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Calorimetry and Thermodynamics in Drug Design

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Key Words

free energy, enthalpy, entropy, binding, rational drug design

Abstract

Modern instrumentation for calorimetry permits direct determination of enthalpy values for binding reactions and conformational transitions in biomolecules. Complete thermodynamic profiles consisting of free energy, enthalpy, and entropy may be obtained for reactions of interest in a relatively straightforward manner. Such profiles are of enormous value in drug design because they provide information about the balance of driving forces that cannot be obtained from structural or computational methods alone. This perspective shows several examples of the insight provided by thermodynamic data in drug design.

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INTRODUCTION

Biological calorimetry dates from the 1780s, when Lavoisier and Laplace used their ice calorimeter to measure the metabolic heats of live animals (35, 36, 40). They studied, for example, the metabolism of a guinea pig by weighing the ice melted in the calorimeter over a given observation period, a value that could then be converted to energy from the knowledge of the latent enthalpy of fusion of water. This metabolic heat was correlated with CO_2 production over the same time period to provide some of the first quantitative data in physiology. Until fairly recently, the use of calorimetry in biophysics and molecular biology was limited to a few expert laboratories that were able to build their own instruments with the requisite sensitivity to measure the heats of reactions of biomolecules that are often only available in limited quantities. This era when calorimetry was in the hands of a few and largely inaccessible to many has been characterized by Ackers & Bolen (1) as the time of the "cult of calorimetry." The cult has expanded. Highly sensitive microcalorimeters for biochemical and biophysical applications became commercially available, leading to a fairly widespread use of the calorimetric methods in biophysics and molecular biology. Such widespread use is perhaps inevitably accompanied by some abuse, a cause for some concern (70).

Recent drug discovery efforts have been dominated by structure-based design concepts. In structure-based design, lead compounds are sought by attempting to match their shapes with the complementary shapes of active sites of receptors using known structures obtained by X-ray crystallography or NMR. It was noted previously, however, that thermodynamic studies are an essential and necessary complement to structural studies in drug design (25). Structural data alone, even when coupled with the most sophisticated current computational methods, cannot fully define the driving forces for binding interactions or even accurately predict their binding affinities. Thermodynamics provides quantitative data of use in elucidating these driving forces and for evaluating and understanding at a deeper level the effects of substituent changes on binding affinity (74).

This review explores several examples in which thermodynamic studies have provided complementary data that serve to enhance drug design efforts. Space limitations do not permit a comprehensive review of thermodynamics in drug discovery, so the examples were selected somewhat arbitrarily. I apologize in advance to those authors whose important work I have neglected.

BASIC THERMODYNAMIC RELATIONSHIPS

Basic thermodynamic relationships (15) are summarized in the sidebar. Equilibrium

constants may be used to calculate standard free energy changes (ΔG°) using the Gibbs relationship (Equation 1.1). The standard free energy change refers to an arbitrary and often unrealistic standard state, typically 1 M reactant concentrations. The free energy change (ΔG) may be calculated from ΔG° for a particular set of reaction conditions defined by the ratio Q (Equation 1.2). The ratio Q is a function of the ratio of actual product and reactant concentrations, the exact mathematical form of which depends on the exact reaction mechanism. Enthalpy values (ΔH) may be determined directly by calorimetry or indirectly from the temperature dependence of equilibrium constants and application of the van't Hoff relationship (ΔH_{VH}; Equation 1.4). Free energy may be parsed into its enthalpic and entropic (ΔS) components (Equation 1.3). Enthalpy values may be temperature dependent, signifying a nonzero heat capacity change (ΔC_p; Equation 1.5). If the heat capacity change is not zero, a more complicated expression for the free energy is needed (Equation 1.8). A complete thermodynamic profile for a reaction of interest requires determination of the free energy, enthalpy, and entropy at a given temperature, and the heat capacity change if the enthalpy varies with temperature.

The free energy ΔG is the key parameter, because its value under a particular set of reactant concentrations dictates the direction of biomolecular equilibria. If its sign is negative, the binding reaction or conformational transition will proceed spontaneously to an extent governed by the magnitude of ΔG. If its sign is positive, the magnitude of ΔG specifies the energy needed to drive the reaction to form product. The free energy is a balance between enthalpy and entropy. The enthalpy change reflects the amount of heat energy required to achieve a particular state, and the entropy measures how easily that energy might be distributed among various molecular energy levels. For binding reactions, negative enthalpy values are common (but not omnipresent), reflecting a tendency for the system to fall to lower energy levels by bond formation. Positive entropy values are common for binding reactions, reflecting a natural tendency for disruption of order, commonly resulting from disruption of solvation interactions and the concomitant release of bound water.

All binding reactions must overcome inescapable entropic penalties (2, 3, 6, 20, 33, 42, 60). The first penalty results from the loss of rotational and translational degrees of freedom as two reacting partners combine to become one complex. At least six degrees of freedom are lost upon complex formation owing to restriction of translational and rotational motions. This penalty may result in an energy cost of up to +15 kcal mol^{-1}, although the exact value is subject to debate (2, 3, 6, 20, 33, 42, 60). The second penalty arises from the restriction of individual bond rotation upon complex formation, for example,

