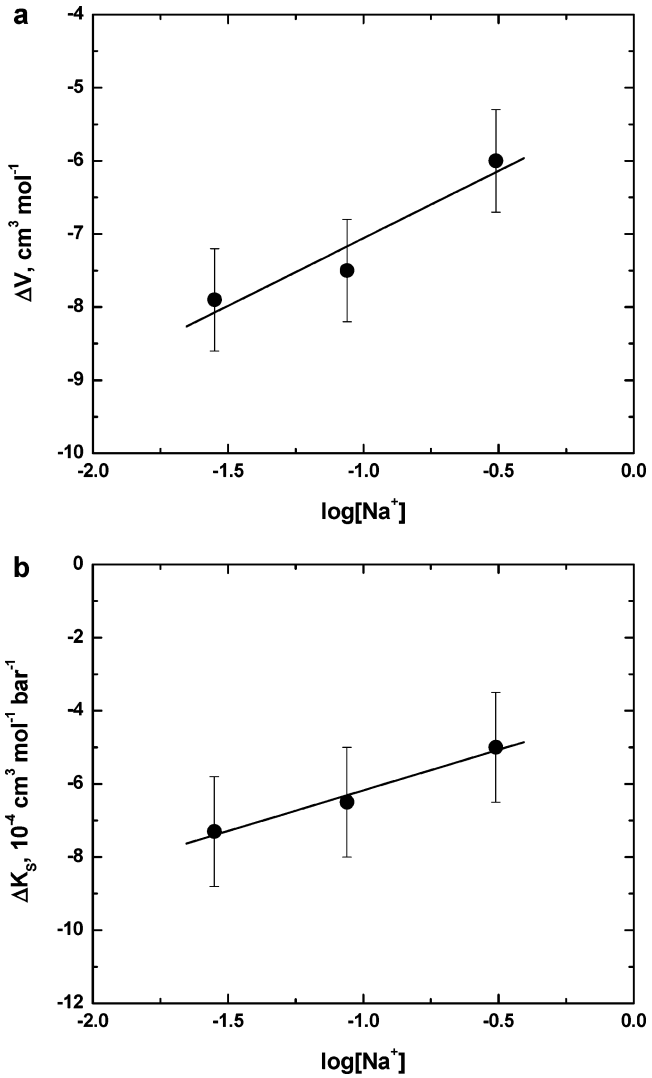

Figure 7. Changes in volume, ΔV (panel A), and adiabatic compressibility, ΔK_S (panel B), accompanying ethidium binding to the poly(rA)poly(rU) duplex versus Na^+ concentration.

(panel A), and adiabatic compressibility, ΔK_S (panel B), accompanying the binding of ethidium to the poly(rA)poly(rU) host duplex. Inspection of Figure 7 reveals that an increase in Na^+ concentration from 28 to 308 mM causes only slight increases in ΔV and ΔK_S. Specifically, ΔV increases from -7.9 ± 0.7 to -5.8 ± 0.7 cm^3 mol^{-1}, whereas ΔK_S increases from $-(7.3 \pm 1.5) \times 10^{-4}$ to $-(5.1 \pm 1.5) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. These changes are practically within experimental uncertainty of ΔV and ΔK_S determination. By extension, it is reasonable to assume that, in general, the effect of salt on the volumetric properties of ethidium association with nucleic acid structures is not strong. Consequently, we have limited our explorations to a single NaCl concentration of 20 mM (which, in 10 mM pH 6.7 cacodylic buffer, corresponds to Na^+ concentration of 28 mM).

Values of ΔV and ΔK_S Depend on the Host DNA or RNA. Table 1 presents the changes in volume, ΔV, and compressibility, ΔK_S, accompanying the binding of ethidium to each of the nucleic acid structures studied. Inspection of data in Table 1 reveals that, for all the intercalation reactions studied in this work, the changes in volume, ΔV, and adiabatic compressibility, ΔK_S, are negative, an observation consistent

Table 1. Changes in Volume, ΔV, and Adiabatic Compressibility, ΔK_S, Accompanying Ethidium Binding to the Nucleic Acid Structures

polynucleotide	ΔV (cm^3 mol^{-1})	ΔK_S 10^{-4} cm^3 mol^{-1} bar^{-1}
poly(rA)poly(rU)	-7.9 ± 0.7	-7.3 ± 1.5
poly(dAdT)poly(dAdT)	-6.8 ± 0.7	-1.0 ± 1.5
poly(dGdC)poly(dGdC)	-7.4 ± 0.7	-3.9 ± 1.5
poly(dIdC)poly(dIdC)	-10.3 ± 0.7	-3.6 ± 1.5
poly(rU)poly(rA)poly(rU)	-2.5 ± 0.7	-7.3 ± 1.5

with previous reports.[31−34] Further inspection of our data presented in Table 1 reveals that disparities exist between the values of ΔV and ΔK_S for different nucleic acid structures. For example, for the poly(dAdT)poly(dAdT) and poly(dIdC)-poly(dIdC) host duplexes, the values of ΔV are equal to -6.8 ± 0.7 and -10.3 ± 0.7 cm^3 mol^{-1}, respectively, while the values of ΔK_S are $-(1.0 \pm 1.5) \times 10^{-4}$ and $-(3.6 \pm 1.5) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. We propose that the observed disparities reflect individual binding-induced changes in hydration of the host structures. In the sections that follow, we describe how our measured changes in volumetric properties can be rationalized in terms of hydration.

