# Netropsin Binding as a Thermodynamic Probe of the Grooves of Parallel DNA†

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**Abstract:** We use the minor groove ligand netropsin as a thermodynamic probe of the grooves of parallel DNA. A combination of circular dichroism spectroscopy, temperature-dependent UV spectroscopy, and isothermal titration calorimetry has been employed to characterize the association of netropsin with two sets of DNA 25-mer duplexes: one set contains duplexes with exclusively dA·dT base pairs; the other, duplexes with four dG·dC base pairs in parallel (ps-D1·D2 and ps-D5·D6) and antiparallel (aps-D1·D3 and aps-D5·D7) orientation. Circular dichroism and calorimetric titration curves show overall stoichiometries of 4:1 ([netropsin]:[duplex]) for the netropsin complexes with the antiparallel duplexes and with ps-D1·D2, and 3:1 with ps-D5·D6. Fully saturated netropsin–DNA complexes melt with transition temperatures 16–28 °C higher than those of the respective free duplexes. These ligand-induced thermal stabilizations correspond to binding affinities, $K_b$, of $\sim 5 \times 10^7$ $M^{-1}$ for the parallel duplexes and $\sim 1 \times 10^8$ $M^{-1}$ for the antiparallel duplexes. Both values correspond to highly specific netropsin binding sites, presumably A·T base pairs. The similarity in values suggests that netropsin recognizes one of the grooves of parallel DNA in a similar manner to the minor groove of antiparallel B-DNA. However, for the set of duplexes containing all A·T base pairs, we obtained binding enthalpies, $\Delta H°_b$, of −6.6 (ps-D1·D2) and −2.2 kcal $mol^{-1}$ (aps-D1·D3), while $\Delta H°_b$ values of −8.9 (ps-D5·D6) and −7.9 kcal $mol^{-1}$ (aps-D5·D7) were obtained for duplexes containing dG·dC base pairs. Binding of netropsin to the first set of duplexes was accompanied by similar release of counterions equal to 1.6 mol of $Na^+$/mol of ligand. Therefore, the $\Delta\Delta H°_b$ of 4.4 kcal in this set can be interpreted as a net hydration difference of three water molecules per base pair, with the parallel duplex being less hydrated.

## Introduction

The polymorphic nature of DNA, which adopts the A, B, or Z conformation, is well established.[1] A recent addition to this family of DNA conformations is that of parallel-stranded DNA (ps-DNA),[2-7] in which the complementary strands have the same 5′ → 3′ orientation, held together by *reverse* Watson–Crick base pairs, forming a double helix with two equivalent grooves.[3]

Previous calorimetric[8] and optical investigations[2-7] of the helix–coil transition of two sets of 25-mers, deoxyoligonucleotide duplexes containing either exclusively dA·dT base pairs or substitutions with four dG·dC base pairs in parallel (ps-D1·D2, ps-D5·D6) and antiparallel orientation (aps-D1·D3, aps-D5·D7) (Figure 1a), reveal a two-state melting behavior for all four duplexes, with similar release of counterions.[8] The parallel duplexes melt with lower transition temperatures than do the corresponding antiparallel control duplexes. These unfavorable differential free energy terms are enthalpically driven and reflect a reduction in both base-stacking and hydrogen-bonding interactions for the set of duplexes with dG·dC base pairs. The cause of the lower stability of parallel DNA in the first set of duplexes has yet to be determined. It might be due to differences in base stacking, hydrogen bonding, and overall hydration.

The interaction of netropsin, Figure 1b, with DNA molecules has been studied by a wide variety of techniques.[9-21] Structural and molecular interaction studies indicate that the ligand binds in the minor groove of the DNA helix in the B conformation, with high specificity for stretches of dA·dT base pairs.[11,20] The complex is stabilized by a combination of hydrogen-bonding, electrostatic, and van der Waals interactions. The specificity of netropsin for dA·dT base pairs is due to (i) the ability of the amide protons of netropsin to become hydrogen bonded to N3 of adenine and O2 of thymine deep in the floor of the minor groove and (ii) the tight fit of the ligand methylpyrrole rings within the minor groove, thus creating additional van der Waals interactions with the sugar–phosphate backbone that constitutes the walls of the minor groove. The deep penetration of netropsin into the minor groove is accompanied by high binding affinities, exothermic enthalpies, and favorable binding entropies.[20,21] Recently, we have used netropsin to probe the hydration of the minor groove of synthetic dA·dT polymers. The minor groove of poly(dA)·poly(dT) is more hydrated than the one in poly[d(AT)]·poly[d(AT)] by eight water molecules per base pair.[21]

In this work, we use netropsin to probe the grooves of parallel DNA by comparing standard thermodynamic binding profiles of netropsin bound to parallel duplexes to those obtained with antiparallel duplexes of identical sequences. We obtain similar binding free energies for all four duplexes, but observe differences in the relative enthalpy and entropy contributions. Comparison with previous binding studies[21] of the interaction of this ligand with poly(dA)·poly(dT) and poly[d(AT)]·poly[d(AT)] allows us to conclude that, for the set containing exclusively A·T base pairs,