BASIC THERMODYNAMIC RELATIONSHIPS

$$\Delta G^0 = -RT \ln K_a \qquad 1.1$$

$$\Delta G = \Delta G^0 + RT \ln Q \qquad 1.2$$

$$Q = \frac{f(\text{product concentrations})}{f(\text{reactant concentrations})}$$

$$\Delta G = \Delta H - T\Delta S \qquad 1.3$$

$$\Delta H_{VH} = -R\left(\frac{\delta \ln K_a}{\delta(1/T)}\right) \qquad 1.4$$

$$\Delta C_p = \left(\frac{\delta \Delta H}{\delta T}\right)_p \qquad 1.5$$

$$\Delta H_T = \Delta H_r + \Delta C_p(T - T_r) \qquad 1.6$$

$$\Delta S_T = \Delta S_r + \Delta C_p \ln\left(\frac{T}{T_r}\right) \qquad 1.7$$

$$\Delta G = \Delta H_r - T\Delta S_r + \Delta C_p\left[(T - T_r) - T\ln\left(\frac{T}{T_r}\right)\right] \qquad 1.8$$

Notes: ΔG, free energy change; ΔH, enthalpy change; ΔS, entropy change; ΔC_p, heat capacity change. T and T_r refer to the temperature and an arbitrary reference temperature, respectively. Thermodynamic parameters subscripted with "T" or "r" refer to those temperatures.

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DSC: differential scanning calorimetry

ITC: isothermal scanning calorimetry

when a small molecule is locked into a particular conformation when bound to its receptor site. The penalty for restriction of bond rotation is estimated to be about 0.6 kcal mol^{-1} per bond (44). This penalty might be reduced by engineering rigid bonds into ligand drug candidates if sufficient structural data exist to guide the design to ensure a precise fit into the receptor site. These inescapable entropic costs must be balanced by, and overcome by, other favorable interactions if binding is to occur. That is one fundamental consideration in drug design.

Although it would appear easy to assign enthalpy and entropy contributions to the small catalog of noncovalent interactions important in biological binding interactions (hydrogen bonding, hydrophobic and electrostatic interactions, and van der Waals forces), it is difficult to disentangle their separate contributions to the energetics of biomolecular interactions and transitions (14). Nonetheless, some tenuous attempts have been made to parse the free energy of drug-DNA binding interactions into component contributions (9, 22, 23, 55). Perhaps a better, and safer, point of view is one proposed by Freire and coworkers, who view binding enthalpy as a measure of bond formation (hydrogen and van der Waals bonding) and therefore of binding specificity, while binding entropy is a measure primarily of the nonspecific hydrophobic force (56, 66, 68). Examples of the utility of this view are discussed below. The interpretation of thermodynamic data in term of component molecular interactions is discussed in several publications (9, 14, 20, 22, 27, 28, 33, 60, 72).

Enthalpy changes can be obtained by either calorimetry or, in principle, from the van't Hoff relationship using equilibrium constants measured as a function of temperature. In an ideal world measured calorimetric and van't Hoff enthalpy values will be equivalent for a given reaction if the proper reaction mechanism is used in data reduction and analysis. The proper reaction mechanism may include coupled processes such as protonation, ion binding and release, or conformational changes. There are reports of discrepancies between calorimetric and van't Hoff enthalpy values (39, 43). The use of van't Hoff analysis to obtain enthalpy values, however, is fraught with error and difficulty (10, 29). A fundamental problem is that, given even modest errors in equilibrium constant determinations, the presence of curvature in van't Hoff plots that would result from nonzero heat capacity changes may not be apparent. In such cases, linear fits to the data that neglect heat capacity changes would lead to systematically biased enthalpy values that would differ significantly from calorimetric values (10).

MODERN MICROCALORIMETRY

The modern microcalorimetry revolution was fueled by the availability of sensitive commercial instruments. Two calorimetric methods, differential scanning calorimetry (DSC) and isothermal scanning calorimetry (ITC), dominate in biophysics and biochemistry (15).

Instruments for both approaches most commonly use a power compensation design. In DSC, the temperature difference between sample and reference cells is monitored as the temperature of both is increased in a precisely controlled fashion. A thermally driven reaction in the sample cell leads to a temperature imbalance between the cells. When that occurs, power to the heaters on the cells is modulated to bring the sample and reference back into balance. The primary data recorded is compensation power as a function of temperature. Power, as a fundamental physical quantity, is readily converted to energy units of joules or calories. Descriptions of the most recent DSC instrument design have been published (49, 54).

Representative DSC data are shown in **Figure 1**. The area under the transition curve defines the enthalpy of the reaction. A difference between the pre- and post-transition baselines results if there is a change in heat capacity. The distance between the two baselines in such cases defines the value of the heat

