Articles

Influence of Drug Binding on DNA Hydration: Acoustic and Densimetric Characterizations of Netropsin Binding to the Poly(dAdT)·Poly(dAdT) and Poly(dA)·Poly(dT) Duplexes and the Poly(dT)·Poly(dA)·Poly(dT) Triplex at 25 °C†

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ABSTRACT: We use high-precision acoustic and densimetric techniques to determine, at 25 °C, the changes in volume, ΔV, and adiabatic compressibility, ΔK_S, that accompany the binding of netropsin to the poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) duplexes, as well as to the poly(dT)·poly(dA)·poly(dT) triplex. We find that netropsin binding to the heteropolymeric poly(dAdT)·poly(dAdT) duplex is accompanied by negative changes in volume, ΔV, and small positive changes in compressibility, ΔK_S. By contrast, netropsin binding to the homopolymeric poly(dA)·poly(dT) duplex is accompanied by large positive changes in both volume, ΔV, and compressibility, ΔK_S. Furthermore, netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex causes changes in both volume and compressibility that are nearly twice as large as those observed when netropsin binds to the poly(dA)·poly(dT) duplex. We interpret these macroscopic data in terms of binding-induced microscopic changes in the hydration of the DNA structures and the drug. Specifically, we find that netropsin binding induces the release of approximately 22 waters from the hydration shell of the poly(dAdT)·poly(dAdT) heteropolymeric duplex, approximately 40 waters from the hydration shell of the poly(dA)·poly(dT) homopolymeric duplex, and about 53 waters from the hydration shell of the poly(dT)·poly(dA)·poly(dT) triplex. In other words, netropsin binding to the homopolymeric duplex, poly(dA)·poly(dT), induces the release of 18 more water molecules than netropsin binding to the heteropolymeric duplex, poly(dAdT)·poly(dAdT). On the basis of apparent molar volume, ϕV, and apparent molar adiabatic compressibility, ϕK_S, values for the initial drug-free and final drug-bound states of the two all-AT duplexes, we propose that the larger dehydration of the poly(dA)·poly(dT) duplex reflects, in part, the formation of a less hydrated poly(dA)·poly(dT)–netropsin complex compared with the corresponding poly(dAdT)·poly(dAdT)–netropsin complex. In conjunction with our previously published entropy data [Marky, L. A., & Breslauer, K. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84,* 4359–4363], we calculate that each water of hydration released to the bulk solvent by ligand binding contributes 1.6 cal K^{-1} mol^{-1} to the entropy of binding. This value correponds to the average difference between the partial molar entropy of water in the bulk state and water in the hydration shells of the two all-AT duplexes. When netropsin binds to the poly(dT)·poly(dA)·poly(dT) triplex, the changes in both volume and compressibility suggest that the binding event induces more dehydration of the triplex than of the duplex state. Specifically, we calculate that netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex causes the release of 13 more waters than netropsin binding to the poly(dA)·poly(dT) duplex. In general, we discuss the basis for relating macroscopic and microscopic properties, particularly emphasizing how measured changes in volume and compressibility can be interpreted in terms of the hydration properties of DNA structures in their ligand-free and ligand-bound states.

The influence of hydration on the properties of nucleic acids has been recognized for quite some time (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). Despite this long-standing recognition, the detailed nature of nucleic acid hydration patterns remains poorly understood. This deficiency is serious since solvent effects frequently are invoked to rationalize experimental observations. The current microscopic picture of nucleic acid hydration primarily derives from the results of X-ray diffraction and NMR investigations. These studies have revealed some intriguing hydration patterns for nucleic acids that appear to depend on base composition, base sequence, and DNA conformation, all of which represent interrelated properties (Drew & Dickerson, 1981; Westhof, 1988; Westhof & Beveridge, 1990; Schneider et al., 1992). Moreover, two relatively recent single-crystal X-ray studies suggest that protein binding to nucleic acids may involve recognition between complementary hydration interfaces rather than direct interaction between protein and nucleic acid functional groups (Otwinowski et al., 1988; Lawson & Carey, 1993). These results raise the interesting possibility that, in addition to conformational preferences, hydration properties may also mediate selective nucleic acid recognition, an event that is

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central to numerous biological control mechanisms.

Unfortunately, macroscopic characterizations of hydration have not kept pace with their microscopic counterparts. Consequently, it is not yet possible to define the thermodynamic contributions that specific hydration patterns make to nucleic acid structures and to their ligand binding properties. For this reason, solvent-based explanations generally tend to be intuitive in nature rather than based on specific quantitative experimental characterizations of hydration. For example, the aberrant properties of the poly(dA)·poly(dT) duplex have been "explained" by postulating that this duplex possesses a greater degree of hydration than standard B-form double helices (Pilet et al., 1975; Leslie et al., 1980; Breslauer et al., 1987; Chuprina, 1985; Teplukhin et al., 1992), a feature that has been used to rationalize its noncanonical structure (Leslie et al., 1980; Alexeev et al., 1987), its bending behavior (Breslauer, 1991), and its unusual ligand binding properties (Breslauer et al., 1987; Marky & Breslauer, 1987; Marky & Kupke, 1989; Marky & Macgregor, 1990; Remeta et al., 1993). With regard to the latter feature, the thermodynamics for drug binding to the poly(dA)·poly(dT) duplex at 25 °C has been shown to be overwhelmingly entropy driven compared to the poly(dAdT)·poly(dAdT) duplex. This difference has been rationalized by proposing that drug binding causes the release of more solvent from the putatively more hydrated heteropolymeric duplex (an event probably coupled with a binding-induced conformational change), thereby resulting in a larger entropy driving force. This hydration-based microscopic explanation of the entropy data subsequently has been reinforced by volumetric studies, which reveal ligand binding to the homopolymeric duplex to be accompanied by a significantly larger increase in volume than binding to the heteropolymeric duplex (Vesnaver & Breslauer, 1990; Marky & Kupke, 1989). However, in the absence of detailed macroscopic characterizations of the properties of the solute-perturbed solvent, relative to bulk water, for the drug, the free DNA, and the drug-bound DNA, such explanations remain intuitive and qualitative in nature.

To obtain the requisite quantitative macroscopic characterizations of hydration, we have begun a program in which the hydration properties of ligand-free and ligand-bound DNA structures are being investigated by a combination of acoustic and densimetric techniques. These two methods yield data that are sensitive to solute–solvent interactions and that can be interpreted to produce thermodynamic characterizations of the solute-perturbed solvent, a domain commonly referred to as the hydration shell (Sarvazyan & Kharakoz, 1977; Buckin et al., 1989; Sarvazyan, 1991; Chalikian et al., 1994c). Recently, we used this combination of experimental techniques to produce a comprehensive characterization of DNA hydration as a function of base composition, base sequence, and duplex structure (Chalikian et al., 1994b). We now expand the applicability of this experimental strategy by demonstrating how it also can be used to resolve and to define the influence of hydration on the ligand binding properties of duplex and triplex DNA structures. Specifically, we report the changes in volume and compressibility that accompany the binding of the oligopeptide antibiotic netropsin, an AT-specific minor groove binding ligand (Wartell et al., 1974; Patel, 1982; Kopka et al., 1985; Zimmer & Wahnert, 1986), to the poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) duplexes and to the poly(dT)·poly(dA)·poly(dT) triplex. We use these data to calculate the number of water molecules released or taken up during the binding event, and we correlate these numbers with the drug binding entropies that we previously have reported (Marky & Breslauer, 1987). Throughout the paper, we discuss how macroscopic and microscopic properties can be related quantitatively, particularly emphasizing how measured changes in volume and compressibility can be interpreted in terms of the hydration properties of DNA structures in their ligand-free and ligand-bound states. Our ultimate goal is to establish a macroscopic nucleic acid database that can be used in conjunction with the corresponding microscopic database to define the role that hydration plays in modulating nucleic acid structure and ligand binding properties.

MATERIALS AND METHODS

Materials. Netropsin was obtained from Boehringer Mannheim (Indianapolis, IN), while the synthetic DNA polymers [poly(dA)·poly(dT), poly(dAdT)·poly(dAdT), and poly(dT)] were purchased from Pharmacia-LKB Biochemicals (Piscataway, NJ). These reagents 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} ohm^{-1}. All measurements were performed in a pH 6.7 buffer consisting of 10 mM cacodylic acid–sodium cacodylate and 0.1 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 sonicated to reduce solution viscosity. All solutions were dialyzed at 4 °C against the same buffer using dialysis tubing with a molecular weight cutoff of 1000 (Spectrum, Houston, TX). Two subsequent changes of buffer were made after equilibration for at least 48 h.