Differential Volumetric Properties of Drug−DNA Complexes and Host Nucleic Acid Structures. According to polyelectrolyte theory, sodium ions are condensed in the vicinity of polyanionic DNA or RNA structures in proportions depending on their charge densities.[35,36] For example, for B-DNA duplex, 0.88 Na^+ ions are associated with each DNA phospahte.[11,36] When a cationic drug binds to DNA, it induces counterion release as reflected in the equilibrium

$$\text{drug} + \text{DNA} \leftrightarrows \text{complex} + n_M Na^+ \qquad (1)$$

where n_M is the number of counterions released to the bulk per molecule of bound drug.

For a monocationic drug, such as ethidium, theoretical calculations predict a value of n_M in eq 1 close to unity (within ±20%) (for review, see ref 11). Hence, for ethidium bromide binding, reaction 1 can be presented as follows (if expressed per mole of binding site)

$$E^+ + Br^- + \text{DNA} \leftrightarrows \text{EDNA} + Na^+ + Br^- \qquad (2)$$

where EDNA denotes the ethidium-DNA complex.

On the basis of reaction 2, the changes in volume, ΔV, and compressibility, ΔK_S, accompanying ethidium complexation with nucleic acids can be presented as follows

$$\Delta V = [V°(\text{EDNA}) - V°(\text{DNA}) + V°(\text{NaBr}) - V°(\text{EB})]/r_b \qquad (3)$$

$$\Delta K_S = [K_S°(\text{EDNA}) - K_S°(\text{DNA}) + K_S°(\text{NaBr}) - K_S°(\text{EB})]/r_b \qquad (4)$$

(31) Macgregor, R. B.; Clegg, R. M.; Jovin, T. M. *Biochemistry* **1985**, *24*, 5503−5510.
(32) Marky, L. A.; Macgregor, R. B. *Biochemistry* **1990**, *29*, 4805−4811.
(33) Kudryashov, E. D.; Buckin, V. A.; Braginskaya, F. I.; Marky, L. A. *Biophysics* **1998**, *43*, 32−36.
(34) Marky, L. A.; Kupke, D. W.; Kankia, B. I. *Methods Enzymol.* **2001**, *340*, 149−165.
(35) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179−246.
(36) Record, M. T.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *11*, 103−178.

Table 2. Differential Volumes and Adiabatic Compressibilities of the Ethidium Complexes and the Drug-Free Host Nucleic Acids

polynucleotide	$V°$(EDNA) − $V°$(DNA), cm^3 mol^{-1}	$K_S°$(EDNA) − $K_S°$(DNA), 10^{-4} cm^3 mol^{-1} bar^{-1}
poly(rA)poly(rU)	212 ± 3	−14 ± 6
poly(dAdT)poly(dAdT)	216 ± 3	11 ± 6
poly(dGdC)poly(dGdC)	214 ± 3	−1 ± 6
poly(dIdC)poly(dIdC)	202 ± 3	−1 ± 6
poly(rU)poly(rA)poly(rU)	229 ± 3	−29 ± 9

where $V°$(DNA), $V°$(EDNA), $V°$(NaBr), and $V°$(EB) are the partial molar volumes of the host nucleic acid (normalized per binding site), the ethidium-nucleic acid complex (normalized per binding site), NaBr, and ethidium bromide, respectively; $K_S°$(DNA), $K_S°$(EDNA), $K_S°$(NaBr), and $K_S°$(EB) are the partial molar adiabatic compressibilities of the host nucleic acid (normalized per binding site), the ethidium-nucleic acid complex (normalized per binding site), NaBr, and ethidium bromide, respectively; and r_b is the binding density. For ethidium association with duplexes and triplexes, the binding density, r_b, roughly corresponds to four (two base pairs) and six nucleotides (two base triplets) per bound drug, respectively.[4,29,30] Hence, for duplexes and triplexes, the binding site includes four and six nucleotides, respectively.

At 25 °C, the values of $V°$(NaBr) and $K_S°$(NaBr) are 23.5 cm^3 mol^{-1} and -43×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.[37] Our measured values of $V°$(EB) and $K_S°$(EB) are 267.1 cm^3 mol^{-1} and -28×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively. With these values, we now use eqs 3 and 4 in conjunction with ΔV and ΔK_S (see Table 1) to calculate the differential volumes [$V°$(EDNA) − $V°$(DNA)] and compressibilities [$K_S°$(EDNA) − $K_S°$(DNA)] for each drug−DNA complex and its host structure. Results of these calculations are presented in Table 2.

