Drug Resistance

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Predicting Drug-Resistant Mutations of HIV Protease**

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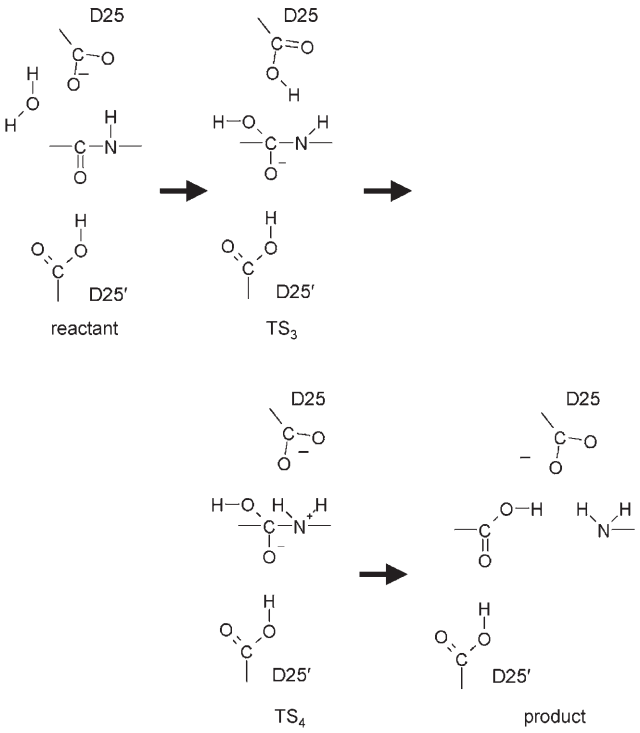
The human immunodeficiency virus (HIV) can acquire drug resistance by mutating a key enzyme that is being used as a drug target, namely HIV protease. Thus, it is extremely important to augment the emerging experimental studies (for example, Ref. [1]) by computational approaches that can predict the most likely drug-resistant mutants. Effective computational screening requires constraints on the ability of the virus to perform its specific function, in addition to the binding energy calculations. With this in mind, we present a new computational strategy for fighting drug resistance by predicting the likely move of the virus through constraints on binding and catalysis. That is, our strategy is focused on the fact that the virus must retain reasonable efficiency (namely, a large k_{cat}/K_M value) for its catalytic reaction while trying to reduce its affinity for the given drug. This requirement can be expressed in terms of the vitality value (γ)[2] and expressed (using the consideration in the Supporting Information) as Equation (1).

$$\ln(\gamma_M/\gamma_N) = \ln[\{K_i(k_{cat}/K_M)\}_M/\{K_i(k_{cat}/K_M)\}_N] \cong \frac{1}{RT}(\Delta\Delta G_{bind}^{N\to M}(\text{drug}) - \Delta\Delta G_{bind}^{N\to M}(\text{TS})) \quad (1)$$

γ_M and γ_N are the vitality values for the mutant and native protein, respectively, K_i is the inhibition constant, R is the gas constant, T is a temperature, and ΔG_{bind}(TS) and ΔG_{bind}(drug) are the transition-state (TS) and drug binding energies, respectively (see Figure S1 in the Supporting Information).

This study focuses on the introduction of our model and on its initial validation, using the resistance of HIV protease to the cyclic urea drug DMP323[3] as a benchmark. Our vitality analysis starts by exploring the effect of mutating ionized and polar residues, which are simpler to model, and then we move to the mutation of nonpolar residues.

By using the group contribution approximation,[4] we performed an initial screening for the mutation of charged and polar residues to nonpolar residues, by calculating the electrostatic contribution of each residue to the ΔG_{bind}(TS) and ΔG_{bind}(drug) values for the native HIV protease (Figure 1a and b, respectively). As seen from Figure 1a, and as was found in Ref. [5], Asp25 contributes significantly to the value of ΔG_{bind}(TS). In this study, we follow Ref. [5] and consider two transistion states in the catalytic reactions of HIV proteases (Scheme 1). It also appears from Figure 1b that DMP323 binds strongly to the same catalytic residue. Thus, mutating Asp25 will reduce the drug affinity to HIV proteases, but will also reduce the ΔG_{bind}(TS) value in a drastic way, thereby leading to the decrease in the k_{cat}/K_M value. Thus, a mutation of Asp25 cannot lead to a large vitality value. In fact, the virus is proteolytically inactive with this mutation.[6] The contribution of Asp30 to the ΔG_{bind}(TS) value (Figure 1a) is due to the interaction of its ionized side chain with the P1 hydroxy group of DMP323 ($O_{DMP323}\cdots O_{Asp30}$ distance: ca. 4 Å[7]). Overall, single mutations of other charged and polar residues to nonpolar residues do not appear to provide an effective way of resisting DMP323 (Figure 1c).