Preparation of Netropsin Complexes with the Poly(dA)·Poly(dT) and Poly(dAdT)·Poly(dAdT) Duplexes. Netropsin, in amounts slightly in excess of saturation, was added to buffer solutions of each all-AT duplex containing a total Na^+ concentration of 16 mM. These solutions were dialyzed exhaustively against the buffer to remove any unbound netropsin. The buffer against which the final dialysis was performed was used as the reference solution in all of the differential acoustic and densimetric measurements. Phosphate analysis (Snell & Snell, 1949) revealed the extinction coefficients at 260 nm, ϵ_{260}, for the complexes of netropsin with the poly(dA)·poly(dT) and poly(dAdT)·poly(dAdT) duplexes to be equal to 8570 and 10 280 M^{-1} cm^{-1}, respectively.

Preparation of Triple-Helix Solutions. Equal amounts of unsonicated poly(dA)·poly(dT) duplex and single-strand poly(dT) were mixed in buffer. Triple-helix formation was detected by its characteristic negative CD band at 217 nm (Park & Breslauer, 1992). The sample then was sonicated to decrease solution viscosity. An extinction coefficient, ϵ_{260}, of 6000 M^{-1} cm^{-1} at ionic strengths between 200 and 300 mM was used to determine the solution concentrations of the poly(dT)·poly(dA)·poly(dT) triplex (Park, 1992).

Concentration Determinations. The concentrations of free netropsin and each drug-free DNA structure were determined spectrophotometrically using the following molar extinction coefficients: netropsin, ϵ_{296} = 21 500 M^{-1} cm^{-1}; poly(dA)·poly(dT), ϵ_{260} = 6000 M^{-1} cm^{-1}; poly(dAdT)·poly(dAdT), ϵ_{260} = 6650 M^{-1} cm^{-1}; poly(dT), ϵ_{264} = 8520 M^{-1} cm^{-1}. These values were provided by the manufacturers and are consistent with those previously reported (Marky & Breslauer, 1987; Park & Breslauer, 1992; Remeta et al., 1993).

For all of the densimetric and ultrasonic velocimetric experiments, DNA concentrations were between 2 and 3 mM in nucleotide. Due to the sensitivity of existing acoustic and

densimetric instrumentation, it is difficult to conduct studies on DNA solutions at lower concentrations. 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 titration experiments, the concentration of netropsin ranged from 5 to 7 mM.

CD Spectroscopy. CD spectra were recorded at 25 °C using an Aviv Model 60 DS spectropolarimeter (Aviv Associates, Lakewood, NJ). CD titration profiles were measured by incrementally adding aliquots of netropsin to a cell containing a known amount of DNA.

Ultrasonic Velocimetry. Solution sound velocities, U, were measured at a frequency of 7.5 MHz using a previously described resonator method (Eggers & Funck, 1973; Sarvazyan, 1982; Eggers, 1992). To achieve the desired relative precision of $\sim 10^{-4}$%, the temperature difference between the sample and reference solutions must be on the order of 10^{-3} °C. This was accomplished using a previously described differential technique (Sarvazyan, 1982), in which two identical resonator cells (sample and reference) are placed in a common thermostated environment, with the difference in the ultrasound velocities in the two cells being measured. In our system, the ultrasonic resonator cells had volumes of 0.8 cm^3 and were thermostated at 25 °C, with an accuracy of ±0.01 °C. The accuracy of all sound velocity measurements achieved with this design is about $\pm 10^{-4}$% (Sarvazyan & Chalikian, 1991; Chalikian & Sarvazyan, 1991).

The characteristic of a solute derived from ultrasonic measurements is the relative molar sound velocity increment, $[U]$, which is equal to $(U - U_0)/(U_0C)$, where C is the molar concentration of a solute, and U and U_0 are the sound velocities in the solution and the solvent, respectively. Acoustic titration experiments were performed by adding equal aliquots of a 5–7 mM netropsin solution (prepared using the same buffer as the DNA solution) to both the sample and the reference cells, each containing 0.80 cm^3 of the DNA solution and the buffer, respectively. Additions were made using a 10-μL Hamilton syringe equipped with a Chaney adaptor (Hamilton Co., Reno, NV), which allows titrant delivery with a relative accuracy of 0.01%. In calculating the relative molar sound velocity increment, $[U]$, we took into account changes in the sound velocity in the solvent, U_0, and in the molar concentration of the solute, C, that result from addition of the netropsin solution. The change in the sound velocity relative increment, $\Delta[U]$, associated with netropsin binding to DNA was calculated using the equation

$$\Delta[U] = (U_{DNA+net} - U_{buf+net})(1 + V'/V'_0)/(U_{buf+net}C) - [U]_0 \quad (1)$$

where $U_{DNA+net}$ is the sound velocity in a solution formed by adding a netropsin solution with a volume of V' to a DNA solution with a volume of V'_0; $U_{buf+net}$ is the sound velocity in a solution formed by adding a netropsin solution with a volume of V' to a buffer solution with a volume of V'_0; C is the initial molar concentration of DNA; and $[U]_0$ is the relative molar sound velocity increment of the initial drug-free DNA.

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, Austria). The apparent molar volumes, ϕV, of DNA were calculated from the following well-known relationship (Millero, 1972):

$$\phi V = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C) \quad (2)$$

where M is molecular weight of DNA (average per nucleotide), and ρ and ρ_0 are the densities of the solution and the solvent (buffer), respectively. The volume changes, ΔV (per mole of DNA), accompanying netropsin binding to DNA were calculated using the equation

$$\Delta V = [(\rho - \rho_0) - (\rho' - \rho'_0)(1 + V'/V'_0)]/(\rho_0 C) \quad (3)$$

where V'_0 is the initial volume of the DNA solution with a concentration of C or the buffer solution in which the same volume of the netropsin solution, V', has been added (the value of V' is chosen so as to be slightly in excess of that needed to saturate the DNA by netropsin); ρ and ρ_0 are the densities of the initial netropsin-free DNA and buffer solutions, respectively; and ρ' and ρ'_0 are, respectively, the densities of the DNA and buffer solutions to which the netropsin solution has been added.

Determination of the Apparent Molar Adiabatic Compressibility. The relative molar sound velocity increments, $[U]$, determined as described above were used in conjunction with the apparent molar volume, ϕV, data to calculate the apparent molar adiabatic compressibility, ϕK_S, of DNA using the relationship (Barnatt, 1952; Owen & Simons, 1957)

$$\phi K_S = \beta_{S0}(2\phi V - 2[U] - M/\rho_0) \quad (4)$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent. Differentiation of eq 4 yields the expression

$$\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U]) \quad (5)$$

where ΔV and $\Delta[U]$ are the changes in the volume and the relative molar sound velocity increments of DNA accompanying saturation by netropsin, respectively. This relationship allows us to calculate the adiabatic compressibility change, ΔK_S, accompanying netropsin binding to a DNA structure.

RESULTS AND DISCUSSION

Molecular Interpretations of Volume and Compressibility Data

General Considerations. To interpret our volume and compressibility data in terms of hydration properties, we have used the following two well-known relationships (Shiio et al., 1955):

$$\phi V = V_M + \Delta V_h = V_M + n_h(V_h - V_0) \quad (6)$$

$$\phi K_S = K_M + \Delta K_h = K_M + n_h(K_{Sh} - K_{S0}) \quad (7)$$

where V_M and K_M are the intrinsic molar volume and the intrinsic 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.

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 a solute atomic group extend to a distance of 3–4 Å, which corresponds to 1–1.5 layers of water molecules (Buckin, 1987; Buckin et

al., 1989; Sarvazyan, 1991; Chalikian et al., 1993, 1994a). Thus, at least from the point of view of acoustodensimetric measurements, the quantity of hydration consists primarily of those water molecules that contact a solute directly. Consequently, the number of water molecules in the first monolayer around a solute can be considered as a lower limit for the hydration number, n_h, in eqs 6 and 7. This number can be defined as the ratio of the accessible surface area of a solute (S_M) to the effective cross section of a water molecule (S_{H_2O}). Thus, n_h is equal to S_M/S_{H_2O}, where the effective cross section of a water molecule, S_{H_2O}, is 9 Å^2. It should be noted here that, in most cases, when estimating the hydration number, n_h, of a low molecular weight solute, such as netropsin, its accessible surface area, S_M, can be approximated by its van der Waals area, S_W.

We already have described in detail how one can use eqs 6 and 7 and the reasoning noted above to interpret volume and compressibility data on DNA double helices in terms of duplex hydration properties as a function of base sequence, base composition, and conformation (Chalikian et al., 1994b). In this study, we consider how ligand binding to DNA structures can influence volume and compressibility data and how such influences can be interpreted in terms of hydration properties.

Specific Considerations for Drug–DNA Binding Events. (1) Counterion Release. When a positively charged ligand such as netropsin binds to duplex DNA, it induces counterion release, as reflected in the equilibrium

$$\text{DNA} + \text{net}^{2+} = \text{DNA–net} + k\text{Na}^+ \quad (8)$$

where k is the number of counterions released per molecule of bound netropsin.

