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Origins of netropsin binding affinity and specificity: Correlations of thermodynamic and structural data

(drug–DNA interactions/minor groove penetration/hydrogen bonding/enthalpy and entropy sources)

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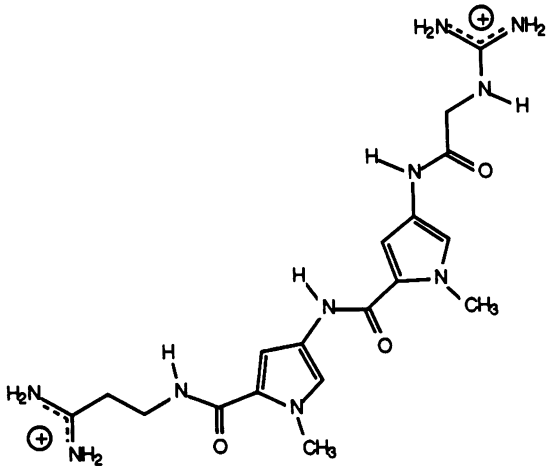
ABSTRACT **We report complete thermodynamic profiles for netropsin binding to an oligomeric and to several polymeric DNA host duplexes. These data allow us to reach the following conclusions: (*i*) netropsin binding by deep penetration into the minor groove is overwhelmingly enthalpy driven and exhibits a very high binding affinity ($K \approx 10^9$ at 25°C); (*ii*) deep penetration into the minor groove is required to form those drug–DNA interactions responsible for the enthalpy-driven high binding affinity of netropsin; (*iii*) I·C base pairs form binding sites for netropsin that thermodynamically are equivalent to those formed by A·T base pairs; (*iv*) the positive binding entropies reflect entropic contributions from molecular events other than just water spine disruption; (*v*) the thermodynamic binding data primarily reflect *local* netropsin–DNA interactions rather than long-range binding-induced conformational changes at regions distant from the binding site; (*vi*) the enhanced binding affinity associated with deep penetration of netropsin into the minor groove does *not* result from more favorable electrostatic interactions; (*vii*) the binding of netropsin to the central AATT core of the decamer duplex [d(GCGAATTCGC)]$_2$ is thermodynamically modeled best by netropsin binding to the poly[d(AT)]·poly[d(AT)] duplex rather than the poly(dA)·poly(dT) duplex. We propose correlations between our thermodynamic data and specific molecular interactions defined by NMR and x-ray structural studies on similar and identical drug–DNA complexes.**

Netropsin is a dicationic and basic oligopeptide that exhibits a wide range of antibiotic activities against bacteria, fungi, and viruses (1). The structure of netropsin is shown below.



To understand the molecular basis for its biological activities, the interaction of netropsin with DNA molecules has been investigated by several laboratories using a variety of experimental techniques (ref. 2 and references cited therein). Particularly noteworthy in these efforts has been the x-ray structure of the Dickerson group, which provides a detailed picture of the molecular interactions present in the netropsin–[d(CGCGAATTCGCG)]$_2$ complex (3–6). Significantly, NMR studies by the Patel group on the identical complex demonstrated that the same structure also exists in solution (7). The relevance of these two biophysical model studies to native DNA was demonstrated by the Dervan group. Their "footprinting" investigations revealed that netropsin binds to similar AT-rich sites of five base pairs in naturally occurring DNA polymers (8–10).

In the aggregate, the studies noted above have produced rather impressive *structural* pictures that show where netropsin binds to DNA and that suggest the formation of specific molecular interactions in the complex. Despite the obvious value of such structural models, their information content is lacking in several important respects. Specifically, the structural picture does not provide us with insight into (*i*) the stability ($\Delta G°$) of the drug–DNA complex in solution; (*ii*) the thermodynamic nature of the overall molecular forces that drive complex formation in solution ($\Delta H°$, ΔC_p, and $\Delta S°$); (*iii*) the relative contributions made by specific molecular interactions to stabilization of the complex in solution; and (*iv*) the temperature-dependent (melting) behavior of the complex. In short, despite their fundamental importance, the structural studies on netropsin–DNA complexes have not characterized the thermodynamic nature of the driving forces nor defined the relative contributions made by each molecular interaction to the DNA binding affinity and specificity of the drug. Such a characterization and ranking of molecular interactions requires thermodynamic data on netropsin binding and the resulting netropsin–DNA complex, preferably as a function of base sequence and drug structure. In recognition of this need, we have been conducting a program in which spectroscopic and calorimetric techniques are being employed to characterize thermodynamically the binding of netropsin and its analogues to selected DNA host duplexes (11–13). In this paper, we describe the results of a study in which we have determined complete thermodynamic binding profiles for the complexation of netropsin to three polymeric DNA host duplexes and to a specially designed oligomeric host duplex. Our investigation has been paralleled and/or preceded by the structural studies of Patel and co-workers using NMR (7, 14–17) and Dickerson and co-workers using single crystal x-ray diffraction (3–6, 18). The results of these structural studies have provided us with a microscopic framework in which to interpret our macroscopic thermodynamic data.