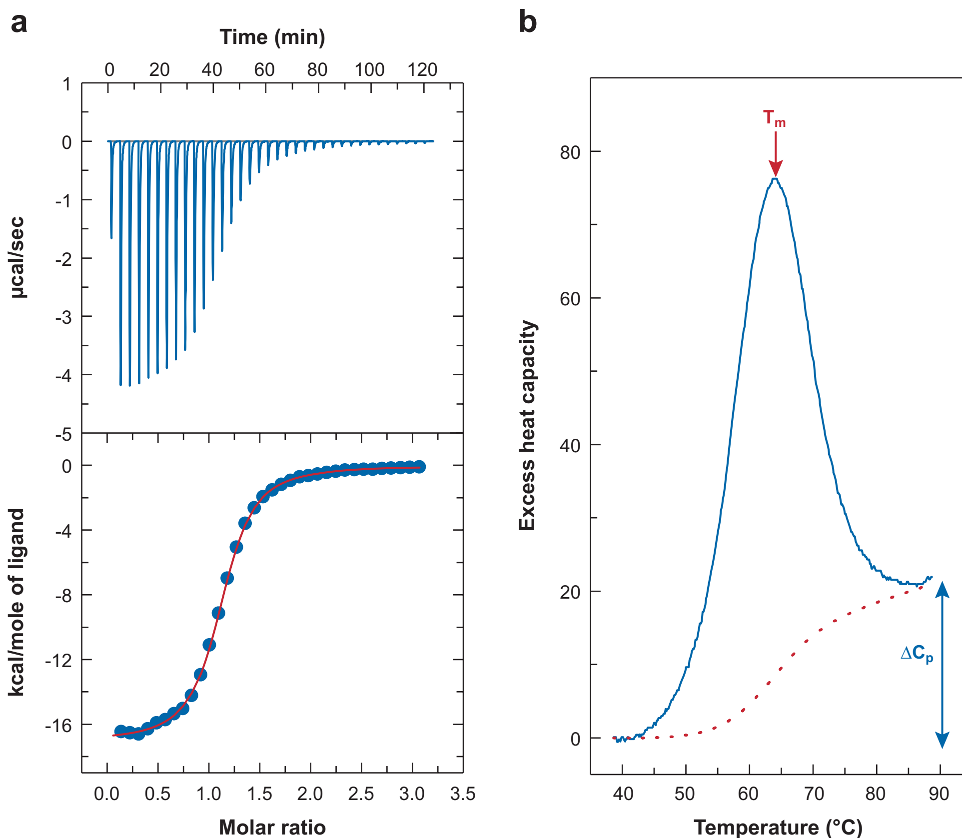

Figure 1

Representative ITC (*a*) or DSC (*b*) data. The ITC data are for the binding of 2′CMP to RNaseA. The top panel shows the primary titration data, and the lower panel shows the binding isotherm constructed from the primary data. The DSC transition shown is for the thermal denaturation of the protein ubiquitin. The diagram indicates the melting transition temperature T_m and the heat capacity change ΔC_p.

capacity change. The degree of the transition at any temperature may be defined by integration of the transition curve. Recommendations for the proper presentation and processing of DSC data were recently published (26).

In ITC, again the temperature difference between a sample cell and a reference cell is monitored. In contrast to DSC, both cells in this case are maintained at a constant temperature. A titration system delivers a reactant to the sample cell. Any heat of reactant binding results in an imbalance between the reference and sample cells, which is compensated for by modulating the power applied to cell heaters. A description of an ITC instrument was published (77).

Representative ITC data are shown in **Figure 1**. The top panel shows primary data, in which power is displayed as a function of time. The programmed titration delivery steps are evident by the series of peaks that return to baseline. The area of each peak is the heat of reaction for each reactant addition. As the receptor binding site becomes saturated, the magnitude of the peak area decreases. The bottom panel shows transformation of the primary ITC data into the form of a binding isotherm. Such a binding isotherm

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may be fit to a particular binding model to obtain a binding constant, binding enthalpy and the stoichiometry of binding. Some necessary considerations in fitting ITC data can be found in references (18, 32, 61–64).

HIGH-THROUGHPUT THERMODYNAMICS AND CALORIMETRY

DSC and ITC are somewhat laborious and time-consuming methods. Even a practiced investigator can usually complete only a few experiments per day. Demands of the pharmaceutical industry require higher throughput than is typically possible with available single sample cell DSC and ITC instruments, which often relegates calorimetry to be used as a secondary screening method or for validation of other assays. To overcome these limitations, several attempts have been made to improve the throughput of calorimetry and thermodynamic measurements.

Perhaps the most impressive attempt is the development of ThermoFluor™ technology (47) by 3D Pharmaceuticals, Inc., now owned by Johnson & Johnson. ThermoFluor™ may be used to conduct high-density miniaturized thermal shift assays as a screen in drug discovery. The technique measures the fluorescence of fluorescent dyes that sense protein unfolding. A 384-well microplate that can be heated at a defined rate is used. Libraries of small molecules can be screened for binding to a particular protein target of interest, exploiting firm underlying thermodynamic principles. Ligand binding is thermodynamically coupled to protein denaturation reactions. Ligands that bind preferentially to the native folded state will stabilize the protein, elevating its melting temperature. The magnitude of the melting temperature shift is a function of the binding affinity, although other binding properties also exert an influence (71). Thus, ThermoFluor™ technology allows characterization of the thermal denaturation of the target protein of interest alone or in the presence of drug candidates, providing a quantitative high-throughput screening method that yields accurate thermodynamic information. By this approach, binding to hundreds of different protein targets by libraries containing tens of thousands of compounds was made possible, greatly facilitating the drug discovery process. An additional novel use of the ThermoFluor™ technology is to identify biological function. For example, a screen for ligand binding to a protein product of an essential gene of unknown function was devised (8). ThermoFluor™ was used to screen the protein against a library of 3000 compounds that were specifically selected to provide information about possible biological functions, and identified binding of only particular ligands from that set, which provided clues to the biological role of the protein.

ThermoFluor™ technology, as powerful as it is, is not true calorimetry and can provide only van't Hoff enthalpy estimates. High-throughput calorimeters are still an urgent need. The best available approach to date is one in which a sensitive DSC instrument is coupled to a robotic system for sample handling (48). This approach remains limited by serial calorimetric determinations, but the robotic system automates cell loading, data acquisition, and cleaning between experiments. Samples are placed in a 96-well plate and kept in a temperature-controlled environment until they are loaded in the calorimeter. The system runs unattended after the initial setup and allows 10 to 50 experiments to be run per day.