In the vicinity of DNA, with its negatively charged phosphate groups, the effective charge on a condensed sodium counterion is substantially reduced, thereby diminishing its influence on surrounding water molecules compared with the corresponding influence it exerts when free in bulk water. Consequently, ligand binding induced release of counterions can be considered as "switching on" the hydration of previously unhydrated (actually less hydrated) sodium ions. In consideration of this effect, measured changes (per mole of nucleotide) in the volume, ΔV, and in the adiabatic compressibility, ΔK_S, upon drug binding to duplex DNA (eq 8) also can be expressed as

$$\Delta V = \phi V_C + (k/N)\Delta V_{hNa^+} - (\phi V_{DNA} + \phi V_{net^{2+}}/N) \quad (9)$$

$$\Delta K_S = \phi K_C + (k/N)\Delta K_{ShNa^+} - (\phi K_{DNA} + \phi K_{net^{2+}}/N) \quad (10)$$

where ϕV_{DNA}, $\phi V_{net^{2+}}$, and ϕV_C are the apparent molar volumes of drug-free DNA, free netropsin, and the netropsin–DNA complex, respectively; ϕK_{DNA}, $\phi K_{net^{2+}}$, and ϕK_C are the apparent molar adiabatic compressibilities of drug-free DNA, free netropsin, and the netropsin–DNA complex, respectively; and N is the binding density (in nucleotides per bound netropsin); while ΔV_{hNa^+} and ΔK_{ShNa^+} are, respectively, the volume and compressibility effects of sodium ion hydration.

(2) Binding-Induced Changes in the Hydration of the Reactants and Products. As noted above, sodium ions influence the surrounding water molecules when they are condensed within the hydration shell of a DNA duplex differently from when they are free in the bulk solvent. In addition, it is generally accepted that netropsin binding to the minor groove of duplex DNA causes the release of bound water molecules from the hydration shells of the free DNA duplex and the free drug. For these reasons, netropsin binding to duplex DNA should be accompanied by significant changes in hydration. Thus, a more complete formulation of eq 8 would be

$$\text{DNA} + \text{net}^{2+} = \text{DNA–net} + k\text{Na}^+ + \sum n_h' \quad (11)$$

in which the term $\sum n_h'$ can be either positive or negative and corresponds to the overall changes in the number of water molecules in the hydration shells of all species.

For each component of the process in which netropsin binds to duplex DNA, one can use eqs 6 and 7 to write an expression for the apparent molar volume and the apparent molar adiabatic compressibility. In this analysis, the sum of the intrinsic volumes, $\sum V_M$, or the intrinsic compressibilities, $\sum K_M$, of the reactants should be equal to the corresponding sum of the products of the reaction. Consequently, as can be seen from eqs 9 and 10, only changes in hydration should contribute to the measured values of ΔV and ΔK_S.

On the basis of the available microscopic and macroscopic data, it is reasonable to assume that netropsin binding to duplex DNA is accompanied by the release of $n_{net^{2+}}$ water molecules from the hydration shell of the drug (netropsin dehydration) and n_{DNA} water molecules from the hydration shell of the DNA (duplex dehydration). In consideration of these binding-induced changes in hydration, one can use eqs 6, 7, 9, and 10 to derive the following two relationships:

$$(\phi V_C - \phi V_{DNA})N - \phi V_{net^{2+}} = n_{DNA}(V_0 - V_{hDNA}) + n_{net^{2+}}(V_0 - V_{hnet^{2+}}) \quad (12)$$

$$(\phi K_{SC} - \phi K_{SDNA})N - \phi K_{Snet^{2+}} = n_{DNA}(K_{S0} - K_{ShDNA}) + n_{net^{2+}}(K_{S0} - K_{Shnet^{2+}}) \quad (13)$$

Combining eqs 9 and 10 with eqs 12 and 13, one obtains:

$$\Delta V = [n_{DNA}(V_0 - V_{hDNA}) + n_{net^{2+}}(V_0 - V_{hnet^{2+}}) + k\phi V_{Na^+}]/N \quad (14)$$

$$\Delta K_S = [n_{DNA}(K_{S0} - K_{ShDNA}) + n_{net^{2+}}(K_{S0} - K_{Shnet^{2+}}) + k\phi K_{Na^+}]/N \quad (15)$$

The use of eqs 9, 10, and 12–15 to calculate/interpret changes in the volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying drug binding to duplex DNA requires knowledge of the apparent molar volumes, ϕV, and the apparent molar adiabatic compressibilities, ϕK_S, of the components in reactions 8 and 11. One also must estimate values for the partial molar volumes, V_h, and the partial molar adiabatic compressibilities, K_{Sh}, of water in the hydration shells of these components. These determinations have been made, as will be described here.

(3) Netropsin. To assess the apparent molar volume, $\phi V_{net^{2+}}$, and the apparent molar adiabatic compressibility, $\phi K_{Snet^{2+}}$, of the dicationic form of netropsin, we measured ϕV and ϕK_S of netropsin dihydrochloride and subtracted from these values twice the partial molar volume, 22.9 ± 0.3 cm^3 mol^{-1} (Takenaka & Arakawa, 1989), and the partial molar adiabatic compressibility, $(-17 \pm 1) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} (Mathieson & Conway, 1974), of a chlorine ion, Cl^-. The apparent molar volume, $\phi V_{net^{2+}}$, derived in this way for dicationic netropsin equals 311.7 ± 1.5 cm^3 mol^{-1}, while the corresponding apparent molar adiabatic compressibility, $\phi K_{Snet^{2+}}$, equals $(11.6 \pm 2.0) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. On the basis of the ratio of the van der Waals surface area, S_W, of netropsin to the effective cross section of a water molecule, we estimated the hydration

Table 1: Absolute Values of the Relative Molar Sound Velocity Increments, $[U]$, the Apparent Molar Volumes, ϕV, and the Apparent Molar Adiabatic Compressibilities, ϕK_S, for the Poly(dA)·poly(dT) and Poly(dAdT)·Poly(dAdT) Duplexes and Their Netropsin Complexes at 16 mM $[Na^+]$

DNA	MW[a]	$[U]$ (cm^3 mol^{-1})	ϕV (cm^3 mol^{-1})	$\phi K_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})
poly(dAdT)·poly(dAdT)	331.7	68.0 ± 0.8[b]	170.0 ± 1.5[b]	−57.5 ± 2.0[b]
poly(dAdT)·poly(dAdT)–netropsin	374.7	67.9 ± 0.8	202.9 ± 1.5	−47.4 ± 2.0
poly(dA)·poly(dT)	331.7	67.0 ± 0.8[b]	169.0 ± 1.5[b]	−57.5 ± 2.0[b]
poly(dA)·poly(dT)–netropsin	374.7	62.8 ± 0.8	205.4 ± 1.5	−40.6 ± 2.0

[a] Molecular weight. [b] From Chalikian et al. (1994b).

number, n_h, of netropsin to be equal to 60. In this treatment, the van der Waals surface area, S_W, of netropsin was calculated as the sum of the accessible surface areas of the constituent atomic groups using the data of Bondi (1964). The partial molar adiabatic compressibility, K_{Sh}, of water in the hydration shell of netropsin then was calculated using eq 7, with the reasonableassumption that the intrinsic compressibility, K_M, of netropsin is negligible, as with other low molecular weight compounds (Sarvazyan, 1991). With these estimates, we calculated K_{Sh} for netropsin to be equal to 8.3×10^{-4} cm^3 mol^{-1} bar^{-1}, which is slightly higher than the compressibility of bulk water (8.1×10^{-4} cm^3 mol^{-1} bar^{-1}).

To estimate the partial molar volume, V_h, of water in the hydration shell of netropsin, we calculated the volume effect of netropsin hydration, ΔV_h, in eq 6 as the sum of the volume effects of hydration of the constituent atomic groups, an approach that has been employed previously (Kharakoz, 1992). Using eq 6, the partial molar volume, V_h, of water in the hydration shell of netropsin then was calculated to be equal to 17 cm^3 mol^{-1}, a value that is about 5% lower than 18.1 cm^3 mol^{-1}, the partial molar volume of bulk water. Thus, for the overall process of netropsin binding to duplex DNA, the dehydration of netropsin makes a positive contribution to the volume change, ΔV, and a small negative contribution to the compressibility change, ΔK_S.

