DNA Conformational Effects on the Interaction of Netropsin with A-tract Sequences†

Natalya N. Degtyareva, Megan J. Fresia, and Jeffrey T. Petty*

Chemistry Department, Furman University, 3300 Poinsett Highway, Greenville, South Carolina 29613

Received August 23, 2007; Revised Manuscript Received October 11, 2007

ABSTRACT: The influence of cosolutes and DNA sequence on the interaction of netropsin with three duplexes has been studied by isothermal titration calorimetry. In buffer, netropsin forms two complexes with a net stoichiometry of 1:1 in the minor groove of the oligonucleotide $(GCGCGAATTCGCGC)_2$. One complex has a weaker affinity and is more enthalpically favored relative to the other one, consistent with previous studies [Freyer, M. W., et al. (2006) *Biophys. Chem. 126*, 186−196]. With the cosolutes betaine and 2-methyl-2,4-pentanediol, the enthalpy and heat capacity changes indicate that the complex with weaker affinity is disfavored relative to the complex with higher affinity. With $(CGCGCAATTGCGCG)_2$, netropsin has one binding mode in buffer, and complex formation is not influenced by the cosolutes. The similarities of the enthalpy and heat capacity changes suggest that netropsin interacts similarly with these two oligonucleotides in the presence of cosolutes. The oligonucleotide $(GCGCAAATTTGCGC)_2$ also forms two complexes with netropsin, and the complex with weaker affinity is again disfavored by the cosolutes. Thus, the interaction of netropsin with these A/T binding sites is influenced both by the bases adjacent to the binding site and by cosolutes. We suggest that these two factors influence the conformation of the minor-groove binding site of DNA.

Cosolutes are a significant aspect of biochemical environments (*1*). To illustrate, the concentrations of nucleic acids and proteins in cells range up to 350 mg/mL and occupy 30−40% of the cellular volume (*2*). Smaller organic compounds such as amino acids and polyol osmolytes also have high concentrations (0.1−1 *m*) in response to stresses such as desiccation (*3*, *4*). Under such conditions, the activities of reactants and products cannot be treated as in dilute solution, and nonideal effects on biochemical reactions are interpreted by use of two models (*5*). First, the volume occluded by the cosolutes imposes an entropic restriction that favors reactions such as folding, condensation, and association. Hard-sphere interactions are a dominant effect on equilibrium and rate constants (*6*). Second, the cosolute and/or the solvent can preferentially associate with the reactants and products, thus perturbing equilibria such as conformational changes. For example, proteins that are unfolded in aqueous solution are induced to fold in the presence of small cosolutes (*7*). The basis of this conversion is the preferential exclusion of the cosolutes from the peptide backbone thus driving folding to reduce solvent exposure. For nucleic acids, preferential associations with cosolutes or water are important factors. To illustrate, preferential interaction of cosolutes occurs when the bases are exposed to the solvent (*8*−*10*). For our studies, these interactions are not expected to be important, as the DNA maintains its duplex state with the bases sequestered from the solvent. A more important factor is the preferential exclusion of the cosolutes, which results in the preferential hydration of the DNA relative to the activity of water in the bulk solution. By use of Wyman linkage relations, this preferential hydration has been used to understand the role of water in reactions involving nucleic acids (*11*−*14*).

In this work, the effect of cosolutes and base sequence on the conformation and reactions of A-tract DNA is considered. These adenine- and thymine-rich sequences are composed of consecutive AA and AT but not TA dinucleotides (5′→3′ notation), and they are distinguished from B-form DNA in several ways (*15*). The bases are propeller-twisted with bifurcated hydrogen bonds between the strands. Axial bending is manifested in the reduced mobility (relative to non-A-tract DNA) during gel electrophoresis (*16*). The minor grooves of A-tracts are narrow and provide a favorable environment for the localization of water and cations (*15*, *17*). A number of small molecules interact preferentially with the minor groove of A-tracts, and we focus on netropsin (Figure S1, Supporting Information). This ligand spans 4−5 bases in the minor groove of A/T-rich sequences, and noncovalent binding is driven by several factors (*18*). First, the cationic ligand is stabilized by the negative electrostatic potential in the minor groove of these sequences. Second, the narrow and flexible minor groove of A-tract sequences provides substantial stabilization via van der Waals interactions. Third, hydrogen bonding between the amines and carbonyls of the adenine and thymine bases and the amides of netropsin contributes to the specificity of binding. Fourth, the expulsion of water from the minor groove provides an entropic advantage for complexation. An important aspect

† Funds for this work were provided by the National Institutes of Health [NIH Grants R15GM071370 and P20 RR-016461 (from the National Center for Research Resource)] and the Henry Dreyfus Teacher-Scholar Awards Program.

* Corresponding author: phone (864) 294-2689; fax (864) 294-3559; e-mail jeff.petty@furman.edu.

10.1021/bi701722x CCC: $37.00
Published on Web 11/29/2007

of these complexes is their heterogeneity, and recent calorimetric studies have demonstrated that netropsin forms two energetically distinct complexes with A/T-rich binding sites (*19*, *20*). One proposed structure involves a complementary interaction of the concave ligand with the minor groove. The other complex has a more linear conformation of netropsin that is stabilized by water-mediated interactions between the ligand and the DNA. Through the effects of small cosolutes and base sequence variations on the enthalpy and heat capacity changes, we suggest that the conformation of the DNA is also an important factor in the interaction of netropsin with the minor groove of A-tract sequences.

EXPERIMENTAL PROCEDURES

The buffer components netropsin, betaine monohydrate (Fluka; St. Louis, MO), and 2-methyl-2,4-pentadiol (Sigma-Aldrich; St. Louis, MO) were used as received. Unless stated otherwise, all calorimetric and spectrophotometric measurements were conducted in a buffer with 10 mM $H_2PO_4^-$/HPO_4^{2-} (pH = 7), 50 mM NaCl, and 1 mM Na_2EDTA. Single strands of 5′-GCGCGAATTCGCGC-3′, 5′-CGCGCAATTGCGCG-3′, and 5′-GCGCAAATTTGCGC-3′ (Integrated DNA Technologies; Coralville, IA) were annealed by slow cooling of the solution from 95 to 10 °C over 48 h. For brevity, hereafter these duplexes are referred to as (-GAATTC-)$_2$[1], (-CAATTG-)$_2$, and (-AAATTT-)$_2$, respectively. The concentration of the single-stranded DNA was measured spectrophotometrically by use of the following extinction coefficients derived from the nearest-neighbor approximation: 127 000 M^{-1} cm^{-1} for (-GAATTC-)$_2$, 125 800 M^{-1} cm^{-1} for (-CAATTG-)$_2$, and 128 800 M^{-1} cm^{-1} for (-AAATTT-)$_2$ at 260 nm, where the concentrations are expressed with respect to the single-stranded oligonucleotide. Concentrations of netropsin were determined by use of the extinction coefficient 21 500 M^{-1} cm^{-1} at 296 nm (*21*). The osmolalities of the solutions were measured on a Wescor 5520 (Wescor; Logan, UT). Absorbance measurements were performed with a Cary 50 spectrometer (Varian; Palo Alto, CA).

