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Energetic effects for observed and unobserved HIV-1 reverse transcriptase mutations of residues L100, V106, and Y181 in the presence of nevirapine and efavirenz

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Abstract—The effect of mutations on amino acid residues L100, V106, and Y181 for unbound HIV-1 reverse transcriptase (RT) and RT bound to nevirapine and efavirenz was investigated using Monte Carlo/free energy perturbation calculations. Using both native and bound crystal structures of RT, mutation of the amino acid residues to both those observed and unobserved in patients was carried out. The results of the calculations revealed that the variant that survives in patients dosed with either nevirapine or efavirenz had a more positive $\Delta\Delta G$ value than other variants that were not observed in patients. These data suggest that the mutation observed in patients is the most effective (the one that binds the drug most weakly) of all possible codon change mutations.

The three FDA-approved non-nucleoside inhibitors (NNRTIs) of HIV-1 reverse transcriptase, nevirapine, efavirenz, and delaviridine,[1] are an important part of combination anti-HIV therapy due to their specificity in targeting the enzyme. However, their ability to be effective is compromised by the emergence of drug resistant mutant viral strains.[2,3] Many mutations, including L100I, V106A, Y181C, G190A, and K103N, are selected for by most of the currently available NNRTIs.[4] Therefore, a new effort has been launched to find compounds that are resistant to the effects of a wider range of single and double mutations.

As a part of the effort to develop new inhibitors, an interesting question arises. Why are certain mutations, such as those listed above, observed in patients out of all other possible single amino acid substitutions? Table 1 lists several key mutations, the codon normally found for each residue, and the mutations possible from single/double nucleotide changes. The known mutants seen in patients are shown in bold.

A sample of the types of questions that can be raised includes the following: what mutation would be most strongly selected by a particular NNRTI? For example, why are L100I and V106A/I chosen over the other possibilities, that is, F, S, or V and E, G, or L options (see Table 1), respectively? Could other, currently unobserved, mutations also interfere with drug binding, and if so, why are they not observed? Do some of the unobserved mutations disrupt the enzyme more than others, thus explaining their appearance (or lack thereof) in patients?

In order to answer these and other questions, free energy perturbation (FEP) calculations can be carried out to predict the effect of each single mutation on the binding of an NNRTI in its respective RT conformation, as determined from X-ray crystallography. In addition, the effect of these mutations on the unliganded (apo) form of the enzyme (based on its crystal structure) can also be ascertained. Thus for each NNRTI and/or mutation of interest, the wild-type to mutant transformation can be evaluated using Monte Carlo simulations. Previously, these types of simulations have been used successfully for the L100I and V106A mutations for nevirapine and efavirenz,[5] the K103N mutation for efavirenz analogs,[6,7] and the L100I and L100I + K103N mutations for etravirine.[8]

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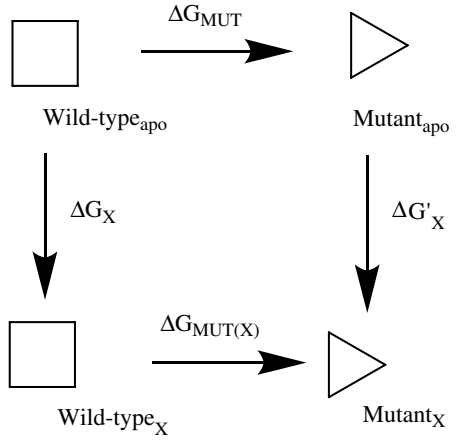
Table 1.

WT number	WT residue	WT codon	Substitution	Mutant residue
100	Leu	TTA	TTT	Phe
			TCA	Ser
			ATA	**Ile**
			GTA	Val
103	Lys	AAA	AAC	**Asn**
			ACA	**Thr**
			AGA	**Arg**
			AUA	Ile
			CAA	**Gln**
			GAA	**Glu**
106	Val	GTA	GAA	Glu
			GCA	**Ala**
			GGA	Gly
			ATA	**Ile**
			CTA	Leu
181	Tyr	TAT	TCT	Ser
			TGT	**Cys**
			TTT	Phe
			AAT	Asn
			CAT	His
			GAT	Asp
			ATT[a]	**Ile**[a]

[a] Denotes double nucleotide substitution necessary.