(4) Netropsin–DNA Complexes. The apparent molar volumes, ϕV, and the apparent molar adiabatic compressibilities, ϕK_S, that we have measured for the complexes of netropsin with the poly(dAdT)·poly(dAdT) duplex and the poly(dA)·poly(dT) duplex are listed in Table 1. For comparison, and for use in our analysis, the corresponding values for the ligand-free DNA duplexes also are included in this table (Chalikian et al., 1994b). Previously, we have shown that, on average, the density of water in the hydration shell of the two DNA duplexes studied here is 20% higher than that of bulk water, while the coefficient of adiabatic compressibility of this water of hydration is 30% lower than that of bulk water (Chalikian et al., 1994b). These data allow us to calculate the partial molar volume, V_{hDNA}, and the partial molar adiabatic compressibility, K_{ShDNA}, of water in the hydration shell of a DNA duplex to be equal to 14.5 cm^3 mol^{-1} and 4.5 $\times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. Thus, the release of water molecules from the hydration shell of a DNA duplex (dehydration of DNA) induced by netropsin binding makes a positive contribution to the values we measure for the change in volume, ΔV, and the change in compressibility, ΔK_S.

(5) Sodium Ions. In aqueous solutions, sodium ions strongly electrostrict surrounding water molecules (Desnoyers et al., 1965). As a result, the partial molar volume and the partial molar adiabatic compressibility of water in the hydration shell of sodium ions are lower than those of bulk water. More specifically, the volume, ΔV_{hNa^+}, and compressibility, ΔK_{hNa^+}, effects of sodium ion hydration have been estimated to be equal to −15.5 ± 1.2 cm^3 mol^{-1} (Hirata & Arakawa, 1973; Conway, 1978; Abraham et al., 1982) and (−33.5 ± 1.0) $\times$ 10^{-4} cm^3 mol^{-1} bar^{-1} (Mathieson & Conway, 1974), respectively. Thus, the release of counterions from the hydration shell of a DNA duplex should contribute negatively to the measured values of both ΔV and ΔK_S.

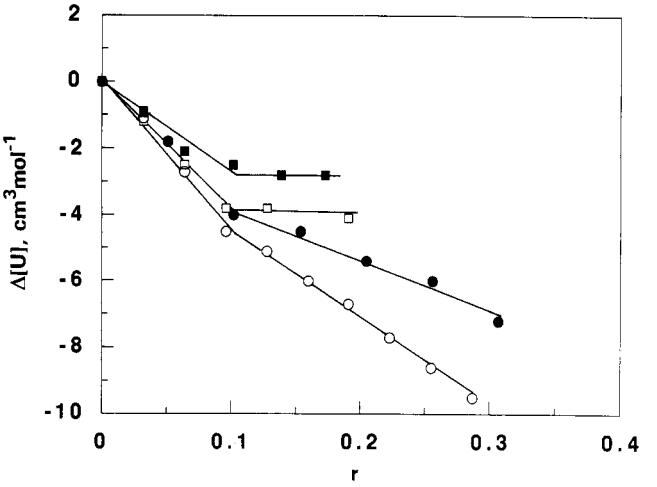

FIGURE 1: Changes in the relative molar sound velocity increment of the poly(dAdT)·poly(dAdT) duplex, $\Delta[U]$, versus the netropsin to DNA ratio, r, at 16 (○), 50 (●), 100 (□), and 200 mM (■) sodium ion concentration.

In the sections that follow, we present the volume and compressibility data we have measured for netropsin binding to duplex DNA and interpret these data in the context of the formalisms described in the previous section.

Netropsin Binding to the Heteropolymeric Duplex Poly(dAdT)·Poly(dAdT)

Figure 1 shows changes in the relative molar sound velocity increment, $\Delta[U]$, for the poly(dAdT)·poly(dAdT) duplex in the absence and presence of netropsin at various drug to DNA binding ratios, r, where $r = C(\text{net})/C(\text{DNA})$. Note that this r dependence of $\Delta[U]$ is shown at four different Na^+ ion concentrations, ranging from 16 to 200 mM. To confirm that netropsin, in fact, binds to duplex DNA under the conditions of our study, we measured CD spectra at 16 and 200 mM Na^+ ions and observed the induced signal at ~315 nm characteristic of bound netropsin (Marky et al., 1985; Park & Breslauer, 1992). In fact, our measured CD spectra (not shown) coincide with similar previously published measurements (Marky et al., 1985; Park & Breslauer, 1992).

Inspection of Figure 1 reveals that, at all ionic strengths, the relative molar sound velocity increment, $[U]$, decreases with increasing r, covering the range from 0 to approximately 0.1. Beyond a netropsin to duplex ratio of 0.1, the character of the $[U]$ versus r plots depends strongly on the sodium ion concentration. At high sodium ion concentrations (100 and 200 mM), the value of $[U]$ remains constant after $r = 0.1$. However, at lower sodium ion concentrations (16 and 50 mM), the value of $[U]$ continues to decrease, but with a less negative slope, $\Delta[U]/\Delta r$. This latter feature probably reflects non-

Table 2: Changes in the Relative Molar Sound Velocity Increments, $\Delta[U]$, Volumes, ΔV, and Adiabatic Compressibilities, ΔK_S, Accompanying Netropsin Binding to the Poly(dAdT)·Poly(dAdT) Duplex

	$C(Na^+)$ (mM)				
	16	50	100	200	1000
ΔV (cm^3 mol^{-1})	−2.5 ± 1.0 (−1.6[a], −2.7[b])	−0.5 ± 1.0	0 ± 1.0 (−0.1[a])	−1.0 ± 1.5	
$\Delta[U]$ (cm^3 mol^{-1})	−5.0 ± 0.5	−4.0 ± 0.5	−4.0 ± 0.5	−3.0 ± 0.8	
$\Delta K_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})	2.0 ± 1.3	3.0 ± 1.3	3.5 ± 1.3	2.0 ± 2.0	
secondary binding, $\Delta[U]/\Delta r$ (cm^3 mol^{-1})	−30 ± 5	−15 ± 5	0	0	

[a] From Marky and Kupke (1989), recalculated per mole of nucleotide. [b] From Vesnaver and Breslauer (1990).

specific secondary netropsin binding at low salt concentrations. Note that the lower the ionic strength of the solution, the more negative the secondary binding slope, $\Delta[U]/\Delta r$, a feature characteristic of an electrostatically driven binding event.

The drug to DNA ratio, r, plotted on the x-axis in Figure 1, is the inverse of the binding density, N. Thus, the r value of 0.1 at which the curves exhibit breaks corresponds to a binding density of 10 nucleotides (5 base pairs) per bound netropsin. This value derived from acoustic measurements coincides with previously published binding densities obtained from optical data (Wartell et al., 1974; Marky et al., 1985), thereby reinforcing the fact that these very different observables are monitoring the same binding event. It should be noted that $\Delta[U]$ measured at an r value of 0.1 corresponds to the change in the relative molar sound velocity increment, $[U]$, of the DNA duplex upon saturation by netropsin via its primary binding motif. These $\Delta[U]$ values were used in eq 5 for calculating the compressibility changes, ΔK_S.

Table 2 lists the changes in the relative molar sound velocity increment, $\Delta[U]$, volume, ΔV, and adiabatic compressibility, ΔK_S, of the poly(dAdT)·poly(dAdT) heteropolymeric duplex upon netropsin binding at sodium ion concentrations ranging from 16 to 200 mM. All of the listed values are normalized per mole of nucleotide. This feature must be kept in mind when comparing these data with those published in the literature, where changes in the corresponding thermodynamic characteristics usually are normalized per mole of bound drug. Inspection of the data in Table 2 reveals that primary netropsin binding to the poly(dAdT)·poly(dAdT) heteropolymeric duplex induces changes in both the volume, ΔV, and the adiabatic compressibility, ΔK_S, and that these changes, within experimental error, are independent of salt concentration. With regard to the volume changes, we find very good agreement between our 16 mM sodium ion data and those obtained by Vesnaver and Breslauer (1990). We also find reasonably good agreement between our data at 16 and 100 mM sodium ion concentrations and another previously published determination, albeit at 20 rather than 25 °C (Marky & Kupke, 1989).

Application of eqs 9 and 10 to the experimental data allows us to calculate the number of sodium ions, k, released per bound netropsin. On the basis of the the volume data and eq 9, we obtain a k value of 2.7 ± 1.0. From the compressibility data and eq 10, we calculate k to be 2.1 ± 1.0. Both values are gratifyingly close to 2, the theoretical number of sodium ions that should be released from a DNA duplex per bound netropsin in order to maintain electroneutrality. This agreement supports the assumptions employed in our analysis, as well as the accuracy of our experimental data.

Netropsin Binding to the Homopolymeric Duplex Poly(dA)·Poly(dT)

Figure 2 shows the changes in the relative molar sound velocity increment, $[U]$, for the poly(dA)·poly(dT) duplex in the absence and presence of netropsin at five different Na^+ ion concentrations, ranging from 16 to 1000 mM. As in the case of the heteropolymeric poly(dAdT)·poly(dAdT) duplex, the binding densities, N, derived from our ultrasonic velocity data coincide with those derived from previously published CD measurements (Marky et al., 1985; Park & Breslauer, 1992) and equal about 10 nucleotides (or 5 base pairs) per bound drug.