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Abbreviation: t_m, melting temperature.

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MATERIALS AND METHODS

DNA Polymers and Oligomers. The DNA polymers and the decameric oligomer were purchased from Pharmacia P-L Biochemicals. Netropsin was kindly provided by F. Arcamone of Farmitalia (Milan, Italy). Solution concentrations were determined spectrophotometrically. All studies were conducted in pH 7 buffer solutions consisting of 0.01 M sodium phosphate/1 mM Na_2EDTA and adjusted to the desired concentration of NaCl.

Calorimetry. The batch calorimeter we used to determine netropsin binding enthalpies is based on the design of Prosen and Berger (19). The instrument has been described in detail (20, 21).

CD Spectroscopy. CD spectra were recorded using a model 60DS spectropolarimeter (AVIV Associates, Lakewood, NJ) equipped with a thermoelectrically controlled cell holder.

UV Absorption Spectrophotometry. Absorbance versus temperature profiles of the free duplexes and the netropsin–duplex complexes were measured and melting temperatures (t_ms) were defined using described protocols (22). Netropsin binding constants were calculated from the differences in t_ms (Δt_m) of the free duplexes and the drug–duplex complexes as described (13).

RESULTS AND DISCUSSION

Netropsin Binding to the Poly[d(AT)]·poly[d(AT)] Duplex: Deep Minor Groove Penetration. Structural studies have shown that netropsin binds to DNA by deep penetration into the minor groove along AT-rich domains (3–7, 14–18). Using a combination of spectroscopic and calorimetric techniques, we have determined the thermodynamic profile for netropsin binding to the poly[d(AT)]·poly[d(AT)] duplex (11–13). To facilitate comparisons with the results reported here, these binding data are listed in the first row of Table 1. At this point, the significant observation is that netropsin binding to poly[d(AT)]·poly[d(AT)] is very strong and is overwhelmingly enthalpy driven.

Netropsin Binding to the Poly[d(GC)]·poly[d(GC)] Duplex: Sterically Blocking the Minor Groove. In the poly[d(GC)]·poly[d(GC)] duplex, deep penetration into the minor groove is sterically blocked by the exocyclic amino group of guanine. By employing a combination of spectroscopic and calorimetric techniques, we have obtained a complete thermodynamic profile for netropsin binding to the poly[d(GC)]·poly[d(GC)] duplex. Our results are listed in the second row of Table 1. The significant observation is that steric blockage of deep penetration into the minor groove results in a substantial decrease in binding affinity relative to poly[d(AT)]·poly[d(AT)]. This decrease in binding affinity results exclusively from a loss of favorable binding enthalpy. This result suggests that deep penetration by netropsin into the minor groove is required to manifest those drug–DNA interactions responsible for the highly favorable binding enthalpy.

Netropsin Binding to the Poly[d(IC)]·poly[d(IC)] Duplex: An Accessible Minor Groove in the Absence of A·T Base Pairs. In the poly[d(IC)]·poly[d(IC)] duplex, deep penetration by netropsin into the minor groove is possible in the absence of A·T base pairs since the exocyclic amino group of guanine is absent in inosine. By employing a combination of spectroscopic and calorimetric techniques, we have obtained a complete thermodynamic profile for netropsin binding to the poly[d(IC)]·poly[d(IC)] duplex. Our results are listed in the third row of Table 1. The significant observation is that when the steric blockage to deep penetration into the minor groove is removed, we return to an enthalpy-driven high binding affinity similar in magnitude to that observed for binding to the the poly[d(AT)]·poly[d(AT)] duplex. This result suggests that deep penetration by netropsin into the minor groove is required to manifest those drug–DNA interactions responsible for the highly favorable binding enthalpy. However, the specific presence of A·T base pairs is not required for netropsin to form those interactions that give rise to the observed enthalpy-driven high binding affinity. Based on these thermodynamic data, we predict that an x-ray study on the complex formed between netropsin and the IC core of the duplex $[d(CGCGIICCCGCG)]_2$ would result in a crystal structure similar to that determined by Dickerson and coworkers for the netropsin–$[d(CGCGAATTCGCG)]_2$ complex (3–6).