ITC studies were carried out on a Microcal VP-ITC instrument (Northampton, MA) controlled by Origin 7.0 software. Typically a 5 or 10 μM solution of the duplex was degassed, loaded into the cell, and titrated with a 400−500 μM netropsin solution. The concentration of netropsin was checked before and after the experiment, and the changes were less than <5%. The injection volume was 1 μL. The reference cell was filled with same buffer solution used for the titration. The heat change associated with the titration was determined by integrating the power required to maintain the reference and sample cells at the same temperature. The heat of dilution due to the addition of the concentrated netropsin to the sample cell was determined by measuring the heat changes well past saturation of the minor-groove binding site. Titrations up to 18 netropsins per oligonucleotide showed no evidence of additional binding modes. The integrated heat changes were fit with either one- or two-site binding models via the functions in the Origin software. The two-site model uses the six adjustable parameters ΔH_1, ΔH_2, N_1, N_2, K_1, and K_2, where the subscripts designate the two types of complexes and ΔH, N, and K are enthalpy change, binding site size of netropsin (relative to the duplex), and equilibrium constant for association, respectively. For the two-site model, N_1 and N_2 were constrained by the empirical net stoichiometry of the complex. Later in our studies, the fitting function of Freyer et al. (*19*) was kindly provided by the authors. No differences were observed in the fitting parameters, so the function available in Origin was used for the studies in this work. All thermodynamic data are expressed relative to the number of moles of netropsin or oligonucleotide for this 1:1 association reaction.

Theoretical estimates of the heat capacity changes (ΔC_p) for the association reactions were made by use of GRASP 1.2 to calculate the changes in the polar and nonpolar solvent-accessible surface areas, ΔSAS_p and ΔSAS_{np}, respectively (*22*). Hydrogen atoms were added to the PDB files 1LEX [netropsin−(-GAATTC-)$_2$], 1LEY [netropsin−(-GAATTC-)$_2$], 1BNA [(-GAATTC-)$_2$], 261D [netropsin−(-CAATTG-)$_2$], 252D [(-CAATTG-)$_2$], 121D [netropsin−(-AAATTT-)$_2$], and 1D65 [(-AAATTT-)$_2$] by use of VegaZZ (*23*−*29*). Note that the two structures for netropsin with the (-GAATTC-)$_2$ sequence are for the two orientations of netropsin in the minor groove of this sequence. The nonpolar atoms were carbon, hydrogen bound to carbon, and phosphorus. The remaining atoms were defined as polar, and a probe radius of 1.4 Å was used. The atomic radii were assigned according to Cornell et al. (*30*). ΔSAS_{np} and ΔSAS_p were calculated from the differences in the surface areas of the DNA−netropsin complex and the summed surface areas of the corresponding native duplex and netropsin. The surface areas of netropsin were calculated by extracting netropsin structural coordinates from the PDB files for the DNA complex. ΔC_p was calculated from the empirical relationship 0.382-($\pm$0.026)ΔSAS_{np} − 0.121($\pm$0.077)ΔSAS_p, which is based on the reactions of DNA association with small molecules, protein folding, and hydrocarbon transfer to water (*31*).

RESULTS

(-GAATTC-)$_2$. Freyer et al. (*19*, *20*) have previously studied the interaction of netropsin with this sequence, and our observations are similar with respect to the shape of the binding isotherms and the heat capacity changes. Two energetically distinct complexes form with this sequence (Figure 1). The net stoichiometry is 1 netropsin:oligonucleotide, thus association occurs in the minor groove because the oligonucleotide contains the favored four-base $(AATT)_2$ binding site for netropsin (Table S1, Supporting Information) (*32*). One binding mode is less exothermic and more favored than the other, and henceforth these are referred to as Complexes I and II, respectively (Figure 1). The heat capacity changes for the reaction illustrate another distinction among the complexes (Figure 2). For Complex I, $\Delta C_p = -178 \pm 12$ cal/K, which is similar to theoretical estimates based on solvent expulsion from the interface (Table 1) (*18c*, *33*). The similarities of the experimental and theoretical estimates of ΔC_p suggest that the complex involves the fully complementary fit of the ligand with the DNA, as in the X-ray crystallography and NMR studies (*34*, *35*). For Complex II, a discontinuity in the variation of ΔH with temperature is

[1] Abbreviations: (-AAATTT-)$_2$, (GCGCAAATTTGCGC)$_2$; (-CAATTG-)$_2$, (CGCGCAATTGCGCG)$_2$; ΔC_p, heat capacity change; (-GAATTC-)$_2$, (GCGCGAATTCGCGC)$_2$; 2-MPD, 2-Methyl-2,4-pentanediol; ΔSAS_{np}, change in nonpolar solvent-accessible surface; ΔSAS_p, change in polar solvent-accessible surface; ITC, isothermal titration calorimetry.

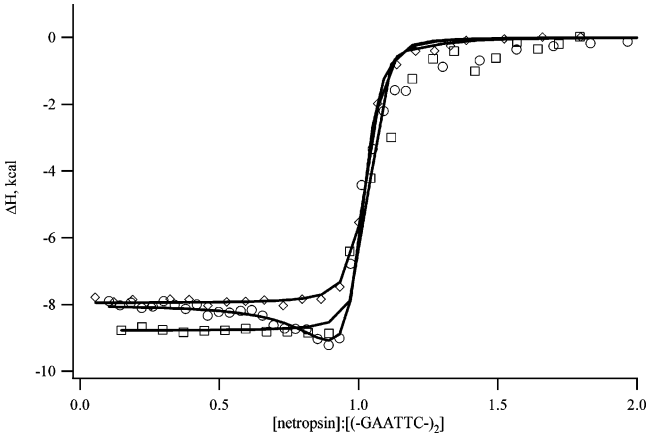

FIGURE 1: Binding isotherms for the titration of netropsin into $(\text{-GAATTC-})_2$ in buffer only (○), buffer with 2.6 *m* betaine (◇), and buffer with 1.3 *m* 2-MPD (□) at 25 °C. In buffer, the enthalpy changes for Complexes I and II are −8.0 and −12.1 kcal, respectively. In betaine and 2-MPD solutions, only one binding mode is observed with enthalpy changes of −7.9 and −9.3 kcal, respectively.

