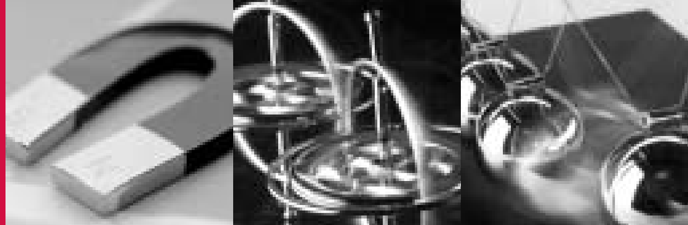
Thermodynamics of Nucleic Acid-Drug Interactions



Application Note

Introduction

Nucleic acids are common targets for antiviral, anticancer, and antibiotic drugs. DNA-binding drugs are designed to modulate gene activity, and RNA-binding drugs inhibit protein translation. In order to optimize the efficacy of drugs, as well as discover new drugs, it is important to fully characterize the drug-nucleic acid interaction, including sequence recognition, structural details and the thermodynamics of binding.

Microcalorimetry is a tool used to determine the thermodynamics of intermolecular binding. Both Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry (DSC) are used to elucidate thermodynamic details of nucleic acid-drug interactions. Thermodynamics, when used in conjunction with structure, sequence, and computational methods, can be used to optimize drugs to bind specific sequences and/or structures of nucleic acid targets.

This application note will review the use of calorimetry to study the thermodynamics of drug-nucleic acid binding. For more information, refer to cited references. Several excellent review articles are also available on this topic (References 1-5).

Overview of Drug-DNA Interactions

There are two principal modes for non-covalent binding to DNA – intercalation and minor groove binding.[1-5] Intercalating drugs have planar, heteroaromatic ring systems which insert between two adjacent base pairs in a helix. The drug-DNA complex is stabilized by π–π hydrophobic and van der Waals interactions between the DNA bases and the drug molecule. Intercalating drugs also cause structural perturbations in the DNA to accommodate the binding, such as the unwinding of the helix and a lengthening of the DNA. Intercalators include ethidium, daunorubicin and actinomycin.

The second mode is minor groove binding. These drugs consist of several aromatic rings (e.g. benzene and pyrrole) and these rings are connected by bonds which have torsional freedom. These drugs typically have a “crescent” shape, so there is a sterically favorable fit during binding and the drug conforms to the target groove of the DNA. Binding is stabilized by hydrophobic interactions, as well as van der Waals interactions and hydrogen bonding. Binding preference is to the A-T base pairs. Minor groove binders do not induce significant structural changes to the DNA. Drugs in this category include Hoechst 33258, netropsin and distamycin.

Thermodynamics of Drug-DNA Interactions

The study of thermodynamics determines the forces for the binding of a drug to its target. To calculate the observed free energy change for an interaction (ΔG_{obs}), the following relationship is used:

$$\Delta G_{obs} = -RT\ln K_B$$

where R is universal gas constant, T is temperature, and K_B is the equilibrium binding constant. The value of K_B is dependent on pH, salt concentration, and other experimental parameters.

ITC directly measures the enthalpy of binding (ΔH_B) and K_B, and the entropy change associated with binding (ΔS_B) can also be calculated:

$$\Delta G_{obs} = \Delta H_B - T\Delta S_B$$

There are several methods available to experimentally determine K_B and ΔG_{obs}. These methods include ITC, surface plasmon resonance, and spectroscopic methods. It is also important to determine ΔH_B and ΔS_B for a binding interaction. ITC is the only experimental method available to directly measure ΔH_B. Two structurally-related drugs can have similar binding affinity for a DNA target. However, one drug can bind via enthalpic mode, while the binding of the other drug is primarily entropic.

When enthalpy is favorable, the driving forces in the drug-DNA interaction are hydrogen bonding, van der Waals interactions, and electrostatic interactions. When entropy is favorable, binding is driven by hydrophobic interactions, while unfavorable entropic changes are due to loss of conformational degrees of freedom. In any interaction there is a balance of opposing factors that result in the observed binding and ΔG_{obs}.

