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The application of thermodynamic methods in drug design

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Abstract

The optimization of lead compounds as viable drug candidates involves the optimization of their binding affinity towards the selected target. The binding affinity, K_a, is determined by the Gibbs energy of binding, ΔG, which in turn is determined by the enthalpy, ΔH, and entropy, ΔS, changes ($\Delta G = \Delta H - T\Delta S$). In principle, many combinations of ΔH and ΔS values can give rise to the same ΔG value and, therefore, elicit the same binding affinity. However, enthalpically dominated ligands do not behave the same as entropically dominated ligands. Current paradigms in drug design usually generate highly hydrophobic and conformationally constrained ligands. The thermodynamic signature of these ligands is an entropically dominated binding affinity often accompanied by an unfavorable binding enthalpy. Conformationally constrained ligands cannot easily adapt to changes in the geometry of the binding site, being therefore highly susceptible to drug resistance mutations or naturally occurring genetic polymorphisms. The design of ligands with the capability to adapt to a changing target requires the introduction of certain elements of flexibility or the relaxation of some conformational constraints. Since these compounds pay a larger conformational entropy penalty upon binding, the optimization of their binding affinity requires the presence of a favorable binding enthalpy. In this paper, experimental and computational strategies aimed at identifying and optimizing enthalpic ligands will be discussed and applied to the case of HIV-1 protease inhibitors. It is shown that a thermodynamic guide to drug design permits the identification of drug candidates with a lower susceptibility to target mutations causing drug resistance.

Keywords: Drug design; HIV-1 protease; Binding affinity

1. Introduction

The completion of the Human Genome Project has created a new scientific reality. About 35,000 genes encoding for as many proteins will be identified. These proteins will have to be characterized, their structure and function determined, and their interactions with other molecules identified. The entire genomes of several pathogens have also been completed and many more will be available in near future. Many of the proteins identified from genomic information will become targets for drug development against a wide variety of diseases. These extraordinary developments underline the need for design strategies that accurately address the issues of binding affinity, specificity, selectivity, bio-availability and toxicology.

Existing methods for lead identification and optimization can be broadly divided into two categories: computational and experimental. Even though the two approaches are used in conjunction with each other either in parallel or sequentially, a real epistemological linkage between the two has been lacking and consequently a rigorous synergism has been absent. It is apparent that thermodynamics and specifically the thermodynamic quantities that define the binding

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affinity (Gibbs energy, enthalpy, entropy and heat capacity changes), which can be measured experimentally and also calculated from structure must provide such a link. Unfortunately, in most cases the link is restricted to a comparison of calculated and experimental binding affinities. Here we discuss a novel integrated approach in which structure-based and experimental thermodynamics play a synergistic role in guiding the design process.

2. Conventional methods for lead optimization

The identification of drug candidates by screening large libraries of potential lead compounds and their optimization by structure-based design is an important step in the development of new pharmaceutical drugs. Modern high throughput screening procedures are able to process thousands of compounds and identify those that exhibit the highest binding affinity with relative accuracy. In structure-based drug design or in lead optimization strategies the goal is the improvement of the binding affinity of the drug candidates. From a thermodynamic point of view, the binding affinity, K_a, is defined in terms of the free energy of binding

$$K_a = e^{-\Delta G/RT} \quad (1)$$

where R is the gas constant and T the absolute temperature. The free energy of binding is, in turn, defined by the enthalpy (ΔH) and entropy (ΔS) changes

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

Therefore,

$$K_a = e^{-(\Delta H - T\Delta S)/RT} = e^{-\Delta H/RT} \times e^{\Delta S/R} \quad (3)$$

It is evident that the binding affinity can be optimized by making ΔH more negative, ΔS more positive or by a combination of both. Even though many combinations of ΔH and ΔS values will elicit the same binding affinity (i.e. the same ΔG and therefore the same K_a), the properties and the response of these compounds to changes in the environment or in the protein target are not the same. The binding enthalpy primarily reflects the strength of the interactions of the ligand with the target protein (e.g. van der Waals, hydrogen bonds, etc.) relative to those existing with the solvent. The entropy change, on the other hand, mainly reflects two contributions: changes in solvation entropy and changes in conformational entropy. Upon binding, desolvation occurs, water is released and a gain in solvent entropy is observed. This gain is particularly important for hydrophobic groups. At the same time, the drug (and certain groups in the protein) loses conformational freedom resulting in a negative change in conformational entropy. Accordingly, from a thermodynamic point of view there are three important factors responsible for improving binding affinity: (1) improving ligand protein interactions over those with the solvent in order to obtain a favorable (negative) enthalpy change; (2) making the ligand more hydrophobic in order to make the solvation entropy large and positive and (3) pre-shaping the ligand to the geometry of the binding site in order to minimize the loss of conformational entropy upon binding.