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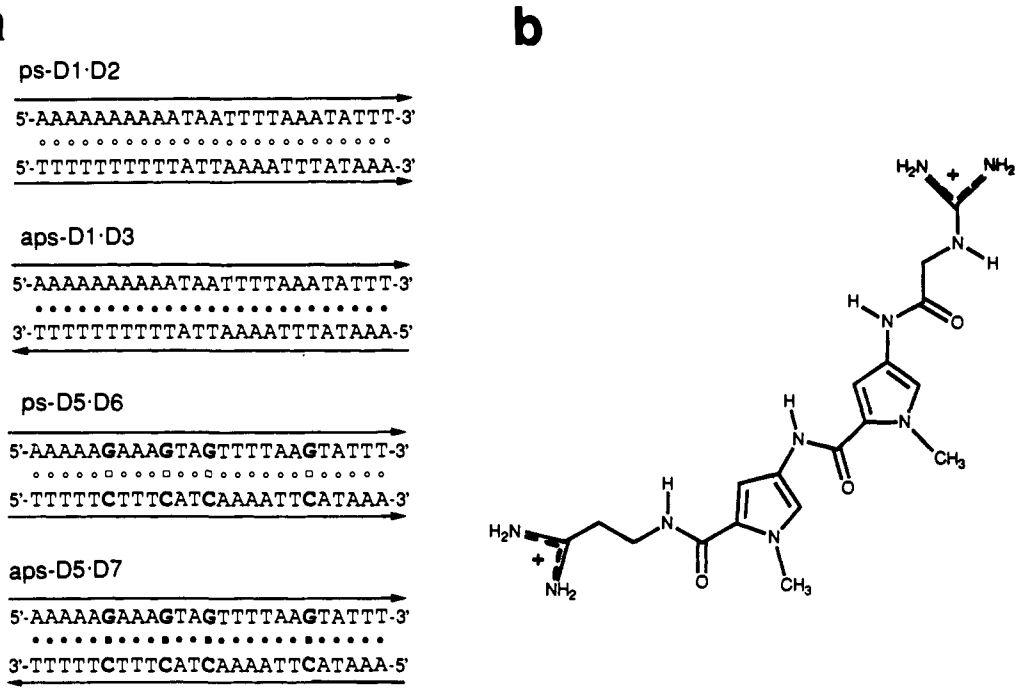


Figure 1. (a) Sequences of deoxyoligonucleotides and their duplexes. The Watson–Crick base pairs of the antiparallel duplexes are denoted by the closed symbols, and the *reverse* Watson–Crick base pairs of the parallel-stranded duplexes are denoted by the open symbols. (b) Structure of netropsin.

the minor groove of antiparallel DNA is more hydrated than the corresponding groove in parallel DNA.

## Experimental Section

**Materials.** Netropsin·2HCl from Serva Inc. was used without further purification. All oligomers were synthesized on an ABI PCR-Mate Model 391 automated synthesizer that uses standard phosphoramidite chemistry,[22] purified by HPLC, and desalted on a Sephadex G-10 exclusion chromatography column. The concentration of the oligomers was determined at 260 nm and 80 °C in water using the following extinction coefficients ($\mu M^{-1}$ $cm^{-1}$): D1, $\epsilon$ = 0.282; D2, $\epsilon$ = 0.250; D3, $\epsilon$ = 0.250; D5, $\epsilon$ = 0.283; D6, $\epsilon$ = 0.245; and D7, $\epsilon$ = 0.240. These extinction coefficients were obtained in $H_2O$ as reported previously.[8] The concentration of netropsin in the buffer solution was determined using an extinction coefficient of 21 500 $M^{-1}$ $cm^{-1}$ at 296 nm. All other chemicals were reagent grade. The buffer solution consisted of 10 mM cacodylic acid and 0.1 mM $Na_2EDTA$, adjusted to the desired NaCl concentration and pH 7. Stock oligomer solutions were prepared by dissolving dry and desalted oligomers in buffer and were used to prepare duplex solutions in equimolar quantities of strands.

**Circular Dichroism (CD).** Spectra were recorded on an AVIV 60DS spectrometer (Aviv Associates, Lakewood, NJ). The temperature of the cell was kept at 5 °C using a Hewlett-Packard 89100-A temperature controller. CD titrations were performed by adding 10-$\mu$L aliquots of a netropsin solution to 2.8 mL of duplex solution in a 1.0-cm cell. Spectra of these solutions were recorded from 220 to 400 nm at 1-nm intervals and corrected for small dilution effects. The stoichiometries of the netropsin–duplex complexes were obtained from the interception of the resulting lines of the plots of ellipticity at 307 or 309 nm versus the molar ratio [netropsin]:[duplex].

**Temperature-Dependence UV Spectroscopy.** Absorbance versus temperature profiles, or melting curves, for the free oligomer duplexes and the netropsin–duplex complexes in appropriate solution conditions were measured at 260 nm with a thermoelectrically controlled Perkin-Elmer 552 spectrophotometer interfaced to a PC-XT computer. The temperature was scanned at a heating rate of 1 °C/min. These melting curves allow us to measure the temperatures, $T_m$, of the midpoints of the helix–coil transition of the free and bound duplexes to single strands[23] for the subsequent measurement of ligand binding affinities.

