Probing the Hydration of the Minor Groove of A·T Synthetic DNA Polymers by Volume and Heat Changes†

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ABSTRACT: The minor-groove ligand netropsin provides a sensitive probe of the hydration difference between poly(dA)·poly(dT) and poly[d(AT)]·poly[d(AT)]. We have measured the volume change ΔV accompanying binding of netropsin to these polymers, using an improved magnetic suspension densimeter. For poly(dA)·poly(dT) we find $\Delta V = +97$ mL/mol of bound netropsin at pH 7.0 and 10 mM sodium phosphate buffer. For poly[d(AT)]·poly[d(AT)] we find $\Delta V = -16$ mL/mol of bound netrospin. This striking differential effect suggests that the poly(dA)·poly(dT) duplex compresses more water (or is more extensively hydrated). From our enthalpy and entropy results we estimate that approximately 10 water molecules, immobilized in the minor groove of this system, are displaced by each netropsin bound. The volume increase, however, is substantially larger than can be explained by a simple melting of these immobilized water molecules in the minor groove. A decompression of at least 40 water molecules must attend the complexation to the poly(dA)·poly(dT) duplex. This suggests that the conformation change attending the binding of the drug to this polymer duplex causes a further dehydration, whereas no such change in dehydration and configuration for the heteropolymer system is indicated.

Water plays a fundamental role in the stabilization of secondary and tertiary structure of both proteins and nucleic acids (Tanford, 1968; Kuntz & Kauzmann, 1974; Edelhoch & Osborne, 1976; Hopfinger, 1977; Finney et al., 1982; Saenger, 1983). The role of solvation by water has been the subject of intensive experimental and theoretical investigations (Falk et al., 1962, 1963, 1970; Hearst, 1965; Wolf & Hanlon, 1975; Hanlon et al., 1975; Pullman, 1975; Perahia et al., 1977; Texter, 1978; Clementi & Corongiu, 1980; Saenger, 1983; Subramanian et al., 1988; Berman et al., 1988). Experimental evidence indicates that helical nucleic acids are heavily hydrated structures (Falk et al., 1963, 1970; Hearst, 1965; Wolf & Hanlon, 1975; Saenger, 1983), with at least two distinct hydration layers: a primary hydration shell of unfreezable water of about 20 water molecules per base pair that is impermeable to ions (Tunis & Hearst, 1968; Falk et al., 1970) and a secondary hydration shell of an undefined number of water molecules that is permeable to ions and freezes into ice-like structures (Wolf & Hanlon, 1975). Sequence-independent DNA hydration sites are presumed to exist near hydrogen-bonding group at the edges of the bases facing the grooves, in the vicinity of the sugars, and around the phosphate groups. Effects at these sites provide a rationale for the conformational plasticity of DNA (Saenger et al., 1986). The particular conformation (A, B, or Z) of a DNA duplex depends directly on the degree of hydration, and changing the water activity by adding organic solvents or varying the concentration (or nature) of counterions can shift the equilibrium to favor one duplex conformation over alternatives (Pohl & Jovin, 1972; Ivanov et al., 1973; Pohl, 1976; Rich et al., 1984). Sequence-dependent hydration has been inferred from X-ray analysis of the crystal structures of oligonucleotides. Examples include the spine of hydration in the B-DNA dodecamer d(CGCGAATTCGCG) (Drew & Dickerson, 1981; Kopka et al., 1983), the water pentagons in the A-type octamer d(GGTATACC) (Kennard et al., 1986), and the minor-groove spine of hydration in the Z-type hexamer d(CGCGCG) (Wang et al., 1979).

The molar volume associated with water of hydration in general is smaller than that of bulk water; volume changes, ΔV, during association reactions are interpreted to reflect changes in electrostriction of water dipoles and/or changes of hydrophobically bound water (Frank & Evans, 1945; Kauzmann, 1959). In the formation of a DNA duplex from single strands, these effects nearly compensate each other (Noguchi et al., 1971). Because of the requirement for large amounts of sample in conventional dilatometric methods (Katz, 1972), volume measurements have not been performed for many association reactions involving nucleic acids (Chapman & Sturtevant, 1969; Noguchi et al., 1971). The development of an improved magnetic suspension technique for precise measurements of density now makes feasible the acquisition of useful values of ΔV on 0.1-mL samples containing ~0.1 μmol of reactant species (Kupke, 1986; Gillies & Kupke, 1988).