3. Thermodynamic consequences of current drug design paradigms

The most important drug design paradigm currently in use is derived from the classic key–lock hypothesis of enzyme specificity originally advanced by Emil Fisher in 1890 (see [1] for a review). The design paradigm is usually referred to as the ''shape complementarity principle'' and essentially consists in the synthesis of a conformationally constrained molecule pre-shaped to the geometry of the target binding site. A molecule that is pre-shaped to the target and conformationally constrained provides specificity and simultaneously enhances the binding affinity. One rotatable bond that becomes immobilized upon binding carries a Gibbs energy penalty close to 2 kJ mol^{-1} due to the loss of conformational entropy [2]. Everything else being equal, a conformationally constrained molecule has a higher binding affinity because it does not carry that entropy penalty. Organic and medicinal chemists have been able to successfully implement this strategy and design conformationally restrained molecules against a variety of targets.

Shape complementarity, however, does not guarantee binding. For binding to occur, a favorable Gibbs energy is required. Since the effective binding energetics is the difference between the magnitude of the drug–target interactions and the interactions of both

with the solvent, it is always possible to generate a significant binding affinity by making the interactions of the drug with the solvent unfavorable, i.e. by increasing the hydrophobicity of the drug. A common strategy has been to design highly hydrophobic compounds that bind to the selected target by combining the proper shape and a strong repulsion from the solvent. This is in fact a common strategy, and as a result the majority of affinity-optimized drug candidates are highly hydrophobic and rigid (pre-shaped to the geometry of the binding site).

The binding of a highly hydrophobic and rigid ligand (conformationally constrained and pre-shaped to the binding site) has a characteristic thermodynamic signature. Fig. 1 summarizes the results obtained for the binding thermodynamics of four HIV-1 protease inhibitors currently in clinical use (Indinavir, Saquinavir, Nelfinavir and Ritonavir) [3]. It is clear that for all inhibitors, the driving force for binding is the entropy change, and that the enthalpy change is unfavorable (Saquinavir, Nelfinavir, Indinavir) or only slightly favorable (Ritonavir). The highly favorable binding entropy of these inhibitors is due to a combination of high hydrophobicity and a minimal conformational entropy loss [3].

4. The response of conformationally constrained inhibitors to target site mutations

A major limiting factor in the treatment of HIV-1 infection has been the emergence of viral strains that exhibit resistance to protease inhibitors [4–11]. The loss of sensitivity usually occurs because the resistant viral strains encode for protease molecules containing specific amino acid mutations that lower the affinity for the inhibitors, yet maintain sufficient affinity for the substrate. For some mutations, the affinity towards the inhibitor might decrease by up to three orders of magnitude while the K_m for the substrate changes by less than one order of magnitude [9,12,13]. The double mutation V82F/I84V has been shown to affect the protease inhibitors in clinical use: Ritonavir, Saquinavir, Nelfinavir, Indinavir and Amprenavir [14–16]. This double mutation is located at the edges of the active site, distorting its wild type geometry without