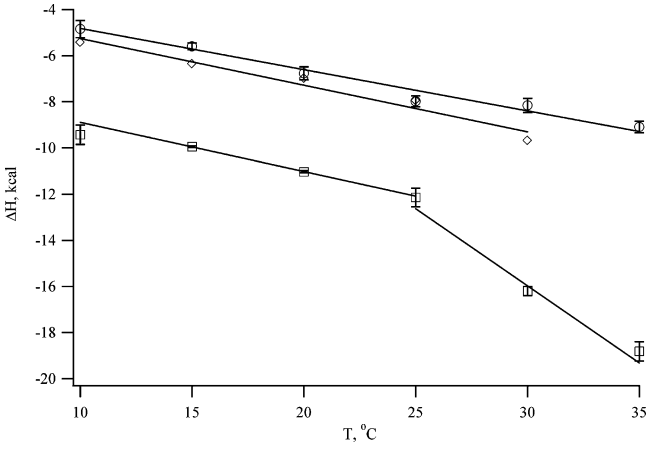

FIGURE 2: Dependence of ΔH on temperature for $(\text{-GAATTC-})_2$ and netropsin binding measured in buffer and buffer with 2.6 *m* betaine. The slope of the linear least-square fit gives $\Delta C_p = -178 \pm 12$ cal/K for Complex I (○). For Complex II (□), ΔC_p is -213 ± 12 cal/K from 10 to 25 °C and -669 ± 58 cal/K from 25 to 35 °C. Only one binding mode was observed in 2.6 *m* betaine (◇) with ΔC_p -202 ± 23 cal/K.

observed at 25 °C, which is similar to the transition temperature observed by Freyer et al. (*19*, *20*) (Figure 2). Relative to Complex I, the slope for Complex II is similar at lower temperatures but is significantly more negative at higher temperatures. Compared with the earlier studies, the slope measured in our studies is less negative, which may be due to refolding of the duplex that is coupled with netropsin binding (*20*). Freyer et al. (*19*) provided structural insight into these complexes by molecular modeling studies. Complex I involves a complementary fit of the concave netropsin into the minor-groove binding site, while Complex II involves a more linear conformation of netropsin. To compensate for the less complementary fit in this latter case, a water molecule was proposed to bridge the amidinium end of the netropsin with the DNA. Based on prior studies of protein−ligand complexes, the enthalpy and entropy changes are consistent with the entrapment of water (*19*, *36*, *37*). The discontinuity in ΔC_p for Complex II occurs at the isoequilibrium temperature for water, thus providing further support for water that is sequestered in the complex (*20*). Based on prior studies of protein−DNA complexes, bound water could also be contributing to the more negative ΔC_p for the formation of Complex II (*38*). Water entrapped in the interfaces of these complexes has a lower heat capacity than bulk water due to restrictions in its vibrational and rotational motions. Such effects extend to the functional groups of the protein and DNA in the vicinity of these buried waters. The net effect of these contributions to the ΔC_p is significant when compared to the contributions due to the changes in the solvent-accessible surface area.

Further insight into the nature of these complexes has been gained by conducting titrations in the presence of 2-methyl-2,4-pentanediol (2-MPD) and betaine (Figure S1, Supporting Information). These cosolutes were chosen for three reasons. First, 2-MPD is a commonly used cosolute that promotes crystallization, yet it also influences the conformation of DNA. For example, the degree of bending of A-tract DNA changes as demonstrated by footprinting, gel electrophoresis, and X-ray diffraction studies (*16*, *39*−*41*). This large-scale conformational change is the consequence of local changes at the base level, a primary concern in this work. Second, both betaine and 2-MPD are excluded from the vicinity of DNA, suggesting that they alter the extent of hydration and thus the conformation of DNA (*42*−*44*). Third, their influences on the electrostatic interactions are opposite, with betaine increasing and 2-MPD decreasing the dielectric constant of the solution with increasing concentrations (*45*, *46*). Relative to studies in buffer, the shapes of the binding isotherms are influenced by the cosolutes. Most importantly, both cosolutes diminish the contribution from Complex II (Figure 1; Table S2, Supporting Information). On the basis of the isotherms, Complex II is absent in solutions with 1.3 *m* 2-MPD or 20% (on a volume ratio basis), a concentration that is comparable to those used for the crystallization of DNA (*39*). Complex II also is not formed in solutions with 1.7 *m* betaine, and subsequent experiments were performed in 2.6 *m* betaine to ensure the absence of this complex. As in buffer, the net stoichiometry is 1 netropsin:oligonucleotide, suggesting that the solution conditions still favor binding in the minor groove (Tables S2 and S3, Supporting Information). The two cosolutes affect the enthalpy changes differently (Figure 1). With 2-MPD, the overall enthalpy changes are more exothermic relative to buffer, which suggests that dielectric constant effects or preferential interactions of the cosolutes with the reactants or products could be important. Studies with a set of cosolutes related to 2-MPD will address the significance of preferential interactions versus electrostatic effects. Our efforts focused on betaine because it has a small impact on the enthalpy changes, relative both to the buffer and to increasing concentrations of betaine (Figure 1; Table S2, Supporting Information). Besides the change in the shape of the binding isotherm, the heat capacity changes provide further evidence that the cosolutes promote the conversion of Complex II to Complex I. In 2.6 *m* betaine solution, $\Delta C_p = -202 \pm 23$ cal/K, which is similar to the $\Delta C_p = -178 \pm 12$ cal/K measured for Complex I in buffer (Figure 2). A discontinuity is not apparent in the ΔH versus temperature plot, but a limited temperature range was used to maintain the stability of the oligonucleotide. We are conducting studies with a more stable oligonucleotide to measure ΔH at higher temperatures.

Table 1: Calculated and Experimental ΔC_p Values of Netropsin−DNA Association

	ΔC_p (cal/K)		
	(-GAATTC-)$_2$	(-CAATTG-)$_2$	(-AAATTT-)$_2$
buffer, Complex I	-178 ± 12[a]	-172 ± 6[a]	-250 ± 6[a]
buffer, Complex II	-213 ± 12,[b] -669 ± 58[c]		-243 ± 26,[b] -592 ± 18[c]
2.6 *m* betaine	-202 ± 23[b]	-156 ± 5[b]	-214 ± 15[b]
2.6 *m* betaine			-303 ± 50[b]
ΔC_p from crystal structures	-195 ± 31 (1LEX)	-201 ± 31 (261D)	-228 ± 33 (121D)
ΔC_p from crystal structures	-187 ± 31 (1LEY)		

[a] ΔH measured from 10 to 35 °C. [b] ΔH measured from 10 to 25 °C. [c] ΔH measured from 25 to 35 °C.

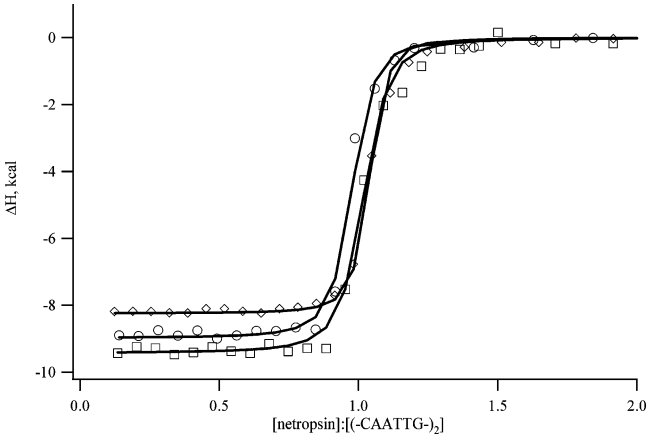

FIGURE 3: Binding isotherms for the titration of netropsin into (-CAATTG-)$_2$ in buffer only (○), buffer with 2.6 *m* betaine (◇), and buffer with 1.3 *m* 2-MPD (□) at 25 °C. The enthalpies calculated from the one-site model fits are −8.6, −8.2, and −9.4 kcal with buffer, betaine, and 2-MPD, respectively.