Inspection of data in Table 2 reveals that each drug−DNA complex exhibits a greater partial molar volume [by 202 to 229 cm^3 (mole of binding site)$^{-1}$] than its free host structure. By contrast, the partial molar adiabatic compressibility of the complex may be either larger or smaller relative to the free host structure. For example, the partial molar adiabatic compressibility of the ethidium-poly(dAdT)poly(dAdT) complex is (11 ± 6) × 10^{-4} cm^3 (mole of binding site)$^{-1}$ bar^{-1} greater than that of the host duplex, whereas the partial molar adiabatic compressibility of the ethidium-poly(rU)poly(rA)poly(rU) complex is (29 ± 9) × 10^{-4} cm^3 (mole of binding site)$^{-1}$ bar^{-1} smaller than that of the host triplex.

Interpretation of Volumetric Observables in Terms of Hydration. In general, the partial molar volume, $V°$, of a solute can be presented as the sum[38−41]

$$V° = V_M + V_T + V_I + \beta_{T0}RT \qquad (5)$$

where V_M is the intrinsic volume which represents the geometric volume of the solute that is not penetrable by solvating water molecules; V_T is the thermal volume which originates from thermally activated mutual vibrations of the solute and solvent molecules; V_I is the interaction volume which represents the solvent contraction around a solute due to hydrogen bonding or electrostriction; β_{T0} is the coefficient of isothermal compressibility of the solvent; R is the universal gas constant; and T is the absolute temperature.

The partial molar adiabatic compressibility, $K_S°$, of a solute can be represented by the sum of the intrinsic, K_M, and hydration, ΔK_h, contributions[18−21]

$$K_S° = K_M + \Delta K_h = K_M + n_h(K_h - K_0) \qquad (6)$$

where K_M is the intrinsic compressibility of a solute; ΔK_h is the hydration-induced change in the solvent compressibility; K_0 and K_h are the partial molar adiabatic compressibilities of water in the bulk state and in the hydration shell of a solute, respectively; and n_h is the "hydration number", which corresponds to the number of water molecules in the hydration shell of a solute.

For small molecules, as well as for nucleic acids and their complexes with drugs, the intrinsic compressibility, K_M, in eq 6 is determined by the compressibility of covalent bonds and the external electron shells, both of which are small and usually can be neglected.[18−22] Consequently, for nucleic acids and drug-nucleic acid complexes, one may neglect for low-molecular weight molecules and nucleic acids, eq 6 can be rewritten as follows

$$K_S° = \Delta K_h = n_h(K_h - K_0) \qquad (7)$$

One important consequence of the near zero value of K_M in eq 6 is that any volume fluctuations or binding-induced changes in volume fluctuations of nucleic acids are small and can be neglected in the analysis below.[42] On the basis of eq 7, the partial molar adiabatic compressibility, $K_S°$, of small molecules, nucleic acids, and their complexes predominantly reflects solute hydration. Changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying drug−DNA binding can be obtained by differentiating eqs 5 and 7

$$\Delta V = \sum_i (\Delta V_{Mi} + \Delta V_{Ti} + \Delta V_{Ii}) \qquad (8)$$

$$\Delta K_S = \Delta\Delta K_h = \sum_i \Delta[n_{hi}(K_{hi} - K_0)] \qquad (9)$$

where the subscript i refers to the number of species participating in reaction 2.

Analogous to eq 9, the value of ΔV_I can be presented to equal $\Delta[n_{hi}(V_{hi} - V_0)]$, where V_0 and V_{hi} are the partial molar volumes of water in the bulk state and the hydration shell of the i-th component of reaction 2, respectively.

Changes in Volume. For an intercalation reaction, one could determine the change in the intrinsic volume, ΔV_M, in eq 8 from structural information on a drug-free host duplex or triplex and its complex with the intercalator. To this end, Connoly's algorithm could be employed.[43] The change in the thermal volume, V_T, in eq 8 could be estimated by multiplying the binding-induced change in solvent accessible surface area, ΔS_A, of the components of reaction 2 by the average thickness, δ, of the thermal volume.[41,44,45] After calculating ΔV_M and ΔV_T, one could determine the change in the interaction volume, ΔV_I, using

(37) Millero, F. J.; Ward, G. K.; Chetirkin, P. V. *J. Acoust. Soc. Am.* **1977**, *61*, 1492−1498.
(38) Pierotti, R. A. *J. Phys. Chem.* **1965**, *69*, 281−288.
(39) Stillinger, F. H. *J. Solution Chem.* **1973**, *2*, 141−158.
(40) Pierotti, R. A. *Chem. Rev.* **1976**, *76*, 717−726.
(41) Kharakoz, D. P. *J. Solution Chem.* **1992**, *21*, 569−595.
(42) Cooper, A. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 181−214.
(43) Connolly, M. J. *Science* **1983**, *221*, 709−713.
(44) Edward, J. T.; Farrell, P. G. *Can. J. Chem.* **1975**, *53*, 2965−2970.
(45) Likhodi, O.; Chalikian, T. V. *J. Am. Chem. Soc.* **1999**, *121*, 1156−1163.

eq 8 and the experimental value of ΔV. Finally, the value of ΔV_I could be used to quantify the release or uptake of water molecules from the hydration shells of the components of reaction 2. Although this is a viable approach, it hardly can be used for studying ethidium complexation with polymeric nucleic acids. Reliable structural data on ethidium-DNA complexes required for calculating ΔV_M ad ΔS_A do not exist. Although X-ray crystallographic data reported for ethidium-dinucleotide complexes[46,47] provide qualitative insights into the structural properties of DNA intercalation, they do not enable one to quantify the geometric features of complexes of ethidium with double- and triple-stranded polynucleotides.