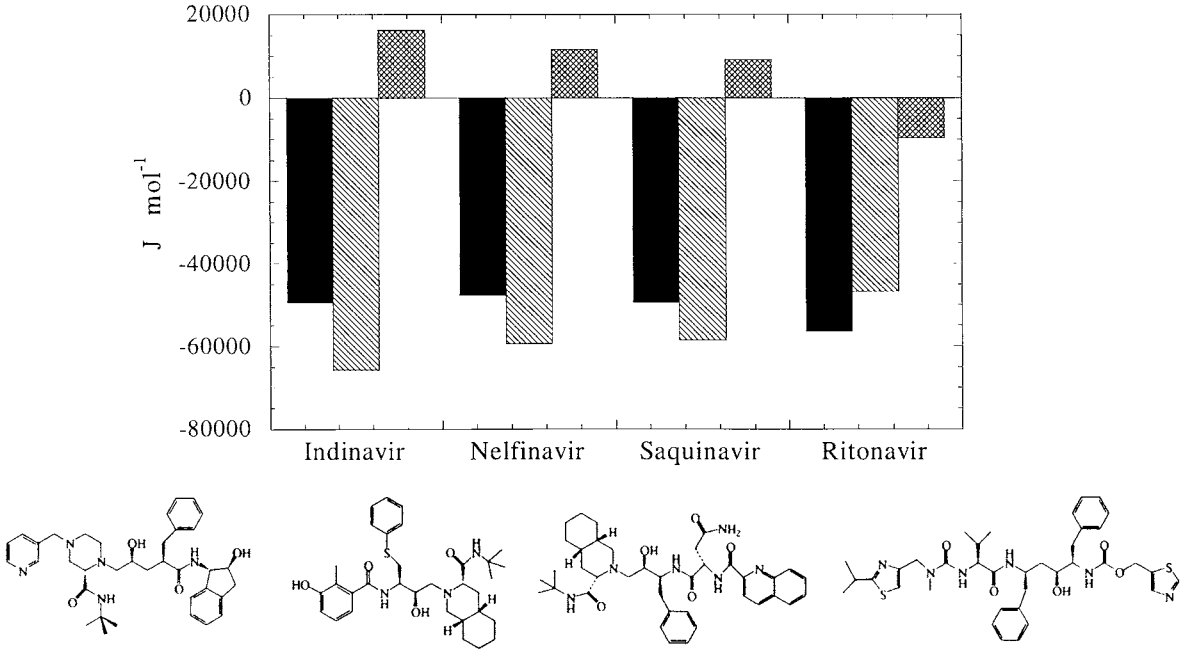

Fig. 1. Dissection of the thermodynamics of inhibitor binding to the HIV-1 protease (pH 5.0, 10 mM acetate buffer, 25°C). Solid bars = ΔG; dashed bars = $-T\Delta S$ and hatched bars = ΔH. The chemical structures of the inhibitors are shown at the bottom. Notice that the binding of the four inhibitors is entropically-driven and that for three of them (Indinavir, Nelfinavir and Saquinavir) the enthalpy change is unfavorable.

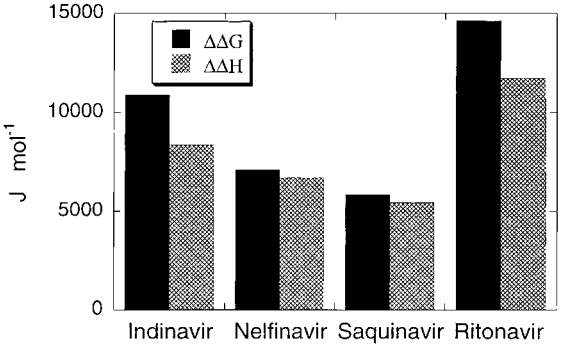

Fig. 2. Effects of the drug resistant mutant V82F/I84V on the thermodynamics of binding of clinical inhibitors to the HIV-1 protease. The V82F/I84V mutation lowers the binding affinity of current inhibitors primarily by making the binding enthalpy more unfavorable. $\Delta\Delta G$ and $\Delta\Delta H$ values were obtained from isothermal titration calorimetry experiments of the inhibitors to the wild type and resistant mutant proteases [3].

changing its polarity or chemical composition. A calorimetric analysis of the effects of this mutation on inhibitor binding reveals that the Gibbs energy of binding becomes less favorable and that the decrease in affinity is primarily due to an even less favorable binding enthalpy as shown in Fig. 2. The positive increase in the binding enthalpy reflects in part the inability of these compounds to adapt to a geometrically distorted binding site.

The calorimetric results obtained with HIV-1 protease inhibitors confirmed our original suggestion that the reduced flexibility of inhibitors pre-shaped to the wild type binding site accentuates their susceptibility to mutations that distort the protease binding site [17]. The difficulty of rigid inhibitors to adapt to an altered binding site geometry lowers the strength of van der Waals, hydrogen bonds and other favorable interactions, resulting in a diminished binding enthalpy and binding affinity. It is apparent that flexible inhibitors will be more accommodating to protease mutations. However, adding flexibility to existing inhibitors will lower their binding affinity because they will pay a higher conformational entropy penalty upon binding. Therefore, flexibility needs to be compensated by additional favorable interactions. These interactions cannot be hydrophobic because the existing inhibitors are already highly hydrophobic and because flexible hydrophobic ligands will lack specificity. The alternative is an enthalpic optimization that will provide the required additional binding affinity and the necessary target specificity. However, lead compounds that bind with a favorable enthalpy have been difficult to find due, among other factors, to the high hydrophobic character of most databases.

5. The need for enthalpically favorable lead compounds

A lead compound that exhibits a favorable binding enthalpy provides several advantages over compounds with similar binding affinities but unfavorable binding enthalpies. A favorable binding enthalpy is a good indicator that the lead compound is establishing good interactions with the target. The binding affinity of an enthalpic lead can always be enhanced by introducing conformational constraints, additional enthalpic interactions or even by introducing hydrophobic groups. The opposite is not true, it is much more difficult to introduce enthalpically favorable interactions into an entropically-driven inhibitor. A conformationally constrained enthalpic inhibitor with very high affinity is the ideal candidate for optimization since it provides an effective scaffold for the introduction of flexible elements without a loss of binding affinity below effective levels.

It is apparent that the identification of high affinity, enthalpically-driven lead compounds or the enthalpic optimization of existing leads are desirable goals. These goals can be accomplished experimentally or computationally as discussed in the following sections.

6. Experimental identification of enthalpically-driven lead ligands or inhibitors

The most immediate difference between a binding process characterized by a positive or negative enthalpy change is in the temperature dependence of the binding affinity. An exothermic binding process will be characterized by a decrease in binding affinity upon a temperature increase while an endothermic binding process will exhibit the opposite behavior. The temperature dependence of the binding constant is given by the van't Hoff equation

$$\frac{\partial \ln K}{\partial T^{-1}} = -\frac{\Delta H}{R} \tag{4}$$

This thermodynamic property can be utilized to design screening methods that select for binding affinity and enthalpy. We will discuss this approach with two types of experimental assays commonly used in drug design, direct binding measurements and enzyme inhibition measurements.