**Determination of Binding Affinities.** Netropsin binding affinities, $K_b$, were measured from the increase in the thermal stability of a fully saturated duplex relative to the free duplex, neglecting any binding to single strands, according to the following equation:[24]

$$\Delta T_m = (RT°_m T_m N/\Delta H°_{hc}) \ln (1 + K_b a_L) \quad (1)$$

where $R$ is the universal gas constant; $T°_m$ and $T_m$ are the transition temperatures of the free and fully saturated duplexes, respectively; $N$ is the total number of bound ligand molecules per duplex and represents the stoichiometry of each netropsin–DNA complex, measured by circular dichroism and calorimetric titrations; $\Delta H°_{hc}$ is the helix–coil transition enthalpy of the free duplex, measured previously by differential scanning calorimetry,[8] and corresponds to the melting of 100% duplex (~20 base pairs) for ps-D1·D2, aps-D1·D3, and aps-D5·D7, and 75% duplex (~15 base pairs) for the ps-D5·D6 duplex; and $a_L$ is the activity of the free ligand, assumed equal to one-half of the solution concentration of netropsin at the transition temperatures of the complexes. These binding affinities correspond to the transition temperatures of the complexes and are extrapolated to the temperature of interest by the integrated van't Hoff equation:

$$\ln K_b \text{ (at 278 K)} = \ln K_b \text{ (at } T_m) - (\Delta H°_b/R)[(1/278) - (1/T_m)] \quad (2)$$

where $\Delta H°_b$ is the binding enthalpy, measured directly with titration calorimetry, assumed to be independent of temperature, i.e., with 0 heat capacity changes, as shown to be correct in studies of the interaction of netropsin with synthetic dA·dT polymers.[18] The binding affinities obtained with this method represent average values of $K_b$ under saturating conditions.

**Calorimetry.** All calorimetric experiments were carried out using the Omega titration calorimeter from Microcal Inc. (Northampton, MA). A detailed description of this instrument has been presented elsewhere.[25] In a typical titration of 23 injections of 5 $\mu$L each, a 100-$\mu$L syringe loaded with ~1.0 mM netropsin solution was used to titrate 1.4 mL of a 40 $\mu$M (in strands) duplex solution in the sample cell. The reference cell, filled with water, acts as a thermal reference to the sample cell. Complete mixing of the solution in the sample cell is effected by stirring the syringe at 400 rpm. The instrument is calibrated by using a known standard electrical pulse, and the precision in the heat of each injection is about 0.5 $\mu$cal. The area under the resulting peak following each injection is proportional to the heat of interaction, $Q$, and when this value is corrected for the ligand dilution heat and normalized by the concentration of added titrant, it is nearly equal to the binding enthalpy, $\Delta H°_b$, at that particular degree of bound ligand. A better determination of $\Delta H°_b$ for a particular site is to average the heats under the intermediate

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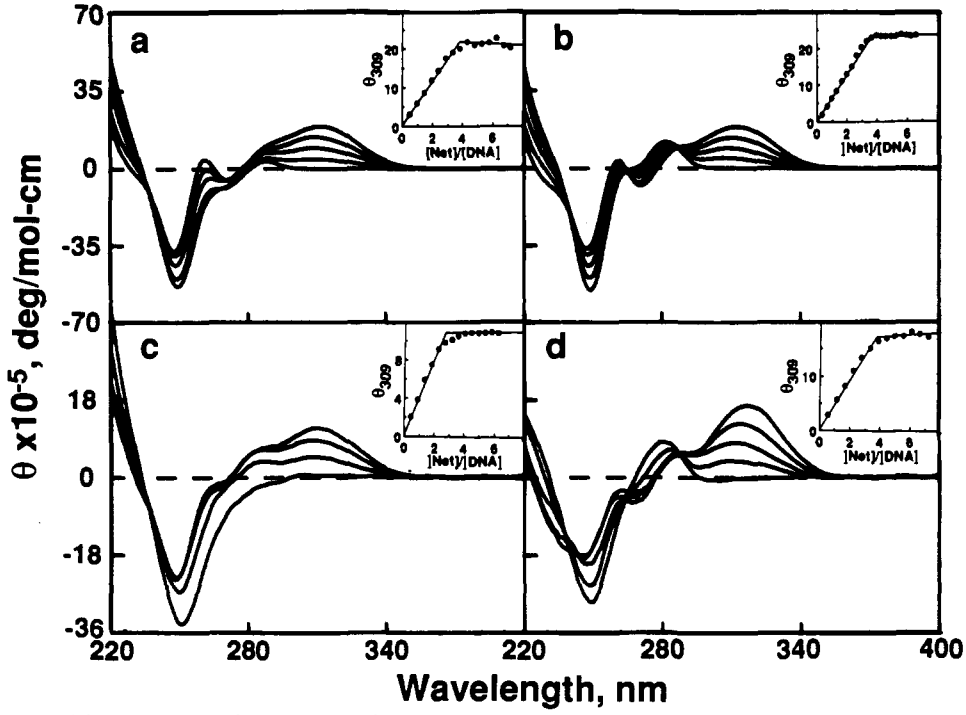