We are currently investigating sequence hydration effects in nucleic acids. One approach is to measure the volume change that accompanies association reactions. These ΔV results together with standard thermodynamic parameters (ΔG, ΔH, ΔS) obtained with the same sample solutions can be correlated with structural information obtained from NMR and X-ray crystallographic data. One such system for which we know a great deal of structural and thermodynamic information consists of the association of netropsin to stretches of A-T base pairs in oligomeric and polymeric molecules.

Netropsin (Figure 1) is an oligopeptide that binds to DNA with a preference for stretches of A-T base pairs (Wartell et

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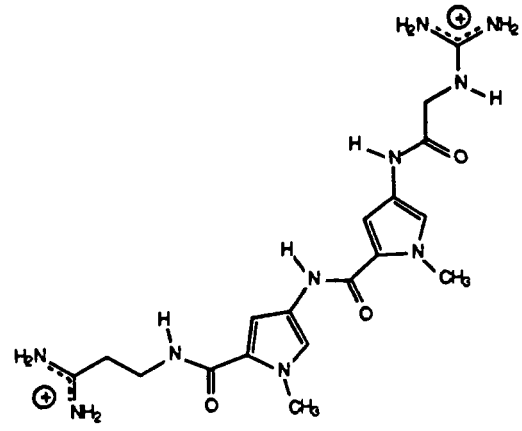

FIGURE 1: Two-dimensional structure of netropsin.

al., 1974; Zimmer, 1975; Zimmer & Wahnert, 1986). The molecular basis of the interactions in these complexes has recently been under intensive study by a wide variety of techniques including NMR (Patel & Canuel, 1977; Patel, 1979, 1982), crystal X-ray diffraction (Berman et al., 1979; Kopka et al., 1985; Coll et al., 1989), footprinting experiments (Taylor et al., 1984; Dervan, 1986; Ward et al., 1988), and thermodynamic studies (Marky et al., 1983a,b, 1985; Marky, 1986; Marky & Breslauer, 1987). The picture emerging from these studies is that netropsin binds in the minor groove of the DNA helix in the B conformation. The complex, encompassing about five base pairs, is stabilized by a combination of interactions including hydrogen-bonding, electrostatic, and van der Waals interactions. The strong specificity for A-T base pairs is due to the ability of netropsin to fully penetrate down to the floor of the minor groove, the amide protons of netropsin being hydrogen bonded to N3 of adenine and O2 of thymine in the floor of this groove. The methylpyrrole rings fit snugly in the groove and create additional van der Waals interactions with the sugar–phosphate backbone that constitutes the walls of the minor groove. Deep penetration of netropsin in the minor groove of synthetic DNA molecules is accompanied by high binding affinities, exothermic enthalpies, or favorable binding entropies (Marky & Breslauer, 1987).

The homopolymer, poly(dA)·poly(dT), has several distinctive features that differentiate it from other B-family DNA helices (Arnott, 1975; Arnott et al., 1983; Hogan et al., 1983; Thomas & Peticolas, 1983; Sarma et al., 1985; Wartell & Harrell, 1986; Behling & Kearns, 1986). The helical repeat in solution is found to be 10 bp per turn, in contrast to 10.5 bp found for random DNA and the alternating poly[d(AT)]·poly[d(AT)] duplex (Rhodes, 1979; Rhodes & Klug, 1981; Peck & Wang, 1981). The axial rise of the helix (3.2 Å) in the former duplex is less than that found for other B-type DNA fibers (3.4 Å). In addition, lowering the water activity of solutions by increasing the ethanol concentration does not cause the homopolymer to undergo a conformational transition to the A form as do the alternating and other DNA sequences (Arnott et al., 1974; Pilet et al., 1975). Short and repeated runs of the homopolymer within DNA that are in phase cause DNA bending (Wu & Crothers, 1984; Hagerman, 1984; Olson et al., 1988), a conformational state of DNA that has recieved a great deal of attention owing to its potential role in gene regulation and nucleosome phasing (Plaskon & Wartell, 1987; Behe, 1987). Unusual properties of this homopolymer have been observed also in its interactions with ligand molecules (Bresloff & Crothers, 1981; Sturm, 1982; Chaires, 1983; Marky et al., 1985; Wilson et al., 1985; Jones et al., 1986; Breslauer et al., 1987; Herrera & Chaires, 1989). The current detailed insight into the structure of poly(dA)·poly(dT) comes from the X-ray crystal structures of dA·dT tracts in oligomeric systems. The features observed in these structures include an optimum base stacking interaction due to a high propeller twist in the base pairs, a system of bifurcated hydrogen bonds, and a narrower minor groove (Coll et al., 1987; Nelson et al., 1987).