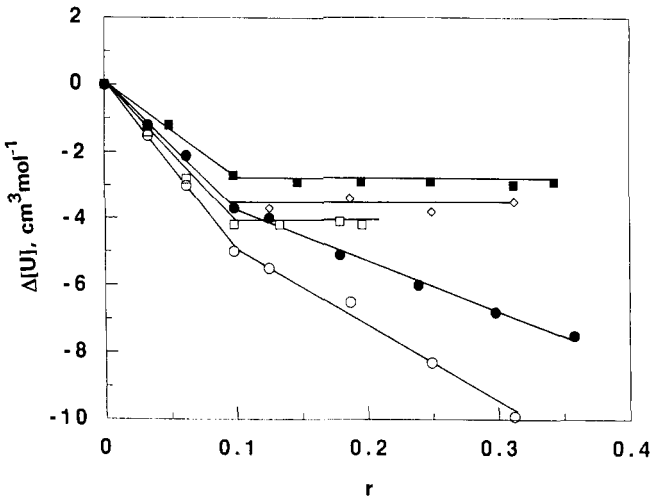

FIGURE 2: Changes in the relative molar sound velocity increment of the poly(dA)·poly(dT) duplex, $\Delta[U]$, versus the netropsin to DNA ratio, r, at 16 (○), 50 (●), 100 (□), 200 (■), and 1000 mM (◇) sodium ion concentration.

Inspection of the trends in Figure 2 reveals that, at low sodium ion concentrations (16 and 50 mM), we once again observe secondary binding. As with the poly(dAdT)·poly(dAdT) duplex, this secondary binding is salt dependent and disappears at Na^+ ion concentrations higher than 100 mM. It is interesting to note that, in contrast to CD, the acoustic observable detects this secondary binding event. This observation suggests that acoustic techniques may provide a more general approach for detecting binding events that do not produce a characteristic optical response.

Table 3 lists the changes in the relative molar sound velocity increment, $\Delta[U]$, volume, ΔV, and adiabatic compressibility, ΔK_S, of the poly(dA)·poly(dT) homopolymeric duplex upon netropsin binding at sodium ion concentrations ranging from 16 to 1000 mM. The ΔV value we measure for this duplex at 16 mM sodium ion is in excellent agreement with the corresponding value reported by Vesnaver and Breslauer (1990). By contrast, apparently poor agreement is found between the volume changes, ΔV, we measure at 16 and 100 mM sodium ion concentrations and the one previously published determination at 20 °C (Marky & Kupke, 1989); the discrepancy at 16 mM $[Na^+]$ exceeds 100% (5.7 cm^3 mol^{-1}). Such disparities, however, can be expected if one recalls that measured values of ΔV correspond to differences between large effects (see eq 9). Furthermore, the value of 5.7 cm^3 mol^{-1} is only 2–3% of the apparent molar volumes of

Table 3: Changes in the Relative Molar Sound Velocity Increments, $\Delta[U]$, Volumes, ΔV, and Adiabatic Compressibilities, ΔK_S, Accompanying Netropsin Binding to the Poly(dA)·Poly(dT) Duplex

	$C(Na^+)$ (mM)				
	16	50	100	200	1000
ΔV (cm^3 mol^{-1})	4.0 ± 1.0 (9.7[a], 4.7[b])	5.0 ± 1.0	5.0 ± 1.0 (6.8[a])	5.0 ± 1.5	4.0 ± 1.5
$\Delta[U]$ (cm^3 mol^{-1})	−5.0 ± 0.5	−4.0 ± 0.5	−4.0 ± 0.5	−3.0 ± 0.8	−3.5 ± 1.0
$\Delta K_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})	8.0 ± 1.3	8.0 ± 1.3	8.0 ± 1.3	7.0 ± 2.0	7.0 ± 2.3
secondary binding, $\Delta[U]/\Delta r$ (cm^3 mol^{-1})	−30 ± 5	−15 ± 5	0	0	0

[a] From Marky and Kupke (1989), recalculated per mole of nucleotide. [b] From Vesnaver and Breslauer (1990).

either netropsin, duplex DNA, or the DNA–netropsin complexes (see Table 1). If these apparent molar volumes have different temperature slopes, the apparent discrepancy simply may reflect the fact that our measurements were performed at a temperature 5 °C higher than those reported in the literature (Marky & Kupke, 1989). Clearly, additional temperature-dependent studies are required to explore this possibility.

Using eq 9 and the volume data listed in Table 3, we calculate a k value of 0.8 ± 1.0 for the number of sodium ions released per netropsin bound. Using eq 10 and the compressibility data listed, we calculate a k value of 2.3 ± 1.0. Considering the assumptions involved in this analysis, as well as experimental error, we are pleased that, with the uncertainties noted, these calculated k values qualitatively agree with the theoretical value of 2 required for electroneutrality.

Poly(dAdT)·Poly(dAdT) versus Poly(dA)·Poly(dT) as Host Duplexes for Netropsin Binding

Inspection of the data in Tables 1–3 reveals some intriguing differences and similarities between the two host duplexes. First, netropsin binding to the heteropolymeric poly(dAdT)·poly(dAdT) duplex is accompanied by negative changes in volume, ΔV, and small positive changes in compressibility, ΔK_S. In contrast, netropsin binding to the homopolymeric poly(dA)·poly(dT) duplex is accompanied by large positive changes in both the volume, ΔV, and the compressibility, ΔK_S. Second, both drug-free duplexes exhibit nearly the same values for their partial molar volumes, ϕV, and their partial molar adiabatic compressibilities, ϕK_S, at 16 mM sodium ion. In contrast, the drug-bound duplexes have different values of ϕV and ϕK_S, with the poly(dA)·poly(dT)–netropsin complex exhibiting higher values of ϕV and ϕK_S than the poly(dAdT)·poly(dAdT)–netropsin complex. Third, we find that the difference between ϕV or ϕK_S of the two drug–DNA complexes coincides, within experimental error, with the difference we measure for the change in volume, ΔV, or compressibility, ΔK_S, that occurs upon netropsin binding at 16 mM Na^+ to the two duplexes.

In the aggregate, the data in Tables 1–3 can be interpreted in terms of the differential hydration effects associated with netropsin binding to the two all-AT DNA duplexes studied here. As will be discussed later, we interpret the higher ϕV and ϕK_S values for the poly(dA)·poly(dT)–netropsin complex compared with the poly(dAdT)·poly(dAdT)–netropsin complex as reflecting, in part, a higher degree of hydration for the latter complex. Thus, as will be elaborated in the next section, we propose that the differences we measure in ΔV and ΔK_S for netropsin binding to the poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) duplexes reflect, in part, differences between the hydration of the final state netropsin–DNA complexes rather than exclusively differences between the hydration of the initial state drug-free homopolymeric and heteropolymeric duplexes.

Resolving Binding-Induced Changes in Hydration. As discussed in the previous section, the volume, ΔV, and the compressibility, ΔK_S, changes we have measured for netropsin binding to duplex DNA can be envisioned as reflecting several effects, some of which are mutually compensating: (i) drug-induced dehydration of the free DNA duplex relative to its drug-bound state; (ii) binding-induced dehydration of free netropsin relative to bound netropsin; and (iii) changes in the hydration of condensed versus free sodium ions. Resolution the contributions of each event to the volume, ΔV, and compressibility, ΔK_S, changes can be accomplished if one accepts the following three stipulations. First, on the basis of structural data and steric considerations (Kopka et al., 1985), one can estimate that netropsin binding to either the poly(dAdT)·poly(dAdT) or the poly(dA)·poly(dT) duplex results in the release of about 75% of the solvent molecules that hydrate the drug (approximately 45 waters). Second, as a first approximation, binding-induced dehydration of netropsin is assumed to exhibit the same contribution to ΔV and ΔK_S for either of the two all-AT duplexes. Third, as already discussed, the binding-induced release of counterions (~2 per bound netropsin) can be considered as switching on the hydration of sodium ions. With these three stipulations, the number of water molecules released from or added to the hydration shells of the DNA duplexes upon netropsin binding can be calculated using eqs 12–15, as will be explained here.