It is worth mentioning that the values of [$V°$(EDNA) − $V°$(DNA)] presented in Table 2 are significantly larger than the differential intrinsic volume of ethidium and sodium ion (Recall that each binding site of drug−DNA complex contains one sodium ion less than that of the free DNA). The intrinsic (molecular) volume of ethidium is 185.5 $cm^3\ mol^{-1}$, as can be calculated based on Connolly's algorithm[43] and structural data presented in refs 46 and 47. The use of the additive approach and group contributions presented by Bondi[48] yields a very similar number of 182.3 $cm^3\ mol^{-1}$. The intrinsic volume of Na^+ is 2.2 $cm^3\ mol^{-1}$ [based on the ionic radius of 0.95 Å[49]]. Thus, the differential intrinsic volume of ethidium and Na^+ is 183.3 $cm^3\ mol^{-1}$, while the values of [$V°$(EDNA) − $V°$(DNA)] range from 202 to 229 cm^3 (mole of binding site)$^{-1}$ (see Table 2). The observed disparity suggests that the host structures and their ethidium complexes are distinct not only with respect to their intrinsic volumes, V_M, but may also differ with respect to the thermal volume, V_T (which reflects binding-induced structural changes), and the interaction volume, V_I (which reflects altered hydration).

Changes in Adiabatic Compressibility. Inspection of eqs 7 and 9 reveals that compressibility is an observable that is more directly linked to DNA hydration than volume. The observed changes in compressibility associated with a drug−DNA binding event can be assigned entirely to the binding-induced alterations in hydration of the interacting species. With this notion, the differential compressibility of each host nucleic acid structure and its complex with ethidium presented in Table 2 reflects their differential hydration properties. Water of DNA hydration exhibits an average partial molar adiabatic compressibility, K_h, that is $\sim 2 \times 10^{-4}$ $cm^3\ mol^{-1}\ bar^{-1}$ smaller than that of bulk water, K_0 ($K_h - K_0 \approx -2 \times 10^{-4}$ $cm^3\ mol^{-1}\ bar^{-1}$).[50]

If one assumes that the values of K_h are similar for the host structures and their ethidium complexes, the differential number of water molecules, n_h(EDNA) − n_h(DNA), solvating each host structure and its complex can be calculated by dividing the value of [$K_S^°$(EDNA) − $K_S^°$(DNA)] by -2×10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$ (see eq 9). Table 3 lists our calculated values of [n_h(EDNA) − n_h(DNA)] for the nucleic acid structures studied here.

Inspection of data in Table 3 reveals that the differential numbers of water molecules solvating the ethidium complexes of poly(rA)poly(rU), poly(dAdT)poly(dAdT), poly(dGdC)-poly(dGdC), poly(dIdC)poly(dIdC), and poly(rU)poly(rA)-poly(rU) and their respective host structures amount to 7.5 ± 3, −5.5 ± 3, 0 ± 3, 0 ± 3, and 14.5 ± 4.5 water molecules (if normalized per binding site), respectively. These values roughly correspond to −1.5 ± 1 to 2.5 ± 1 water molecules per nucleotide. This is a rather insignificant difference since the number of molecules solvating double stranded nucleic acids range between 18 (genomic duplexes) and 46 [poly(dIdC)-poly(dIdC)] per nucleotide.[50,51] Our estimates are in qualitative agreement with "osmotic stress" results of Qu and Chaires[52,53] who found that, within experimental error, no net water uptake or release accompanies ethidium association with DNA.

Table 3. Differential Hydration of the Ethidium Complexes and the Drug-Free Host Nucleic Acids

polynucleotide	n_h(EDNA) − n_h(DNA)
poly(rA)poly(rU)	7 ± 3
poly(dAdT)poly(dAdT)	−5.5 ± 3
poly(dGdC)poly(dGdC)	0 ± 3
poly(dIdC)poly(dIdC)	0 ± 3
poly(rU)poly(rA)poly(rU)	14.5 ± 4.5

Net Changes in Hydration Accompanying the Association of Ethidium with Host Nucleic Acid Structures. The overall change in the number of water molecules in the hydration shells of all components of reaction 2 (total change in hydration), Δn_h, accompanying the complexation of ethidium with nucleic acids can be defined as the difference in the number of water molecules solvating the species in the right (Na^+ and EDNA) and left (ethidium and DNA) of reaction 2

$$\Delta n_h = n_h(\text{EDNA}) + n_h(Na^+) - n_h(\text{DNA}) - n_h(E^+) \quad (10)$$