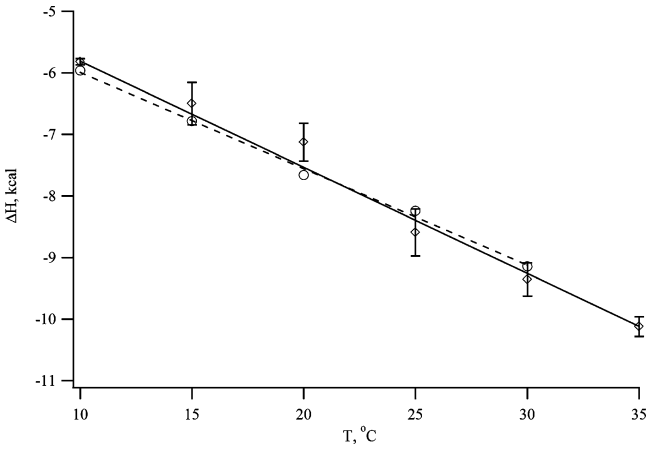

FIGURE 4: Plot of ΔH as a function of temperature for the reaction between (-CAATTG-)$_2$ and netropsin in buffer (◇) and buffer with 2.6 *m* betaine (○). ΔC_p is -172 ± 6 cal/K (solid line) in buffer and -156 ± 5 cal/K (dashed line) in betaine solution.

(-CAATTG-)$_2$. To consider how cosolutes influence other DNA sequences, the interaction of netropsin with the (-CAATTG-)$_2$ sequence was studied. Structural studies demonstrate that netropsin binds similarly to the (-GAATTC-)$_2$ and (-CAATTG-)$_2$ sequences, with specific preference for the (AATT)$_2$ binding site (*25*, *34*, *47*) From an energetic standpoint, we observed that netropsin interacts differently with these sequences in two respects. First, in buffer, only one binding mode in the minor groove of (-CAATTG-)$_2$ is observed, and the stoichiometry is 1 netropsin:oligonucleotide (Figure 3; Table S4, Supporting Information). Second, the heat capacity plot shows no discontinuities and is linear from 10 to 35 °C (Figure 4). ΔC_p of -172 ± 6 cal/K is similar to the theoretical estimate of -201 ± 31 cal/K, which suggests that the same type of complex forms both in solution and in crystals (Table 1) (*25*, *34*). In addition, ΔC_p is also comparable to that for the formation of Complex I with the (-GAATTC-)$_2$ sequence (Table 1). These results suggest that the complex formed with the (-CAATTG-)$_2$ sequence is most similar to the Complex I formed with the (-GAATTC-)$_2$ sequence.

To consider the effect of cosolutes, the association reaction was conducted in the presence of 2-MPD and betaine (Figure 3). To be consistent with the (-GAATTC-)$_2$ studies, solutions with 2.6 *m* betaine were also used for the titration of (-CAATTG-)$_2$ with netropsin (Figure 4). In this solution, the complexes still bind in the minor groove as 1:1 complexes (Table S5, Supporting Information). Relative to buffer, the enthalpy changes in the presence of betaine are within experimental error over the temperature range of 10−25 °C, resulting in $\Delta C_p = -156 \pm 5$ cal/K, which is similar to the -172 ± 6 cal/K measured in buffer (Table 1). Thus, the similarities of the ΔH and ΔC_p in buffer relative to a solution with 2.6 *m* betaine suggest that preferential interactions of the reactants and products with betaine are not important. As further points of comparison, the ΔH and ΔC_p values for the reaction of netropsin with the (-GAATTC-)$_2$ and (-CAATTG-)$_2$ sequences in the presence of betaine are similar, which suggests that netropsin associates in a comparable manner with the two sequences in this environment (Table 1; Tables S3 and S5, Supporting Information).

(-AAATTT-)$_2$. Base sequence and cosolute effects were further considered with (-AAATTT-)$_2$. As with the previous two sequences, structural studies show that netropsin forms a Class I structure with this sequence (*47*). The calorimetry studies yield a net stoichiometry of 1 netropsin:oligonucleotide, again demonstrating that association occurs in the minor groove (Table S6, Supporting Information). The thermodynamic features of the reaction of netropsin with (-AAATTT-)$_2$ and (-GAATTC-)$_2$ are compared because two complexes form in both cases (Figures 1 and 5). Three differences are observed and may be attributed to the slight differences in the binding mode (*27*). First, relative to the reaction with (-GAATTC-)$_2$, the enthalpy changes are less exothermic with (-AAATTT-)$_2$. Second, in contrast to (-GAATTC-)$_2$, the enthalpy changes with (-AAATTT-)$_2$ are more negative in the betaine than in buffer. Third, ΔH_1 becomes more negative and ΔH_2 becomes less negative in betaine for (-AAATTT-)$_2$, which suggests that Complex I is converting to Complex II. These latter two distinctions between the two sequences may be related to the extent of hydration of the two sequences or preferential interactions

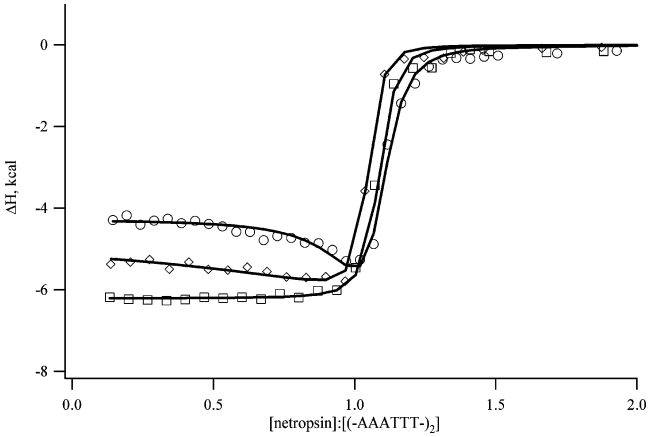

FIGURE 5: Binding isotherms for the titration of netropsin into (-AAATTT-)$_2$ in buffer only (○), buffer with 2.6 *m* betaine (◇), and buffer with 1.3 *m* 2-MPD (□) at 25 °C. The enthalpy changes are −4.2 (Complex I) and −10.4 (Complex II) kcal in buffer, −4.8 (Complex I) and −6.7 (Complex II) kcal in 2.6 *m* betaine, and −6.2 kcal in 1.3 *m* 2-MPD.