There are two reported attempts to produce miniature calorimeters using microscale technology. An enthalpy array device allows mixing of two 250 nanoliter drops, facilitating the measurement of heats of binding or of enzymatic reactions (65). Arrays of such reactors could be fabricated, allowing parallel enthalpy measurements for true high-throughput screening. A micromachined thermoelectric flow calorimeter with a sample volume of 5 microliters was described (69), a design that would be amenable to the fabrication of an array for parallel data acquisition. Although both of these devices

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represent promising initial steps toward high-throughput calorimetry and can operate with greatly reduced sample volumes, their performance and sensitivity appear to be mediocre at best when compared with existing commercial microcalorimeters.

CASE STUDIES: BINDING TO NUCLEIC ACIDS

Isostructural Is Not Isoenergetic

The anthracycline antibiotics doxorubicin and daunorubicin are mainstays of cancer chemotherapy, having been in clinical use for over three decades (52, 75). The two compounds differ only by the presence of a hydroxyl group present in doxorubicin at the C14 position. Doxorubicin binds more tightly to DNA than does daunorubicin, with about 1 kcal mol^{-1} more favorable binding free energy (11). These compounds present a quandary for structure-based design. High-resolution X-ray crystallographic studies show that doxorubicin and daunorubicin are essentially isostructural when bound to their DNA binding site (19, 30). The molecular interactions visualized in their respective complexes are identical, and the added hydroxyl group in doxorubicin does not appear to participate in any additional specific interactions. The structures of the two compounds bound to DNA are shown in **Figure 2**. Where does the additional binding free energy come from in the doxorubicin complex? The thermodynamic profiles for the two compounds (**Figure 2*c***) shed light on the problem. When the binding free energies are parsed into their enthalpic and entropic components, distinctive patterns are revealed (11). Doxorubicin binding results from favorable contributions from both enthalpy and entropy. In contrast, daunorubicin binding contains an unfavorable entropic component. The distinctive thermodynamic profiles reveal subtle differences not evident in their high-resolution structures. The difference in entropy between two compounds points toward important contributions that are not readily visualized, perhaps differences in solvent or ion organization that cannot be seen even in high-resolution structures (30). Even though the doxorubicin and daunorubicin DNA complexes are isostructural, they are not isoenergetic. That

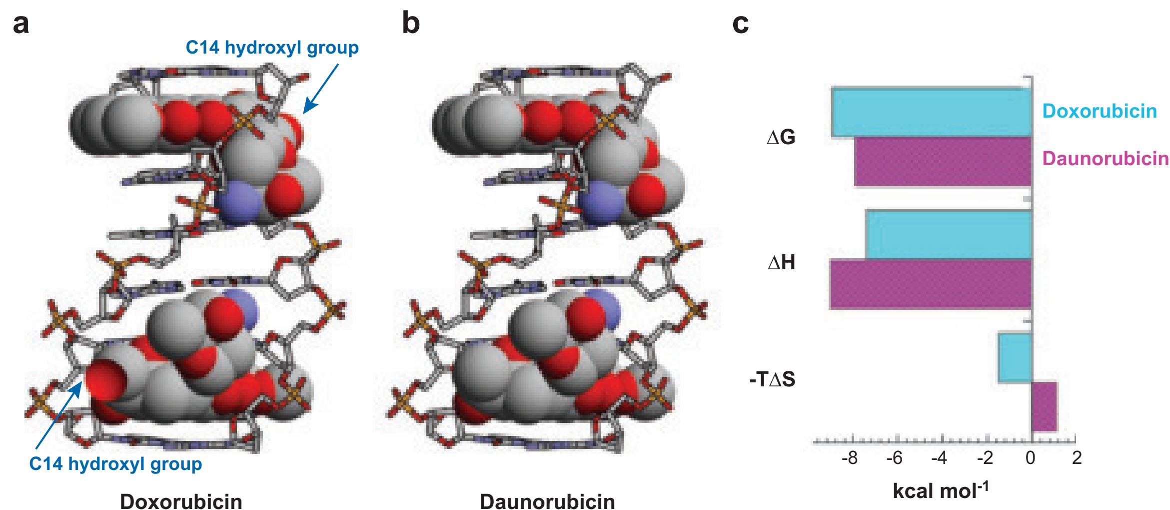

Figure 2

Structures of the DNA complexes of (*a*) doxorubicin and (*b*) daunorubicin. The arrows in panel *a* indicate the C14 hydroxyl group present in doxorubicin. Panel *c* shows the thermodynamic profiles for doxorubicin and daunorubicin binding to DNA.