Scheme 1. A schematic description of the reacting system in the catalytic reactions of HIV proteases.

The above analysis restricts itself to single mutations. However, HIV protease can acquire drug resistance by simultaneously mutating multiple sites.[8] Thus, we also investigated double mutants and found low vitality values for all the double mutants of charged and polar residues, except those involving Asp29 and 30 (Figure 2). This finding

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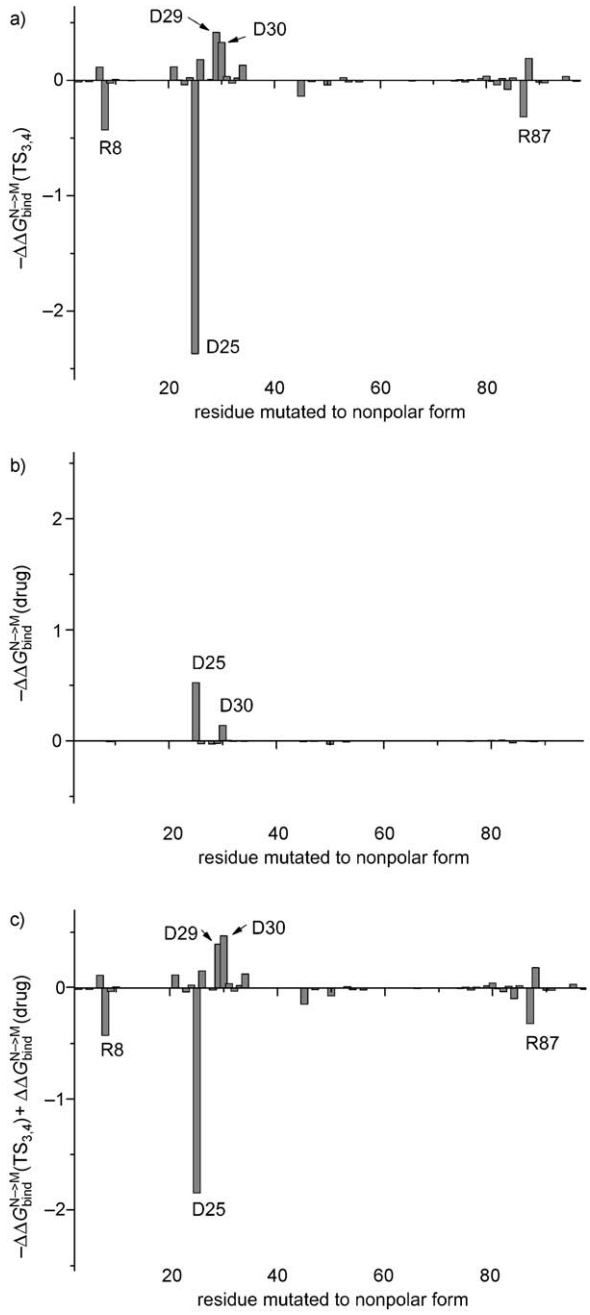

Figure 1. Electrostatic contributions (in kcal mol^{-1}) of the protein side chains of HIV protease to a) $-\Delta\Delta G_{bind}^{N\rightarrow M}$(TS) (one of the two Asp25 residues is ionized as in Refs. [5, 13]), where M corresponds to the nonpolar form of the given residue N, whose contribution is zero. The calculations represent the average from the binding of TS_3 and TS_4. b) $-\Delta\Delta G_{bind}^{N\rightarrow M}$(drug) (where the two Asp25 residues are ionized), and c) $\Delta\Delta G_{bind}^{N\rightarrow M}$(drug)$-\Delta\Delta G_{bind}^{N\rightarrow M}$(TS). The electrostatic contributions of the protein backbones to the binding energies are excluded from the figures, since the backbone contributions cancel out in the determination of the $\Delta\Delta G$ value when the same main-chain structure is used for the native and mutant protein in this specific method. D25(prot.) and (ion.) stand for protonated and ionized D25 in the TS, respectively. Residues 100–198 correspond to residues 1–99 in the second monomer unit of HIV protease. The residue contributions to the ΔG_{bind}(drug) value are averaged over the two monomer units of the HIV protease. The calculations used $\varepsilon_p = 40$ for charged residues and $\varepsilon_p = 4$ for the other residues (see the Supporting Information).