Netropsin Binding to the Poly(dA)·Poly(dT) Duplex Induces Release of about 18 More Water Molecules Than Netropsin Binding to the Poly(dAdT)·Poly(dAdT) Duplex. By applying eq 12 to the volume data, we calculate that netropsin binding to the poly(dAdT)·poly(dAdT) duplex causes an uptake of 9 waters into the hydration shell of this all-AT heteropolymeric duplex; that is, $n_{DNA} = -9$. A similar negative n_{DNA} value of −12 is obtained by applying eq 14. Thus, on the basis of these numbers, netropsin binding to the poly(dAdT)·poly(dAdT) duplex appears to induce a net increase in hydration. For the homopolymeric poly(dA)·poly(dT) duplex, eqs 12 and 14 yield n_{DNA} values of 1 and 6, respectively, which imply small decreases in the hydration of this DNA duplex upon netropsin binding. However, the absolute magnitudes of these n_{DNA} values should be viewed with caution since they are highly dependent on the estimate of the partial molar volume, V_h, of water in the hydration shell of netropsin. Instead, we will focus on differences between n_{DNA} values, since such Δn_{DNA} data are not dependent on the accuracy of the additivity estimate used to calculate the partial molar volume, $V_{hnet^{2+}}$, of water in the hydration shell of netropsin. In fact, as is implicit in eq 14 and explicit in eq 16, Δn_{DNA} can be calculated directly from the difference between the values of ΔV corresponding to the netropsin binding to the poly(dA)·poly(dT) and poly(dAdT)·poly(dAdT) duplexes ($\Delta\Delta V$). If $\Delta\Delta V$ is expressed per mole of drug bound, then the following relationship can be written:

$$\Delta n_{DNA} = \Delta\Delta V/(V_0 - V_{hDNA}) \quad (16)$$

Using this expression, we calculate the differences between n_{DNA} for the poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) duplexes to be about 18 water molecules. In other words, netropsin binding to the homopolymeric poly(dA)·poly(dT) duplex causes the release of 18 more water molecules than netropsin binding to the heteropolymeric poly(dAdT)·poly(dAdT) duplex.

A similar analysis can be performed using the compressibility data. Specifically, by applying eq 13, we calculate that netropsin binding to the poly(dAdT)·poly(dAdT) duplex causes the release of 22 waters from the hydration shell of this all-AT heteropolymeric duplex. The same result is obtained by applying eq 15. For the homopolymeric poly(dA)·poly(dT) duplex, eqs 13 and 15 yield similar values of n_{DNA} equal to 41 and 38, respectively. In contrast to the volume-based calculations, we believe the absolute numbers derived from the compressibility data to be more reliable. The reason for this expectation is that, in the compressibility-based calculations, the negligibility of the intrinsic compressibility is the only assumption employed to estimate the partial molar adiabatic compressibility, $K_{Shnet^{2+}}$, of water in the hydration shell of netropsin. This assumption has been shown to be correct for low molecular weight solutes such as netropsin (Buckin et al., 1989; Sarvazyan, 1991; Chalikian et al., 1994c). Thus, analysis of our compressibility data suggests that netropsin binding causes dehydration of both all-AT host duplexes. As with the volume data, the compressibility data can be analyzed using eq 17 to calculate Δn_{DNA}, the difference between the number of waters released from the hydration shells of the poly(dA)·poly(dT) and poly(dAdT)·poly(dAdT) duplexes upon netropsin binding:

$$\Delta n_{DNA} = \Delta\Delta K_S/(K_{S0} - K_{ShDNA}) \quad (17)$$

where $\Delta\Delta K_S$ is expressed per mole of bound drug. Using this expression, we calculate a Δn_{DNA} value of 17. It is gratifying that this differential estimate agrees well with the value of 18 calculated from the volume data.

In summary, analysis of our compressibility data reveals that netropsin binding to the homopolymeric poly(dA)·poly(dT) duplex causes the release of about 40 water molecules from its hydration shell, while netropsin binding to the heteropolymeric poly(dAdT)·poly(dAdT) duplex causes the release of about 22 water molecules from its hydration shell. In other words, netropsin binding to the poly(dA)·poly(dT) duplex causes the release of about 18 more water molecules than netropsin binding to the poly(dAdT)·poly(dAdT) duplex.

The Netropsin-Bound Duplex Is More Hydrated When Poly(dAdT)·Poly(dAdT) Serves as the Host Structure. As noted above, our data reveal a larger net change in hydration for netropsin binding to the poly(dA)·poly(dT) duplex than binding to the poly(dAdT)·poly(dAdT) duplex. Generally speaking, this conclusion is consistent with previously published interpretations of entropy, enthalpy, and volumetric data associated with netropsin binding to these two all-AT polymeric duplexes (Marky & Breslauer, 1987; Marky & Kupke, 1989). In these previous studies, changes in observables were interpreted exclusively in terms of differences in the properties of the initial drug-free duplex states, while equivalent properties for the final drug-bound duplex states were implicitly assumed. These microscopic interpretations of the entropy (Marky & Breslauer, 1987) and volume (Marky & Kupke, 1989) data assumed the initial drug-free poly(dAdT)·poly(dAdT) duplex to exist in a B-conformation, with the poly(dA)·poly(dT) duplex existing as a more hydrated altered B′-conformation (Leslie et al., 1980; Thomas & Peticolas, 1983; Alexeev et al., 1987), which may undergo a solvent-releasing, helix-to-helix conformational transition to the B-form upon drug binding (Zimmer & Wahnert, 1986; Herrera & Chaires, 1989; Breslauer et al., 1987).

In short, these previous studies rationalized their overall differences in observables by focusing exclusively on the differences in the extent of hydration of the initial drug-free duplexes. On the other hand, we find that at 16 mM Na^+ the two drug-free duplexes exhibit nearly the same values for their partial molar volumes, ϕV, and their partial molar adiabatic compressibilities, ϕK_S. Since these two observables are sensitive to solute–solvent interactions, our data suggest that the two drug-free duplexes exhibit similar overall hydration properties. In contrast, the two drug-bound duplexes exhibit different values of ϕV and ϕK_S. Thus, in contrast to previous interpretations by us and others, we now propose that the greater change in hydration associated with netropsin binding to the poly(dA)·poly(dT) duplex versus the poly(dAdT)·poly(dAdT) duplex in large part reflects the formation of a less hydrated final poly(dA)·poly(dT)–netropsin complex rather than a more hydrated initial drug-free poly(dA)·poly(dT) duplex. In connection with this interpretation, it should be emphasized that the observables ϕV and ϕK_S are sensitive to the entire population of solute-perturbed water molecules, not just those associated with the minor groove. Thus, the differences in hydration properties of the two all-AT duplexes in their final drug-bound states should be understood in terms of differences in the total hydration rather than exclusively in terms of differences in minor groove hydration, the feature on which most previous discussions have focused. The differences we observe for the drug-bound states also, in part, may reflect differences in the conformations adopted by the poly(dA)·poly(dT) and poly(dAdT)·poly(dAdT) duplexes upon netropsin binding (Breslauer et al., 1987; Herrera & Chaires, 1989).

In summary, the interpretation described above contrasts with previous proposals in which differences in $\Delta S°$ and ΔV for netropsin binding to the poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) duplexes were interpreted exclusively in terms of differences between the hydration of the initial drug-free states of the two all-AT duplexes (Marky & Breslauer, 1987; Marky & Kupke, 1989). In general, when developing hydration-based interpretations of $\Delta S°$, ΔV, and ΔK_S data, one also should consider differences in the hydration of the final states rather than focusing exclusively on the initial states.

Relationships between the Volume, Compressibility, and Entropy Changes Accompanying Netropsin Binding to the Two All-AT Host Duplexes. We previously have reported the entropy changes, $\Delta S°$, associated with netropsin binding to the same two all AT-duplexes studied here. Consequently, we now can evaluate how ΔV and ΔK_S changes correlate with $\Delta S°$ changes and assess how these three observables interrelate in their capacities to report on changes in hydration. To facilitate these comparisons, Table 4 lists the differential values of these three observables for the two binding events studied here, along with the associated differences in the number of water molecules released.

Inspection of the data in this table reveals the intuitively satisfying result that positive changes in ΔV and ΔK_S ($\Delta\Delta V$ = +65 cm^3 mol^{-1} and $\Delta\Delta K_S$ = +60 × 10^{-4} cm^3 mol^{-1} bar^{-1}) are associated with positive changes in $\Delta S°$ ($\Delta\Delta S°$ = +28 cal K^{-1} mol^{-1}) and that these positive changes are associated with the net release of water molecules (Δn_{DNA} = +18 water

Table 4: Differences in the Changes in Volume, $\Delta\Delta V$, Adiabatic Compressibility, $\Delta\Delta K_S$, Entropy, $\Delta\Delta S°$, and the Number of Water Molecules Released upon Netropsin Binding to the Poly(dA)·Poly(dT) and Poly(dAdT)·Poly(dAdT) Duplexes at 16 mM [Na^+]

process	$\Delta\Delta V$ (cm^3 mol^{-1})	$\Delta\Delta K_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})	$\Delta\Delta S°$ [a] (cal mol^{-1} K^{-1})	Δn_{DNA} (mol)
[poly(dA)·poly(dT)–netropsin] – [poly(dAdT)·poly(dAdT)–netropsin]	65	60	28	18

[a] From Marky and Breslauer (1987).

molecules). More quantitatively, we find that an entropy differential of +28 cal K^{-1} (mol of bound netropsin)$^{-1}$ is associated with the net release of 18 water molecules. Thus, each water of hydration released to the bulk solvent contributes ~1.6 cal K^{-1} mol^{-1} to the observed entropy change.