Heat capacity change due to binding (ΔC_p) can also be measured by ITC, by determining the dependence of ΔH on temperature. The heat capacity change can also be estimated from change in accessible surface area (ΔASA), and one can correlate calculated heat capacity values to experimentally-determined values.

Scientists have identified the energetic contributions to the binding free energy for drug-DNA interactions, and these free energy terms can be estimated with ITC data.[1-5] The observed binding constant and ΔG_{obs} is dependent on buffer, temperature, ionic strength, concentration, etc., and need to take into account the effects of the experimental conditions. The following five free energy components have been identified for DNA-drug interactions:

- Unfavorable contribution from conformational changes in the drug or DNA due to binding (ΔG_{conf})

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- Unfavorable contribution due to loss of rotational and translational degrees of freedom upon binding (ΔG_{r+t})
- Contribution from hydrophobic transfer of the unbound drug to the DNA binding site (ΔG_{hyd}). This value can be determined from heat capacity change.
- Contribution from coupled polyelectrolyte effects due to binding of cationic ligands (ΔG_{pe}). DNA is a polyanion, so it binds cations. Binding a cationic drug to DNA effectively expels cations from the DNA. Binding constant and observed ΔG are dependent on salt concentration. ΔG_{pe} can be determined experimentally by measuring the binding constant at different salt concentrations.
- Contribution due to non-covalent interactions, such as hydrogen bond formation and van der Waals interactions (ΔG_{mol})

These free energy terms can be experimentally determined by ITC, in conjunction with established empirical relationships from other experimental data. For a full review of the energetics of binding, see References 1-5.

Knowledge of these terms will determine the energetics of drug-DNA binding, and this information is used to modify drug structure to improve binding affinity and selectivity.

Minor Groove Recognition of Duplex DNA

Hoechst 33258 is a well-characterized minor groove binding ligand. ITC studies where Hoechst 33258 bound to a short oligonucleotide containing a single specific binding site, showed that binding below 35°C was entropically driven.[6] ΔH_B determined calorimetrically differed in magnitude and size compared to van't Hoff enthalpies determined by fluorescence spectroscopy.[7] Calorimetric and crystallographic studies indicate that binding of Hoechst 33258 to the oligonucleotide model sequence is a rigid body interaction with very little conformational changes to DNA and drug (ΔG_{conf} =0). Using ΔC_p determined by ITC, as well as previously derived values for entropy changes related to rotation and translation, the thermodynamics for binding of Hoechst 33258 at 25ºC was determined (Figure 1 and Table I).

Other groove binders have been studied, and some of these results are summarized in Table I. All of these drug-DNA interactions demonstrate a negative heat capacity change; however, binding is enthalpically driven (negative ΔH) for some drugs. One trend is that drugs related to Hoechst 33258 are entropically driven with unfavorable enthalpy. In all cases of drugs binding to minor groove of duplex DNA, favorable entropy is due to hydrophobic interaction and release of bound water when the drug binds to DNA, as well as release of counterions when cationic drugs bind to DNA. Thermodynamic profiles of minor groove binders to DNA are dependent upon the sequence at the binding site.

ITC and DSC data has shown that berenil preferentially binds to A-T rich regions in DNA, but it can also bind to G-C rich regions of DNA.[8]

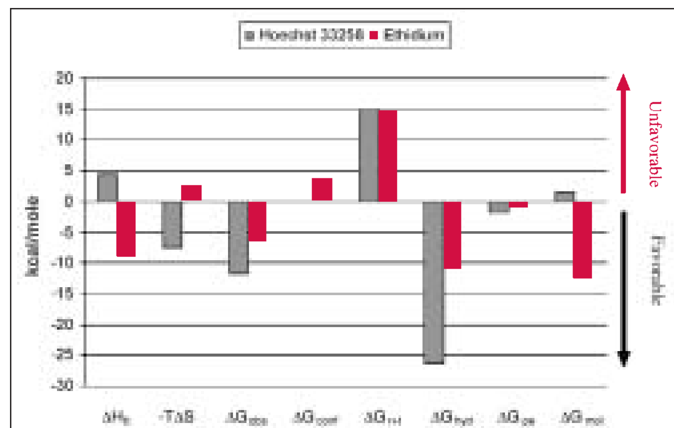

FIGURE 1. Thermodynamic profiles for the interaction of Hoechst 33258 (from Reference 6) and ethidium (from Reference 5) with DNA. Binding at 25°C. Positive values are unfavorable, negative values are favorable forces. Note that ΔG_{conf} for Hoechst 33258 is 0.