In this work we present volume change measurements on the association of netropsin to two synthetic DNA polymers, poly[d(AT)]·poly[d(AT)] and poly(dA)·poly(dT), in an attempt to understand the role of water and the overall molecular forces that control the affinity and specificity of netropsin to A-T binding sites and, indirectly, to understand sequence hydration effects. Complementary studies by calorimetry and melting protocols were performed for correlating entropy changes with the volume change data.

Materials and Methods

Materials. Netropsin from Serva Inc. was used without further purification; poly[d(AT)]·poly[d(AT)] was purchased from Pharmacia-LKB Biochemicals; poly(dA)·poly(dT) was purchased from Boeringer Mannheim. Both polymers were obtained in dried form and were used without further purification. All salts were of reagent grade. Polymer samples were dissolved in buffer, sonicated, and dialyzed exhaustively against the same buffer and were of similar viscosities. The concentration of each compound in solution was determined spectrophotometrically with the following molar extinction coefficients: netropsin, ϵ_{296} = 21 500 $M^{-1}\cdot cm^{-1}$; poly[d(AT)]·poly[d(AT)], ϵ_{260} = 6500 $M^{-1}\cdot cm^{-1}$ in phosphate; poly(dA)·poly(dT), ϵ_{260} = 6000 $M^{-1}\cdot cm^{-1}$ in phosphate. For these experiments the final concentration of netropsin was 1–2 mM; a small excess of polymer was maintained by assuming 1 netropsin = 10 polymer phosphate groups. All measurements were performed in buffer solutions consisting of 10 mM sodium phosphate–1 mM Na_2EDTA adjusted to pH 7 and to an overall NaCl concentration of 0 or 100 mM.

Magnetic Suspension Densimetry. The volume change, ΔV, that accompanies the binding of netropsin with each polymer was determined by measuring the density on weighed samples in a magnetic suspension densimeter. The instrument used in this study is an improved version of the original biochemical design by Senter (1969) and has been previously described (Gillies & Kupke, 1988). This instrument requires only 100 μL per measurement, and its sensitivity is such that it can deliver volume differences with a precision of <0.5 nL. The calculation of ΔV is done by measuring the mass and the equilibrium density of solutions before and after mixing; the observed change in volume, Δv, upon adding reactant A to reactant B is given by

$$\Delta v = m_{A+B}/\rho_{mix} - (m_A/\rho_A + m_B/\rho_B) \qquad (1)$$

where m is the mass in grams and ρ is the density of the solution in grams per milliliter. The two terms within parentheses refer to the initial volume before mixing. The value of Δv is then reduced to that per mole of the limiting reagent, to give ΔV. According to eq 1 small weighing errors have no appreciable effect on ΔV. The three density values, while independent, need not be of high absolute accuracy since it is their differences which are required for ΔV. The densimeter is calibrated with aqueous KCl solutions of known density. The density of the sample is obtained by relating the measured voltage to the straight line calibration equation of voltage versus density since the electrical current required to stably support the tiny permanent magnet (jacketed) at a preset height below the meniscus is directly proportional to the density

Table I: Volume Change Measurements for the Binding of Netropsin to A·T Polymers at 20 °C

DNA duplex	$\Delta V°$ (mL/mol of bound drug) 16 mM Na^+	116 mM Na^+
poly[d(AT)]·poly[d(AT)]	−16	−1
poly(dA)·poly(dT)	+97	+68

of the surrounding fluid (Kupke & Beams, 1972). With repetitive samples the density is measured with a precision of better than 5×10^{-6} g/mL. The temperature was kept at 20 °C.

Calorimetry. All calorimetric experiments were carried out on the OMEGA titration calorimeter from Microcal Inc. (Northampton, MA). A detailed description of this instrument has been presented elsewhere (Wiseman et al., 1989). Solutions of netropsin were used to titrate both polymers with a 100-μL syringe, mixing being effected by stirring of the syringe at 400 rpm. The concentration of netropsin in the syringe was generally 20–25 times higher than that of any of the polymer solution in the reaction cell. Typically, 10–14 injections of 7 μL each were done in a single titration. Since the reference cell of the calorimeter acts only as a thermal reference to the sample cell, this cell was filled with water containing 0.01% azide. The instrument was calibrated by means of a known standard electrical pulse.