6.1. Direct binding measurements

In this approach, a property of the protein target or ligand that is sensitive to binding is usually used to estimate the amount of ligand bound. This property can be the optical absorbance, fluorescence emission, resonance energy transfer, fluorescence quenching or some other property not necessarily of a spectroscopic origin. High-throughput screening techniques can be readily adapted to select for affinity and enthalpy by performing the screen at a minimum of two temperatures. Ligands characterized by a favorable binding enthalpy will have a lower binding affinity at increasing temperatures and consequently will exhibit a reduced degree of binding.

6.2. Enzyme inhibition measurements

The binding of an enzyme inhibitor is usually estimated by measuring the amount of substrate depleted or product formed after certain time (enzyme velocity). A decrease in the amount of substrate depleted or product formed is indicative of an increase in the amount of inhibitor bound. In order to select for binding affinity and enthalpy, enzyme initial velocity measurements can be performed at a minimum of two temperatures in the absence and presence of a known concentration of inhibitor.

The initial velocity $v(\mathrm{I})$ in the presence of a concentration [I] of inhibitor is given by standard rate equations. It can be shown that at low substrate concentrations ($[\mathrm{S}] \ll K_\mathrm{m}$), both competitive and non-competitive inhibitors will exhibit the same apparent kinetic behavior. For competitive inhibition,

$$v(\mathrm{I}) = \frac{k_\mathrm{cat}[\mathrm{E}]_\mathrm{T}[\mathrm{S}]}{K_\mathrm{m}(1 + ([\mathrm{I}]/K_\mathrm{I})) + [\mathrm{S}]} \approx \frac{k_\mathrm{cat}[\mathrm{E}]_\mathrm{T}[\mathrm{S}]}{K_\mathrm{m}(1 + ([\mathrm{I}]/K_\mathrm{I}))} \tag{5}$$

and for non-competitive inhibition

$$v(\mathrm{I}) = \frac{k_\mathrm{cat}[\mathrm{E}]_\mathrm{T}[\mathrm{S}]}{K_\mathrm{m}(1 + ([\mathrm{I}]/K_\mathrm{I})) + [\mathrm{S}](1 + ([\mathrm{I}]/K_\mathrm{I}))} \approx \frac{k_\mathrm{cat}[\mathrm{E}]_\mathrm{T}[\mathrm{S}]}{K_\mathrm{m}(1 + ([\mathrm{I}]/K_\mathrm{I}))} \tag{6}$$

The above equations indicate that the observed rate $v(\mathrm{I})$ is dependent on several kinetic parameters that can be affected by temperature. Therefore, the effect of temperature on the inhibition constant, K_I, must be isolated from the temperature effects on the other parameters. This can be done by normalizing the measured activity to that obtained in the absence of the inhibitor. The relative decrease in activity due to the presence of inhibitor ($v(\mathrm{I})/v(0)$) is simply

$$\frac{v(\mathrm{I})}{v(0)} = \frac{K_\mathrm{I}}{K_\mathrm{I} + [\mathrm{I}]} \tag{7}$$

indicating that under these conditions, the temperature dependence of K_I can be isolated from that of K_m. According to the above equations, measuring $v(\mathrm{I})/v(0)$ at several temperatures under conditions in which the substrate concentration is much lower than K_m will give the enthalpic contribution to inhibitor binding. This approach is illustrated in Fig. 3 for two different HIV-1 protease inhibitors, Glu-Asp-Leu and acetyl-pepstatin, characterized by negative and positive binding enthalpies, respectively [17–19]. It is evident that the binding affinity of Glu-Asp-Leu is lower at 35°C than at 15°C whereas acetyl-pepstatin exhibits a higher affinity at 35°C. Analysis of the data in terms of Eq. (7) (solid lines) yield inhibition constants of 9 and 16 μM for Glu-Asp-Leu; and 1.0 and 0.3 μM for acetyl-pepstatin at 15 and 35°C, respectively. These values are consistent with those obtained by direct calorimetric titrations and confirm the validity of the approach. The van't Hoff enthalpies calculated from the data in Fig. 3 are −21 and 44 kJ mol^{-1} for Glu-Asp-Leu and acetyl-pepstatin, respectively, which are close to those measured directly by calorimetric titrations (−15 and 31 kJ mol^{-1}, respectively).

