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Enthalpy–entropy compensations in drug–DNA binding studies

(thermodynamic driving forces/drug-induced changes/probes of DNA conformation)

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ABSTRACT We present a comparative study of calorimetrically derived thermodynamic profiles for the binding of a series of drugs with selected DNA host duplexes. We use these data to demonstrate that comparisons between *complete* thermodynamic profiles (ΔG°, ΔH°, ΔS°, ΔCp) are required before drug binding can be used as a probe of DNA conformation, since enthalpy–entropy compensations can cause two drug–DNA binding events to exhibit similar binding free energies (ΔG°) despite being driven by entirely different thermodynamic forces (ΔH°, ΔS°). In this work, we employ a combination of spectroscopic and calorimetric techniques to characterize thermodynamically the DNA binding of netropsin and distamycin (two minor groove-directed ligands), ethidium (an intercalator), and daunomycin (a combined intercalator/groove binder). Our free energy data (ΔG°) show that each drug exhibits similar binding affinities at 25°C for the alternating copolymer duplex poly[d(A-T)]·poly[d(A-T)] and for the homopolymer duplex poly(dA)·poly(dT). However, our calorimetric measurements reveal that the nature of the thermodynamic forces (ΔH°, ΔS°) that drive drug binding to these two host duplexes at 25°C are entirely different, despite similar binding free energies (ΔG°) and similar salt dependencies ($\partial \ln K/\partial \ln[Na^+]$). Specifically, the 25°C binding of all four drugs to the alternating copolymer poly[d(A-T)]·poly[d(A-T)] is overwhelmingly *enthalpy driven*, whereas the corresponding binding of each drug to the homopolymer duplex poly(dA)·poly(dT) is overwhelmingly *entropy driven*. Thus, the similar binding free energies (ΔG°) we measure for complexation of each drug with poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT) result from compensating changes in the enthalpy and entropy terms. Comparison with the thermodynamic profiles for the complexation of these drug molecules to other DNA host duplexes at 25°C reveals that the binding of each is strongly enthalpy driven, except when the poly(dA)·poly(dT) homopolymer serves as the host duplex. This comparison allows us to conclude that poly[d(A-T)]·poly[d(A-T)] behaves thermodynamically as the more "normal" host duplex toward drug binding, whereas the entropy-driven binding to the poly(dA)·poly(dT) duplex represents "aberrant" behavior. Furthermore, since each of the four drugs exhibits different modes of DNA binding, we conclude that the observed entropy-driven behavior for binding to poly(dA)·poly(dT) reflects an intrinsic property of the homopolymer duplex that is perturbed in a common manner upon ligation rather than a common property of all four binding ligands. To rationalize the large positive entropy changes that drive drug complexation with the poly(dA)·poly(dT) duplex, we propose a model that emphasizes binding-induced perturbations of the more highly hydrated, altered B conformation of the homopolymer. Our results suggest that an aberrant thermodynamic binding profile may reflect an unusual DNA conformation in the host duplex. However, before such a conclusion can be reached, *complete* thermodynamic binding profiles must be examined, since enthalpy–entropy compensations can cause two binding events to exhibit similar binding constants even when they are driven by very different thermodynamic forces.

Parallel structural and thermodynamic studies on drug binding to DNA can yield insights into the origins of drug binding affinities and specificities that neither study alone could provide (1–9). We already have demonstrated the power of such parallel investigations in our characterization of the molecular origins of the DNA binding affinity and binding specificity exhibited by the oligopeptide netropsin (Net) (6, 7). Specifically, we have used the structural picture derived from NMR and x-ray studies to develop a microscopic interpretation of our macroscopic thermodynamic binding data.