Table 1. Netropsin binding to DNA host duplexes at 25°C

Host duplex	$\Delta G°$, kcal/mol	$\Delta H°$, kcal/mol	$\Delta S°$, cal/mol·K
Poly[d(AT)]·poly[d(AT)]	−12.7	−11.2	+5.0
Poly[d(GC)]·poly[d(GC)]	−7.1	−4.3	+9.3
Poly[d(IC)]·poly[d(IC)]	−11.1	−9.9	+4.0
$[d(GCGAATTCGC)]_2$	−11.5	−9.3	+7.5

One calorie = 4.184 J.

Deep Minor Groove Penetration by Netropsin Does Not Result in Enhanced Electrostatic Interactions. We measure very different thermodynamic profiles for netropsin binding to the poly[d(AT)]·poly[d(AT)] duplex and to the poly-d(GC)]·poly[d(GC)] duplex (see data in Table 1). Do these differences simply reflect differences in electrostatic interactions when the dicationic drug can and cannot penetrate deeply into the minor groove of DNA? To answer this question, we have determined and compared how the netropsin binding constants vary with salt concentration for these two host duplexes. The resulting $\partial \log K/\partial \log[Na^+]$ values, which reflect the electrostatic contributions to the binding constants (23, 24), are listed in Table 2. The significant observation is that netropsin binding to both duplexes is driven by essentially equal electrostatic contributions. This result suggests that deep penetration into the minor groove is *not* required to manifest those interactions that give rise to the electrostatic contribution to the binding free energy. This conclusion is consistent with a structural picture in which the charged ends of netropsin are able to penetrate the duplex sufficiently to form all favorable electrostatic interactions, even when the body of the drug cannot penetrate the minor groove, as with the sterically blocked poly[d(GC)]·poly[d-(GC)] duplex. In this latter case, we envision the body of the drug to be bowed out with the two charged ends still interacting with the duplex. In connection with this structural picture, it should be noted that the magnitude of $\partial \log K/\partial \log[Na^+]$ reflects the involvement of both charged ends of netropsin in the binding event (23, 24). In other words, netropsin binds as a dication to both host duplexes, independent of whether the body of the drug can penetrate deeply into the minor groove.

Oligomer versus Polymer Host Duplexes: Local versus Long-Range Effects. Does our thermodynamic profile for netropsin binding to poly[d(AT)]·poly[d(AT)] reflect *local* drug–DNA interactions or do drug-induced conformational changes distant from the binding site contribute to the binding thermodynamics? To make this assessment, we determined the thermodynamic profile for netropsin binding to a short oligomer containing a single binding site (25). The length of a sufficiently short oligomeric host duplex precludes potential contributions from long-range, drug-induced conformational changes distant from the binding site. Thus, by com-

Table 2. Salt dependence of netropsin binding constant at 25°C

Host duplex	$\partial \log K/\partial \log[Na^+]$
Poly[d(AT)]·poly[d(AT)]	−1.6
Poly[d(GC)]·poly[d(GC)]	−1.5

paring polymer and oligomer binding profiles, we can assess if our thermodynamic data result from local drug–DNA interactions or if long-range effects must be considered. To this end, we determined the binding profile for netropsin complexation with the decamer duplex [d(GCGAATTCG-C)]$_2$. This oligomeric host duplex contains the identical binding site and flanking sequences present in the oligomeric duplex used in the x-ray work of Dickerson and co-workers (3–6) and the NMR study of Patel (7). We used this truncated decamer form of the "Dickerson dodecamer" since, in contrast to the 12-mer duplex (26), the 10-mer duplex melts in a two-state manner and its netropsin complex exhibits a lower and therefore more convenient t_m (22, 25).

Netropsin and [d(GCGAATTCGC)]$_2$ Form a 1:1 Complex. Upon binding, netropsin exhibits an induced CD signal at 310 nm where DNA is optically silent (2, 11–13). We took advantage of this feature to determine the stoichiometry of the complex formed between netropsin and the decamer duplex. Specifically, we monitored the binding-induced ellipticity at 310 nm as we titrated netropsin into a CD cell containing a solution of the oligomeric duplex. The resulting CD titration curve exhibits a break at a netropsin-to-duplex ratio of 1:1. This result establishes that our host duplex contains a single binding site. In conjunction with the NMR solution studies of Patel (7) and the x-ray structure of Dickerson and co-workers (3–6), we conclude that the central AATT core of the duplex serves as the binding site.