	T (ºC)	ΔG_{obs} (kcal/mol)	ΔH_B (kcal/mol)	$T\Delta S_B$ (kcal/mol)	ΔC_p (cal/mole-K)	Ref.
Groove Binders:						
Hoechst 33258	25	-7.7	+4.4	+12.1	-330	5,6
Netropsin	25	-8.7	-5.8	+2.9	-213	5
Distamycin	20	-10.1	-12.3	-2.2	na	2
Berenil	25	-8.0	+0.6	+8.6	-146	5
Intercalators:						
Daunorubicin	20	-7.9	-9.0	+1.1	-160	2
Doxorubicin	20	-8.9	-7.4	+1.5	-150	2
Ethidium	25	-6.7	-9.0	-2.3	-140	2
Propidium	25	-7.5	-6.8	+0.7	-150	2
Actinomycin	10	-8.5	-2.7	+5.8	-364	2

Table I. Thermodynamic parameters for minor groove binding drugs and intercalators binding to DNA. For detailed information refer to cited reference.

Intercalating Drug Recognition of Duplex DNA

Daunomycin and ethidium are well-characterized DNA intercalators. ITC has been used to look at the thermodynamics of intercalators binding to double-stranded DNA.[1-5] In general, intercalation is driven by hydrophobic binding of the drug to the intercalation site. Also, for ethidium and propidium, there were significant structural changes to the DNA, including an increase in base pair rise by approximately 3.4Å, as well as DNA unwinding. Using data obtained by other techniques and ITC, the free energy components were determined for several intercalators (see Figure 1 for ethidium, and Table I for ethidium and other drugs).

For intercalators shown in Table I, there is a free energy cost due to the structural changes in DNA to bind to the intercalator, as well as loss in translational and rotational degrees of freedom in the drug and the DNA after binding (see Figure 1 for free energy contributions to ethidium binding). The other free energy contributions were sufficiently favorable to result in binding. As in minor groove binding drugs, there is a favorable contribution from hydrophobic interactions, as well as polyelectrolyte release. One difference between free energy of Hoechst 33258 binding,

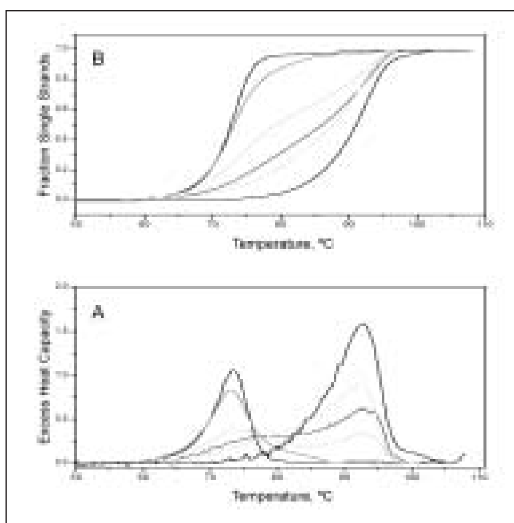

FIGURE 2. DSC studies of daunorubicin binding to *E. coli* DNA. The bottom panel A shows the primary DSC data. The top panel shows the integrated DSC data, normalized to show the fraction of single strands as a function of temperature. The integrated curves in the top panel B correspond to molar ratios of added drug to DNA base pairs of (from left to right): 0, 0.05, 0.067, 0.111, 0.167, 0.333. The DNA concentration in all experiments was 0.84 mM base pairs.

compared to intercalators, is that Hoechst 33258 binding is driven by hydrophobic interactions, while other intercalators bind due to hydrophobic and non-covalent interactions.