In developing correlations between thermodynamic and structural data, it is essential to understand potential thermodynamic contributions from binding-induced changes in solvation and conformation (4–10). Such information is needed before one can dissect thermodynamic binding profiles into contributions from general solvent effects and from specific drug–DNA interactions. In this connection, we have shown for Net binding that a comparison of free energy data alone can be misleading due to enthalpy–entropy compensations (6, 7). Specifically, we demonstrated that despite nearly identical binding free energies (ΔG°) and binding salt dependencies ($\partial \log K/\partial \log[Na^+]$), Net complexation to the poly[d(A-T)]·poly[d(A-T)] duplex and to the poly(dA)·poly(dT) duplex is dictated by entirely different driving forces (ΔH°, ΔS°). We found Net binding to the alternating copolymer duplex poly[d(A-T)]·poly[d(A-T)] to be overwhelmingly enthalpy driven, whereas binding to the homopolymer duplex is overwhelmingly entropy driven. The nearly identical binding free energies we measured for Net complexation with the two host duplexes resulted from compensating enthalpy and entropy changes. Initial efforts to explain this behavior focused on positive entropy contributions from binding-induced disruption of the unique water spine that lines the minor groove around AT-rich domains (11–13). These explanations implied that such enthalpy–entropy compensation effects would be a unique feature of AT-specific minor groove binding ligands. However, subsequent Net binding studies on DNA host duplexes void of A·T base pairs revealed that disruption of AT-specific minor groove bound water is not required to yield positive binding entropies (6). This intriguing new result motivated us to ask the following question: Does the large positive entropy change we observe for Net binding to the poly(dA)·poly(dT) duplex reflect a general property of the homopolymer host duplex or does it reflect a unique property of AT-specific minor groove binding ligands?

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Abbreviations: t_m, melting temperature; EtdBr, ethidium bromide; Dau, daunomycin; Net, netropsin; Dis A, distamycin A.

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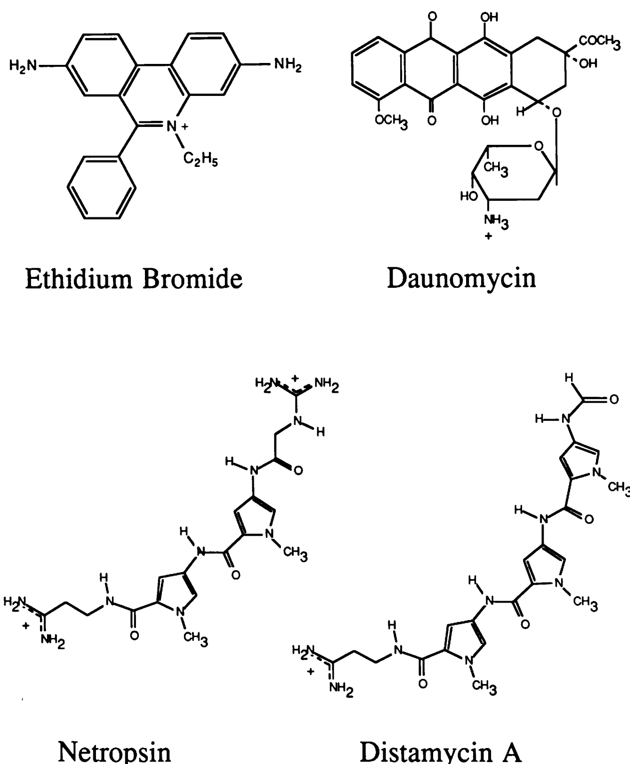
To address this question, we have used a combination of spectroscopic and calorimetric techniques to characterize thermodynamically the binding of an intercalator [ethidium bromide (EtdBr)] (4, 14), an intercalator/groove binder [daunomycin (Dau)] (15, 16) and a minor groove binder [distamycin A (Dis A)] (5, 17) to the same two all-AT host duplexes used in our study of the minor groove binding ligand Net (6, 7, 9, 11). The resulting thermodynamic binding data allow us to assess if the large positive entropy change and the associated enthalpy–entropy compensation behavior observed for Net binding to poly(dA)·poly(dT) at 25°C (6, 7, 9, 11) is a unique property of AT-specific minor groove binding ligands or if it is a general feature of ligand binding to the homopolymer duplex. Significantly, structural studies already have defined the DNA binding modes of all four drugs that we thermodynamically characterize in this work (18–23). Consequently, the results reported here also will assist us in our general quest to develop correlations between the macroscopic world of thermodynamic data and the microscopic world of structural data.