Monte Carlo/FEP calculations were performed using binding site models for the RT/nevirapine (1VRT), RT/efavirenz (1FK9), and RT (1HMV) using standard simulation protocols. The final models for the unliganded RT included 123 amino acid residues within 15 Å of the NNRTI binding site and for the complex sites also included the inhibitor. Using the MCPRO program,[8,9] each protein/ligand complex was energy-minimized prior to the MC simulations using a distance-dependent dielectric constant of 4 to relieve unfavorable contacts.

The MC/FEP calculations were performed to compute free energy changes associated with the mutation of residue X to Y for each inhibitor according to Scheme 1.



Scheme 1. Thermodynamic cycle. Apo is the uncomplexed enzyme and subscript X indicates drug–enzyme complex.

The difference in the free energy changes for each inhibitor X (nevirapine or efavirenz), $\Delta\Delta G_X = \Delta G_{MUT(X)} - \Delta G_{MUT}$, is a measure of the effectiveness of the drug against the mutated form of the enzyme versus wild-type. A positive value of $\Delta\Delta G_X$ would suggest that, in the presence of drug X, a mutant form of the enzyme would be more resistant than wild-type and thus would be likely to appear in the clinic. For comparisons between two drugs A and B, a positive value for $\Delta\Delta G_{A-B}$ (i.e., $\Delta\Delta G_A - \Delta\Delta G_B$) would mean that drug A is less effective against the mutant than is drug B, and thus mutant forms of the enzyme would be more likely to appear in the presence of drug A. Since both drugs in this study inhibit WT RT and both suffer loss of activity against mutants, changes in the $\Delta\Delta G$ values can be primarily attributed to changes in the structure of the protein upon mutation, and thus calculations may be able to address the question of emergence of some, but not other, mutants.

The following selected mutations were considered in this study: L100 I, F, and S; V106 A, G, and L; Y181 C, F, I, and H. Most mutations were carried out in two steps; for example, leucine to norvaline and isoleucine to norvaline. The total ΔG for the transformation was thus $\Delta G_{L\rightarrow I} = (\Delta_{GL\rightarrow NV}) - (\Delta G_{I\rightarrow NV})$. For computational ease, all mutations were set up to mutate the larger side chain to a smaller one. Both the ligand and the protein were solvated with a 22 Å water cap. Each FEP calculation entailed 10 windows with double-wide sampling (20 free-energy increments) and covered 20 M configurations of equilibration and 15–30 configurations of averaging. Separate MC simulations were performed for each mutation for all inhibitor/RT complexes or unbound protein. The backbone of the protein was fixed and each inhibitor and all side chains within ~10 Å of the center of the water cap were fully flexible during the simulations. All energy evaluations used the OPLS-AA force field,[10] except that CM1A atomic charges scaled by 1.14 were used for the inhibitors.

For the perturbations using nevirapine and efavirenz, the results of the various mutations are listed in Table 2. For each amino acid mutation, the variant that survives to emerge in patients following drug treatment should be the most common mutation. In Table 2, these are the first mutations in the list for each amino acid residue. As can be seen, the calculated and experimental $\Delta\Delta G$ trends are in accord with the most positive value corresponding to the mutation found in patients. The more negative the value of $\Delta\Delta G$, the better the binding between the enzyme and the drug.

In general, the agreement between the calculated and average experimental values is acceptable (Table 2). For the L100I mutation for nevirapine, the $\Delta\Delta G_{Calc}$ value of −0.83 kcal is very close to a previous determination of −0.48 kcal[11] and to the average experimental value of 0.68 kcal. The $\Delta\Delta G_{Calc}$ value of 0.24 for efavirenz is close to the previous value of 0.82 kcal[11] as well as to the $\Delta\Delta G_{Expt}$ value of 1.21 kcal. Furthermore, the better inhibitor against a given mutation should have a more negative $\Delta\Delta G_{Calc}$ value, and it does. Within each

Table 2. Calculated and experimental $\Delta\Delta G$ values (kcal/mol) for various HIV-1 reverse transcriptase mutations for nevirapine relative to efavirenz