Netropsin Binding to the Decamer Duplex Is Strong and Enthalpy Driven at 25°C. We determined the binding constant for the drug–decamer association reaction by measuring the effect of netropsin binding on the thermal stability (t_m) of the host duplex. We observed that netropsin binding induces a 31°C increase in the thermal stability of the complex relative to the free duplex. We have combined this Δt_m of 31°C with our calorimetric enthalpy data to calculate a netropsin binding constant, K, of 2.84×10^8 at 25°C (13, 25). This K value corresponds to a binding free energy, $\Delta G°$, of −11.5 kcal/mol of bound drug at 25°C.

We used batch calorimetry to measure directly the enthalpy change at 25°C for the binding of netropsin to the decamer duplex. After minor corrections for heats of dilution, we obtained a binding enthalpy of −9.3 kcal/mol of bound drug. In conjunction with our experimentally determined binding free energy, this binding enthalpy allows us to calculate a binding entropy of +7.5 cal/mol·K of bound netropsin. For an association reaction in aqueous solution, such a positive entropy change may reflect binding-induced release of water from the drug and/or the duplex as well as binding-induced release from the duplex of condensed sodium counterions (12, 23, 24).

Netropsin Binding to the Oligomeric Duplex [d(GCGAATTCGC)]$_2$ Is Thermodynamically Similar to Netropsin Binding to Polymeric Duplexes with Accessible Minor Grooves. Inspection of the data in Table 1 reveals the similarity between the thermodynamic profiles we have determined for netropsin binding to the decamer host duplex and to polymer host duplexes with accessible minor grooves. This similarity suggests that these thermodynamic data result primarily from *local* drug–DNA interactions when netropsin binds by deep penetration into the minor groove. Consequently, we can interpret our oligomer and polymer binding profiles in terms of the specific *local* drug–DNA interactions defined by Dickerson and co-workers' x-ray structure (3–6) and Patel's NMR studies (7, 14–17).

Poly[d(AT)]·poly[d(AT)] versus Poly(dA)·poly[d(AT)] as Host Duplexes. Inspection of the data in Table 3 reveals that netropsin binding to poly[d(AT)]·poly[d(AT)] is overwhelmingly enthalpy driven, whereas netropsin binding to poly(dA)·poly(dT) is overwhelmingly entropy driven. The similarity in the binding free energies simply results from

Table 3. Netropsin binding to two AT polymers at 25°C

Host duplex	$\Delta G°$, kcal/mol	$\Delta H°$, kcal/mol	$\Delta S°$, cal/mol·K
Poly[d(AT)]·poly[d(AT)]	−12.7	−11.2	+5.0
Poly(dA)·poly(dT)	−12.2	−2.2	+33.0

impressive enthalpy–entropy compensations. We previously have shown that the differences in the polymer binding data listed in Table 3 are not due to heat capacity effects (13). We have suggested that these differences may reflect binding-induced changes in solvation due to the more highly hydrated nature of the non-B conformation of the homopolymer duplex. Independent of the details of our interpretation, comparison of the data in Tables 1 and 3 reveals that netropsin binding to the decamer duplex [d(GCGAATTGC-G)]$_2$ is thermodynamically modeled best by binding of the drug to the duplex formed by the alternating rather than the homopolymer sequence.

Macroscopic–Microscopic Correlations. Dickerson and co-workers' x-ray structure (3–6) and Patel's NMR picture (7) of the netropsin–[d(CGCGAATTCGCG)]$_2$ complex reveal the following significant drug–DNA interactions: (*i*) three intermolecular hydrogen bonds between the amide hydrogen atoms of netropsin and the N-3 of adenine and the O-2 of thymine that face the minor groove; the bifurcated nature of these hydrogen bonds proposed by Dickerson has been confirmed by the elegant work of the Dervan group at California Institute of Technology (10); (*ii*) four intermolecular van der Waals contacts; two of these contacts occur between the C-2 hydrogens of the adenine and the pyrrole H-3 protons; the other two contacts occur between the C-2 hydrogens of adenine and the methylene groups adjacent to each terminal amide linkage; (*iii*) two intermolecular electrostatic interactions between the two charged amino ends of netropsin and the phosphate groups and/or the base pair edges in the minor groove. In addition to these interactions, contributions to the thermodynamic binding data also may result from hydrophobic contacts that occur when the drug is transferred from the aqueous surroundings of the bulk solvent to the local environment within the minor groove. In the sections that follow, we propose potential microscopic sources of our thermodynamic data.