The experiments in Fig. 3 demonstrate the feasibility of screening for binding affinity and enthalpy without excessively increasing the time required for the assays. This approach permits the selection of a relatively small number of compounds with the

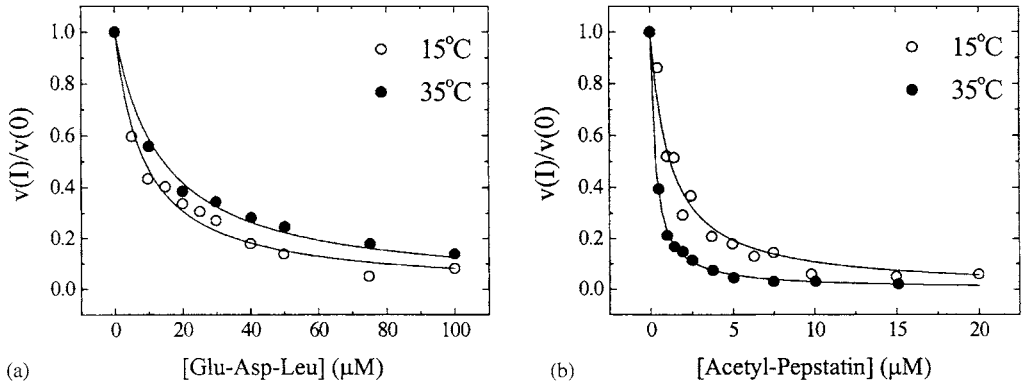

Fig. 3. Temperature dependence of HIV-1 protease inhibition for an exothermic and endothermic inhibitor. Fractional activity of the protease, measured at increasing concentrations of the inhibitors Glu-Asp-Leu (A) or acetyl-pepstatin (B), were determined at 15°C (solid squares) or 35°C (open triangles). Inhibitors with favorable enthalpy (Glu-Asp-Leu) show decreased affinity as the temperature is increased. Inhibitors with unfavorable enthalpy (acetyl-pepstatin) show increased affinity with increasing temperature. In both cases $[S] \ll K_m \sim 2.5$ mM (adapted from [19]). This experimental setup can be implemented in high throughput screening protocols for the simultaneous identification of compounds with high binding affinity and favorable binding enthalpy.

desired enthalpy sign that can be further characterized by isothermal titration calorimetry.

7. Structure-based computational analysis of binding enthalpy

The enthalpic optimization of an existing lead requires the ability to predict binding enthalpies from structure. It has been shown that one of the most accurate ways of calculating enthalpy changes associated with protein stability from structure is by means of an empirical parameterization that includes changes in solvent accessibility and the atomic packing density (δ) of the native structure [20]

$$\Delta H(T,\delta) = a(T,\delta)\,\Delta\mathrm{ASA}_{ap} + b(T,\delta)\,\Delta\mathrm{ASA}_{pol} \quad (8)$$

where $\Delta\mathrm{ASA}_{ap}$ and $\Delta\mathrm{ASA}_{pol}$ are the changes in solvent accessible surface area for non-polar and polar atoms and $a(T,\delta)$ and $b(T,\delta)$ are empirically determined coefficients. Since the packing density of proteins is highly homogeneous, the parameters $a(T,\delta)$ and $b(T,\delta)$ are essentially a function of temperature only (Fig. 4). This figure presents a correlation plot for the calculated and experimental enthalpies of denaturation for 36 proteins. The calculated values were obtained by using the following parameters: $a(60) = -5.45 \pm 1.7$ J mol^{-1} Å^{-2} and $b(60) = 85.15 \pm 18.2$ J mol^{-1} Å^{-2}. The regression coefficient is 0.95 with a zero intercept and a slope of 0.99.

While the structural parameterization of the enthalpy change derived from protein denaturation data has been successfully applied to the binding of some small ligands, it cannot be expected to be successful in all cases. The standard error in the prediction of the enthalpy of protein stability is on the order of 20 kJ mol^{-1} which is acceptable when compared to the magnitude of the enthalpy changes for protein denaturation (~500 kJ mol^{-1}). For ligand binding, the absolute magnitude of binding enthalpies is on the order of 20 kJ mol^{-1} and, therefore, the error level could be significant. Also, there are additional factors that become relevant in the structural analysis of the enthalpy change for ligand binding.

7.1. Reference temperature

The structural parameterization of the enthalpy change for protein stability was obtained at 60°C, which is the median temperature for protein heat denaturation and also the temperature at which the experimental data is obtained. Ligand binding is usually measured at or close to 25°C which requires a 35°C extrapolation. So, in the application of the stability parameterization to ligand binding, the total error is compounded by both the intrinsic error in