UV Melting Curves. Absorbance versus temperature profiles (melting curves) for the polymer duplexes and the netropsin-bound polymers in appropriate solution conditions were measured at 260 nm with a thermoelectrically controlled Perkin-Elmer 552 spectrophotometer interfaced to a PC-XT computer for acquisition and analysis of experimental data. The temperature was scanned at a heating rate of 0.5 °C/min. These melting curves allow us to measure the transition temperatures, T_m, which are the midpoint of the helix–coil transition of the free and netropsin-bound duplexes to single strands (Cantor & Schimmel, 1980). Netropsin association constants K are calculated from the observed increase in the thermal stability of the bound duplex by the following equation (Crothers, 1971):

$$1/T_m - 1/T'_m = 1/[n\Delta H° \ln (1 + Ka_d)] \quad (2)$$

where T_m and T'_m are the transition temperatures of the free and bound duplexes, respectively; $\Delta H°$ is the helix–coil transition enthalpy of the free duplex, measured directly by differential scanning calorimetry; a_d is the activity of the free netropsin, assumed equal to half of the total concentration of netropsin in the solution at the transition temperature; and n is the neighbor exclusion parameter (equal to 5). Ligand binding constants obtained in this way refer to high temperatures and are extrapolated to the temperature of interest by the van't Hoff equation, using the binding enthalpy $\Delta H°_b$ measured directly by titration calorimetry assuming zero heat capacity change:

$$\partial \ln K/\partial(1/T) = -\Delta H°_b/R \quad (3)$$

Results

Volume Change. Using a magnetic suspension densimeter, we have measured directly the equilibrium volume change, ΔV, associated with the binding of netropsin to poly(dA)·poly(dT) and to poly[d(AT)]·poly[d(AT)] at 20 °C. The results are listed in Table I for the binding of netropsin to each polymer at two different salt concentrations as indicated in the table. In these experiments the molar ratio of polymer (as nucleotide) per netropsin was 10–12. Each entry represents an average

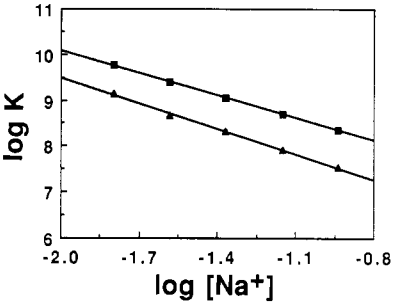

FIGURE 2: Salt dependence of binding constants for the association of netropsin to poly[d(AT)]·poly[d(AT)] (■) and poly(dA)·poly(dT) (▲). In 10 mM sodium phosphate buffer–1 mM Na_2EDTA adjusted to pH 7.0 and to the required NaCl concentration.

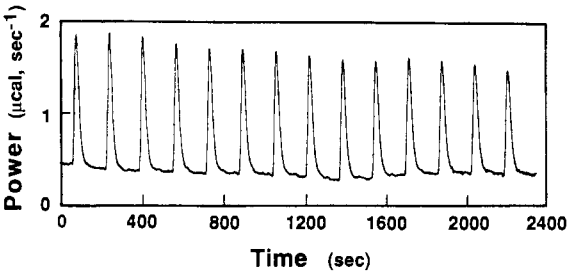

FIGURE 3: Typical calorimetric titration curve: 1.4 mL of poly[d(AT)]·poly[d(AT)] (0.47 mM in phosphate) was titrated with a netropsin solution (0.44 mM). All reagents were in a 10 mM sodium phosphate buffer containing 1 mM Na_2EDTA at pH 7 and 0.1 M NaCl. Each peak corresponds to an injection of 7 μL of the drug and an exothermic heat of ~36 μcal.

of at least two determinations. Similar results were obtained with different lot samples as well as with unsonicated polymers having higher viscosities. The results of Table I indicate that at low salt concentration binding of netropsin to the poly(dA)·poly(dT) duplex is accompanied by a substantial release or decompression of water molecules, while binding to poly[d(AT)]·poly[d(AT)] indicates a small or marginal uptake of water molecules; this striking differential effect suggests that the homopolymer duplex compresses more water or is more extensively hydrated. This effect is decreased with a 10-fold increase in salt concentration as expected for processes that are driven by electrostatic interactions in which a larger counterion concentration screens the effective charge density of the polyion and softens the compression of water dipoles.