As is seen from eq 10, in addition to the differential number of water molecules solvating EDNA and DNA [n_h(EDNA) − n_h(DNA)], the net change in hydration involves dehydration of ethidium and hydration of Na^+ ion. To estimate the number of water molecules in the hydration shells of ethidium and Na^+ ions, we use the previously developed two-state model of solute hydration.[50] In the model, liquid water is presented as consisting of two structural species: the high density/high enthalpy species, structurally similar to ice III, and the low density/low enthalpy species, structurally similar to ice I. It is assumed that the structural and thermodynamic distinctions between bulk and hydration water originate solely from the differential fractional composition, f_{1h}, whereas the two species and thermodynamic parameters associated with each of them are identical for bulk and hydration water. At 25 °C, the fractional composition, f_{1h}, of bulk water is 0.27, which signifies that 27% of water molecules are in the high density/high enthalpy (ice III-like) state with the remaining 73% being in the low density/low enthalpy (ice I-like) state. For water solvating charged and polar groups, the equilibrium between the two species is shifted toward the high density/high enthalpy species.[50] In contrast, for water solvating nonpolar groups, the equilibrium is shifted toward the low density/low enthalpy species.[50] For nucleic acids, the value of f_{1h} is within the range of 0.80 to 0.84, which characterizes the hydration shell of DNA and RNA as being dominated by electrostatic solute−solvent interactions.[50]

The model and related formalism described in ref 50 enables one to determine the number, n_h, and fractional composition,

(46) Tsai, C.-C.; Jain, S. C.; Sobell, H. M. *J. Mol. Biol.* **1977**, *114*, 301−315, 1977.
(47) Jain, S. C.; Sobell, H. M. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1179−1194.
(48) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−451.
(49) Hirata, F.; Arakawa, K. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3367−3369.
(50) Chalikian, T. V. *J. Phys. Chem. B* **2001**, *105*, 12 566−12 578.
(51) Chalikian, T. V.; Völker, J.; Srinivasan, A. R.; Olson, W. K.; Breslauer, K. J. *Biopolymers* **1999**, *50*, 459−471.
(52) Qu, X.; Chaires, J. B. *J. Am. Chem. Soc.* **1999**, *121*, 2649−2650.
(53) Qu, X.; Chaires, J. B. *J. Am. Chem. Soc.* **2001**, *123*, 1−7.

f_{1h}, of water of solute hydration based on its interaction volume, V_I, and the hydration contribution to compressibility, ΔK_h. As mentioned above, for small molecules and nucleic acids, ΔK_h coincides with K_S°. For Na^+, the values of V_I and K_S° have been estimated to be -15 cm^3 mol^{-1} [49] and -33.5×10^{-4} cm^3 mol^{-1} bar^{-1},[54] respectively. Using the formalism presented in ref 50, we calculate for Na^+ the hydration number, $n_h(Na^+)$, of 5.5 and the fractional composition of water of hydration, f_{1h}, of 0.99.

The partial molar adiabatic compressibility, K_S°, of the cationic form of ethidium can be found by subtracting the partial molar adiabatic compressibility of a Br^- ion [-9.5×10^{-4} cm^3 mol^{-1} bar^{-1} [54]] from that of ethidium bromide (-28×10^{-4} cm^3 mol^{-1} bar^{-1}). Thus, we calculate the partial molar adiabatic compressibility, K_S°, of cationic ethidium to be 18.5×10^{-4} cm^3 mol^{-1} bar^{-1}. Similarly, the partial molar volume, V°, of the cationic form of ethidium can be obtained by subtracting the partial molar volume of Br^- ion [30.4 cm^3 mol^{-1} [55]] from that of ethidium bromide (267.1 cm^3 mol^{-1}). Thus, the partial molar volume, V°, of cationic ethidium is 236.7 cm^3 mol^{-1} bar^{-1}. According to eq 5, the interaction volume, V_I, of ethidium can be obtained by subtracting the sum ($V_M + V_T + \beta_{T0}RT$) from V°. Recall that the intrinsic volume, V_M, of ethidium is 185.5 cm^3 mol^{-1}. To determine its thermal volume, V_T, we have presented the molecule of ethidium as a "barrel" [as described by Kharakoz[41]]. The thermal volume, V_T, has been calculated based on geometric considerations as a void volume of the thickness of 0.50 Å surrounding the barrel. We have recently described in great detail the application of this procedure to evaluating the thermal and interaction volumes of heterocyclic bases and nucleosides.[56] Now, we employ the same approach to evaluate the interaction volume, V_I, for cationic ethidium and calculate the value of -41.9 cm^3 mol^{-1}. Using the values of ΔK_h and V_I and the formalism described in ref 50, we determine that cationic ethidium contains in its hydration shell 23.5 water molecules (n_h) with the fractional composition, f_{1h}, of 0.75.