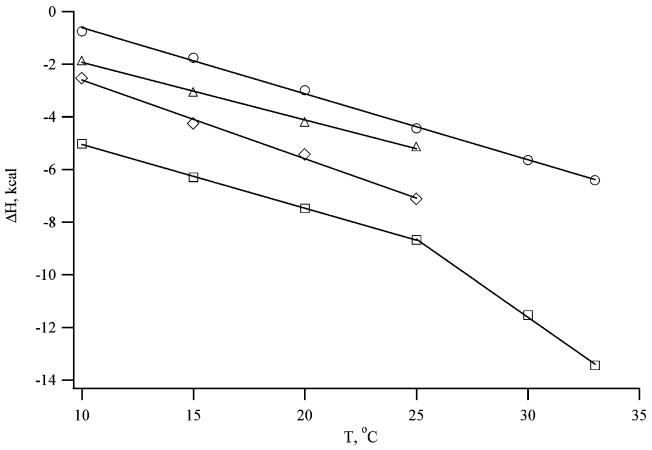

FIGURE 6: Dependence of Δ*H* for Complexes I and II on temperature for (-AAATTT-)$_2$ and netropsin binding in buffer and buffer with 2.6 *m* betaine. ΔC_p is the slope of the linear least-square fit. ΔC_p measured in buffer is −250 ± 6 cal/K for Complex I (○). For Complex II (□), ΔC_p is −243 ± 26 cal/K in the range from 10 to 25 °C and −592 ± 18 cal/K from 25 to 33 °C. ΔC_p measured with betaine is −214 ± 15 cal/K for Complex I (△) and −303 ± 50 cal/K for Complex II (◇).

of the cosolutes with DNA, netropsin, and/or the complex. A series of experiments with cosolutes that vary in structure and dielectric constant effects on the solvent will be used to resolve these issues. From the standpoint of the heat capacity changes in buffer and betaine, netropsin binds similarly to the two sequences. For the formation of Complex I with (-AAATTT-)$_2$, ΔC_p is −250 ± 6 cal/K, which is similar to the theoretical estimate of −228 ± 33 cal/K (Figure 6 and Table 1). As with (-GAATTC-)$_2$, a discontinuity is observed in the Δ*H* versus temperature plot, and ΔC_p for Complex II with (-AAATTT-)$_2$ is more negative at higher temperatures (−592 ± 18 cal/K). The differences in the ΔC_p values for the two sequences may again reflect the differences in the binding modes (Table 1). For both sequences, the addition of cosolute changes the shape of the binding isotherm, and the heat capacity changes were again measured in 2.6 *m* betaine (Figures 5 and 6). The two complexes are observed in this solution, and their ΔC_p values are −214 ± 15 and −303 ± 50 cal/K for Complexes I and II, respectively. Both values are comparable to the value for Complex I in buffer. Thus, relative to the buffer, the small changes in ΔC_p for the formation of Complex I and the significantly less negative ΔC_p for the formation of Complex II suggests that Complex II is more similar to Complex I in betaine. These observations are consistent with the (-GAATTC-)$_2$ results.

DISCUSSION

These studies have focused on how cosolutes and base sequence influence the interaction of netropsin with A-tract binding sites. The possible origins of the two minor-groove binding modes are considered. Heterogeneity could originate within the complex. For example, netropsin is asymmetric and can adopt two orientations in the minor-groove binding site (Figure S1, Supporting Information). The sequences considered in our studies all form Class I complexes with netropsin, and X-ray diffraction shows that the favored orientation has the guanidinium directed toward the 3′ end of the Watson strand in the duplex (*34*, *48*). From NMR studies, the rate of exchange is rapid (>2/s); thus flipping is an unlikely contribution due to the long 1 s filter period used for the ITC studies (*35*, *49*, *50*). Likewise, sliding of the ligand within the binding site is not significant based on NMR studies (*49*). Another possibility is that netropsin can adopt alternate conformations within the binding site. By comparing published X-ray diffraction structures of netropsin−DNA complexes, the guanidinium end was shown to have little variation among the different studies, which was attributed to the rigidity of this group and its tighter fit in the minor groove (*51*). In contrast, the amidinium end exhibits greater variation, which results in different degrees of curvature of the netropsin. It was concluded that the tendency to form hydrogen bonds between the netropsin and DNA is balanced by conformational changes that accompany binding. These concepts are also important for understanding previous calorimetry studies (*19*, *20*). On the basis of enthalpy, entropy, and heat capacity changes for the association of netropsin with different DNA sequences, the different binding modes were attributed to one with a complementary fit and another with a more linear conformer. The latter one involves water contacts between the amidinium and the functional groups in the minor groove, thus compensating for the less complementary fit. Such water-based interactions are abundant in crystal structures of small molecule complexes with DNA, and their energetic significance is now being appreciated (*52*, *53*).

Our studies with the cosolutes provide further insight into the binding modes of netropsin with A-tract sequences, and three possible effects are now evaluated. First, by altering the dielectric constant of the solution, cosolutes can perturb ionic interactions that are associated with particular conformational states, as illustrated by studies of quadruplex folding (*54*). Of particular interest to our studies, cations localize in the minor groove of A-tract sequences and promote narrowing of the minor groove (*55*, *56*). Because the nonpolar 2-MPD and zwitterionic betaine have opposite effects on the dielectric constant of the solution yet both induce the conversion of Complex II to Complex I, electrostatic changes should not be important for our studies. A second influence could arise from excluded volume, whereby the volume occupied by the cosolutes promotes reactions that reduce the solvent exposure. Because netropsin is comparable in size

to the cosolutes, this effect should not be significant (*57*, *58*). Furthermore, prior studies of DNA denaturation demonstrate that a more significant factor for smaller cosolutes is hydration changes, a third factor that is now considered (*59*). Cosolutes can be treated on an equal basis as the solvent, and the distribution of cosolute and solvent in the vicinity of DNA often differs from that in the bulk solution. For both 2-MPD and betaine, their preferential exclusion from duplex DNA has been demonstrated, so that the DNA is preferentially hydrated in comparison to the activity of water in the bulk solution (*42*, *43*). In further support of the preferential hydration of DNA in the presence of 2-MPD and betaine, the two cosolutes have different chemical structures and properties, yet both eliminate Complex II at comparable concentrations. Finally, the similarities of ΔH and ΔC_p in buffer and in cosolute solutions for the (-CAATTG-)$_2$ sequence indicate that preferential interactions with the cosolutes do not influence complex formation.

These considerations suggest that cosolutes indirectly influence the association of netropsin with DNA via hydration effects, and water is an important feature of the structure and function of DNA. For example, large-scale conformational changes such as B-form → Z-form and B-form → A-form are induced by cosolutes that alter the activity of water in the bulk solution (*54*, *60*). Such conversions are favored because the excluded cosolutes promote the formation of less hydrated structures. The solvent environment also influences A-tract DNA, which is distinguished by a network of water molecules and cations that emanate from the minor groove (*17*, *34*, *55*, *61*). Cosolutes, as well has high temperatures, perturb this spine of hydration and cations, thereby promoting the formation of normal B-form DNA (*62*). A-tracts are also bent with respect to the helical axis, and a range of experimental techniques have been used to study how the structure and conformation of A-tract DNA respond to cosolutes. X-ray crystallography studies show that 2-MPD diminishes the extent of bending (*41*). Information is derived from gel electrophoresis studies because the bent duplexes have anomalously slow migration in the gels. Mobility is enhanced in the presence of sufficient amounts of cosolutes, because the cosolutes induce the conformational transition to a normal B-form (*16*, *63*). Local probes of the conformation of A-tract sequences are provided by chemical and enzymatic cleavage of the DNA strands. For example, hydroxyl radicals attack the deoxyriboses to generate footprinting patterns that depend on the solvent accessibility (*64*). Cleavage becomes increasingly inhibited in the 5′ → 3′ direction and suggests that the minor groove becomes increasingly narrow along the DNA strand. With increasing concentrations of 2-MPD, the asymmetry is removed, and a more uniform width comparable to B-form DNA results (*39*). More recently, strand cleavage with uranyl cations demonstrates that DNA flexibility is also an important factor that distinguishes A-tract from other A/T-rich and random sequence DNA (*40*). The uniform cleavage observed at sufficient 2-MPD concentrations suggests that this cosolute induces conformational changes in the DNA that result in a more flexible minor groove.