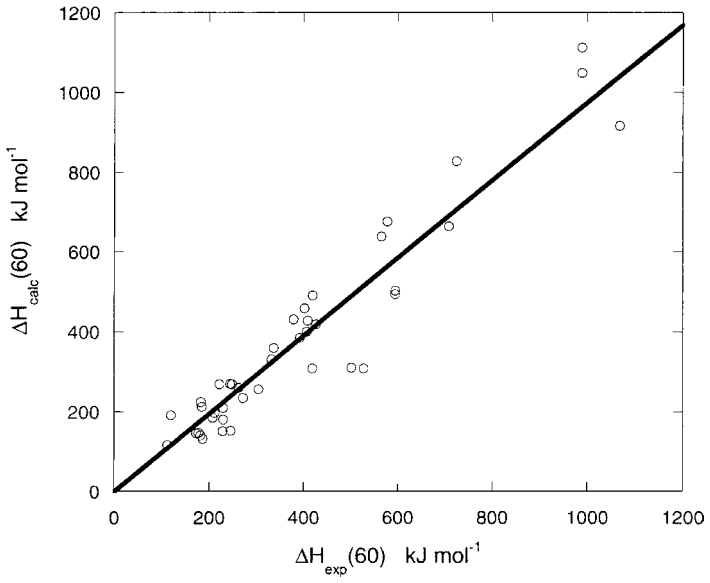

Fig. 4. Correlation plot between calculated and experimental enthalpies of denaturation for 36 proteins. The regression coefficient is 0.95 with a zero intercept and a slope of 0.99. The proteins are: α-chymotrypsin (5cha), acyl carrier protein (1acp), arc repressor (1arr), B1 domain of protein G (1pgb), B2 domain of protein G (1pgx), barnase (1bnr), barstar (1bta), BPTI (5pti), carbonic anhydrase B (2cab), CI2 (1coa), cyt b5 (tryp frag) (1cyo), GCN4 (2zta), HIV-1 protease (1hhp), HPr (2hpr), IL-1 (6ilb), lac repressor headpiece (1lcd), lysozyme (apo equine) (2eql), lysozyme (hen) (1lzt), lysozyme T4 (1lzt, 2lzm), met repressor (1cmb), OMTKY3 (2ovo), papain (9pap), parvalbumin (5cpv), pepsin (5pep), pepsinogen (3psg), plasminogen K4 domain (1pmk), RNase T1 (8rnt, 9rnt), RnaseA (3rn3), Sac7d (1sap), SH3 spectrin (1shg), Staphylococcus nuclease (1stn), stefin A (1cyv), tendamistat (3ait), thioredoxin (2trx), trp repressor (3wrp), ubiquitin (1ubq). The experimental thermodynamic parameters have been tabulated by Robertson and Murphy [29]. The calculated values are computed from the structure using the empirical parameterization of the enthalpy described in the text. The pdb file entries are in parenthesis after each protein.

the enthalpy and heat capacity parameterizations. The parameterization of the binding enthalpy needs to be tested and refined at 25°C, which is the temperature at which most binding experiments are performed.

7.2. Protein conformation

It has become evident that the binding of small ligands is usually associated with a change in protein conformation. This change in protein conformation is not necessarily of a global character; in fact, most of the time it only involves a local rearrangement or stabilization of unstructured regions near the binding site [18,21,22]. In addition, the ligand itself may occupy enthalpically different states in the free and bound states. Since binding enthalpies are usually small, contributions due to conformational changes, especially of the protein, need to be considered explicitly even if they are only of a local nature.

7.3. Linkage to protonation–deprotonation reactions

Ligand binding is often coupled to the protonation or deprotonation of certain ionizable groups either in the protein or the ligand itself [23]. Ionization enthalpies are often of the same order of magnitude of the intrinsic binding enthalpy itself and, therefore, need to be considered explicitly. Calculation of the ionization contribution to the binding enthalpy requires knowledge of the pK's and protonation enthalpies of the ionizable groups. At the present time, this information can only be obtained experimentally. Explicit methods to dissect proton linkage contributions to the binding enthalpy can be found in [23,24].

7.4. Protein-specific refinement of structural parameterization

According to the above discussion, the measured binding enthalpy, $\Delta H_{\text{binding}}$, can be considered as the

sum of a minimum of three terms

$$\Delta H_{\text{binding}} = \Delta H_{\text{intrinsic}} + \Delta H_{\text{conformation}} + \Delta H_{\text{ionization}} \quad (9)$$

The intrinsic enthalpy is the term arising from the molecular interactions (including changes in solvation) between ligand and protein assuming that they exist in the same conformation in the free and bound states. This term is given by Eq. (8) and only requires knowledge of the structure of the protein–ligand complex. By definition, this term does not include any energetics associated to conformational changes induced by binding. Even though the intrinsic binding enthalpy cannot be compared directly to measured binding enthalpies, this is the term that needs to be optimized in structure-based design since it reflects the strength of the interactions between ligand and target. In the absence of a high resolution structure for the free protein, obtained under identical conditions as the structure of the complex (a rather common situation), the enthalpy contribution due to changes in conformation can be left as a fitting parameter to be estimated from structure–thermodynamic correlations for the specific system under consideration. Contributions due to protonation–deprotonation coupling must be identified and isolated experimentally by performing calorimetric measurements at different pH values and buffers with different ionization enthalpies [23,24].

Fig. 5. The free and bound conformations of the HIV-1 protease. Upon substrate or inhibitor binding the flaps close upon the ligand. The energetics associated with this conformational change needs to be included in the effective binding energetics. Mutations that affect the energetics of this change will affect the binding affinity even if the mutations are away from the binding site.