MATERIALS AND METHODS

DNA Polymers. The synthetic DNA polymers used in the drug binding studies reported here were obtained from PL Biochemicals (Piscataway, NJ) with the exception of poly(dA)·poly(dT), which was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). The concentrations of the DNA solutions were determined by using the following extinction coefficients ($M^{-1}\cdot cm^{-1}$): ε_{260} of poly[d(A-T)]·poly[d(A-T)] = 6650; ε_{251} of poly[d(I-C)]·poly[d(I-C)] = 6900; ε_{254} of poly[d(G-C)]·poly[d(G-C)] = 8400; ε_{260} of poly(dA)·poly(dT) = 6000; and ε_{260} of salmon testes DNA = 6550.

Drug Molecules. EtdBr and Dau were obtained from Sigma and used without further purification. Net and Dis A were kind gifts from F. Arcamone of Farmitalia (Milano). The concentrations of drug solutions were determined by using the following extinction coefficients: $\varepsilon_{EtdBr,480}$ = 5850 $M^{-1}\cdot cm^{-1}$; $\varepsilon_{Dau,477}$ = 11,500 $M^{-1}\cdot cm^{-1}$; $\varepsilon_{Net,296}$ = 21,500 $M^{-1}\cdot cm^{-1}$; $\varepsilon_{Dis\ A,303}$ = 34,000 $M^{-1}\cdot cm^{-1}$. The structures of these drugs are shown below.



Buffer System. All solutions were prepared by using a buffer consisting of 10 mM sodium phosphate and 1 mM EDTA. This buffer was adjusted to a final pH of 7.0 with aliquots of NaOH. This procedure resulted in a total sodium ion concentration of 16 mM.

UV Melting Curves. Absorbance versus temperature profiles for each drug-free and drug-bound duplex were determined by using a thermoelectrically controlled Perkin Elmer 575 programable spectrophotometer interfaced with a Tektronix 4051 computer. Samples were heated at a rate of 0.5°C/min while the temperature and the absorbance at or near 260 nm were recorded every 30 sec. Differential melting curves [$(\Delta A/\Delta t)$ versus t] were obtained by taking the difference in absorbance every degree. Melting temperatures were derived from these curves by using previously described methods (9, 42).

Job Plots. Drug–DNA binding densities were determined by monitoring the change in absorbance of either the DNA or the drug as each drug was titrated into solutions of each host duplex. The resulting profiles of absorbance versus mol % of drug are called Job plots. Such plots are biphasic and exhibit a discontinuity at the mol % drug that reflects the stoichiometry of the drug–DNA complex. We used Job plots as well as CD titration curves and UV melting curves to determine saturation levels for each drug with each host duplex.

Determination of the Binding Constants, K_b, by Using UV Melting Curves. The thermal stability of a nucleic acid duplex can be characterized by its melting temperature (t_m), which can be derived from the differentiated absorbance versus temperature profile as previously described (7, 42). Drugs that bind more strongly to the initial duplex state compared with the final single-stranded state will cause an increase in t_m for the host duplex. All drugs studied here exhibit this behavior. Fig. 1 shows a typical family of differentiated melting curves that we obtained for the helix-to-coil transitions of poly[d(A-T)]·poly[d(A-T)] in the absence (top panel) and in the presence (lower three panels) of Dis A at the indicated DNA-to-drug ratios. Note that the saturation bind-

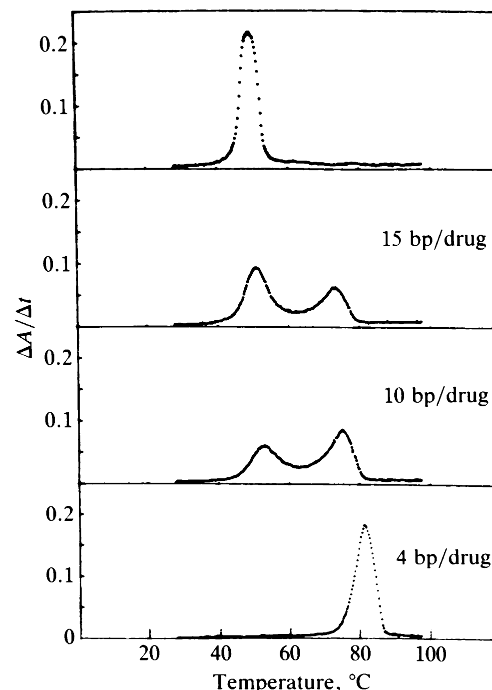


FIG. 1. Differentiated melting curves ($\Delta A/\Delta t$ versus t) for the helix-to-coil transitions of poly[d(A-T)]·poly[d(A-T)] in the absence and presence of Dis A at the indicated DNA-to-drug ratios. bp, base pairs.

