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The Role of 2′,3′-Unsaturation on the Antiviral Activity of Anti-HIV Nucleosides against 3TC-Resistant Mutant (M184V)

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Abstract—Molecular modeling studies show that the 2′,3′-double bond of the sugar moiety of various 2′,3′-unsaturated nucleosides interacts with the aromatic moiety of Tyr115 of HIV-1 reverse transcriptase (RT) by hydrophobic π–π interaction. In 3TC-resistant mutant (M184V) RT, 2′-fluoro-2′,3′-unsaturated nucleosides with a bulky 4′-substituent experience significant steric hindrance with the side chain of Val184.
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Concerns about the development of the high level viral resistance to 3TC have prompted studies of the molecular mechanism of resistance, which is conferred by a single mutation at codon 184 in the catalytic domain of HIV-1 reverse transcriptase (RT).[1,2] The sterically demanding nature of Val184 imposes severe restriction to the conformation of nucleoside reverse transcriptase inhibitor (NRTI) triphosphates, in which only a limited number of NRTIs maintain the antiviral activity against M184V RT. It is noteworthy that no unnatural L-nucleosides have been reported to be active against M184V RT. Until now, only three classes of NRTIs have been reported to be active against M184V RT, which include dioxolane/oxathiolane (DAPD[3]/dOTC[4]), acyclic (tenofovir disoproxil)[5] and 2′,3′-unsaturated nucleosides [D4T,[6] D4FC,[7] D-2′,3′-unsaturated-2′-fluorocytidine (2′Fd4C)[8]]. Among these, antiviral activities of various 2′-fluoro-2′,3′-unsaturated nucleosides[8,9] against M184V RT have been extensively studied, in which D-2′,3′-unsaturated cytidine analogues show potent activity against M184V RT[8] but the isosteric replacement of 4′-oxygen with a sulfur atom (D-2′,3′-unsaturated 2′-fluoro-4′-thiocytidine, 2′F-4′Sd4C)[9] develops significant cross-resistance. On the other hand, abacavir, which has a 4′-CH_2 group instead of an oxygen atom, exhibits only 2- to 5-fold cross resistance to M184V RT.[10] Taken together, it is clear that the antiviral activity of 2′,3′-unsaturated nucleosides against M184V RT is significantly dependent on the 4′-substituent of the sugar moiety. Thus, it was of interest to find out how the 2′,3′-unsaturated moiety and its substitution contribute to the binding mode of the unsaturated nucleosides at the active site of M184V RT.

Six NRTI (3TC, D4T, D4FC, 2′Fd4C, 2′F-4′Sd4C, and carbovir) triphosphates were docked into the active site of HIV-1 RT, and the resulting enzyme–ligand complex was studied by the energy minimization method (Fig. 1).[11] Carbovir triphosphate was included in this study because the carbovir triphosphate is known to be the active metabolite of the abacavir.[10]

Conformational Analysis

The initial conformations of the NRTIs were constructed by builder module in Spartan 5.1.1 (Wave-

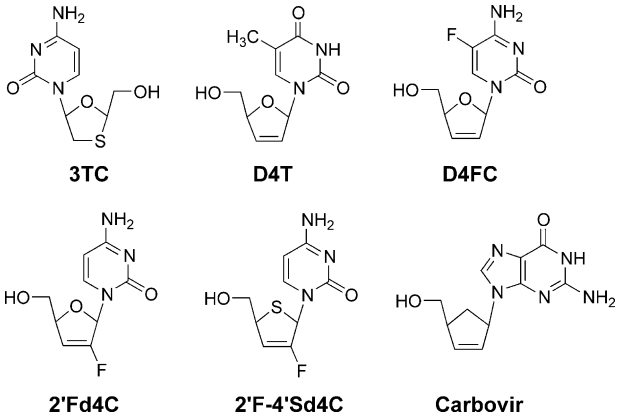

Figure 1. 3TC and five 2′,3′-unsaturated NRTIs under study.

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functions, Inc. Irvine, CA). The initial conformations were cleaned up and geometrically optimized through quantum mechanical ab initio calculations using RHF/3-21G* basis in Spartan 5.1.1.

Binding Affinity Study to HIV-1 Reverse Transcriptase

All molecular modeling of the enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO, USA) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme–ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd).[12] A model of the NRTI binding site was constructed, which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7/4 (template/primer) duplex.[11] The geometry-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of 5th nucleotide in the template of the 7/4 (template/primer) duplex was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray structure (1rtd)[12] was modified to guanine or cytosine. The inhibitor triphosphates were manually docked to the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure.[12] Gästeiger–Hückel charge was given to the enzyme–ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges were loaded to enzyme site from the biopolymer module in Sybyl. Fluorine parameters were obtained from the literature[13,14] and MM2 parameters, and put to the parameter files. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and the mutated residue (Val 184) were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme–inhibitor complexes were minimized by using Kollman-All-Atom Force Field until the iteration number reached 5000.

The energy-minimized structures were analyzed to provide the characteristic binding modes of the 2′,3′-unsaturated D-nucleosides to HIV-1 RT.

Although 3TC is one of the most commonly used nucleoside analogues in first-line combination therapy for the treatment of HIV-1 infections,[15] 3TC monotherapy results in the high-level resistance conferred by a single mutation at codon 184, which increases the EC_{50} value of 3TC at least 1000-fold.[1] As reported previously, the crystal structure of M184V RT without bound DNA template–primer duplex and deoxyribonucleoside triphosphate shows apparently no significant difference from that of the wild type (WT) RT.[16] In addition, the active site of HIV-1 RT is tight enough not to allow any significant conformational change.[17] Therefore, as the Met184 in the wild-type RT directly faces the nucleoside triphosphate binding site, the structural difference between the Met184 and Val184 should exert its effect on the binding event of the nucleoside triphosphate at the active site of M184V RT to discriminate the NRTIs, which causes steric hindrance with Val184.