Before further consideration of conformational factors, direct osmotic effects are considered (*11*). Increasing amounts of cosolutes decrease the activity of water in the bulk solution. Because the cosolutes are excluded from the vicinity of DNA, waters associated with DNA experience increasing osmotic pressure as the concentrations of cosolutes in the bulk solution increase. Thus, complexes with sequestered water are disfavored at sufficient concentrations of cosolutes. However, only one binding mode is observed with (-CAATTG-)$_2$ with and without cosolutes, so we suggest that the base sequence and the cosolutes influence the DNA conformation and thus the types of netropsin complexes. Prior studies demonstrate that conformational heterogeneity is an inherent feature of A-tract sequences. For example, a comprehensive analysis of the X-ray scattering and NMR data of a sequence analogous to (-GAATTC-)$_2$ concluded that this sequence is an ensemble of at least four structures (*65*). These structures vary with respect to the conformation of the bases, sugars, and phosphates. Local conformational differences are also evident at the dinucleotide level, and these suggest why two binding modes are observed with the (-GAATTC-)$_2$ and (-AAATTT-)$_2$ sequences but not with the (-CAATTG-)$_2$ sequence. Of all the dinucleotide steps in these sequences, the 5′CA step is most flexible and dynamic, based on conformational parameters such as the roll, slide, and twist of the base pairs (*66*). Importantly, the distributions of the deviations in these parameters suggest that no particular conformation is favored and that a wide range of conformations is accessed. We suggest that the CA step is adaptable, which is relevant because the termini of the binding sites are important for complex formation (*19*, *55*, *67*) Further distinctions among the sequences are also present in the minor-groove width. For (-GAATTC-)$_2$ and (-AAATTT-)$_2$, the groove narrows in the 5′→ 3′ direction, which is also suggested on the basis of hydroxyl radical footprinting (*64*). In contrast, (-CAATTG-)$_2$ has a symmetric minor groove (*25*, *68*) While crystal packing forces may be involved, similar conclusions from independent X-ray diffraction studies indicate that the shapes of the grooves are inherent features of these sequences (*69*).

In aggregate, calorimetric studies demonstrate that the association of netropsin with A-tract sequences is influenced by the conformation of netropsin, the base sequence of the binding site, and the solution environment. Netropsin can adopt conformations that are not fully complementary to the minor-groove binding site yet still have high affinity (*19*, *20*). Sequestered water is a critical factor in stabilizing such complexes. Our studies show that the conformation of the DNA is also important, as demonstrated by comparison of the (-GAATTC-)$_2$, (-AAATTT-)$_2$, and (-CAATTG-)$_2$ sequences. These finding are consistent with other studies that have considered the influence of the base sequence on the shape and adaptability of the binding sites for ligands (*70*, *71*). Cosolute effects are indirect because they are related to the extent of hydration. Betaine and 2-MPD are excluded from the vicinity of DNA; thus changing their concentrations alters the extent of hydration of the DNA. These changes impact the conformation of A-tract DNA, as evidenced by the straightening of the helical axis and the widening of the minor groove. Thus, calorimetric studies provide further insight into the direct and indirect role of water in the association of small-molecule ligands with DNA.

CONCLUDING REMARKS

The interaction of netropsin with A-tract sequences is an excellent model system for understanding the factors that

promote the selective and specific binding of small molecules with DNA. This study considers how the minor-groove binding of netropsin depends on the conformation of DNA. In accord with previous studies, netropsin forms two complexes with the (-GAATTC-)$_2$ and (-AAATTT-)$_2$ sequences. However, only one complex forms with the (-CAATTG-)$_2$ sequence. These differences are attributed to the flexibility of the flanking sequences. In addition, cosolutes alter the hydration and conformation of the DNA, thus influencing the types of complexes that form with netropsin. These results complement previous calorimetry studies by showing that conformation of both the DNA and the netropsin are important factors in the binding of netropsin with A-tract sequences. The impact of the cosolutes is also important from two other standpoints. First, the cellular environment is nonideal, which perturbs the structure of nucleic acids, and our studies illustrate how cosolutes remove distinctions between the sequences. Second, recent studies have considered the role of water in the binding of small molecules with DNA, and our studies suggest that DNA conformational changes could contribute to hydration changes.

ACKNOWLEDGMENT

We are grateful for discussions with M. Freyer, R. Buscaglia, E. Lewis, and N. Hud and for the insightful comments of the reviewers.

SUPPORTING INFORMATION AVAILABLE

Structures of netropsin and cosolutes (Figure S1), enthalpy changes and netropsin binding ratios for (-GAATTC-)$_2$ in the presence of buffer (Table S1), various concentrations of betaine (Table S2), and 2.6 *m* betaine (Table S3), enthalpy changes and netropsin binding ratios for (-CAATTG-)$_2$ in the presence of buffer (Table S4) and 2.6 *m* betaine (Table S5), and enthalpy changes and netropsin binding ratios for (-AAATTT-)$_2$ in the presence of buffer (Table S6) and 2.6 *m* betaine (Table S7). This material is available free of charge via Internet at http://pubs.acs.org.