ing of Dis A induces a large increase in the thermal stability of the host duplex. Specifically, we measure a Δt_m value [t_m(drug-bound duplex) $- t_m°$(drug-free duplex)] of ≈30°C for Dis A binding to the poly[d(A-T)]·poly[d(A-T)] duplex at a base pair-to-drug ratio of 4:1. This approach allowed us to determine Δt_m values for the binding of Dis A, Dau, and EtdBr to each host duplex. Following the theoretical treatment of Crothers (24), we used these Δt_m values in conjunction with optically derived binding densities and calorimetrically determined transition enthalpies to calculate binding constants (K_b) for the association of each drug with its corresponding host duplex at 25°C. We previously have described the details of this calculation (7). These K_b values then were used in conjunction with calorimetrically determined binding enthalpies to calculate binding free energies at 25°C.

Determination of $\partial \ln K_b/\partial \ln[Na^+]$ Values. We have used the Δt_m method noted above to determine K_b values over a range of salt concentrations for the binding of each drug to each host duplex. The resulting data allow us to construct ln–ln plots of K_b versus $[Na^+]$. The slopes of such plots provide values for $\partial \ln K/\partial \ln[Na^+]$. We have used this approach to determine $\partial \ln K/\partial \ln[Na^+]$ values for the DNA binding of each drug to the two all-AT polymer duplexes. The resulting data are listed in Table 3. These values are typical of what one might expect for the binding of mono- and dications to DNA duplexes (25, 26).

Batch Calorimetry. The batch calorimeter used in this study is based upon the design of Prosen and Berger and has been described in detail (7, 9, 27, 28). We determined the binding enthalpy, ΔH_b, at 25°C for complexation of each drug with the corresponding host duplexes by mixing in the calorimeter solutions of each drug with each nucleic acid duplex. Fig. 2 shows a typical heat burst curve that results from the binding of Net with poly[d(I-C)]·poly[d(I-C)]. Significantly, we worked at low enough drug concentrations so that nearly all heats of dilution (due to drug aggregation) were negligible. The lone exception was Dau, which aggregates at low concentrations and therefore required a correction for the heat of dilution. For this correction, we used a noncalorimetrically determined van't Hoff enthalpy of aggregation (9). Consequently, the Dau binding enthalpies listed in Tables 1 and 2 may differ from their absolute values, depending on the accuracy of the van't Hoff correction term. However, the relative values of the Dau binding enthalpies remain unaltered since the van't Hoff correction term was applied equally to each measurement. For this reason, the general duplex-dependent trends observed in the thermodynamic binding profiles of Dau are not dependent on the accuracy of the correction term.

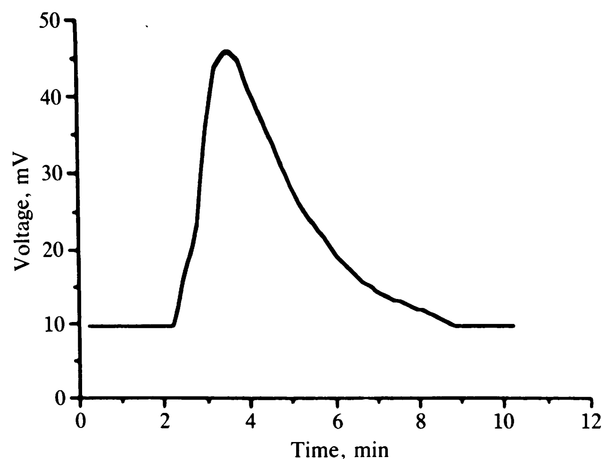


FIG. 2. Typical calorimetric heat burst curve. This curve was produced upon mixing a solution of Net (0.757 mM) and a solution of poly[d(I-C)]·poly[d(I-C)] at a phosphate-to-drug ratio of 10:1. The area under the curve corresponds to a total heat production of 1.177 mcal (1 cal = 4.184 J).