Equilibrium Binding Constants. Binding constants for the association of netropsin to both polymers at several salt concentrations are plotted in Figure 2; for a given polymer, these binding constants decreased with increasing salt concentration. A linear regression analysis of the lines in the log K–log $[Na^+]$ plots allow us to estimate the slopes, which are directly proportional to the number of salt contacts that are present in the complex. These slopes are equal to −1.64 and −1.81 for the netropsin–poly[d(AT)]·poly[d(AT)] and netropsin–poly(dA)·poly(dT) complexes, respectively. These results are in good agreement with the theoretical value of 1.76 that is obtained from polyelectrolyte theory (Manning, 1978; Record et al., 1978) and are strongly indicative that both positive charges of the drug are involved in the complex, which release 2 mol of Na^+ ions to the solvent per mole of bound netropsin.

Calorimetry. In order to help with the interpretation of our densimetric results, we carried out titration calorimetric experiments on the same solutions used in densimetry for equivalence of states and at different salt concentrations. A typical titration curve for the addition of netropsin to the poly[d(AT)]·poly[d(AT)] duplex is shown in Figure 3. The

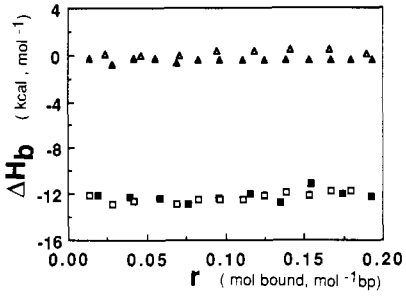

FIGURE 4: Dependence of the binding enthalpies, ΔH_b, with the amount of bound netropsin, r. Netropsin–poly[d(AT)]·poly[d(AT)] (squares); netropsin–poly(dA)·poly(dT) (triangles). open symbols correspond to 10 mM sodium phosphate buffer containing 1 mM Na_2EDTA at pH 7.0; closed symbols correspond to the same buffer and additional 0.1 M NaCl.

Table II: Calorimetric Measurements for the Binding of Netropsin to A·T Polymers at 20 °C

DNA duplex	$\Delta H°$ (kcal/mol of bound drug) 16 mM Na^+	116 mM Na^+
poly[d(AT)]·poly[d(AT)]	−12.2	−12.3
poly(dA)·poly(dT)	−0.4	+0.2

heats obtained for each injection are independent of the total concentration of added ligand. After a small correction for dilution heats of netropsin, molar binding enthalpies are calculated, as shown in Figure 4 as a function of the bound netropsin per base pair. Association enthalpies at two different total salt concentrations are summarized in Table II. The significant observation is that we measured a high exothermic enthalpy for the binding of netropsin to poly[d(AT)]·poly[d(AT)] and near zero enthalpy with poly(dA)·poly(dT). For both polymers these enthalpies are independent of the amount of bound netropsin; thus, no cooperative effects were found. Increasing the salt concentration 10-fold had little effect on the binding enthalpies. These enthalpic values are in good agreement with the ones previously obtained at higher DNA concentrations by batch calorimetry (Marky et al., 1985). Thus, both the volume and the heat changes show striking differences upon binding of this drug to these two isomeric DNA duplexes. The significance of this differential effect will be discussed in the following sections.

Complete Thermodynamic Profiles. In order to compare directly our thermodynamic results, the independent and observed variables, $\Delta V°$ and $\Delta H°$, along with the dependent and calculated variables, $\Delta G°$ and $\Delta S°$, are tabulated in Table III at two different salt concentrations. The free energy values are calculated by the standard thermodynamic relation $\Delta G° = -RT \ln K$, while the entropies are from the equation $\Delta G° = \Delta H° - T\Delta S°$; all values refer to a common temperature of 20 °C. Very similar free energy values are obtained for the association of netropsin with both polymers; however, a fundamental difference is seen in the nature of the forces that contribute to the overall observed free energy change. The driving force is enthalpic in the case of poly[d(AT)]·poly[d(AT)] and entropic for poly(dA)·poly(dT). These results are in good agreement with the volume change measurements that indicate an increase in disorder (increase in entropy) due to a large release or decompression of water moleules in the binding of netropsin to poly(dA)·poly(dT).