**Figure 2.** Typical circular dichroism spectra of netropsin–duplex complexes at several [netropsin]:[duplex] ratios in 10 mM sodium cacodylate buffer containing 0.1 mM $Na_2EDTA$ and 0.1 M NaCl at pH 7.0 and 5 °C. To obtain each spectrum, 2.7 mL of duplex solution in a 1-cm quartz cell at a concentration of 2.7 μM was titrated with 10-μL aliquots of a 0.34 mM netropsin solution. Insets correspond to the resulting titration curves at 309 nm. Spectra shown are for netropsin complexes with (a) ps-D1·D2, (b) aps-D1·D3, (c) psD5·D6, and (d) aps-D5·D7.

peaks that correspond to the titration of a site. In addition, the overall stoichiometry of the netropsin–DNA complexes was obtained with this technique by following the integral heat as a function of ligand concentration.

## Results

**CD Spectra and Overall Stoichiometry of Netropsin–DNA Complexes.** The CD spectra of each free duplex are shown in Figure 2. Spectral differences between parallel and antiparallel are evident, as has been reported previously.[5] The addition of netropsin to an oligomer duplex solution results in changes in the overall CD spectrum; in particular, an induced Cotton effect of the bound ligand is best indicated by the presence of an extra band that centers at ~309 nm. At this wavelength, we have followed the changes in ellipticity as each of the duplexes is saturated with the addition of ligand. The resulting titration curves are shown in the insets of Figure 2. The intersection of the two lines in each titration curve corresponds to stoichiometries of 3:1 (netropsin molecules per duplex) for the complex of netropsin with the ps-D5·D6 duplex and 4:1 for the netropsin complexes with the other three duplexes.

**Stability of Complexes.** Typical differential melting curves of the complexes, at several ligand to DNA molar ratios, are shown in Figure 3. An increase in ligand concentration results in both a change in the shape of the melting curves and a shift to higher temperatures by 16–28 °C of the helix–coil transition of these complexes. The free duplex melts in a monophasic transition, changing to a biphasic transition with an increase in ligand concentration due to the increase in the relative population of bound DNA base pairs, and returning to a monophasic transition at saturating conditions.[17] The $T_m$ values of the biphasic transitions of unsaturated complexes are similar to those of the free and fully bound duplexes. This melting behavior (two separate domains of free and bound base pairs) is characteristic of ligand–DNA binding processes with $K_b$ values in excess of $10^7$, as has been characterized previously in the binding of this ligand to synthetic dA·dT polymers.[17] The molar ratios at saturation correspond roughly to the above mentioned stoichiometries; a further addition of ligand, to 7–8 ligands per duplex, has the effect of a 0.5–1 °C increase on the $T_m$ values of these transitions. The main observation is that the fully saturated duplexes melt with transition

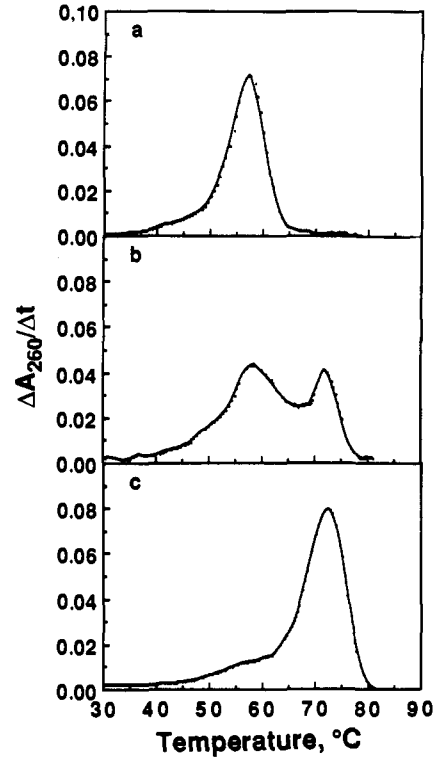


**Figure 3.** Typical differential melting curves of aps-D5·D7 (1 μM in duplex concentration) in 10 mM sodium cacodylate buffer containing 0.1 mM $Na_2EDTA$ and 0.1 M NaCl at pH 7.0 and 5 °C at [netropsin]:[duplex] ratios of 0 (a), 1:1 (b), and 4:1 (c).

temperatures 16–28 °C higher than those of the free duplexes.