If one makes the reasonable assumption that the difference in entropy changes, $\Delta S°$, predominantly reflects differences in hydration, then in a manner similar to eqs 16 and 17, one can write the expression

$$\Delta\Delta S° = \Delta n_{DNA}(S_0 - S_{hDNA}) \quad (18)$$

where S_0 and S_{hDNA} are the partial molar entropies of water in the bulk state and in the hydration shell of DNA, respectively. This treatment assumes that the partial molar entropy, S_{hDNA}, of the solvating water is the same for both the homopolymeric and heteropolymeric all-AT duplexes.

Equation 18 allows us to calculate the difference between the partial molar entropies of water in the bulk state and in the hydration shell of DNA, $S_0 - S_{hDNA}$. To avoid computational coupling of parameters, eq 18 should be applied only when $\Delta\Delta S°$ and Δn_{DNA} are determined by independent means, as has been done here. Using the Δn_{DNA} value of 18 derived from our volume and compressibility data and the $\Delta\Delta S°$ value (28 cal K^{-1} mol^{-1}) obtained calorimetrically (Marky & Breslauer, 1987), we calculate the partial molar entropy difference, $S_0 - S_{hDNA}$, to be 1.6 cal K^{-1} mol^{-1}. Thus, water in the hydration shell of DNA duplexes (more specifically, of the two all-AT duplexes studied here) is characterized not only by a lower partial molar volume and a lower partial molar compressibility (Chalikian et al., 1994b) but also by a lower partial molar entropy relative to bulk water.

This value of $S_0 - S_{hDNA}$ may prove to be independent of the type of bound ligand. If so, it will provide a basis for calculating the difference in the number of waters released upon the binding of any ligand to the poly(dA)·poly(dT) and poly(dAdT)·poly(dAdT) duplexes simply from measured values of $\Delta\Delta S°$. Additional studies are underway to assess the generality of the $S_0 - S_{hDNA}$ value determined in this work.

Netropsin Binding to the Poly(dT)·Poly(dA)·Poly(dT) Triplex

As previously described, a negative CD band at 217 nm can be used to detect the formation and existence of the poly(dT)·poly(dA)·poly(dT) triple helix (Park & Breslauer, 1992). Using this criterion, we confirmed triple-helix formation at 200 mM sodium ion concentration and 25 °C, when equal amounts of the poly(dT) single strand and the poly(dA)·poly(dT) duplex were mixed. However, under these conditions, netropsin binding induces displacement of the third strand to produce the netropsin-bound poly(dA)·poly(dT) duplex and the free poly(dT) single strand. This process causes an increase of 6 cm^3 mol^{-1} in the relative molar sound velocity increment, $[U]$ (see Figure 3), and a volume decrease of 28 cm^3 mol^{-1}. Using eq 4, we calculate that this displacement reaction is accompanied by a decrease in compressibility of 31 × 10^{-4} cm^3 mol^{-1} bar^{-1}. On the basis of eqs 6 and 7, these changes

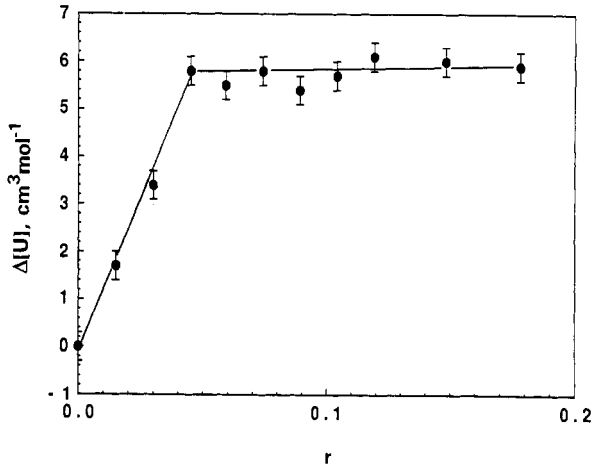

FIGURE 3: Changes in the relative molar sound velocity increment of the poly(dT)·poly(dA)·poly(dT) triplex, $\Delta[U]$, versus the netropsin to DNA ratio, r, at 200 mM sodium ion concentration. Under these conditions, netropsin binding induces displacement of the third strand (see text).

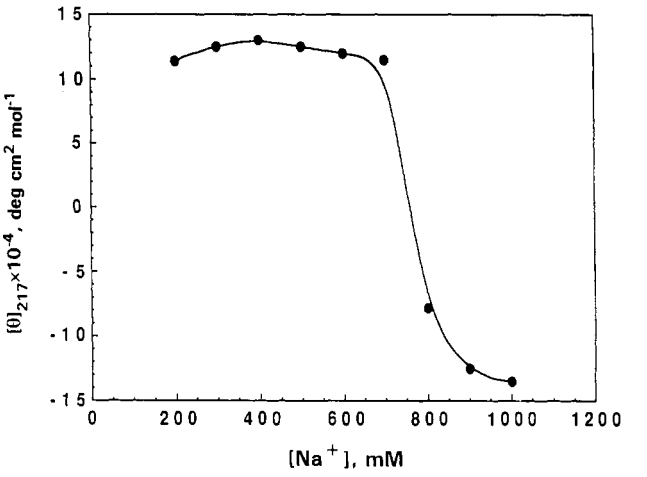

FIGURE 4: Molar ellipticity (per mole of nucleotides) versus sodium ion concentration for a solution containing the poly(dA)·poly(dT) duplex saturated by netropsin and a free poly(dT) strand at 217 nm. At 800 mM sodium ion, a sharp duplex-to-triplex transition occurs (see text).

can be interpreted as suggesting an increase in the overall hydration due to an increase in the total accessible surface area of DNA upon expulsion of the third strand.

Inspection of Figure 5 reveals that increasing the ionic strength to 800 mM sodium ion results in a sharp duplex-to-triplex transition for the DNA–netropsin complex. On the basis of this observation, we have chosen a 1 M sodium ion concentration as a solution condition under which the poly(dT)·poly(dA)·poly(dT) triplex will maintain its triplex structure, even when netropsin binds to its minor groove. Table 5 lists the changes in the relative molar sound velocity increment, $\Delta[U]$, volume, ΔV, and adiabatic compressibility, ΔK_S, we have determined for netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex at 1 M Na^+.

Figure 5 shows the changes in the relative molar sound velocity increment, $[U]$, of the poly(dT)·poly(dA)·poly(dT)

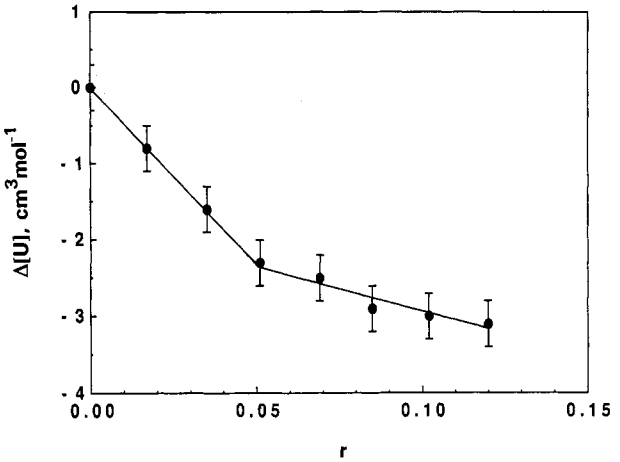

FIGURE 5: Changes in the relative molar sound velocity increment of the poly(dT)·poly(dA)·poly(dT) triplex, $\Delta[U]$, versus the netropsin to DNA ratio, r, at 1 M sodium ion concentration.

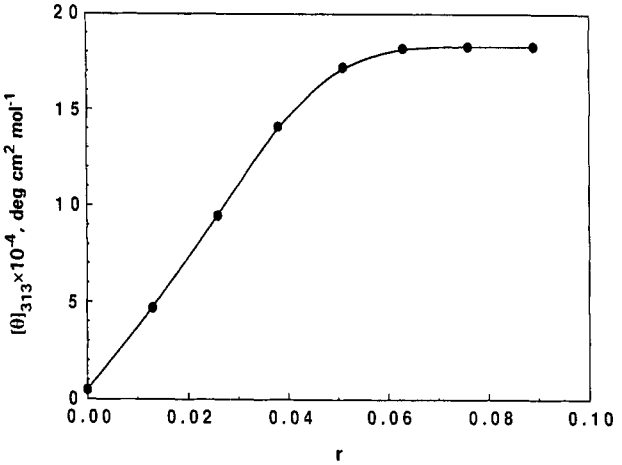

FIGURE 6: Molar ellipticity (per mole of nucleotides) at 313 nm of a solution containing the poly(dT)·poly(dA)·poly(dT) triplex versus the netropsin to DNA ratio, r, at 1 M sodium ion concentration.