RESULTS AND DISCUSSION

Poly[d(A-T)]·poly[d(A-T)] versus Poly(dA)·Poly(dT) as Host Duplexes. By using a combination of spectroscopic and calorimetric techniques (see *Materials and Methods*), we have obtained complete thermodynamic profiles for the 25°C binding of EtdBr, Dau, and Dis A to two all-AT DNA host duplexes—namely, poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT). Our results are summarized in Table 1. For comparative purposes, we also include in Table 1 our previously published results on Net binding (6, 7). Inspection of the data in Table 1 reveals the following two significant features: (*i*) For each drug, the binding affinity ($\Delta G°$) at 25°C qualitatively is similar for complexation to the alternating copolymer duplex and the homopolymer duplex. (*ii*) For each drug, binding to the alternating copolymer duplex at 25°C is overwhelmingly *enthalpy driven*, whereas binding to the homopolymer duplex at 25°C is overwhelmingly *entropy driven*. Thus, despite different structures and different modes of binding, enthalpy–entropy compensations at 25°C cause all four drugs to exhibit similar binding affinities ($\Delta G°$) when they complex with the two all-AT DNA host duplexes (see data in Table 1). The inescapable conclusion is that the origin of these compensating enthalpy and entropy changes resides in the differential properties of the two all-AT host duplexes rather than in the nature of the binding ligand. Furthermore, since minor groove binding ligands (Net and Dis A) as well as intercalators (EtdBr and Dau) exhibit similar compensation behavior, it is unlikely that the "water spine" in the minor grove contributes significantly to the compensation phenomenon (6, 11–13). For the case of Net binding to the two all-AT host duplexes, we already have shown that the enthalpy and entropy changes as well as the associated compensation phenomenon we observe at 25°C also exist at 42°C (e.g., $\Delta C_p \approx 0$) (7). In other words, the compensation behavior observed for Net binding is not a peculiar property of the temperature (25°C) at which we conducted our drug binding studies.

Which of the two all-AT host duplexes {poly[d(A-T)]·poly[d(A-T)] or poly(dA)·poly(dT)} exhibits "normal" DNA binding thermodynamics and which is "aberrant"? To answer this question, we have determined the binding profiles for all four drugs to several other DNA host duplexes. The results of these studies are listed in Table 2. Comparison of the data in Tables 1 and 2 reveals that the alternating copolymer poly[d(A-T)]·poly[d(A-T)] behaves thermodynamically as the more normal host duplex for all of the drugs studied. Specifically, the binding of all four drugs at 25°C to the DNA host duplexes listed in Table 2 is overwhelmingly enthalpy driven, as we observe for binding to the poly[d(A-T)]·poly[d(A-T)] duplex. By contrast, only the poly(dA)·poly(dT) duplex exhibits the strong entropic driving force for drug binding. We therefore conclude that the poly(dA)·poly(dT) duplex possesses properties that cause it to yield anomalous thermodynamic profiles for drug binding.

Influence of Electrostatics. Could the thermodynamic differences noted in Table 1 simply be electrostatic in origin? To address this question, we have determined the salt dependence for the binding constants of each drug to the poly[d-(A-T)]·poly[d(A-T)] duplex and to the poly(dA)·poly(dT) duplex. Our results are listed in Table 3. Inspection of these data reveal that EtdBr, Dau, and Dis A exhibit $\partial \ln K/\partial \ln[Na^+]$ values that are consistent with monocation binding, whereas Net exhibits a $\partial \ln K/\partial \ln[Na^+]$ value that is consistent with dication binding. In other words, these data demonstrate that independent of the host duplex, the electrostatic contribution

Table 1. Comparison of thermodynamic profiles for drug binding at 25°C to the alternating copolymer duplex poly[d(A-T)]·poly[d(A-T)] and the homopolymer duplex poly(dA)·poly(dT)