Molecular Origins of the Enthalpy Data. Our salt-dependent binding data (Table 2) reveal that electrostatic interactions alone cannot be responsible for the enthalpy-driven high binding affinity we observe when netropsin penetrates deeply into the minor groove. Consequently, the molecular origins for the observed enthalpy-driven binding affinity must reside in the hydrogen bonds, the van der Waals contacts, and/or the hydrophobic interactions noted above. All these interactions require deep penetration into the minor groove to bring the relevant groups close enough. We can estimate the sum of these short-range contributions to the binding event from the differences in the thermodynamic binding profiles for poly[d(AT)]·poly[d(AT)] (where deep minor groove penetration is possible) and poly[d(GC)]·poly[d(GC)] (where the deep penetration is sterically blocked). As shown in Table 4, this difference yields a $\Delta\Delta G°$ of −5.6 kcal/mol, a $\Delta\Delta H°$ of −6.9 kcal/mol, and a $\Delta\Delta S°$ of −4.3 cal/mol·K. These thermodynamic driving forces need to be explained in terms of differences in H bonding, van der Waals contacts, and/or solvent effects when netropsin does and does not penetrate deeply into the minor groove. The conventional wisdom

Table 4. Estimate of netropsin–DNA short-range interactions

"Host duplex"	$\Delta\Delta G°$, kcal/mol	$\Delta\Delta H°$, kcal/mol	$\Delta\Delta S°$, cal/mol·K
Poly[d(AT)]$_2$·poly[d(GC)]$_2$	−5.6	−6.9	−4.3

argues that hydrogen bonding would not provide a significant enthalpic driving force for association reactions in aqueous solution. This argument is based on the notion that in such association reactions one simply is exchanging solute–solvent hydrogen bonds for solute–solute hydrogen bonds. Consequently, the resulting differential hydrogen bonding effect should be small. We believe that this reasoning is flawed since it assumes that the microenvironment within the minor groove possesses a local dielectric constant similar to that of the bulk medium. In fact, if the local environment within the minor groove possessed a substantially reduced dielectric constant, then one could argue that such drug–DNA hydrogen-bonding interactions could contribute substantially to the binding event through an enthalpic driving force (30). In this connection, we recently have obtained results using a fluorescent probe that reveal that the microenvironment within the minor groove in fact possesses a reduced dielectric constant compared with the bulk solvent (R. Jin and K.J.B., unpublished results).

The favorable van der Waals contacts noted above represent another candidate for the molecular origins of our enthalpy-driven high binding affinity. These contacts undoubtedly contribute to the observed thermodynamic driving forces we measure. However, we are unable at this time to quantify their influence. Future experiments involving synthetically altered derivatives of netropsin and homologues of distamycin are designed to address this question.

Molecular Origins of the Entropy Data. Inspection of the entropy data listed in Table 1 reveals that netropsin binding is accompanied by a positive entropy change for all of the host duplexes we have studied. Since association reactions intrinsically exhibit negative *statistical* entropy changes, the positive binding entropies we observe suggest compensating positive entropic contributions from binding-induced release of solvent and/or counterions. In addition, the binding entropies we measure exhibit very similar magnitudes for *all* of the host duplexes (see Table 1). This observation suggests that the binding entropy is not particularly sensitive to whether netropsin penetrates or does not penetrate deeply into the minor groove. By contrast, the binding enthalpy clearly is sensitive to the mode of interaction. Inspection of the thermodynamic data in Table 1 reveals that changes in the binding enthalpies rather than the binding entropies are responsible for the different binding affinities (ΔG°) that we measure. In the paragraphs that follow, we discuss possible molecular origins for the positive binding entropies we measure as well as their similar magnitudes.

Poly[d(AT)]·poly[d(AT)] duplex served as our first host for netropsin binding studies (11, 13). Based exclusively on the thermodynamic data obtained from this binding study, it was tempting to propose that the positive binding entropy resulted from drug-induced disruption of the Dickerson water spine in the minor groove around A·T base pairs. However, inspection of the expanded data base reported here reveals that we now observe a qualitatively similar binding entropy for netropsin complexation with the poly[d(GC)]·poly[d(GC)] duplex (see Table 1). Since the exocyclic amino group of guanine in this all-GC host duplex prevents formation of a minor groove water spine (3–6, 29), the positive entropy change we observe for netropsin binding to the poly[d(GC)]·poly[d(GC)] duplex cannot reflect water spine disruption. In short, in the absence of fortuitous compensations, our data suggest that the similar binding-induced positive entropy changes we measure for these two host duplexes probably reflect molecular events other than water spine disruption. Several alternative explanations are discussed below.