REFERENCES

1. Kornberg, A. (2000) Ten commandments: Lessons from the enzymology of DNA replication, *J. Bacteriol. 182*, 3613−3618.
2. Pielak, G. J. (2005) A model of intracellular organization, *Proc. Natl. Acad. Sci.U.S.A. 102*, 5901−5902.
3. Cayley, S., and Record, M. T., Jr. (2003) Roles of cytoplasmic osmolytes, water, and crowding in the response of *Escherichia coli* to osmotic stress: Biophysical basis of osmoprotection by glycine betaine, *Biochemistry 42*, 12596−12609.
4. Rosgen, J., Pettitt, B. M., and Bolen, D. W. (2004) Uncovering the basis for nonideal behavior of biological molecules, *Biochemistry 43*, 14472−14484.
5. Davis-Searles, P. R., Saunders, A. J., Erie, D. A., Winzor, D. J., and Pielak, G. J. (2001) Interpreting the effects of small uncharged solutes on protein-folding equilibria, *Annu. Rev. Biophys. Biomol. Struct. 30*, 271−306.
6. Minton, A. P. (2006) Macromolecular crowding, *Curr. Biol. 16*, R269−R271.
7. Baskakov, I., and Bolen, D. W. (1998) Forcing thermodynamically unfolded proteins to fold, *J. Biol. Chem. 273*, 4831−4834.
8. Nordstrom, L. J., Clark, C. A., Andersen, B., Champlin, S. M., and Schwinefus, J. J. (2006) Effect of ethylene glycol, urea, and N-methylated glycines on DNA thermal stability: The role of DNA base pair composition and hydration, *Biochemistry 45*, 9604−9614.
9. Spink, C. H., Garbett, N., and Chaires, J. B. (2007) Enthalpies of DNA melting in the presence of osmolytes, *Biophys. Chem. 126*, 176−185.
10. Lambert, D., and Draper, D. E. (2007) Effects of osmolytes on RNA secondary and tertiary structure stabilities and RNA-Mg^{2+} interactions, *J. Mol. Biol. 370*, 993−1005.
11. Parsegian, V. A., Rand, R. P., and Rau, D. C. (1995) Macromolecules and water: probing with osmotic stress, *Methods Enzymol. 259*, 43−94.
12. Rand, R. P., Parsegian, V. A., and Rau, D. C. (2000) Intracellular osmotic action, *Cell. Mol. Life Sci. 57*, 1018−1032.
13. Qu, X., and Chaires, J. B. (2001) Hydration changes for DNA intercalation reactions, *J. Am. Chem. Soc. 123*, 1−7.
14. Kiser, J. R., Monk, R. W., Smalls, R. L., and Petty, J. T. (2005) Hydration changes in the association of Hoechst 33258 with DNA, *Biochemistry 44*, 16988−16997.
15. Hud, N. V., and Plavec, J. (2003) A unified model for the origin of DNA sequence-directed curvature, *Biopolymers 69*, 144−159.
16. Dlakic, M., Park, K., Griffith, J. D., Harvey, S. C., and Harrington, R. E. (1996) The organic crystallizing agent 2-methyl-2,4-pentanediol reduces DNA curvature by means of structural changes in A-tracts, *J. Biol. Chem. 271*, 17911−17919.
17. Shui, X., McFail-Isom, L., Hu, G. G., and Williams, L. D. (1998) The B-DNA dodecamer at high resolution reveals a spine of water on sodium, *Biochemistry 37*, 8341−8355.
18. (a) Marky, L. A., and Breslauer, K. J. (1987) Origins of netropsin binding affinity and specificity: Correlations of thermodynamic and structural data, *Proc. Natl. Acad. Sci. U.S.A. 84*, 4359−4363. (b) Marky, L. A., and Kupke, D. W. (1989) Probing the hydration of the minor groove of AT synthetic DNA polymers by volume and heat changes, *Biochemistry 28*, 9982−9988. (c) Haq, I. (2002) Thermodynamics of drug-DNA interactions, *Arch. Biochem. Biophys. 403*, 1−15. (d) Haq, I., Ladbury, J. E., Chowdhry, B. Z., Jenkins, T. C., and Chaires, J. B. (1997) Specific binding of Hoechst 33258 to the d(CGCAAATTTGCG)$_2$ duplex: Calorimetric and spectroscopic studies, *J. Mol. Biol. 271*, 244−257.
19. Freyer, M. W., Buscaglia, R., Cashman, D., Hyslop, S., Wilson, W. D., Chaires, J. B., and Lewis, E. A. (2006) Binding of netropsin to several DNA constructs: Evidence for at least two different 1:1 complexes formed from an -AATT-containing ds-DNA construct and a single minor groove binding ligand, *Biophys. Chem. 126*, 186−196.
20. Freyer, M. W., Buscaglia, R., Hollingsworth, A., Ramos, J., Blynn, M., Pratt, R., Wilson, W. D., and Lewis, E. A. (2007) Break in the heat capacity change at 303 K for complex binding of netropsin to AATT containing hairpin DNA constructs, *Biophys. J. 92*, 2516−2522.
21. Rosu, F., Gabelica, V., Houssier, C., and De Pauw, E. (2002) Determination of affinity, stoichiometry and sequence selectivity of minor groove binder complexes with double-stranded oligodeoxynucleotides by electrospray ionization mass spectrometry, *Nucleic Acids Res. 30*, e82−e86.
22. Nicholls, A., Sharp, K. A., and Honig, B. (1991) Protein folding and association: Insights from the interfacial and thermodynamic properties of hydrocarbons, *Proteins: Struct., Funct., Genet. 11*, 281−296.
23. Goodsell, D. S., Ng, H. L., Kopka, M. L., Lown, J. W., and Dickerson, R. E. (1995) Structure of a dicationic monoimidazole lexitropsin bound to DNA, *Biochemistry 34*, 16654−16661.
24. Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. (1981) Structure of a B-DNA dodecamer: Conformation and dynamics, *Proc. Natl. Acad. Sci. U.S.A. 78*, 2179−2183.
25. Nunn, C. M., Garman, E., and Neidle, S. (1997) Crystal structure of the DNA decamer d(CGCAATTGCG) complexed with the minor groove binding drug netropsin, *Biochemistry 36*, 4792−4799.
26. Wood, A. A., Nunn, C. M., Trent, J. O., and Neidle, S. (1997) Sequence-dependent crossed helix packing in the crystal structure of a B-DNA decamer yields a detailed model for the Holliday junction, *J. Mol. Biol. 269*, 827−841.
27. Tabernero, L., Verdaguer, N., Coll, M., Fita, I., Vandermarel, G. A., Vanboom, J. H., Rich, A., and Aymami, J. (1993) Molecular-structure of the A-tract DNA dodecamer d(CGCAAATTTGCG) complexed with the minor-groove binding-drug netropsin, *Biochemistry 32*, 8403−8410.
28. Edwards, K. J., Brown, D. G., Spink, N., Skelly, J. V., and Neidle, S. (1992) Molecular structure of the B-DNA dodecamer d(CG-