Table 5: Changes in the Relative Molar Sound Velocity Increments, $\Delta[U]$, Volume, ΔV, and Adiabatic Compressibility, ΔK_S, of the Poly(dT)·Poly(dA)·Poly(dT) Triplex upon Netropsin Binding at 1 M $[Na^+]$

ΔV (cm^3 mol^{-1})	3.5 ± 1.5
$\Delta[U]$ (cm^3 mol^{-1})	−3.0 ● 1.0
$\Delta K_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})	6.0 ± 2.3
secondary binding, $\Delta[U]/\Delta r$ (cm^3 mol^{-1})	−15 ± 5

Table 6: Changes per Mole of Bound Drug in the Relative Molar Sound Velocity Increments, $\Delta[U]$, Volumes, ΔV, and Adiabatic Compressibilities, ΔK_S, of the Poly(dT)·Poly(dA)·Poly(dT) Triplex and the Poly(dA)·Poly(dT) Duplex upon Netropsin Binding at 1 M $[Na^+]$

	duplex	triplex
ΔV (cm^3 mol^{-1})	40	75
$\Delta[U]$ (cm^3 mol^{-1})	−35	−65
$\Delta K_S \times 10^4$ (cm^3 mol^{-1} bar^{-1})	70	125

triplex at 1 M Na^+ as a function of r, the netropsin to DNA ratio. Figure 6 presents the corresponding CD titration curve. Note that both the acoustic and CD titration data reveal the primary ligand binding density, N, to be about 20 nucleotides (7 triplets) per bound netropsin. This reduced binding density of netropsin for the triplex versus the duplex state is consistent with a previous optical study (Park & Breslauer, 1992). Further inspection of Figure 5 reveals that netropsin also exhibits a secondary binding mode to the triplex structure, even at a salt concentration as high as 1 M. Recall that, for the two all-AT duplexes, secondary binding disappeared at sodium ion concentrations above 100 mM. This differential behavior may be due to the higher charge density of triplex versus duplex DNA. It is interesting to note that, in contrast to CD, the acoustic observable detects this secondary binding event, as it does with duplex DNA. This observation suggests that acoustic techniques may provide a more general approach for detecting triplex binding events that do not produce a characteristic optical response.

Differential Impact of Netropsin Binding to the Duplex and Triplex States

Comparison of the data in Tables 3 and 5 reveals that the volume and compressibility changes per mole of nucleotide for netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex at 1 M Na^+ are similar to those that accompany netropsin binding to the poly(dA)·poly(dT) duplex. However, if one expresses these changes per mole of bound drug rather than per mole of nucleotide, the changes in volume and/or compressibility upon netropsin binding to the triplex and duplex states become distinguishable (see data in Table 6). Specifically, we find that netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex causes changes in both volume and compressibility that are nearly twice as large as those observed when netropsin binds to the poly(dA)·poly(dT) duplex. This large differential can be interpreted as suggesting that netropsin binding induces more dehydration of the triplex structure than the duplex structure. This difference may reflect the larger netropsin binding site size associated with ligand binding to the triplex (7 triplets) versus the duplex (5 base pairs) state. Equations 14 and 15 can be used to obtain a quantitative estimate of this differential dehydration. For such an analysis, we assume that netropsin binding to the triplex and duplex states results in the same degree of dehydration of the drug and induces the same number of released counterions. We also assume that water in the hydration shell of both the duplex and the triplex exhibits the same partial molar volume, V_h, and partial molar adiabatic compressibility, K_{Sh}. Given these assumptions, eqs 14 and 15 can be recast into eqs 19 and 20, which enable us to calculate Δn_{DNA}, the difference in the number of water molecules released from the hydration shells of the triplex and duplex states upon netropsin binding:

$$\Delta n_{DNA} = (\Delta V_T N_T - \Delta V_D N_D)/(V_0 - V_{hDNA}) \quad (19)$$

$$\Delta n_{DNA} = (\Delta K_T N_T - \Delta K_D N_D)/(K_{Sh0} - K_{ShDNA}) \quad (20)$$

where Δn_{DNA} is as defined previously; ΔV_T and ΔV_D are the volume changes upon netropsin binding to the triplex and duplex, respectively; ΔK_T and ΔK_D are the compressibility changes upon netropsin binding to the triplex and duplex, respectively; and N_T and N_D are the binding densities for the triplex and duplex, respectively.

Netropsin Binding to the Poly(dT)·Poly(dA)·Poly(dT) Triplex Results in the Release of 13 More Water Molecules Than Netropsin Binding to the Poly(dA)·Poly(dT) Duplex. Applying eqs 19 and 20 to the data listed in Table 5, we calculate that netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex causes the release of 13 more water molecules than netropsin binding to the poly(dA)·poly(dT) duplex. Consequently, netropsin binding to the triplex structure causes

the release of 53 water molecules from its hydration shell. Further work is required to explain this intriguing observation in terms of differential conformations/hydration of the initial and final states. However, our observation provides an important result/constraint with which any hydration-based explanation must be consistent.

CONCLUDING REMARKS

We have used acoustic and densimetric techniques to measure the changes in volume, ΔV, and adiabatic compressibility, ΔK_S, that accompany netropsin binding to duplex and triplex DNA structures. We describe how these data can be used in conjunction with apparent molar volume and compressibility data to define the relative hydration properties of the initial and final states. Specifically, the following general features emerge from our interpretation of the data.

1. DNA binding induced dehydration of netropsin makes a positive contribution to ΔV and a small negative contribution to ΔK_S.

2. Netropsin binding induced dehydration of DNA makes positive contributions to both ΔV and ΔK_S.

3. Netropsin binding induced release of DNA-condensed sodium ions makes negative contributions to both ΔV and ΔK_S.

4. Changes in the relative molar sound velocity increment, $\Delta[U]$, as a function of the netropsin to duplex DNA ratio, r, define salt-independent binding densities, equivalent to those determined by optical titration curves, while also revealing a secondary binding event at low salt that is not detected by optical measurements.

5. Netropsin binding to the heteropolymeric poly(dAdT)·poly(dAdT) duplex is accompanied by negative changes in volume, ΔV, and small positive changes in compressibility, ΔK_S. In contrast, netropsin binding to the homopolymeric poly(dA)·poly(dT) duplex is accompanied by large positive changes in both the volume, ΔV, and the compressibility, ΔK_S.

6. Both drug-free duplexes exhibit nearly the same values for their partial molar volumes, ϕV, and their partial molar adiabatic compressibilities, ϕK_S, at 16 mM sodium ion. In contrast, the poly(dA)·poly(dT)–netropsin complex exhibits higher values of ϕV and ϕK_S than the poly(dAdT)·poly(dAdT)–netropsin complex. We interpreted the higher ϕV and ϕK_S values for the poly(dA)·poly(dT)–netropsin complex compared with the poly(dAdT)·poly(dAdT)–netropsin complex as reflecting, in part, a higher degree of hydration for the latter complex. We therefore proposed that the differences we measured in ΔV and ΔK_S for netropsin binding to the poly(dAdT)·poly(dAdT) and poly(dA)·poly(dT) duplexes reflect, in part, differences between the hydration of the final state netropsin–DNA complexes rather than exclusively differences between the hydration of the initial state drug-free homopolymeric and heteropolymeric duplexes.

7. We found that netropsin binding to the poly(dA)·poly(dT) duplex causes the release of about 40 water molecules, which is about 18 more waters than are released when netropsin binds to the poly(dAdT)·poly(dAdT) duplex.

8. We calculated that each water of hydration released to the bulk solvent by netropsin binding contributes 1.6 cal K^{-1} mol^{-1} to the observed drug binding entropy, $\Delta S°$. This value corresponds to the average difference between the partial molar entropies of water in the bulk state and in the hydration shell of the two all-AT duplexes studied here.

9. We found that netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex causes changes in both the volume and compressibility that are nearly twice as large as those observed when netropsin binds to the poly(dA)·poly(dT) duplex. We interpreted this large differential as suggesting that netropsin binding induces more dehydration of the triplex structure than of the duplex structure. Specifically, we calculated that netropsin binding to the poly(dT)·poly(dA)·poly(dT) triplex causes the release of about 53 water molecules, which is 13 more waters than are released when netropsin binds to the poly(dA)·poly(dT) duplex.

We discussed the relationships between macroscopic and microscopic properties, particularly how measured changes in volume, ΔV, and compressibility, ΔK_S, can be interpreted in terms of the hydration properties of DNA structures in their ligand-free and ligand-bound states.

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