Discussion

In order to discuss our observed thermodynamic parameters in terms of the available structural information and molecular

Table III: Complete Thermodynamic Profiles for the Binding of Netropsin to A·T Polymers at 20 °C[a]

DNA duplex	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)	$\Delta V°$ (mL/mol)
poly[d(AT)]·poly[d(AT)]	−13.1 (−11.2)	−12.2 (−12.3)	+0.9 (−1.1)	−16 (−1)
poly(dA)·poly(dT)	−12.3 (−10.1)	−0.4 (+0.2)	+11.9 (+10.3)	+97 (+68)

[a] In 10 mM sodium phosphate buffer at pH 7.0; inclusion of 0.1 M NaCl for values in parentheses.

events, it is best to cast the association of netropsin to each of the DNA polymers in the following form: DNA(helix,-aNa$^+$,bH$_2$O) + drug(cH$_2$O) → complex(xNa$^+$,yH$_2$O) + mNa$^+$(zH$_2$O) + nH$_2$O. Each of the thermodynamic parameters reported in this paper corresponds to the above reaction. The free and drug-bound DNA duplex will have associated sodium ions and bound water molecules. The initial poly[d(AT)]·poly[d(AT)] free duplex is in a B-helical conformation and the poly(dA)·poly(dT) in a B′-helical conformation and presumed to undergo a conformation change to the B form upon binding of this drug. The binding of netropsin will release counterions with a change in the overall hydration state of each of the species involved; we assume, however, that the same amount of sodium ion is displaced by the binding reaction for both polymers according to our salt dependence studies.

Binding Affinities. Netropsin binds to these two polymers with similar and strong binding affinities ($\sim 10^9$), which indicates that the two types of sequences present in poly[d(AT)]·poly[d(AT)], ATATA/TATAT and TATAT/ATATA, and that in poly(dA)·poly(dT), AAAAA/TTTTT, provide identical binding sites. We still do not have available NMR or crystal X-ray structures of netropsin with all the possible binding sites mentioned above. From the available structures (Patel, 1982; Kopka et al., 1985; Coll et al., 1989) it is reasonable to assume that the structures of the final drug complexes with these two polymers are very similar with perhaps small changes in the specific hydrogen bonds (single vs bifurcated) between the amide protons of netropsin and the DNA proton acceptor groups (N3 of adenine and O2 of thymine) facing the minor groove of the DNA.

Binding Enthalpies. The observed binding enthalpies for the association of netropsin to both polymers are remarkably different (Table II). These measured enthalpies will comprise exothermic contributions from specific H-bonding and van der Waals interactions and an endothermic contribution if a decrease in hydration accompanies the binding and/or a change in the conformation of the polymer–drug complex. Ion pair formation apparently contributes negligibly according to our salt dependence experiments, and the transition from a B′ to the B conformation does not add measurably to the heat change as has been measured for other conformational transitions (Marky et al., 1981; Chaires & Sturtevant, 1986). We may consider the total enthalpy change of binding, ΔH_b, as the sum of $\Delta H_{intrinsic} + \Delta H_{conformation} + \Delta H_{hydration}$. We propose that the binding of netropsin to both polymers contributes identically to $\Delta H_{intrinsic}$ and that no contribution arises from changes in polymer and drug conformation. Hence, the observed difference in ΔH_b for the binding of this drug to these two polymers depends primarily upon heat differences arising from hydration changes.

Binding Entropies. In a similar fashion the derived entropy change, ΔS_b, is equal to the sum of the following contributions: $\Delta S_{molecularity}$ (the loss of entropy due to a bimolecular association reaction), $\Delta S_{conformational}$ (changes in the polymer con-

Table IV: Differential Thermodynamic Profiles for the Binding of Netropsin to A·T Polymers at 20 °C[a]

	$\Delta\Delta G°$ (kcal/mol)	$\Delta\Delta H°$ (kcal/mol)	$\Delta(T\Delta S°)$ (kcal/mol)	$\Delta\Delta V°$ (mL/mol)
16 mM Na^+	0.8	11.8	11.0	113
116 mM Na^+	1.1	12.5	11.4	69

[a] These values were obtained by subtracting each thermodynamic parameter of poly(dA)·poly(dT) from the corresponding values of poly[d(AT)]·poly[d(AT)].