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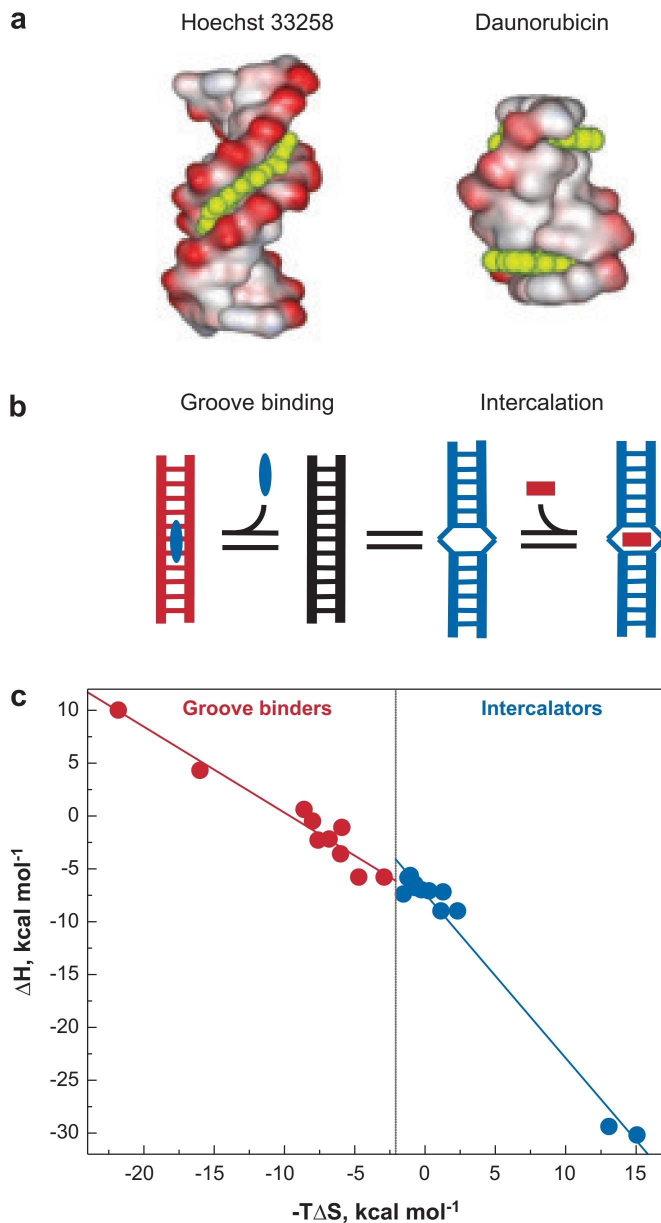

Figure 3

Thermodynamic signatures for the DNA binding mode of small molecules. (*a*) The structures the DNA complexes of the groove binder Hoechst 33258 (*left*) and daunorubicin (*right*). (*b*) A schematic of the binding mechanism. (*c*) The enthalpic and entropic contributions to DNA binding for groove binders and intercalators.

isostructural does not imply isoenergetic was first articulated by the Breslauer laboratory, who found that a variety of DNA lesions had little effect on the global structure of duplex DNA yet had profound effects on the thermodynamic stability (50).

A Thermodynamic Signature for DNA Binding Mode

Small molecules bind to duplex DNA by two predominant binding modes, intercalation and groove binding. These binding modes are illustrated in **Figure 3**. In groove binding, the DNA structure is largely unperturbed (76). Groove binders fit into the minor groove of B-form DNA without changing its shape, in analogy with a lock-and-key mechanism. In contrast, intercalators bind by insertion between base pairs, and their binding is coupled to an obligatory conformational change in DNA in which the helix is unwound and lengthened to form an open intercalation cavity (4, 73). Intercalation is analogous to an induced-fit mechanism. Distinctive thermodynamic signatures for these two binding modes were recently elucidated using thermodynamic data obtained from 20 compounds whose mechanisms of binding were well characterized (12). The dataset was assembled using only compounds for which calorimetrically determined enthalpy values were available. The results revealed that binding of groove binders was entropically driven, whereas intercalator binding was driven by large, favorable enthalpy contributions. These trends are illustrated in **Figure 3**, in which data are presented as a plot of enthalpy versus the entropy contribution, $-T\Delta S$.

Groove binders all lie in the quadrant of the graph with small, negative to positive enthalpy values, and negative, favorable entropy contributions. Intercalators, in contrast, have favorable, negative enthalpy values, and near-zero unfavorable, positive values for the $-T\Delta S$ term. For intercalation, the DNA must undergo reorganization to a form that is more

rigid and entropically less favorable. The entropic penalty must be overcome by enthalpy, arising at least in part from favorable van der Waals stacking interactions between the base pairs and the intercalated chromophore. For the case of DNA binding proteins, a different trend was elucidated (34). Proteins that bend or distort DNA were entropically driven, whereas proteins that bound without major alteration of the DNA structure were enthalpically driven.

Measuring Ultratight DNA Binding by DSC

One advantage of DSC is its capability to measure association constants for ultratight binding reactions (7). Thermal denaturation methods offer a particularly powerful approach for studying both the specificity and affinity of nucleic acid binding ligands (58, 59). We exploited this capability in studies of a new class of bisintercalating anthracycline antibiotics (31, 37, 51, 53). The novel bisanthracyclines were designed on the basis of structures such as those shown in **Figure 2** (13). In those structures, anthracycline monomers are seen to bind to DNA in a tail-to-tail orientation, with reactive amine groups separated by only 7 angstroms in the minor groove. The simple design strategy was to covalently link those amine groups with a moiety that would fit precisely into the minor groove and would not interfere with intercalation of the anthraquinone rings between base pairs or of the fit of the daunosamine moiety in the minor groove. The structure of one such bisintercalating anthracycline is shown in **Figure 4** (51). The binding affinity of such a bisintercalator was expected to be approximately the square of the binding constant of the monomer (17). Because the binding constant of daunorubicin is approximately 10^6 M^{-1}, bisdaunorubicin would be expected to bind with picomolar affinity. For such tight binding, traditional spectroscopic approaches to measuring binding become prohibitively difficult for a variety of reasons, chief among them is the difficulty