8. The binding enthalpy of protease inhibitors

The binding of inhibitors to the HIV-1 protease is coupled to a conformational change of the protein as illustrated in Fig. 5 and also to protonation–deprotonation processes [3,23]. As such, it represents an important case to illustrate the implementation of a protein-specific refinement of the structural parameterization. The goal of this protein-specific parameterization is to obtain a highly refined and accurate set of parameters from a limited data set of inhibitors and then utilize the resulting values in the search for or in the optimization of new inhibitors. According to Eqs. (8) and (9), after

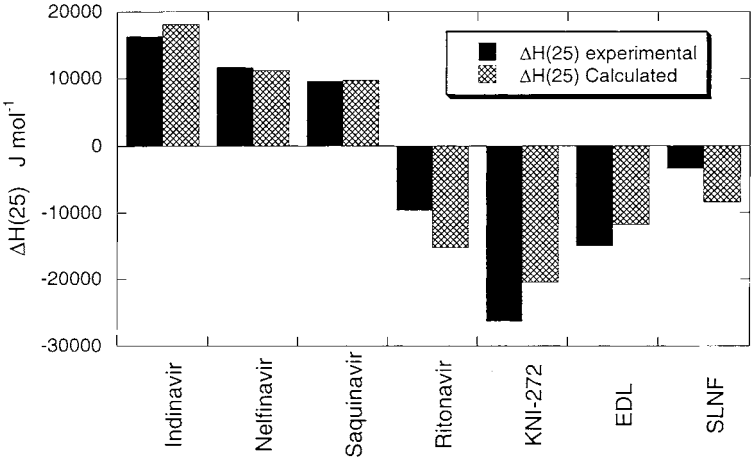

Fig. 6. Experimental and calculated enthalpies for seven inhibitors of the HIV-1 protease. This graph illustrates the accuracy of a protein-specific refinement of the structural parameterization of the enthalpy change. The enthalpy can be calculated from structure with a standard error smaller than 1.5 kJ mol^{-1} which permits discrimination between exothermic and endothermic inhibitors.

subtracting ionization contributions, the enthalpy for inhibitor binding at 25°C can be written as

$$\Delta H = \Delta H_{\text{conformation}} + a(\Delta\text{ASA}_{\text{ap}}) + b(\Delta\text{ASA}_{\text{pol}}) \quad (10)$$

We have developed a database of seven HIV-1 protease inhibitor complexes for which high resolution structures and thermodynamic data are available (Fig. 6). If this joint database is analyzed by non-linear least squares in terms of Eq. (10), the following parameters are obtained:

$$\Delta H(25) = 17455 - 54.0(\Delta\text{ASA}_{\text{ap}}) + 135.5(\Delta\text{ASA}_{\text{pol}})\ (\text{J mol}^{-1}) \quad (11)$$

The accuracy of the procedure is illustrated in Fig. 6. It is evident that the analysis accounts for the experimental enthalpy with a standard error of ± 1.6 kJ mol^{-1} and that it correctly identifies those inhibitors that bind with a favorable or unfavorable enthalpy. Furthermore, the analysis indicates that the