Prior to binding, the free drug is hydrated. In addition, any host duplex will possess solvation layers other than an AT-specific groove water spine. These additional levels of solvation have been noted by Kopka *et al.* in their x-ray picture (29). It seems reasonable to suggest that these "non-water spine" DNA solvation shells will be less sensitive to sequence and therefore similar for all of our host duplexes. Thus, netropsin binding might disturb these common solvation domains in a similar manner for all of our host duplexes. Such a picture would be consistent with the similar and positive binding entropies that we measure for all host duplexes studied (see Table 1).

Another explanation for the similar and positive entropies focuses on the binding-induced release of counterions. Specifically, the dicationic nature of netropsin causes its DNA binding to induce the release of two sodium cations (23, 24). This event results in an increase in mole number, thereby making a positive contribution to the binding entropy (23, 24). In principle, the magnitude of this contribution should be similar for all of the host duplexes. The nearly identical salt dependencies we measure for binding to the AT and GC polymers support this expection (note the similar values of $\partial \log K/\partial \log[Na^+]$ listed in Table 2). Consequently, a molecular interpretation of our entropy data in terms of counterion release not only is consistent with the sign of the binding entropies but also with their similar magnitudes. Clearly, alternative interpretations are possible. Experiments on systematically altered netropsin derivatives and their homologues are necessary to help differentiate between possible molecular interpretations of our entropy data.

CONCLUSIONS

Comparisons between our thermodynamic binding profiles for netropsin complexation with an oligomeric and selected polymeric host duplexes permit us to reach the following conclusions: (*i*) the thermodynamic data we measure primarily reflect *local* netropsin–DNA interactions rather than long-range binding-induced conformational changes at regions distant from the binding site; this conclusion is based on the fact that we measure very similar thermodynamic profiles for binding of netropsin to the polymeric host duplex poly[d(AT)]·poly[d(AT)] and to the AT-rich core of the oligomeric host duplex $[d(GCGAATTCGC)]_2$; (*ii*) netropsin binding by deep penetration into the minor groove of DNA is overwhelmingly enthalpy driven and exhibits a very high binding affinity; (*iii*) deep penetration into the minor groove is required to form those molecular interactions that result in the enthalpy-driven high binding affinity of netropsin (e.g., H bonding, van der Waals contacts, etc.); this conclusion is based on a comparison between the thermodynamic profiles for netropsin binding to DNA duplexes in which deep minor groove penetration is and is not possible; (*iv*) I·C base pairs form binding sites for netropsin that thermodynamically are equivalent to those formed by A·T base pairs; in the absence of fortuitous compensations, this equivalence suggests that I·C base pairs also can manifest those molecular interactions responsible for the enthalpy-driven high binding affinity exhibited by A·T base pairs; (*v*) the *positive* binding entropies we measure for netropsin complexation with all host duplexes are consistent with favorable entropic contributions from binding-induced release of solvent and/or counterions. The similarity in the magnitude of the binding entropies when alternating AT and GC copolymers serve as hosts suggests favorable entropy contributions from solvent effects other than water spine disruption; (*vi*) the enthalpically driven strong binding of netropsin to the poly[d(AT)]·poly[d(AT)] duplex compared with its binding to the poly[d(GC)]·poly[d(GC)] duplex does *not* reflect enhanced electrostatic interactions when the drug deeply penetrates the minor groove of the all-AT polymer. This conclusion is based on the fact that we measure essentially identical salt dependencies for the binding constants of netropsin to both the alternating AT and GC host duplexes. This result is consistent with the conven-

tional wisdom that electrostatics do not substantially contribute to the enthalpy term; (*vii*) netropsin binding to the AATT central site in the $[d(GCGAATTCGC)]_2$ oligomeric duplex is thermodynamically best modeled by netropsin binding to poly[d(AT)]·poly[d(AT)] rather than to poly(dA)·poly(dT).

In this paper we have demonstrated the power of parallel thermodynamic and structural studies. Such parallel studies allow correlations between macroscopic and microscopic phenomena that are not possible based on the results of either study alone. Future studies on structural homologues of the netropsin will allow us to more specifically define the microscopic origins of our macroscopic data. Such information is required for developing a rational approach to the design of ligands that bind to DNA with a particular affinity and a desired specificity.

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