		Poly[d(A-T)]·poly[d(A-T)]			Poly(dA)·poly(dT)		
Drug	Binding mode	ΔG°, kcal/mol	ΔH°, kcal/mol	ΔS°, cal/K·mol	ΔG°, kcal/mol	ΔH°, kcal/mol	ΔS°, cal/K·mol
Net	Minor groove	−12.7	−11.2	+5	−12.2	−2.2	+33
Dis A	Minor groove	−12.6	−18.5	−20	−11.4	−4.2	+24
EtdBr	Intercalator	−9.1	−10.0	−3	−7.2	−1.2	+18
Dau	Intercalator/minor groove	−9.4	−8.9*	+2	−8.4	−2.1*	+21

*Refer to batch calorimetry discussion in *Materials and Methods*.

to the binding of each drug qualitatively is consistent with the charge on the drug (25, 26). Thus, the differences in the thermodynamic binding data listed in Table 1 for poly[d(A-T)]·poly[d(A-T)] versus poly(dA)·poly(dT) do not reflect differences in electrostatic contributions to the binding event for each host duplex.

Origin of Unusual Binding Thermodynamics Exhibited by the Poly(dA)·poly(dT) Host Duplex. So far we have shown that the large positive entropy changes that drive drug binding to the poly(dA)·poly(dT) duplex are not a function of either the binding ligand, the binding mode, or the electrostatics of the binding event. Furthermore, from comparisons with other DNA host duplexes (Table 1 versus Table 2) we have demonstrated that the all-AT homopolymer is the host duplex that exhibits aberrant, entropy-driven drug binding thermodynamics. This aberrant drug binding behavior suggests that the homopolymer duplex possesses features that distinguish it from classic, B-DNA double helices. This conclusion, which is based exclusively on thermodynamic data, is consistent with the results of structural studies that suggest that the poly(dA)·poly(dT) duplex exists in an altered B conformation (29–35) that is more highly hydrated (36). These structural studies provide us with a microscopic framework in which to develop a molecular interpretation for the entropy-driven process we observe for ligand binding to the poly(dA)·poly(dT) duplex.

Our molecular "explanation" for the reduction in enthalpic and increase in entropic driving forces associated with drug binding to the poly(dA)·poly(dT) duplex focuses on two unusual properties of the host duplex—namely, its altered B conformation and its greater degree of hydration. As described below, these two properties (conformation and hydration) are not independent and therefore may change in a coupled manner upon drug binding to the poly(dA)·poly(dT) duplex, so as to give rise to the observed enthalpy–entropy compensation.

A large number of studies suggest that poly(dA)·poly(dT) exists in solution in a non-B conformation (29–35). Drug binding to this altered conformation may induce the host duplex into a B conformation. Such binding-induced intramolecular conformational changes have been reported (37). If such a drug-induced intramolecular transition is endothermic, then it will reduce the observed exothermicity of the drug binding enthalpy relative to its intrinsic B-conformation value. We observe such a reduced exothermicity when poly(dA)·poly(dT) serves as the host duplex. However, existing data on helix-to-helix transitions suggest that such an intramolecular transition may not yield an enthalpy change large enough to rationalize the reduction in binding enthalpy that we observe (38, 39).

Alternatively, we can focus on the unusual hydration of the poly(dA)·poly(dT) duplex in our effort to develop an explanation for the compensating enthalpy and entropy changes we observe when drugs bind to the homopolymer duplex compared with binding to other host duplexes. [Recall that the altered conformation of the poly(dA)·poly(dT) duplex is more highly hydrated than classic B-form DNA duplex structures (36)]. In this picture, drug binding to the homopolymer duplex disrupts some of the DNA hydration sphere, thereby releasing water to the bulk solvent and making a positive contribution to the binding entropy. Berman (40) and Breslauer and coworkers (6, 7, 9) independently have proposed that such solvent-induced positive entropy contributions may be a general feature of the thermodynamic forces that drive ligand–DNA interactions. Consistent with this picture, we observe a more positive entropy change for drug binding to the more highly hydrated homopolymer [poly(dA)·poly(dT)] compared with drug binding to the alternating copolymer {poly[d(A-T)]·poly[d(A-T)]}. This proposed disruption or "melting" of the hydration domains can be envisioned as an endothermic process, thereby reducing the exothermicity of the observed binding enthalpy. These two coupled, drug-induced solvent effects ["melting" of hydration layers ($+\Delta H$) and release of the "melted" water to the bulk medium ($+\Delta S$)] may account for the reduced enthalpic and increased entropic driving forces we observe for drug binding to the poly(dA)·poly(dT) duplex. In other words, these two coupled solvent effects could "explain" the enthalpy–entropy compensation phenomenon we observe for drug binding to the poly(dA)·poly(dT) duplex.