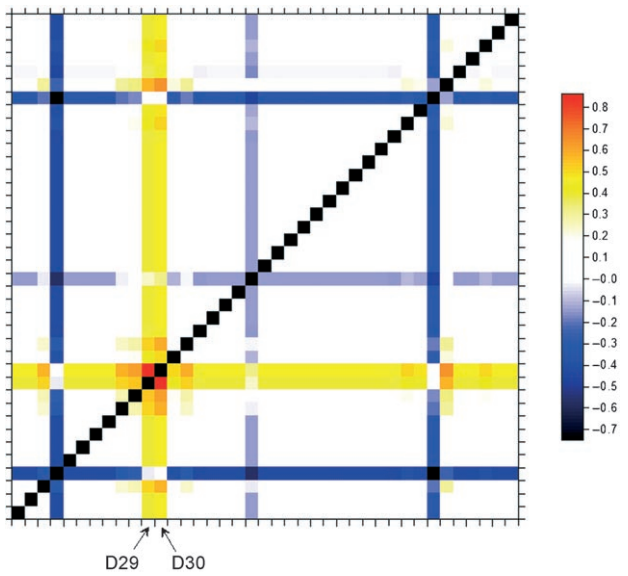

Figure 2. Vitality diagram for double mutants of charged and polar residues with nonpolar residues. The axes refer to the different mutants (from left to right and bottom to top, the original residues are: Q2, T4, Q7, R8, T12, R14, Q18, K20, E21, T26, D29, D30, T31, E34, E35, N37, K41, K43, K45, K55, R57, Q58, Y59, D60, Q61, E65, C67, H69, K70, T74, T80, N83, R87, N88, T91, Q92, C95, T96, and N98). D25 was excluded for simplicity. The figure excludes the main-chain contributions. The calculations used $\varepsilon_p = 40$ and $\varepsilon_p = 4$ for charged and polar residues, respectively. The colors refer to the calculated energy values in kcal mol^{-1} unit, where the mutation with the largest vitality value is shown in red. The diagonal elements (black) contain no data, since a double mutant of a single residue does not exist.

indicates that mutations of charged and polar residues are unlikely to be used by the virus as the most effective drug-resistance strategy in the case of DMP323.

The above use of group contributions provides an initial screening that is fully consistent with the fact that current drug-resistant mutants do not involve mutations of charged and polar residues. However, in the absence of any observed drug-resistant mutations we needed to validate our approach on the much more challenging cases of mutations of nonpolar residues. More specifically, since it has been observed that the resistance to DMP323 involves the V82F and I84V mutants,[1,9] we validated our approach by calculating the change in vitality associated with mutations at residues 82 and 84. The corresponding results are depicted in Figure 3. The computed ΔG_{bind}(drug) values of DMP323 in the native and the V82F and I84V mutants of HIV proteases are in excellent agreement with the measured values[9,10] (Figure 3b). The present LRA/α computational values of ΔG_{bind}(drug) are similar to the values reported in Ref. [11]. Similarly, the computed $\Delta\Delta G_{bind}$(TS) values are comparable to the $\Delta\Delta G_{bind}$(TS) value derived from the experimental k_{cat}/K_M values[2] (see the Supporting Information). Most significantly, the vitality values obtained in the present study reproduce the experimentally obtained values (Figure 3c) and the corresponding drug resistance.[1,9]