Hence, our estimates suggest that the binding of ethidium to the nucleic acid structures is accompanied by the release of 23.5 water molecules from the hydration shell of the drug, the uptake of 5.5 water molecules by the released Na^+ ion, and, depending on the host structure, the uptake (up to 14.5) or release (up to 5.5) of water molecules from the nucleic acid hydration shell (see Table 3). Using eq 10, we determine that the overall change in hydration, Δn_h, for each intercalation reactions studied in this work is positive. Specifically, ethidium association with poly(rA)poly(rU), poly(dAdT)poly(dAdT), poly(dGdC)-poly(dGdC), poly(dIdC)poly(dIdC), and poly(rU)poly(rA)-poly(rU) is accompanied by the net release of 11 ± 3, 23.5 ± 3, 18 ± 3, 18 ± 3, and 3.5 ± 4.5 water molecules, respectively.

Entropic Contribution of Hydration to the Energetics of Ethidium Binding. The binding entropy, ΔS°, for macromolecular association can be presented as the sum of the intrinsic (configurational), ΔS_{conf}, hydrational, ΔS_{hyd}, and translational, ΔS_{trans}, terms[57]

$$\Delta S_b = \Delta S_{conf} + \Delta S_{hyd} + \Delta S_{trans} \qquad (11)$$

For one-to-one stoichiometric binding, the value of ΔS_{trans} has been estimated to be on the order of -8 cal K^{-1} mole^{-1}.[57] As a first approximation, however, for the process in which ethidium binds to DNA, the ΔS_{trans} term can be ignored, because a decrease in S_{trans} due to the association of the drug with DNA should be roughly counterbalanced by an increase in S_{trans} due to dissociation of Na^+ ion. Therefore, in our analysis below, we assume that $\Delta S_{trans} \approx 0$.

The hydrational change in entropy, ΔS_{hyd}, can be estimated by multiplying the total number of water molecules released to the bulk, Δn_h, by $-(S_h - S_0)$, the average difference in the partial molar entropy between water of solute hydration and bulk water. The data on entropy of hydration of a variety of protein groups presented by Makhatadze and Privalov[58] suggest that, at 25 °C, the values of $(S_h - S_0)$ for water molecules solvating charged, polar, and hydrophobic groups are quite similar and equal to -1.3 ± 0.4 cal K^{-1} mol^{-1}. Significantly, in a previous study, we have obtained a similar estimate for the entropic contribution (-1.6 cal K^{-1} mol^{-1}) for water molecules solvating DNA duplexes.[59] At 25 °C, the average difference between the partial molar entropies of water in the bulk state and water in the hydration shells of a solute does not appear to strongly depend on the chemical nature of solvent-exposed atomic groups or, even, on the type of solute. This experimental result is in qualitative agreement with theoretical calculations of Rashin and Bukatin.[60,61]

On the basis of the value of $(S_h - S_0)$ of -1.3 ± 0.4 cal K^{-1} mol^{-1}, we calculate for ethidium complexation with poly(rA)-poly(rU), poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), poly(dIdC)poly(dIdC), and poly(rU)poly(rA)poly(rU) the hydrational contributions to the binding entropy, ΔS_{hyd}, of 14.3 (11×1.3), 30.6 (23.5×1.3), 23.4 (18×1.3), 23.4 (18×1.3), and 4.6 (3.5×1.3) cal mol^{-1} K^{-1}, respectively. Note that changes in hydration make favorable contributions to the energetics of ethidium binding. The configurational change in entropy, ΔS_{conf}, can be calculated from eq 11 using the values of ΔS_{hyd} and available data on the binding entropy, ΔS°, for ethidium complexation with DNA structures. To this end, we have used the ΔS° data reported by Chou et al.[62] Specifically, for poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), and poly-(dIdC)poly(dIdC), the values of ΔS° at 25 °C were found to be -3, 8, and 1 cal mol^{-1} K^{-1}, respectively.[62] For these duplexes, we calculate ΔS_{conf} of -33.6 ($-3-30.6$), -15.4 ($8-23.4$), and -22.4 ($1-23.4$) cal mol^{-1} K^{-1}, respectively.

In contrast to ΔS_{hyd}, the binding-induced changes in configurational entropy, ΔS_{conf}, are unfavorable. The value of ΔS_{conf} reflects binding-induced structural alterations of the host duplex and concomitant reduction in its conformational degrees of freedom. Interestingly, congfigurational penalty is highest for the poly(dAdT)poly(dAdT) duplex followed by the poly(dIdC)-poly(dIdC) and poly(dGdC)poly(dGdC) duplexes. This order may correlate with the extent of binding-induced structural changes of the three DNA duplexes.