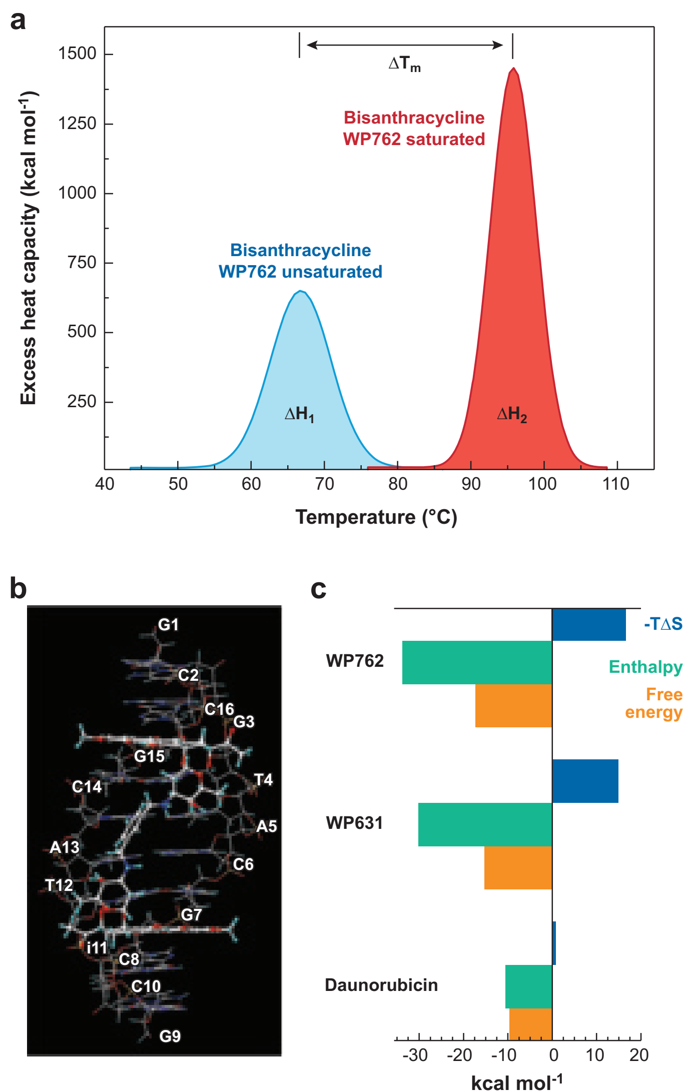

Figure 4

Ultratight binding of a bisanthracycline to DNA. (*a*) A schematic of the DSC thermograms observed for DNA in the absence and presence of saturating amounts of the bisanthracycline WP762. (*b*) The structure of the WP762-DNA complex. (*c*) The thermodynamic profiles for the binding to DNA of the parent monomer daunorubicin and two bisanthracyclines, WP631 and WP762, which differ in their linker geometry. Free energy, enthalpy, and -TΔS are shown.

of working at low enough ligand concentrations to avoid stoichiometric binding yet still retaining a measurable signal. A number of well-developed statistical mechanical theories show that binding constants can be reliably

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measured from shifts in melting temperatures of the receptor macromolecule (7, 16, 41, 57), although caution is needed in the application of these approaches (71).

Figure 4 shows the application of that approach to the characterization of bisanthracycline binding to DNA. Upon binding, the DSC thermogram is shifted upward by over 30°. That T_m shift may be used to calculate a binding constant for the bisanthracycline at the elevated T_m. DSC offers the added advantage that the binding enthalpy may also be estimated from the difference in the areas of the thermograms in the presence and absence of bound ligand. The enthalpy obtained from the area of the thermogram for DNA alone, along with the total enthalpy for melting of the bisanthracycline-DNA complex may be used with application of Hess's law to infer the enthalpy of the bisanthracycline binding reaction. A complete thermodynamic profile may then be derived for the binding reaction.

Comparative binding profiles are shown in **Figure 4** for daunorubicin and two bisdaunorubicins that differ only in their linker geometry. The binding free energy for the bisdaunorubicin molecules is nearly double that of the parent monomer, as predicted and intended. The thermodynamic profiles, however, show that the bisanthracyclines suffer from a large, unfavorable entropic contribution. The source of this unfavorable entropy is likely due to restriction of rotation around the linker bonds upon binding (44). The thermodynamic data suggest an improved design in which rotation around the linker bonds is restricted, while retaining the correct geometry for bisintercalation into DNA. If such a design were to be realized, binding affinity would be predicted to increase from the current picomolar range to femtomolar or greater.

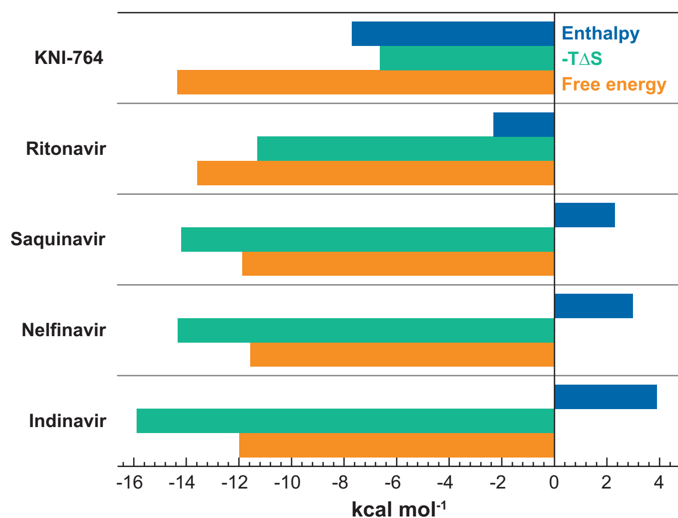

Figure 5

Enthalpy optimization of HIV protease inhibitors. Thermodynamic profiles for the binding of first- and second-generation (KNI-764) HIV protease inhibitors. Free energy, enthalpy, and -TΔS are shown.