Significantly, our proposed binding-induced disturbance of poly(dA)·poly(dT) hydration does not depend on the mode of drug binding since both intercalators (e.g., EtdBr) and groove binders (e.g., Net) exhibit similar enthalpy–entropy compensations (see data in Table 1). We therefore conclude that the compensating enthalpy and entropy changes we observe when drugs bind to the poly(dA)·poly(dT) homopolymer duplex do not uniquely reflect disruption of a water spine in the minor groove of the host duplex. Instead, we propose that the compensation behavior we observe results from funda-

Table 2. Thermodynamics of drug binding to selected DNA host duplexes at 25°C

Drug and host duplex	ΔG°, kcal/mol	ΔH°, kcal/mol	ΔS°, cal/K·mol
Net + poly[d(I-C)]·poly[d(I-C)]	−11.1	−9.9	+4
EtdBr + poly[d(I-C)]·poly[d(I-C)]	−9.3	−9.2	+1
EtdBr + salmon testes DNA	−9.5	−12.4	−10
Dau + poly[d(G-C)]·poly[d(G-C)]	−9.0	−10.4*	−5
Dau + salmon testes DNA	−9.0	−9.9*	−3
Dis A + $[d(GCGAATTCGC)]_2$	−11.5	−15.8	−16

*Refer to batch calorimetry discussion in *Materials and Methods*.

Table 3. Salt dependence of the 25°C binding constants for drug complexation with the poly[d(A-T)]·poly[d(A-T)] and the poly(dA)·poly(dT) host duplexes

Drug	Poly[d(A-T)]·poly[d(A-T)] $\partial \ln K/\partial \ln[Na^+]$	Poly(dA)·poly(dT) $\partial \ln K/\partial \ln[Na^+]$
Net	−1.63	−1.51
Dis A	−0.79	−0.97
EtdBr	−1.06	—
Dau	−0.90	−1.20

mental differences in hydration [at levels beyond the minor groove (41)] of the poly(dA)·poly(dT) duplex compared with poly[d(AT)]·poly[d(AT)] and other B-form duplexes.

Drug Binding as a Probe of DNA Conformation. An interesting application of drug binding studies is their use as a probe of unusual DNA conformational domains, including DNA bending. The reasoning behind this application is that a drug will exhibit a differential binding affinity ($\Delta G°$) for DNA regions that assume normal B-like conformations compared with those regions which adopt non-B conformations. The thermodynamic binding studies reported here reveal that this application of drug binding could be misleading if one reached conclusions based exclusively on comparisons of binding free energy data ($\Delta G°$) and the salt dependence of the binding constant ($\partial \ln K/\partial \ln[Na^+]$). Specifically, we have demonstrated that binding events that are driven by entirely different thermodynamic forces ($\Delta H°$ versus $\Delta S°$) can exhibit nearly identical binding free energies ($\Delta G°$) due to impressive enthalpy–entropy compensations. Consequently, differences in binding profiles that reflect differences in the conformation of the host duplex are best detected by comparing binding enthalpies and entropies rather than binding free energies. Thus, when using a drug as a probe of DNA structure, *complete* thermodynamic profiles should be determined and compared to avoid erroneous conclusions based exclusively on comparisons of free energy data.

Note Added in Proof. By using a newly developed more sensitive stopped-flow microcalorimeter, we recently determined binding enthalpies for Dau at drug concentrations below the aggregation limit, thereby precluding the need to correct for self-association of the drug (D.P.R., K.J.B., R. L. Berger, and C. P. Mudd, unpublished results). These directly determined Dau binding enthalpies are somewhat more endothermic than the corresponding values listed in Tables 1 and 2. Significantly, however, the relative values of the Dau binding enthalpies are not altered so that the trends we observe in the thermodynamic binding profiles for Dau remain valid.

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