**Binding Affinities and Their Dependence on Concentration of NaCl.** We use eq 1 to calculate netropsin binding affinities from the increased thermal stability of the complexes. The relevant parameters are listed in Table I. We obtain netropsin binding affinities of ~5 × $10^7$ $M^{-1}$ for the parallel duplexes and ~1.0 × $10^8$ $M^{-1}$ for the antiparallel duplexes. The magnitude of these

Table I. Parameters Used for the Calculation of $K_b$ from Optical Melts[a]

| oligomer | $T^\circ_m$ (K) | $T_m$ (K) | $N$ | $\Delta H^\circ_{hc}$ (kcal/mol) | $\Delta H^\circ_b$ (kcal/mol) | $K_b$ ($M^{-1}$) |
|---|---|---|---|---|---|---|
| ps-D1·D2 | 304.7 | 325.3 | 4 | 117 | −6.6 | $(5.9 \pm 2.7) \times 10^7$ |
| aps-D1·D3 | 322.5 | 350.1 | 4 | 151 | −2.2 | $(1.1 \pm 0.5) \times 10^8$ |
| ps-D5·D6 | 295.5 | 318.0 | 3 | 87 | −8.9 | $(3.3 \pm 1.8) \times 10^7$ |
| aps-D5·D7 | 329.7 | 345.1 | 4 | 167 | −7.9 | $(1.7 \pm 0.9) \times 10^8$ |

[a] Values were taken in 10 mM sodium cacodylate buffer containing 0.1 mM $Na_2EDTA$ and 0.1 M NaCl at pH 7.0. Values for $T^\circ_m$ and $T_m$ are within ±0.5 K; $N$, ±5%; $\Delta H^\circ_{hc}$, ±3%; and $\Delta H^\circ_b$, ±3%.

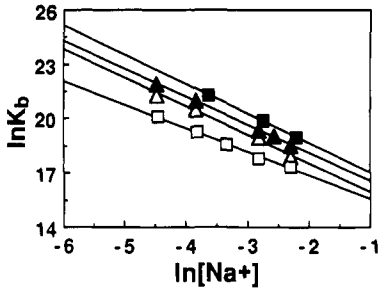


Figure 4. Salt dependence of netropsin–duplex complexes in 10 mM sodium cacodylate buffer containing 0.1 mM $Na_2EDTA$ at pH 7.0 and 5 °C and adjusted to the desired NaCl concentration. The symbols for each duplex are ps-D1·D2 (Δ), aps-D1·D3 (▲), ps-D5·D6 (□), and aps-D5·D7 (■).

binding affinities increases with a decrease in the salt concentration, a result consistent with the role of electrostatic interactions in the overall formation of the complexes. The number of salt contacts in the netropsin–DNA complexes is obtained from the slopes of the lines of the ln $K_b$ vs ln [$Na^+$] plots of Figure 4. These slopes are −1.6 for the complexes of netropsin with both antiparallel duplexes and −1.6 and −1.3 for the complexes of netropsin with ps-D1·D2 and ps-D5·D6 duplexes, respectively. The first three values are in good agreement with the theoretical value of −1.76 predicted for a double-charged ligand from polyelectrolyte theory.[26,27] Small deviations from the theoretical values may be attributed to the lower overall charge density of the free oligomer duplexes.[8] These values suggest that both positively charged ends of the ligand are involved in the complex, releasing an average of 2 mol of $Na^+$ to the solvent/mol of bound netropsin. However, the value of the slope for the netropsin–ps-D5·D6 complex is much lower than predicted and suggests structural perturbations, due to the inclusion of four dG·dC base pairs, resulting from changes in hydrogen-bonding and base-stacking interactions of the G·C and neighboring base pairs.[28]

**Netropsin Binding Results in Exothermic Heats.** The heat of netropsin interacting with each duplex was determined directly via titration calorimetric experiments. The total heat as a function of the total concentration of added ligand for each netropsin–oligomer system is shown in Figure 5a, b. We measured exothermic heats for all four duplexes that level off under saturating conditions. These saturation ratios correspond to the stoichiometries obtained in CD titrations. After a small correction for the heat of dilution of the ligand, the corresponding molar binding enthalpies are calculated as a function of the [netropsin]:[duplex] ratio (Figure 5a′,b′). The overall magnitude of the enthalpies depends upon the particular duplex conformation, sequence, and degree of ligand binding. For each set of duplexes, binding to the first site in each duplex (the strongest site) results in dramatic enthalpic differences that disappear at higher degrees of binding. These differences are more pronounced with the set containing all A·T base pairs, consistent with the overall differential hydration of the free duplexes. The resulting average binding enthalpies are summarized in Table II.

Table II. Thermodynamic Profiles for the Association of Netropsin to Duplexes at 5 °C[a]

| duplex | $\Delta G^\circ$ (kcal/mol) | $\Delta H^\circ$ (kcal/mol) | $T\Delta S^\circ$ (kcal/mol) | d(ln $K_b$)/d(ln [$Na^+$]) |
|---|---|---|---|---|
| ps-D1·D2 | −9.9 | −6.6 | 3.3 | −1.57 |
| aps-D1·D3 | −10.2 | −2.2 | 8.0 | −1.56 |
| ps-D5·D6 | −9.6 | −8.9 | 0.7 | −1.32 |
| aps-D5·D7 | −10.5 | −7.9 | 2.6 | −1.63 |

[a] All values were taken in 10 mM sodium cacodylate buffer containing 0.1 mM $Na_2EDTA$ and 0.1 M NaCl at pH 7.0. The $\Delta G^\circ$ values are within ±5%; $\Delta H^\circ$, ±3%; and $T\Delta S^\circ$, ±8%.