CAATTGCG)$_2$: An examination of propeller twist and minor-groove water structure at 2.2 A resolution, *J. Mol. Biol. 226*, 1161−1173.
29. Pedretti, A., Villa, L., and Vistoli, G. (2004) VEGA - an open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming, *J. Comput.-Aided Mol. Des. 18*, 167−173.
30. Cornell, W. D., Cieplak, P., Bayly, C. I., Gould, I. R., Merz, J., Kenneth, M., Ferguson, D. M., Spellmeyer, D. C., Fox, T., Caldwell, J. W., and Kollman, P. A. (1995) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules, *J. Am. Chem. Soc. 117*, 5179−5197.
31. Ren, J., Jenkins, T. C., and Chaires, J. B. (2000) Energetics of DNA Intercalation Reactions, *Biochemistry 39*, 8439−8447.
32. Abu-Daya, A., Brown, P., and Fox, K. (1995) DNA sequence preferences of several AT-selective minor groove binding ligands, *Nucleic Acids Res. 23*, 3385−3392.
33. Spolar, R. S., and Record, M. T., Jr. (1994) Coupling of local folding to site-specific binding of proteins to DNA, *Science 263*, 777−784.
34. Goodsell, D. S., Kopka, M. L., and Dickerson, R. E. (1995) Refinement of netropsin bound to DNA: Bias and feedback in electron density map interpretation, *Biochemistry 34*, 4983−4993.
35. Patel, D. J., and Shapiro, L. (1985) Molecular recognition in noncovalent antitumor agent-DNA complexes: NMR studies of the base and sequence dependent recognition of the DNA minor groove by netropsin, *Biochimie 67*, 887−915.
36. Clarke, C., Woods, R. J., Gluska, J., Cooper, A., Nutley, M. A., and Boons, G.-J. (2001) Involvement of water in carbohydrate-protein binding, *J. Am. Chem. Soc. 123*, 12238−12247.
37. Cooper, A. (2005) Heat capacity effects in protein folding and ligand binding: A re-evaluation of the role of water in biomolecular thermodynamics, *Biophys. Chem. 115*, 89−97.
38. Bergqvist, S., Williams, M. A., O'Brien, R., and Ladbury, J. E. (2004) Heat Capacity Effects of Water Molecules and Ions at a Protein-DNA Interface, *J. Mol. Biol. 336*, 829−842.
39. Ganunis, R. M., Guo, H., and Tullius, T. D. (1996) Effect of the crystallizing agent 2-methyl-2,4-pentanediol on the structure of adenine tract DNA in solution, *Biochemistry 35*, 13729−13732.
40. Mollegaard, N. E., and Nielsen, P. E. (2003) Increased temperature and 2-methyl-2,4-pentanediol change the DNA structure of both curved and uncurved adenine/thymine-rich sequences, *Biochemistry 42*, 8587−8593.
41. Dickerson, R. E., Goodsell, D., and Kopka, M. L. (1996) MPD and DNA bending in crystals and in solution, *J. Mol. Biol. 256*, 108−125.
42. Hong, J., Capp, M. W., Anderson, C. F., Saecker, R. M., Felitsky, D. J., Anderson, M. W., and Record, M. T., Jr. (2004) Preferential interactions of glycine betaine and of urea with DNA: Implications for DNA hydration and for effects of these solutes on DNA stability, *Biochemistry 43*, 14744−14758.
43. Hultgren, A., and Rau, D. C. (2004) Exclusion of alcohols from spermidine-DNA assemblies: Probing the physical basis of preferential hydration, *Biochemistry 43*, 8272−8280.
44. Stanley, C., and Rau, D. C. (2006) Preferential hydration of DNA: The magnitude and distance dependence of alcohol and polyol interactions, *Biophys. J. 91*, 912−920.
45. Cohn, E. J., and Edsall, J. T. (1943) *Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions*, pp 140−154, Reinhold, New York.
46. Travers, F., and Douzou, P. (1974) Dielectric constant of mixed solvents used for a low temperature biochemistry, *Biochimie 56*, 509−514.
47. Neidle, S. (1997) Crystallographic insights into DNA minor groove recognition by drugs, *Biopolymers 44*, 105−121.
48. Neidle, S. (2001) DNA minor-groove recognition by small molecules, *Nat. Prod. Rep. 18*, 291−309.
49. Lee, M., Chang, D. K., Hartley, J. A., Pon, R. T., Krowicki, K., and Lown, J. W. (1988) Structural and dynamic aspects of binding of a prototype lexitropsin to the decadeoxyribonucleotide d(CG-CAATTGCG)$_2$ deduced from high-resolution proton NMR studies, *Biochemistry 27*, 445−455.
50. Klevit, R. E., Wemmer, D. E., and Reid, B. R. (1986) ^{1}H NMR studies on the interaction between distamycin A and a symmetrical DNA dodecamer, *Biochemistry 25*, 3296−3303.
51. Goodwin, K. D., Long, E. C., and Georgiadis, M. M. (2005) A host-guest approach for determining drug-DNA interactions: An example using netropsin, *Nucleic Acids Res. 33*, 4106−4116.
52. Ladbury, J. E. (1996) Just add water! The effect of water on the specificity of protein-ligand binding sites and its potential application to drug design, *Chem. Biol. 3*, 973−980.
53. Miao, Y., Lee, M. P. H., Parkinson, G. N., Batista-Parra, A., Ismail, M. A., Neidle, S., Boykin, D. W., and Wilson, W. D. (2005) Out-of-shape DNA minor groove binders: Induced fit interactions of heterocyclic dications with the DNA minor groove, *Biochemistry 44*, 14701−14708.
54. Smirnov, I. V., and Shafer, R. H. (2007) Electrostatics dominate quadruplex stability, *Biopolymers 85*, 91−101.
55. Hamelberg, D., McFail-Isom, L., Williams, L. D., and Wilson, W. D. (2000) Flexible structure of DNA: Ion dependence of minor-groove structure and dynamics, *J. Am. Chem. Soc. 122*, 10513−10520.
56. Hud, N. V., and Feigon, J. (2002) Characterization of divalent cation localization in the minor groove of the A_nT_n and T_nA_n DNA sequence elements by ^{1}H NMR spectroscopy and manganese(II), *Biochemistry 41*, 9900−9910.
57. Ellis, R. J. (2001) Macromolecular crowding: Obvious but underappreciated, *Trends Biochem. Sci. 26*, 597−604.
58. Minton, A. P. (2006) Macromolecular crowding, *Curr. Biol. 16*, R269−R271.
59. Spink, C. H., and Chaires, J. B. (1999) Effects of hydration, ion release, and excluded volume on the melting of triplex and duplex DNA, *Biochemistry 38*, 496−508.
60. Malenkov, G., Minchenkova, L., Minyat, E., Schyolkina, A., and Ivanov, V. (1975) The nature of the B to A transition of DNA in solution, *FEBS Lett. 51*, 38−42.
61. Kubinec, M. G., and Wemmer, D. E. (1992) NMR evidence for DNA bound water in solution, *J. Am. Chem. Soc. 114*, 8739−8740.
62. Herrera, J. E., and Chaires, J. B. (1989) A premelting conformational transition in poly(dA)-poly(dT) coupled to daunomycin binding, *Biochemistry 28*, 1993−2000.
63. Lu, Y., Stellwagen, E., and Stellwagen, N. C. (2006) Effect of organic cosolvents on the free solution mobility of curved and normal DNA molecules, *Electrophoresis 27*, 1462−1470.
64. Burkhoff, A. M., and Tullius, T. D. (1987) The unusual conformation adopted by the adenine tracts in kinetoplast DNA, *Cell 48*, 935−943.
65. Schwieters, C. D., and Clore, G. M. (2007) A physical picture of atomic motions within the Dickerson DNA dodecamer in solution derived from joint ensemble refinement against NMR and large-angle X-ray scattering data, *Biochemistry 46*, 1152−1166.
66. ElHassan, M. A., and Calladine, C. R. (1997) Conformational characteristics of DNA: Empirical classifications and a hypothesis for the conformational behaviour of dinucleotide steps, *Philos. Trans. R. Soc. London, Ser. A 355*, 43−100.
67. Degtyareva, N. N., Wallace, B. D., Bryant, A. R., Loo, K. M., and Petty, J. T. (2007) Hydration changes accompanying the binding of minor groove ligands with DNA, *Biophys. J. 92*, 959−965.
68. Vlieghe, D., Sponer, J., and Van Meervelt, L. (1999) Crystal structure of d(GGCCAATTGG) complexed with DAPI reveals novel binding mode, *Biochemistry 38*, 16443−16451.
69. Dickerson, R., Goodsell, D., and Neidle, S. (1994) "... The tyranny of the lattice ...", *Proc. Natl. Acad. Sci. U.S.A. 91*, 3579−3583.
70. Dixit, S. B., Andrews, D. Q., and Beveridge, D. L. (2005) Induced fit and the entropy of structural adaptation in the complexation of CAP and {lambda}-repressor with cognate DNA sequences, *Biophys. J. 88*, 3147−3157.
71. Zacharias, M. (2006) Minor groove deformability of DNA: A molecular dynamics free energy simulation study, *Biophys. J. 91*, 882−891.

BI701722X