Mutation		Nevirapine				Efavirenz			
		Apo[b]	ΔG[c]	$\Delta\Delta G_{Calc}$	$\Delta\Delta G_{Expt}$[d]	Apo[b]	ΔG[c]	$\Delta\Delta G_{Calc}$	$\Delta\Delta G_{Expt}$
L100	**I**[a]	15.88	15.05	−0.83	0.68	15.88	16.12	0.24	1.21
	F	12.09	8.56	−4.15	nd[e]	12.09	8.76	−3.33	nd
	S	44.03	43.01	−1.02	nd	44.03	40.15	−3.88	nd
V106	**A**	−17.58	−14.18	3.40	2.48	−17.58	−16.18	1.4	2.21
	G	4.84	8.14	3.31	nd	4.84	5.16	0.32	nd
	L	−2.1	−3.47	−1.37	nd	−2.1	−7.33	−5.23	nd
Y181	**C**	6.90	14.12	7.22	2.95	6.9	12.57	5.67	0.35
	F	5.24	3.3	−1.99	nd	5.24	2.66	−2.58	nd
	I	15.92	15.9	−0.02	nd	15.92	10.39	−5.53	nd
	H	−13.17	−12.16	1.01	nd	−13.17	−13.85	−0.68	nd

[a] Known mutations are in bold.
[b] ΔG_{MUT} in thermodynamic cycle.
[c] $\Delta G_{MUT(X)}$ in thermodynamic cycle.
[d] Determined from averages from Refs. 13 and 14.
[e] nd, not determined.

series of possible mutations at a given site (L100, V106, or Y181) and with a given drug (nevirapine or efavirenz), the most positive $\Delta\Delta G_{Calc}$ value is observed for the specific mutation which has been observed in the clinic; for example, −0.83 for the L100I mutation for nevirapine. While not reflected in the $\Delta\Delta G$ values, it is also interesting to note that the drug–protein interaction energy is reduced by 1.5 and 3.6 kcal/mol for the mutations that are not observed in patients.

For the L100 residue, the isoleucine mutant is the one found in patients. The more negative $\Delta\Delta G$ values (indicating better binding) for both the L100F and L100S mutations in Table 2, as compared to that for L100I, are what would be expected. The L100I mutation, which involves geometric rearrangement of one carbon atom in the residue, generally confers resistance by destabilizing the formation of a hydrogen bond between the K101 carbonyl oxygen and a NH donor group. This interaction is common in most NNRTIs,[11] including nevirapine and efavirenz. In addition, the shifted methyl group can adversely affect other favorable non-bonded ligand–protein interactions.[12] There seems to be some systematic overestimation of the strength of drug binding in the L100 mutants, for example, L100I appears to bind slightly better than wild-type. But among the three mutants, the most poorly binding is the mutant that is observed for both drugs.

For the V106 residue, the alanine variant is the most common mutation and the data show that it binds the drugs most poorly. The V106A variant is thought to confer resistance by decreasing favorable van der Waals interactions with the inhibitor.[5] What is surprising is that the V106G mutation is not observed in patients treated in particular with nevirapine.

For Y181, C is the mutation most commonly observed in patients and the data in Table 2 clearly show that the drug binding ($\Delta\Delta G$) is substantially reduced for this mutation. The loss of π-stacking interactions between the aromatic tyrosine ring and the aromatic rings of nevirapine and other inhibitors is thought to explain the decreased activity caused by the tyrosine to cysteine change. The loss of the interaction of the tyrosine and the smaller, less polarizable cyclopropyl group of efavirenz is expected to be less detrimental.[5] Significantly, data from patients[15] indicate that nevirapine causes the Y181I mutation to grow out, while efavirenz does not; this difference is reflected in our $\Delta\Delta G$ values of −0.02 for nevirapine versus −5.5 for efavirenz.

In conclusion, the Monte Carlo/FEP simulations show that as predicted, among the possible variants that could occur at a given site through single base replacement, the variant that is observed in patients following drug treatment is the one that is the most successful at decreasing the drug binding. It should be noted, however, that other effects might also be operating. For example, mutations could result in van der Waals interactions that stabilize or destabilize the unbound form of the enzyme relative to the bound complex. Our calculations were not designed to evaluate effects such as these. In spite of this limitation, our approach appears to provide a means of predicting which specific mutations might arise as a result of the development of drug resistance, and gives indications of which drugs might be less likely to lead to resistant forms of the virus.

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