In the aggregate, the hydrational and configurational terms represent major contributors to the binding entropy. Highly favorable changes in the hydrational entropy, ΔS_{hyd}, are opposed

(54) Mathieson, J. G.; Conway, B. E. *J. Solution Chem.* **1974**, *3*, 455−477.
(55) Conway, B. E. *J. Solution Chem.* **1978**, *7*, 721−770.
(56) Lee, A.; Chalikian, T. V. *Biophys. Chem.* **2001**, *92*, 209−227.
(57) Luque, I.; Freire, E. *Methods Enzymol.* **1998**, *295*, 100−127.
(58) Makhatadze, G. I.; Privalov, P. L. *Adv. Protein Chem.* **1995**, *47*, 307−425.
(59) Chalikian, T. V.; Plum, G. E.; Sarvazyan, A. P.; Breslauer, K. J. *Biochemistry* **1994**, *33*, 8629−8640.
(60) Rashin, A. A.; Bukatin, M. A. *Biophys. Chem.* **1994**, *51*, 167−192.
(61) Rashin, A. A.; Bukatin, M. A. *J. Phys. Chem.* **1994**, *98*, 386−389.
(62) Chou, W. Y.; Marky, L. A.; Zaunczkowski, D.; Breslauer, K. J. *J. Biomol. Struct. Dyn.* **1987**, *5*, 345−359.

by unfavorable changes in the configurational entropy, ΔS_{conf}. To appreciate the vital role water plays in modulating the energetics of ethidium binding, note that the values of $-T\Delta S_{hyd}$ are on the order of changes in free energy, $\Delta G°$. Specifically, for poly(dAdT)poly(dAdT), poly(dGdC)-poly(dGdC), and poly(dIdC)poly(dIdC), the values of $-T\Delta S_{hyd}$ are equal to -9.1, -7.0, and -7.0 kcal mol^{-1}, respectively, whereas the $\Delta G°$ values are -9.1, -8.6, and -9.3 kcal mol^{-1}, respectively.[62] Clearly, further investigations, involving a combination of volumetric and calorimetric measurements on larger sets of ligands and nucleic acids, are required for developing a more complete understanding of the role of hydration in modulating the energetics of drug−DNA recognition. Such investigations are underway in our lab with the results presented in this work laying foundation for future studies.

Concluding Remarks

We have used acoustic and densimetric techniques to measure the changes in volume, ΔV, and adiabatic compressibility, ΔK_S, that accompany the binding of the prototypical intercalator ethidium bromide to the poly(rA)poly(rU), poly(dAdT)poly-(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC) duplexes as well as the poly(rU)poly(rA)poly(rU) triplex. For all the drug−DNA binding events studied in this work, we have measured negative changes in volume, ΔV, and adiabatic compressibility, ΔK_S. We discuss the basis for relating our measured macroscopic results and microscopic properties, particularly, emphasizing how measured changes in volume and compressibility can be interpreted in terms of the differential hydration properties of DNA and RNA structures in their ligand-free and ligand-bound states.

Differential numbers of water molecules solvating the ethidium complexes of poly(rA)poly(rU), poly(dAdT)poly-(dAdT), poly(dGdC)poly(dGdC), poly(dIdC)poly(dIdC), and poly(rU)poly(rA)poly(rU) and their respective host structures amount to 7 ± 3, -5.5 ± 3, 0 ± 3, 0 ± 3, and 14.5 ± 4.5 (normalized per binding site), respectively. Thus, the complex may be more, equally, or less hydrated than the host structure. For evaluating the total change in hydration associated with the binding of ethidium to nucleic acids, one should take into account dehydration of the drug and hydration of the released sodium ion. Ethidium association with poly(rA)poly(rU), poly-(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), poly(dIdC)poly-(dIdC), and poly(rU)poly(rA)poly(rU) is accompanied by the net release of 11 ± 3, 23.5 ± 3, 18 ± 3, 18 ± 3, and 3.5 ± 4.5 water molecules, respectively. We have estimated the entropic contributions of these changes in hydration. For ethidium complexation with poly(rA)poly(rU), poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), poly(dIdC)poly(dIdC), and poly(rU)-poly(rA)poly(rU), the hydrational contributions to the binding entropy, ΔS_{hyd}, equal 14.3, 30.6, 23.4, 23.4, and 4.6 cal mol^{-1} K^{-1}, respectively. These changes are favorable and quite significant being on the order of the binding free energy. Favorable changes in the hydrational entropy are opposed by unfavorable changes in the configurational entropy, ΔS_{conf}. For poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC), and poly-(dIdC)poly(dIdC), the values of ΔS_{conf} equal -33.6, -15.4, and -22.4 cal mol^{-1} K^{-1}, respectively.

In general, our results emphasize the vital role of hydration in drug−DNA binding, while also underscoring the fact that hydration must be carefully taken into account in analysis and prediction of the energetics of nucleic acid recognition. In particular, this notion is central for rational design of novel drugs that are specifically targeted to selected nucleotide sequences. This work lays foundation for expansion of such studies to larger sets of drugs and nucleic acid structures.

Experimental Section

Materials. Ethidium bromide was obtained from Sigma-Aldrich Canada (Mississauga, Ontario, Canada) whereas the synthetic DNA and RNA polymers [poly(rA)poly(rU), poly(rU), poly(dAdT)poly-(dAdT), poly(dGdC)poly(dGdC), and poly(dIdC)poly(dIdC)] were purchased from Amersham Biosciences Corp. (Baie d'Urfé, Québec, Canada). These reagents were of the highest grade commercially available and used without further purification.