CASE STUDIES: PROTEIN BINDING INTERACTIONS

Optimizing Affinity in HIV-1 Protease Inhibitors

HIV protease inhibitors are competitive inhibitors, so their potency is directly correlated with their binding affinity. The Freire laboratory has pursued a program to optimize HIV inhibitor binding by consideration of the thermodynamics of the binding interactions (45, 46, 67, 68). Their starting point is the realization that the binding free energy is a function of both enthalpy and entropy, and that many combinations of ΔH and TΔS values can yield the same value of ΔG. Enthalpically or entropically optimized inhibitors, however, might have different specificities or different pharmacological properties, even though their interaction with the target protease may have similar free energies. Enthalpic contributions reflect mostly the strength of inhibitor bonding interactions with protease (hydrogen bonds; van der Waals interactions) relative to solvent bonding. Entropic contributions to the free energy reflect mostly changes in solvent entropy arising from the hydrophobic transfer of inhibitor to its binding site.

Figure 5 shows thermodynamic profiles for the first-generation HIV protease inhibitors indinavir, nelfinavir, saquinavir, and ritonavir. For all of these, binding is

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entropically driven, and for three of the four, binding is accompanied by an unfavorable positive enthalpy change. Because these inhibitors are conformationally constrained, the binding entropy reflects primarily a large solvation entropy gain due to a hydrophobic transfer. Differences in the thermodynamic binding profile of the newer-generation inhibitors are evident, as shown by one example in **Figure 5**. KNI-764 binds more tightly than any of the first-generation inhibitors, by at least 1 kcal mol^{-1} in free energy. The favorable free energy results from almost equal favorable enthalpic and entropic contributions. KNI-764 thus has improved bonding interactions with the protease. Analysis of the thermodynamic profiles of a series of HIV protease inhibitors led to the conclusion that extremely high binding affinity requires a favorable binding enthalpy, and to the concept of enthalpic optimization as a drug design principle (46). Optimal binding enthalpy does not correlate simply with the number of hydrogen bond donors or acceptors, but instead results from the quality of the hydrogen bond formed within the binding pocket relative to inhibitor-solvent hydrogen bonding interactions.

The Enthalpy Funnel

The value of enthalpy optimization was reinforced by additional studies from the Freire laboratory on inhibitors of plasmepsin II, a hemoglobin-degrading enzyme that is a key component in the life cycle of the *Plasmodium* parasites responsible for malaria (56). Plasmepsin II represents a novel target for antimalarial drug development. A lead compound was developed, based on an allophenylnorstatine scaffold that mimics the main cleavage site in the hemoglobin molecule of infected victims. Compounds evolved from the lead that optimized the contribution of the enthalpy to the binding free energy. The affinity of 71 allophenylnorstatine inhibitors was measured and parsed into enthalpic and entropic components, with the results shown in **Figure 6**. The results define a rather dramatic and distinctive enthalpy funnel, in which the enthalpy/entropy combinations narrow as affinity (expressed as log K_a) increases. The optimal balance in this case appears to arise with the free energy partitioned as one-third enthalpy and two-thirds entropy. Being mindful of the need to achieve such a thermodynamic balance throughout the optimization process is crucial for a successful design effort.

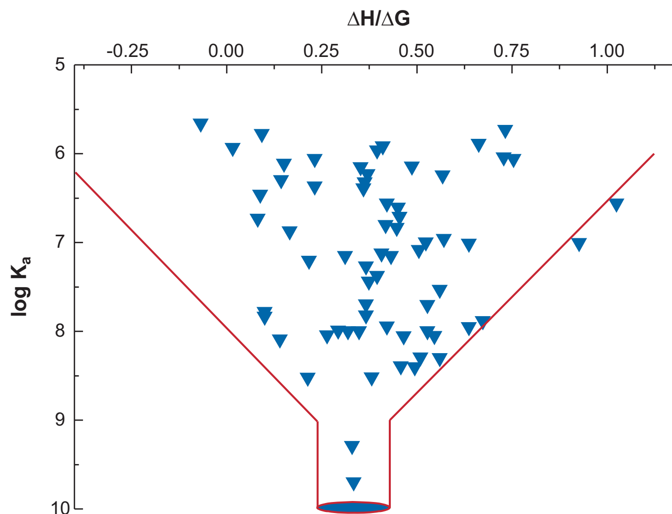

Figure 6

The enthalpy funnel. Thermodynamic data for the binding of 71 plasmepsin inhibitors are shown. The ratio of the binding enthalpy to free energy is shown as a function of the logarithm of the association constant.

CASE STUDY: HIGH SELECTIVITY DOES NOT DEMAND HIGH AFFINITY

Although high affinity is often a major goal in drug design, it is not an absolute requirement for selectivity and specificity. It is instructive to review the results obtained from a DNA aptamer that illustrates this point. A DNA aptamer that recognized L-argininamide was discovered (24) by the SELEX (Systematic Evolution of Ligands by Exponential enrichment system) (21) approach. The aptamer was approximately 100-fold more selective for L-argininamide over several other arginine analogs and other amino acids. A

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high-resolution NMR structure of a truncated version of the aptamer showed that a large portion of the DNA was unstructured in the absence of argininamide, but in the presence of argininamide a highly ordered structure formed in which the amino acid was encapsulated into a unique DNA fold (38). Our laboratory attempted the first detailed thermodynamic characterization of aptamer binding for this system, coupled with molecular dynamics simulations (5).