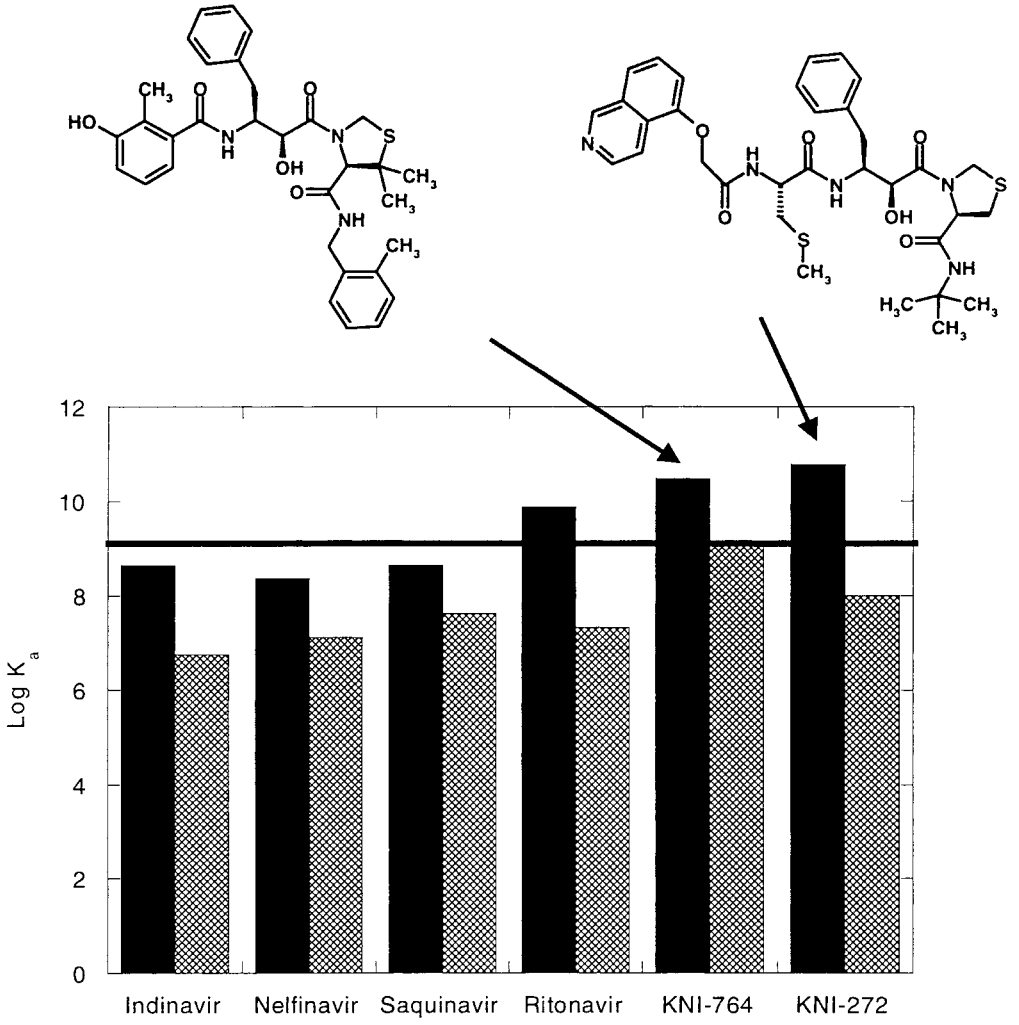

Fig. 7. The binding constant (logarithmic scale) of different inhibitors against the wild type HIV-1 protease (dark bars) and the resistant mutant V82F/I84V (hatched bars). The horizontal solid bar represents the binding constant of KNI-764 against the resistant mutant. The chemical structures of KNI-272 and KNI-764 are shown in top of the graph. Adapted from [30].

enthalpy change associated with the conformational change upon binding is on the order of 17 kJ mol^{-1}. In this analysis, the calculated changes in solvent accessibility explicitly consider water molecules that are immobilized at the protease inhibitor complex. In our calculations, we use a cutoff distance of 5 Å from the inhibitor and consider only water molecules that are completely buried from the solvent. The non-polar and polar coefficients per unit area are within the range of those derived for protein stability (Fig. 4) when extrapolated to 60°C.

The ability to predict from structure the binding enthalpy and other thermodynamic functions associated with inhibitor binding permits the implementation of accurate scoring functions for docking and binding optimization that explicitly include affinity as well as binding enthalpy.

9. HIV-1 protease inhibitors with lower susceptibility to mutations

We have shown that some members of a family of allophenylnorstatine-based HIV-1 protease inhibitors developed by Kiso and coworkers [25–28] bind to the protease with favorable binding enthalpies close to -25 kJ mol^{-1} and favorable entropies [23]. One of these inhibitors (KNI-272) is conformationally constrained, loses very little conformational entropy upon binding and consequently binds to the protease with extremely high affinity $K_a = 6.3 \times 10^{10}$ M^{-1} ($K_d = 16$ pM). Because this inhibitor is highly constrained it is also highly susceptible to mutations in the target molecule. For example, the binding affinity of KNI-272 is diminished 550-fold by the resistant mutant V82F/I84V. As discussed above, an inhibitor characterized by an extremely high binding affinity provides the opportunity to accommodate certain degree of flexibility and still exhibit an acceptable binding affinity. This is the situation with KNI-764, an inhibitor that displays significant activity against several resistant mutants including V82F/I84V. One of the most significant features of this inhibitor is the presence of an additional rotatable bond at the P2′ position that permits the inhibitor to accommodate to the binding site distortions created by the V82F/I84V mutation. As a result, KNI-764 binds to the wild type protease with a slightly lower binding affinity (36 pM) but the V82F/I84V mutation only lowers its affinity by a factor of 26 [30]. The binding of KNI-764 to the HIV-1 protease is also characterized by a favorable enthalpy of -32 kJ mol^{-1} at 25°C [30]. Fig. 7 shows the chemical structure of KNI-272 and KNI-764 and illustrates the different responses of various inhibitors to the resistant mutant V82F/I84V. It is clear that KNI-764 is not only significantly affected by the mutation but also its binding affinity against the resistant mutant is still higher than that of three clinical inhibitors against the wild type.

10. Conclusions

The results presented above clearly illustrate that inhibitors with built-in flexibility at critical locations are less susceptible to resistant mutations. However, since flexibility lowers the binding affinity, an important condition in their design is that the conformationally constrained version of that inhibitor exhibits extremely high affinity. In this way, the conformationally relaxed inhibitors will maintain enough affinity to be effective. The most straightforward way of achieving high binding affinity is by combining a favorable binding enthalpy with a favorable binding entropy. Unfortunately, current drug design strategies predominantly favor entropic optimization. In this paper, we have summarized experimental and computational strategies aimed at identifying and optimizing enthalpically favorable inhibitors.

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