DSC is also used to study the binding of drugs to DNA[9-12] (Figure 2). When a drug is added to DNA, the transition midpoint (T_m) increases, as does the area under the thermogram. When drug concentration is high enough to saturate the binding sites on the DNA, so drug binding is only to the duplex form, McGhee[13] showed that:

$$\frac{1}{T_m^0} - \frac{1}{T_m} = \frac{R}{\Delta H_{DNA}} \ln[(1 + K_h L)^{1/n}$$

DSC and UV melting profiles have been used to measure the binding affinity of a new bisintercalating antibiotic, while other methods were not successful. This new drug had a binding affinity of 3.1 (±0.2) x 10^{11} M^{-1} at 20°C.[11] DSC was also used to characterize the binding of echinomycin to DNA, a bisintercalator that is difficult to study due to its poor solubility.[12]

Polyamide Binding to Duplex DNA

The discovery and development of drugs to bind specific sequences of duplex DNA is of interest to modulate gene expression. Polyamide DNA binding agents have been extensively studied for this application. Polyamides have pyrroles, imidazoles, and other heterocyclic rings, and are able to recognize specific sequences, both Watson-Crick and non-Watson-Crick base pairs. Polyamides can also be used to detect single nucleotide polymorphisms, to screen for mutations which could lead to cancers.

ITC has been used to study the binding of polyamides to DNA.[14-16] Wilson's group showed that imidazole-rich polyamides, designed to bind T-G mismatched base pairs, have large negative binding enthalpies, indicating that interactions are driven by hydrogen bonding, and that enhanced binding to the T-G site is due to favorable entropic contributions.[16]

Drug Binding to Triplex DNA

Triplex DNA is formed by sequence-specific hydrogen bond formation between a single-stranded triplex-forming oligonucleotide and purine bases in the major groove of a target sequence. Formation of triplex DNA can also modulate gene expression. However, triplex structures tend to be unstable, and can be stabilized by binding of triplex-specific drugs, such as intercalating drugs (e.g. anthraquinones).

Most aminoglycoside groove binders tend to destabilize triplex DNA. Neomycin is a groove-binding drug which stabilizes the triplex structure. The thermodynamics of neomycin binding to triplex DNA had been studied by ITC.[17] Stoichiometry was approximately 2 drug molecules per triplex, the binding was driven by enthalpy and there was little, if any, entropic contribution under experimental conditions.[17]

RNA-Drug Interactions

RNA can also serve as a sequence and/or structural target of drugs. Many RNA-binding drugs bind to ribosomal RNA and interfere with protein translation. Other RNA targets include ribozymes and aptamers. RNA binding drugs include aminoglycoside antibiotics (e.g. neomycin B and tobramycin), cyclic peptides, and intercalators (e.g. ethidium). Thermodynamics of binding of tobramycin (an aminoglycoside antibiotic) to polymeric RNA duplex polyr(I)-polyr(C) was studied with ITC, and binding affinity depended on pH and salt concentration.[18] The thermodynamics of binding depended on pH: at pH≤6.0, tobromycin binding was driven by entropy, but at pH >6, binding was enthalpically driven.[17] Thermodynamics of binding of neomycin, paromomycin, and ribostamycin to the ribosomal RNA recognition site were also studied by ITC. As the pH increased, the binding became more enthalpically favorable, and binding affinity depended on pH and salt concentration.[19]

Summary

ITC and DSC are well-established methods for studying the thermodynamic forces driving DNA-drug interactions. Successful drug development programs include gathering critical lead optimization information early in the process. Before a drug enters clinical trials, it is necessary to know the molecular basis of the drug-target interaction, including structure and function. Thermodynamic data have been shown to be a powerful adjunct to these molecular characteristics in fully understanding DNA binding interactions with intercalating, groove recognition, and sequence-specific drugs. Designing and optimizing DNA-targeted drugs which are thermodynamically more favorable can lead to final drug selections with improved binding affinity, as well as sequence and/or structure specificity.

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