**Standard Thermodynamic Profiles.** Standard thermodynamic profiles, $\Delta G^\circ$, $\Delta H^\circ$, and $T\Delta S^\circ$, are summarized in Table II. The free energies are calculated by using the standard thermodynamic relationship, $\Delta G^\circ = -RT \ln K_b$, and the entropy contribution is obtained from the Gibbs equation, $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$. All values refer to a common temperature of 5 °C. Very similar free energy values result from the association of netropsin with both sets of duplexes. A fundamental difference, however, is seen in the nature and magnitude of the forces that contribute to the overall observed free energy change. The driving force is primarily entropic for the binding of netropsin to the aps-D1·D3 duplex and enthalpic for the other three duplexes.

## Discussion

Netropsin spans about five base pairs in the complexation with a DNA duplex,[10,18] so our 25-mer duplexes contain up to five heterogeneous binding sites of variable sequence. The measured stoichiometries of 3:1 and 4:1 for these duplexes allow for a large number of combinations of possible binding sites with variable sequence. Simple inspection of the sequence of the duplexes containing exclusively A·T base pairs, for instance, yields up to 13 potential binding sites, in principle with similar binding affinities in the range of $10^8$, making thermodynamic discrimination of the individual sites impossible. For this reason, all the reported thermodynamic binding profiles correspond to the average of the thermodynamic parameters for all sites.

**Stoichiometry of Complexes.** Netropsin complexes with the duplexes containing 100% A·T base pairs have a stoichiometry of 4:1. The substitution of four G·C base pairs for four A·T base pairs in these duplexes brings a change in the overall stoichiometry to 3:1 in the complexation with the ps-D5·D6 parallel duplex, and the stoichiometry remains 4:1 for the complex with the isomeric antiparallel duplex aps-D5·D7. From the length of the duplexes, one expects a value of 5 for the maximum number of bound ligands, assuming the formation of all 25 base pairs. The lower values of the stoichiometries obtained here may simply reflect the presence of frayed ends in all four duplexes which are closed by A·T base pairs,[29,30] as seen in previous calorimetric studies.[8,31] The further loss of one bound ligand in the netropsin complex with the ps-D5·D6 duplex may be explained in terms of structural changes created by the substitution of four dG·dC for four dA·dT base pairs, which results in different base-pairing and base-stacking interactions and may induce additional perturbations of neighboring base pairs (see Figure 6).[28]

**All Sites Have Similar Binding Affinities.** In buffer solutions containing 100 mM NaCl, netropsin binds to all four duplexes with similar and strong binding affinities ($\sim 10^8$), characteristic of the binding of netropsin to sites containing all A·T base pairs.[17-21] Despite the sequence heterogeneity of the binding sites and differences in the proton-acceptor groups facing the floor of the grooves, O2 (antiparallel duplexes) or O4 (parallel duplexes) of thymine as shown in Figure 6, our results indicate that these

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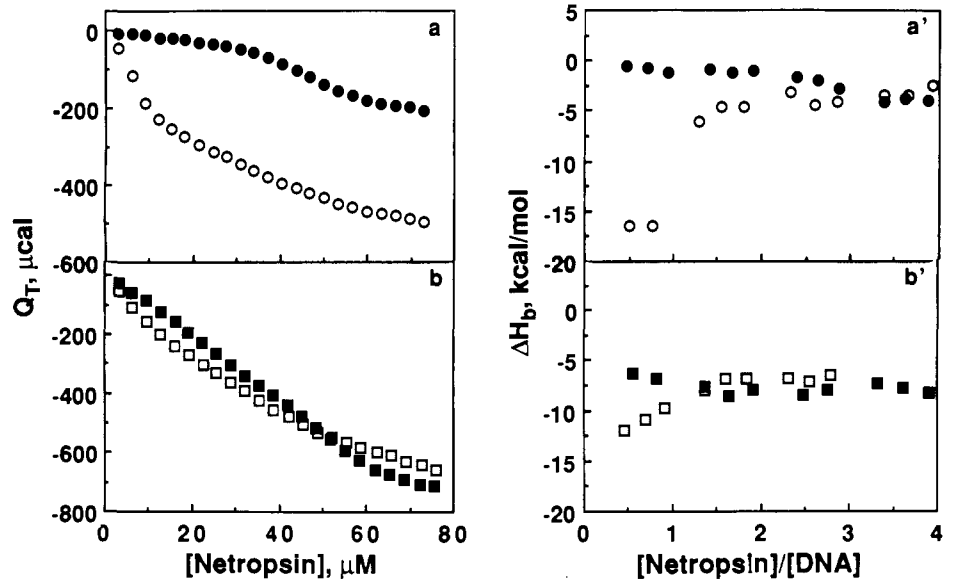


**Figure 5.** (Left) Calorimetric titration curves for netropsin–duplex complexes in 10 mM sodium cacodylate buffer containing 0.1 mM $Na_2EDTA$ at pH 7.0 and 5 °C. To obtain each curve, 2 mL of duplex solution (25 μM in strands) was titrated with 5-μL aliquots of a 0.85 mM netropsin solution. Curves shown are for netropsin complexes with (a) ps-D1·D2 (○) and aps-D1·D3 (●) and (b) ps-D5·D6 (□) and aps-D5·D7 (■). (Right) Derived curves of the dependence of molar binding enthalpies on degree of netropsin binding. Curves shown are for netropsin complexes with (a') ps-D1·D2 (○) and aps-D1·D3 (●) (b') ps-D5·D6 (□) and aps-D5·D7 (■).