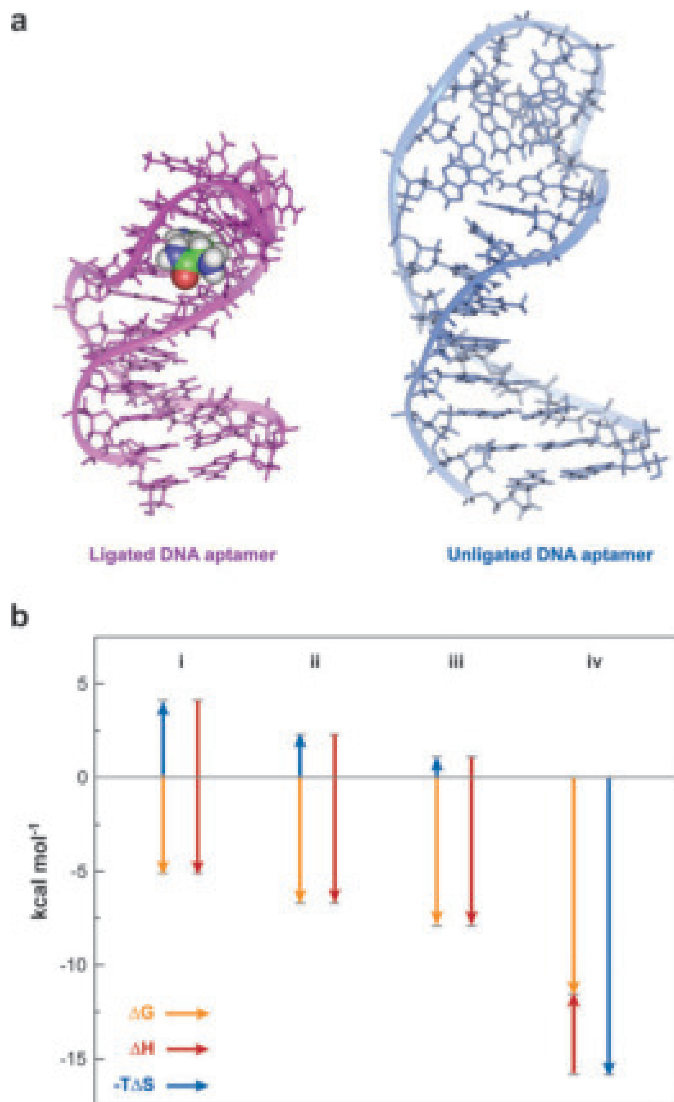

Figure 7

Binding of L-argininamide to its DNA aptamer. (*a*) The structures of the (*left*) ligated and (*right*) unligated DNA aptamers. (*b*) Thermodynamic profiles (i) for the binding of L-argininamide to the aptamer, (ii) for ethidium intercalation into DNA, (iii) for daunorubicin intercalation into DNA, and (iv) for the groove binding of Hoechst 33258 to DNA.

Figure 7 shows the structures of the ligated and unligated argininamide aptamer. In the absence of argininamide, our thermodynamic and molecular dynamics simulations indicated that the DNA formed a classic hairpin-loop structure, with the loop containing 10 unpaired bases. The highly selective binding of L-argininamide to the DNA had a free energy change of only -5.1 kcal mol^{-1}, corresponding to an association constant of only 6000 M^{-1}. Parsing the free energy into its component enthalpy and entropy contributions showed that binding was accompanied by a large, favorable enthalpy of -8.7 to -9.2 kcal mol^{-1}. Binding was opposed by an unfavorable -TΔS contribution of $+3.6$ to $+4.1$ kcal mol^{-1} (**Figure 7**). This case represents a clear illustration of an induced-fit mechanism, in which binding is tightly coupled to a large conformational change in the receptor DNA. The otherwise disordered and dynamic loop structure undergoes a major conformational change to form a stable binding pocket to which the argininamide is anchored. Such ordering is entropically unfavorable, as was measured, and must be overcome by specific bonding interactions that are enthalpically favorable. The opposing enthalpy and entropy contributions yield a modest favorable free energy change. High specificity results, without high affinity.

Figure 7 shows a progression in the thermodynamic profiles of small molecules that bind to DNA. The progression begins with an entropically driven groove-binder that does not perturb the B-DNA structure and that binds with positive enthalpy. Two DNA intercalators with different unwinding angles show proportionally larger unfavorable entropic contributions, with roughly equal binding enthalpy values. Finally, the argininamide aptamer with its massive conformational change shows the largest opposing entropy contribution.

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CONCLUSIONS

These few examples illustrate how thermodynamic data complement structural data, if they exist for a particular system. The thermodynamic profile shows the enthalpic and entropic contributions to the binding free energy and indicates the nature of forces that drive complex formation. Such information can guide drug development by suggesting the nature of modification that could most optimize binding to the macromolecular target.

SUMMARY POINTS

1. Thermodynamic data are an essential complement to structural data in drug development and for the optimization of lead compounds.
2. A complete thermodynamic profile for a binding interaction includes the binding free energy (ΔG), enthalpy (ΔH), entropy (ΔS), and heat capacity change (ΔC_p).
3. The thermodynamic profile indicates the predominant forces that drive the binding interaction.
4. Isostructural complexes need not be isoenergetic.
5. Favorable enthalpy contributions arise from favorable hydrogen bonding and van der Waals interactions, and favorable entropic contributions arise primarily from hydrophobic interactions and desolvation.
6. Thermal denaturation methods provide valuable tools for rapid screening of the binding compound libraries and for quantitative measurement of ultratight binding interactions.
7. High specificity does not demand high affinity.

FUTURE ISSUES

1 Full integration of calorimetry and thermodynamics into the drug discovery process requires development of high-throughput calorimeters to facilitate the acquisition of quantitative data.

2. A more fundamental thermodynamic understanding of the molecular interactions (e.g., hydrogen bonds, van der Waals forces, hydrophobic forces, water and ion binding interactions, electrostatic forces) that govern noncovalent binding of drugs to their receptor targets is urgently needed.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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