figuration during binding), $\Delta S_{counterions}$ (release of Na^+), and $\Delta S_{hydration}$ (partial and/or complete release or uptake of water molecules). Complexation of the drug to each polymer covers about five A-T base pairs and brings similar counterion release (i.e., similar slopes from the log K–log [Na^+] plots); thus, each of the first three terms is considered to be identical for both reactions. The fourth term is substantially different for the two systems according to our ΔV results; there appears to be a small increase in the hydration state for the netropsin–poly[d(AT)]·poly[d(AT)] system and a large decompression of water molecules for the netropsin–poly(dA)·poly(dT) system. The large release of water molecules cannot be accounted for from hydrophobic effects, and we note that ionic changes appear to be the same for both systems. When the observed thermodynamic parameters are considered in terms of a differential effect, that is, if the parameters of netropsin–poly(dA)·poly(dT) are substracted from those of netropsin–poly[d(AT)]·poly[d(AT)] (as a reference state), we obtained the values in Table IV. These results strongly suggest that poly(dA)·poly(dT) in the free state is more hydrated or binds water more tightly. Upon binding of netropsin there is a release of water molecules that correlates well with the differential binding entropy.

Entropy Correlation with Differential Hydration. The difference in the entropy term for the binding reaction of the two polymer systems in Table IV is similar in magnitude and sign to the difference in the enthalpies. Since relatively large volume changes in aqueous systems, as observed here, reflect primarily changes in solvent structure and because we have no reason to suppose that the solutes in this case can contribute significant void volume changes, we consider our data in terms of changes in water structure. It has been suggested that a spine of immobilized water molecules lies along the minor groove of poly(dA)·poly(dT) duplexes (Chuprina, 1987). Via hydrogen bonding, such a spine of frozen-in water molecules may stabilize a warped B-type structure (as B′) and also narrow the groove. Assuming that the final structures and drug–oligonucleotide contacts are similar in both polymer systems, the substitution of the drug in the minor groove of the poly(dA)·poly(dT) duplex should release this spine of water molecules. The calculations of Chuprina (1987) indicate there may be one primary and one secondary water molecule in the spine for each A-T base pair of the homopolymer. Thus, as many as five water molecules lie in the primary hydration sheath for the A-T stretch encompassing the netropsin binding site. An obvious analogy arises to the heat absorbed per mole of normal ice when melted into the liquid state (ΔH = 1.4 kcal·mol^{-1} = $T\Delta S$ at 0 °C, or 2.3 kcal·mol^{-1} at 20 °C). According to this limited view, the frozen-in water in the spine along the minor groove of the A_n/T_n sequence would be melted into the bulk solvent phase upon interaction with drug. Using the value of 11.0 kcal·mol^{-1} for the $T\Delta S$ difference of the two interactions in low salt (Table IV), the difference in this entropy term (20 °C) for the putative melting of 10 water molecules is 1.1 kcal·(mol of water)$^{-1}$ (or 1.5 kcal·mol^{-1} if the secondary water molecules are assumed to be only partially frozen-in). This near correspondence with the entropy gained upon releasing immobilized water in normal ice is very likely a coincidence. Even if we consider the extreme scenario of 10 water molecules compressed as in a close-packed array (12 mL·mol^{-1}), the volume would be raised by 60 mL·mol^{-1} per netropsin site. This amount is approximately half the difference in volume observed (113 mL·mol^{-1}). More realistically, if we assume 2–3 mL·mol^{-1} compression (Millero et al., 1974) as an average for water molecules in the primary and secondary sheath in the minor groove of the poly(dA)·poly(dT) polymer, these can only raise the volume by 25 ± 5 mL·mol of netropsin bound)$^{-1}$. Barring differences in ionic changes and in void spaces of those two systems, we propose that the concurrent conformation change attending the binding to the homopolymer entails a decompression of other hydrated regions as well. The proposed propeller twist (Chuprina, 1987) in the homopolymer upon transition to the B configuration may cause a significant decompression of the 20 unfreezable water molecules surrounding helical nucleic acids (Tunis & Hearst, 1968; Falk et al., 1962, 1963). Our result indicates that a minimum of 40 water molecules is involved in the homopolymer binding of netropsin and in the subsequent change in the configuration of the poly(dA)·poly(dT)–drug complex. The entropic gain for this more generalized decompression must, of course, agree with that deduced from the heat-transfer and affinity measurements. Thus, the melting of the spine waters in the minor groove of the poly(dA)·poly(dT) duplex, while more immobile than those hydration waters at the exterior sites, cannot be as organized as that of the tetrahedrally bonded waters in normal ice.