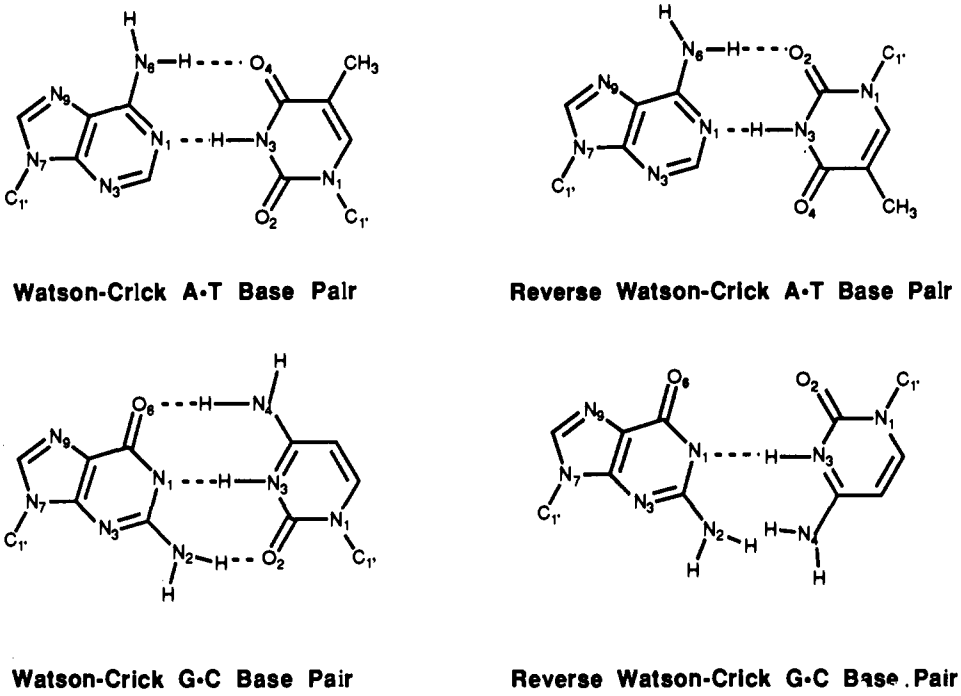


**Figure 6.** Some of the types of base-pairing schemes found in nucleic acids.

sequences constitute similar binding sites. NMR or crystal X-ray structures of netropsin with all the possible binding sites are still unavailable. The available structures of netropsin–DNA complexes containing sites consisting of stretches of A·T base pairs, GAATTC/GAATTC, AAATTT/AAATTT, and AAAAA/TTTTT,[12-14] indicate changes in the type and number of hydrogen bonds between the peptide protons and the O2 of thymine and N3 of adenine. Therefore their hydrogen-bonding contributions to the overall binding strength are similar. The lower $K_b$ values for netropsin binding to the parallel duplexes may reflect small differences in the van der Waals contributions due to changes in groove width.

**Contributions to the Binding Enthalpies and Binding Entropies.** The binding of netropsin to all four duplexes is accompanied by exothermic enthalpies with magnitudes that depend upon the amount of bound ligand and the duplex conformation (Table I). The average magnitude of these enthalpy values depends upon the relative contributions of specific H-bonding, van der Waals interactions, and overall hydration of the unligated duplex. If we assume similar enthalpic contributions from hydrogen-bonding and van der Waals interactions and negligible contributions from any binding-induced conformational changes of the parallel duplexes, then the observed enthalpy differences arise from hydration changes due to the high duplex content of the AA/TT base pair stack, as is the case of the differential hydration of dA·dT synthetic polymers.[21]

The overall $T\Delta S°$ term is equal to the sum of several contributions: (1) the unfavorable entropy due to a bimolecular association reaction, which is identical for all reactions; (2) the release of counterions, which is identical for the ligand association with ps-D1·D2 and with antiparallel duplexes and slightly lower with the ps-D5·D6 duplex; and (3) the release or uptake of water

molecules, which will depend upon the overall hydration of the free duplexes. Therefore, the observed entropic differences between the duplexes of the set containing all A·T base pairs should correspond entirely to hydration changes; for the second set it should correspond to changes in both hydration and release of counterions.