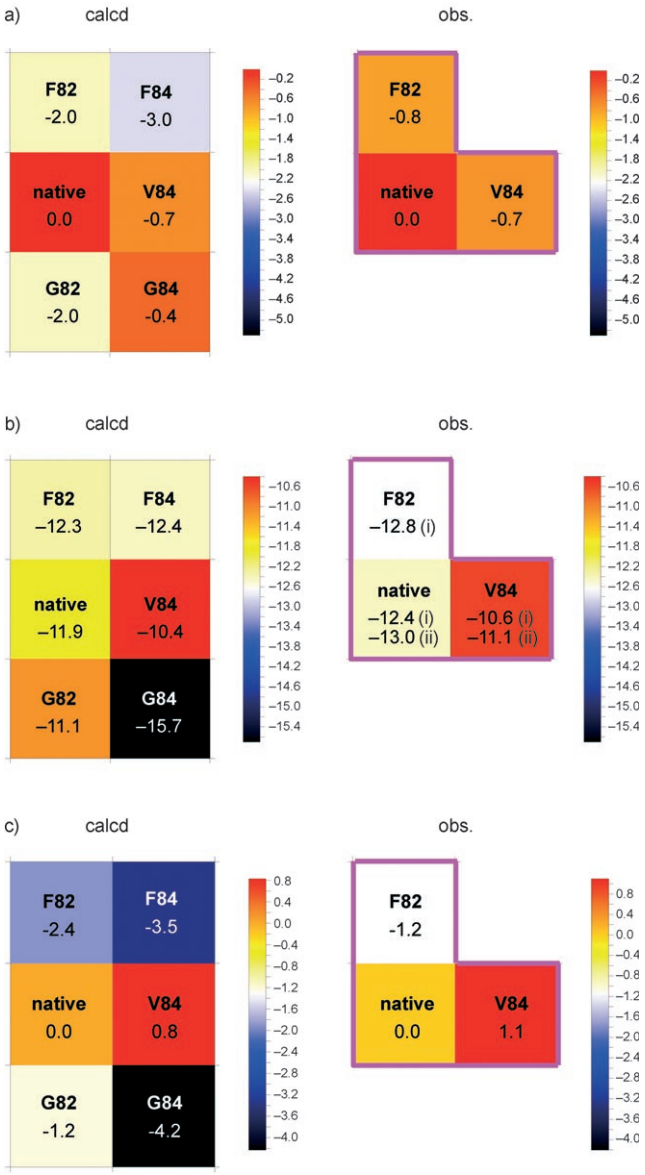

Figure 3. Calculated (left) and observed (right) values for a) $-\Delta\Delta G_{bind}^{N\to M}$(TS), obtained with the PDLD/S-LRA method[11,14] (the results represent the average for TS_3 and TS_4), b) ΔG_{bind}(drug) obtained with the LRA/α method[11] (experimental values (i) and (ii) were taken from Refs. [10] and [9], respectively), and c) $\Delta\Delta G_{bind}^{N\to M}$(TS) + $\Delta\Delta G_{bind}^{N\to M}$(drug) for single mutations at 82 and 84. The color in each element refers to the calculated energy (in kcal mol^{-1}). The observed values are only available for the native protein (with V82 and I84) and the V82F and I84V mutants, which are boxed by a pink solid line. Drug-resistant mutants are expected when $\Delta\Delta G_{bind}^{N\to M}$(TS) − $\Delta\Delta G_{bind}^{N\to M}$(drug) > 0.

The decrease in the van der Waals contact between DMP323 and the I84V mutant has been proposed[8] as the molecular origin of the corresponding drug resistance. The present study establishes that the selection of Val at position 84 provides the largest vitality value and thus the optimal drug resistance. Mutations at site 84 by a much smaller (for example, Gly) or larger (for example, Phe) residue than Val do not lead to effective resistance to DMP323 (Figure 3c). The present study also suggests that the large vitality value of the I84V mutant is mainly a result of the poor binding of DMP323 (Figure 3b).

Interestingly, we found that only a few specific charged residues are crucial for the drug binding (see Figure 1 and Ref. [8]). However, most of those residues are also directly involved in the enzymatic reaction,[12] and thus, the virus is unlikely to mutate them. The other charged residues are not likely to affect the ΔG_{bind}(TS) and ΔG_{bind}(drug) values effectively (see Figure 1). Thus, it appears that the most effective drug-resistance strategy of HIV protease involves the mutation of uncharged residues that are able to impose steric constraints on the given drug without reducing the k_{cat}/K_M value significantly. The present approach seems to provide a way to predict drug resistance even for mutations of uncharged residues. In fact, the most important new element in our approach is the ability to predict mutations that are unlikely to occur (for example, the mutants with low $\ln(\gamma_M/\gamma_N)$ values in Figure 3c).

Methods Section

This work used the crystal structures of the native HIV protease without a drug (PDB 4HVP)[15] and with the DMP323 drug (PDB 1QBS)[7] for the calculations of the ΔG_{bind}(TS) and ΔG_{bind}(drug) values. The atomic partial charges of the amino acids and the transition-state model (Scheme 1) were adopted from the MOLARIS force field[14] and from previous studies,[16] respectively. The atomic partial charges of DMP323 are listed in the Supporting Information (Table S1). All the acidic and basic residues were considered to be ionized, except for one of the two Asp25 residues in TS_4, as was done in previous studies.[13] The screening reported in Figures 1 and 2 was done by using the electrostatic group contributions (see the Supporting Information) and Ref. [5] The TS binding energy at positions 82 and 84 of HIV protease (Figure 3a) was evaluated by the PDLD/S-LRA[11,14] method (see the Supporting Information). The calculations of the ΔG_{bind}(drug) values for mutations at positions 82 and 84 (Figure 3) were performed by the microscopic all atom linear response approximation (the microscopic LRA/α) method.[11] All the PDLD/S-LRA and microscopic LRA/α calculations were performed by the automated procedure of the MOLARIS program.[14] The PDLD/S-LRA simulations involved the generation of 5 configurations in the charged and uncharged forms by molecular dynamics simulations of 10 ps with a 1-fs time step at 300 K. The calculations use the SCAAS spherical boundary condition[14] and the LRF long range treatment[14] (see the Supporting Information for further details).

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