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Two-Dimensional NMR Studies on the Anthramycin–d(ATGCAT)$_2$ Adduct†

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ABSTRACT: Two-dimensional NMR experiments were performed on the adduct of anthramycin with d-(ATGCAT)$_2$ to obtain the assignments of the nucleotide base and sugar protons as well as the anthramycin protons. Anthramycin is covalently attached to a guanine 2-amino group, forming the d(AT^{am}GCAT)·d-(ATGCAT) modified duplex. The anthramycin protons in the minor groove exhibit NOEs to several nucleotide protons. The network of anthramycin-nucleotide NOEs and the measurement of the 10-Hz coupling constant between the anthramycin H11 and H11a protons shows that anthramycin is covalently attached as the *S* stereoisomer at the anthramycin C11 position with the side chain of anthramycin oriented toward the 5′ end of the modified strand. The NOE data show that the anthramycin-modified duplex is in a right-handed conformation with all bases in an anti conformation. Analysis of the $J_{1'-2'}$ coupling constants for the resolved H1′ resonances shows that the S-type conformation of the sugars is highly preferred.

Anthramycin (Figure 1) is a potent antitumor antibiotic which reacts covalently with guanosine-containing duplex DNA to form an adduct that spans a four base pair region [for a review of pyrrolo[1,4]benzodiazepine antibiotics, see Hurley and Needham-VanDevanter (1986)]. Hurley and Petrusek (1979) and Petrusek et al. (1981) concluded that anthramycin forms a minor groove adduct with the exocyclic amino group of guanine. Carbon-13 and proton NMR experiments confirmed this conclusion and demonstrated the anthramycin C11 position as the reactive site (Graves et al., 1984). Subsequent ^{1}H NMR studies were performed on an anthramycin-modified deoxyhexanucleotide, d(AT^{am}G-CAT)·d(ATGCAT), in which anthramycin is covalently attached to the exocyclic amino group of one of the two guanines in the duplex. The other guanine is sterically blocked from reaction with anthramycin, and thus only a 1:1 anthramycin–duplex adduct is formed. These experiments showed that adduct formation had not significantly perturbed the B-DNA conformation and provided insight into solution geometry and dynamics (Graves et al., 1985).

The C11 position of anthramycin could be attacked by the exocyclic amino group of guanine from either face of the molecule, leading to potential formation of *R* and *S* isomers of the adduct. Furthermore, each of these isomers has two potential orientations of the anthramycin side chain in the minor groove (Figure 1). Our previous NMR studies showed that one form of the complex predominates in solution, although the NOESY data were not definitive as to which stereoisomer was formed. Rather, we assumed the formation of the 11(*S*) stereoisomer, as predicted from the model building studies (Petrusek et al., 1981). Subsequent molecular mechanics calculations (Rao et al., 1986; Remers et al., 1986; Zakrewska & Pullman, 1986) found that the lowest energy conformer is one in which anthramycin is bound in the minor groove as the 11(*S*) stereoisomer with the side chain pointing toward the 5′ end of the modified strand.

In the present report, we extend the two-dimensional NMR experiments on the d(AT^{am}GCAT)·d(ATGCAT) modified duplex using a combination of TOCSY and NOESY experiments at 500 MHz [e.g., for reviews see van de Ven and Hilbers (1988) and Nerdal et al. (1988)]. We present coupling constant and NOE data which demonstrate formation of the 11(*S*) adduct and provide assignments for each of the base and most of the sugar protons. In addition, anthramycin-DNA contacts are observed which show that the anthramycin side chain is oriented toward the 5′ end of the modified strand.

Experimental Procedures

Sample Preparation. Anthramycin methyl ether was generously supplied by Professor L. H. Hurley. The deoxyribonucleotide d(ATGCAT)$_2$ was purchased from Pharmacia/P-L Biochemicals. The sample was prepared as described previously (Graves et al., 1985). Briefly, an excess of anthramycin methyl ether was added as a solid to a solution of d-(ATGCAT)$_2$ dissolved in 10 mM sodium phosphate, pH 7.0, 0.1 mM disodium EDTA, and 100 mM sodium chloride. The solution was vigorously mixed for 4 days at 5 °C in the absence of light. The solid anthramycin was then removed from the sample by centrifugation, and unreacted anthramycin was removed via repeated extraction with equal volumes of buffer-saturated 1-butanol. After five extractions, no free an-

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