**Differential Thermodynamic Profiles Suggest a Lower Hydration State for the Ligand-Free ps-D1·D2 Duplex.** If each of the thermodynamic parameters for the association of netropsin to the ps-D1·D2 duplex are subtracted from the corresponding parameters of netropsin binding to the aps-D1·D3 duplex, the resulting differential thermodynamic profiles correspond to the reaction

$$\text{aps-D1·D3}_{\text{free duplex}} \rightarrow \text{ps-D1·D2}_{\text{free duplex}} + \Delta n\text{H}_2\text{O} \quad (3)$$

We can use this thermodynamic cycle because binding of netropsin to the duplexes is accompanied by similar $K_b$ values, stoichiometries, and release of counterions. In addition, we are assuming that the structures of both complexes are stabilized by similar molecular interactions, so that any differences in the type of hydrogen bonds and/or van der Waals contacts would contribute negligibly to the overall energetics. We obtain a small $\Delta\Delta G°$ of −0.3 kcal mol$^{-1}$, which is the result of a compensation of an unfavorable $\Delta\Delta H°$ of +4.4 kcal mol$^{-1}$ and a favorable $\Delta(T\Delta S°)$ of +4.7 kcal mol$^{-1}$, and a marginal differential counterion release, $\Delta\Delta n_{Na^+}$ of +0.01 mol of $Na^+$/mol of bound ligand. The positive sign of this enthalpy–entropy compensation is the same as in the differential parameters obtained on binding this ligand to poly(dA)·poly(dT) and to poly[d(AT)]·poly[d(AT)], in which we measured a higher hydration state for the homopolymer. Therefore, the $\Delta n$ term in eq 3 is a positive quantity that corresponds to a release of water molecules and suggests that the unligated parallel duplex is less hydrated. Furthermore, to estimate this decrease in differential hydration of the parallel duplex relative to the antiparallel duplex, we use the average value of 0.3 kcal mol$^{-1}$ for the enthalpy of releasing 1 mol of electrostricted water form the native DNA molecule[32,33] and $\Delta\Delta H = \Delta(T\Delta S°) = 4.7$ kcal mol$^{-1}$. We obtain 16 ± 1 mol of $H_2O$/mol of complex, or 3 to 4 mol of $H_2O$/base pair. If instead we use the thermodynamic profiles for the formation of the same duplexes reported previously[8] and a similar thermodynamic cycle, we obtain $\Delta\Delta H = \Delta(T\Delta S°) = 23$ (±7) kcal/mol of duplex, corresponding to a differential hydration of 3 to 5 water molecules/base pair. The similarity of these numbers strongly suggests that the groove in the ps-D1·D2 duplex is less hydrated than the minor groove in aps-D1·D3. This is consistent with the increased hydration of AA/TT stretches,[21,34] which contain a narrower minor groove as observed in X-ray crystal structure studies.[35] Perhaps it is this lower hydration state of parallel DNA that is responsible for its lower stability. We are currently measuring volume changes for these association reactions, for which our calculations predict a significant differential volume change of 10 mL/mol of base pair.

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# Structure–Reactivity Correlations for Reactions of Substituted Phenolate Anions with Acetate and Formate Esters†

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**Abstract:** The reactions of substituted phenolate anions with *m*-nitrophenyl, *p*-nitrophenyl, and 3,4-dinitrophenyl formates follow nonlinear Brønsted-type correlations that might be taken as evidence for a change in the rate-limiting step of a reaction that proceeds through a tetrahedral addition intermediate. However, the correlation actually represents two different Brønsted lines that are defined by meta- and para-substituted phenolate anions and by meta- and para-substituted *o*-chlorophenolate anions. A concerted mechanism for both acetyl- and formyl-transfer reactions is supported by the absence of a detectable change in the Brønsted slope at $\Delta pK = 0$ for the attacking and leaving phenolate anions within each class of Brønsted correlations. Regular increases in the dependence of log $k$ on the $pK_a$ of the nucleophile with increasing $pK_a$ of the leaving group correspond to a positive interaction coefficient $p_{xy} = \partial\beta_{lg}/\partial(pK_{nuc}) = \partial\beta_{nuc}/\partial(pK_{lg})$. The observation of two different Brønsted lines for the reactions of substituted phenolate anions with phenyl acetates is attributed to a steric effect that decreases the rate of reaction of substituted *o*-chlorophenolate anions by 25–50%. The reactions of meta- and para-substituted phenolate and *o*-chlorophenolate anions with substituted phenyl acetate esters follow values of $\beta_{nuc} = 0.53$–$0.66$ and $-\beta_{lg} = 0.50$–$0.63$. The reactions of meta- and para-substituted phenolate anions with formate esters are $\sim 10^3$ times faster and follow smaller values of $\beta_{nuc} = 0.43$–$0.64$ and $-\beta_{lg} = 0.31$–$0.48$. However, the reactions of meta- and para-substituted *o*-chlorophenolate anions with the same formate esters follow larger values of $\beta_{nuc} = 0.63$–$0.90$ and $-\beta_{lg} = 0.46$–$0.90$. The large values of $\beta_{nuc}$ and $-\beta_{lg}$ for the reactions of substituted *o*-chlorophenolate anions with formate esters may arise from destabilization by the *o*-chloro group of a stacking interaction that is present in the transition state for reactions of formate esters, but not acetate esters.

## Introduction

Acyl-transfer reactions can occur through a stepwise mechanism, with a tetrahedral addition intermediate that has a significant lifetime, or through a concerted, one-step mechanism with a single transition state and no addition intermediate. The change from a stepwise to a concerted mechanism with changing structure of the reactants presumably occurs when the addition compound becomes so unstable that it does not have a signficiant lifetime; there is no significant barrier for its decomposition, and it does

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