All measurements were performed in a pH 6.7 buffer consisting of 10 mM cacodylic acid-sodium cacodylate and 1 mM Na_2EDTA. The ionic strength of the buffer was adjusted to the desired level by adding known amounts of NaCl. DNA samples were dissolved in the buffer and dialyzed at room temperature against the same buffer using dialysis tubing with a molecular weight cut off of 1000 Da (Spectrum, Houston, TX). Two subsequent changes of buffer were made after equilibration for at least 48 h.

Preparation of Triple Helix Solutions. Equimolar amounts of the poly(rA)poly(rU) duplex and the poly(rU) single strand were mixed in buffer. Triple helix formation was detected by CD spectroscopic measurements.

Concentration Determinations. The concentrations of free ethidium bromide and each drug-free DNA structure were determined spectrophotometrically using the following molar extinction coefficients: ethidium bromide, $\epsilon_{480} = 5850$ M^{-1} cm^{-1}; poly(rA)poly(rU), $\epsilon_{260} = 6300$ M^{-1} cm^{-1}; poly(dAdT)poly(dAdT), $\epsilon_{260} = 6650$ M^{-1} cm^{-1}; poly-(dGdC)poly(dGdC), $\epsilon_{254} = 8400$ M^{-1} cm^{-1}; poly(dIdC)poly(dIdC), $\epsilon_{251} = 6900$ M^{-1} cm^{-1}; poly(rU)poly(rA)poly(rU), $\epsilon_{257} = 5900$ M^{-1} cm^{-1}; and poly(rU), $\epsilon_{261} = 9500$ M^{-1} cm^{-1}. These values were either provided by the manufacturer or taken from the literature.[23,62]

For all the densimetric and ultrasonic velocimetric experiments, DNA concentrations were between 2 and 3 mM in nucleotide. For CD measurements, the DNA concentrations were in the range of 0.8−1.0 mM in nucleotide. Throughout this paper, all DNA concentrations are expressed per mole of nucleotide, unless otherwise indicated. For acoustic and densimetric titration experiments, the concentration of ethidium bromide ranged from 5 to 7 mM.

CD Spectroscopy. CD spectra were recorded at 25 °C using an Aviv model 62 DS spectropolarimeter (Aviv Associates, Lakewood, NJ). CD titration profiles were measured by incrementally adding aliquots of ethidium bromide to a cell containing a known amount of DNA.

Volumetric Measurements. 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-5000, Anton Paar, Graz, Austria). The partial molar volume, $V°$, of the nucleic acids in the presence and absence of the drug was calculated from density values using the relationship[63]

$$V° = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C) \qquad (12)$$

where ρ and ρ_0 are, the densities of the nucleic acid solution and the solvent, respectively; C is the molar concentration of the nucleic acid; and M is the nucelic acid's molecular weight (expressed as per mole of nucleotide).

Solution sound velocity measurements were carried out at 7.2 MHz by analyzing the amplitude-frequency characteristics of an ultrasonic resonator as described previously.[64−67] In our system, we used an

(63) Millero, F. J. In *Water and Aqueous Solutions*; Horne, R. A., Ed.; John Wiley & Sons: New York, 1972; pp 519−595.
(64) Eggers, F.; Funck, Th. *Rev. Sci. Instrum.* **1973**, *44*, 969−978.
(65) Sarvazyan, A. P. *Ultrasonics* **1982**, *20*, 151−154.

ultrasonic resonator with lithium niobate resonators and the minimum sample volume of 0.8 mL.[65] The analysis of the frequency characteristics of the resonator was performed by a Hewlett-Packard model HP4195A network/spectrum analyzer (Mississauga, Ontario, Canada).

The key characteristic of a solute directly derived from ultrasonic velocimetric measurements is the relative molar sound velocity increment, [*U*]

$$[U] = (U - U_0)/(U_0C) \tag{13}$$

where U and U_0 are the sound velocities in the protein solution and the neat solvent, respectively.

Differential densimetric and ultrasonic titrations were performed at 25 °C by adding equal aliquots of a 5 to 7 mM ethidium bromide solution (prepared using the same buffer as the DNA solution) to both the sample and reference cells as previously described.[59] Values of the relative molar sound velocity increment, [U], were used in conjunction with the measured partial molar volume data, $V°$, to calculate the partial molar adiabatic compressibility, $K_S°$, of the nucleic acids using the following relationship[68,69]

$$K_S° = \beta_{S0}(2V° - 2[U] - M/\rho_0) \tag{14}$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent. The densimetric and ultrasonic velocimetric experiments have been performed at least three times with the average values of [*U*] and $V°$ being used in eq 14.

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(66) Eggers, F. *Acustica* **1992**, *76*, 231−240.
(67) Eggers, F.; Kaatze, U. *Meas. Sci. Technol.* **1996**, *7*, 1−19.
(68) Barnatt, S. *J. Chem. Phys.* **1952**, *20*, 278−279.
(69) Owen, B. B.; Simons, H. L. *J. Phys. Chem.* **1957**, *61*, 479−482.