Significant efforts have been directed toward the 2′,3′-unsaturated nucleosides because of their potent antiviral activity and, particularly, their antiviral potency toward M184V RT (Table 1). Among those, D4FC and 2′Fd4C show potent antiviral activity against M184V RT.[7,8] On the other hand, abacavir has moderate cross-resistance to M184V RT (2- to 5-fold cross resistance),[10] and 2′F-4′Sd4C shows significant cross-resistance.[9]

In order to investigate the binding mode of each NRTI to RT, the six NRTI structures, obtained from the quantum mechanical ab initio calculations using RHF/3-21G* basis in Spartan 5.1.1, were superimposed and compared. 3TC has the largest van der Waals radius of the sugar moiety which is directly projected to Val184 in the HIV RT. The van der Waals radii of 4′-CH_2 in carbovir and 4′-sulfur in 2′F-4′Sd4C are similar, and the 4′-oxygen atoms in D4T, D4C and 2′Fd4C have the smallest van der Waals radius (Fig. 2). As the 4′-substituent of the sugar moiety of NRTI triphosphate is in close contact to Val184, it might be possible to correlate the cross-resistance of NRTIs to M184V RT with the relative sizes of the 4′-substituents. D4T, D4FC and 2′Fd4C with an oxygen atom at the 4′-position are still active or more active against M184V RT than against WT RT, while ABC (abacavir) with 4′-CH_2 substituent has a little cross-resistance to M184V RT. However, different

Table 1. In vitro antiviral activity (EC_{50}, μM) of the NRTIs against the wild type (WT) and M184V RT

	WT	M184V	FI
3TC[3]	0.042	>50	>1200
D4T[6]	0.45	0.5	1.1
D4FC[7]	0.18	0.14	0.8
2′Fd4C[8]	8.0[a]	1.8[a]	0.23
2′F-4′Sd4C[9]	5.0[a]	125[a]	25
ABC[10]	5.3	15.5	2.9

[a]EC_{90} in μM.

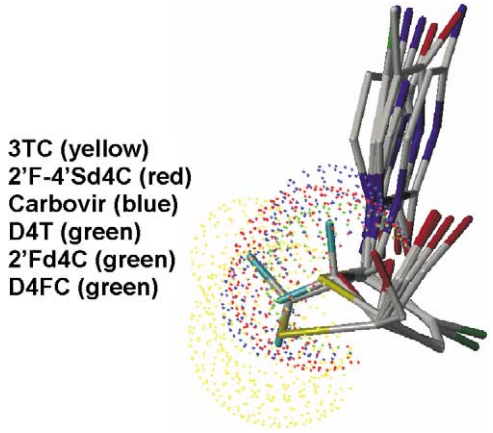

Figure 2. Superimposed structures of 3TC/D4T/2′F4′Sd4C/2′Fd4C/carbovir/D4FC.

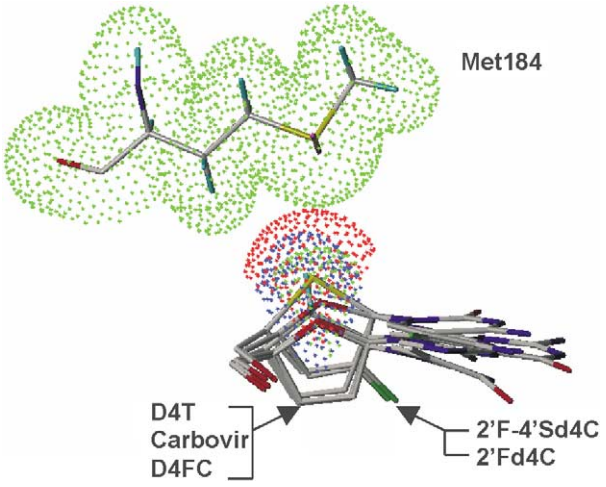

Figure 3. The rotation of the sugar moiety of 2′Fd4C and 2′F-4′Sd4C pushes the 4′-substituent close to Met184. The 4′-oxygen in 2′Fd4C (green) is far away from Met184, but the 4′-sulfur atom in 2′F-4′Sd4C (red) is in close contact to Met184.

resistance profiles of 2′F-4′Sd4C and ABC (abacavir), which have the 4′-substituents similar in size, deserved more investigation.

Comparison of the minimized structures of ternary complexes (NRTI-DNA-WT RT) shows several interesting features: (a) The 2′-fluoro 2′,3′-unsaturated nucleosides (2′Fd4C and 2′F-4′Sd4C) show their sugar moieties slightly rotated to locate their 4′-substituents closer to Met184 (Fig. 3). This conformational difference is believed to be the result of the possible steric hindrance between the 2′-fluorine atom and the adjacent amino acid (Gln151). (b) Every 2′,3′-unsaturated nucleoside studied shows their 2′,3′-double bond at close proximity (~3.5 Å) with right orientation to the aromatic side chain of Tyr115, which can be understood as a result of hydrophobic π–π interaction (Fig. 4).[9] This π–π interaction strengthens the binding between the HIV RT and the triphosphates, and may partially explain the significant anti-HIV activity of the d4Ns against the M184V mutant.

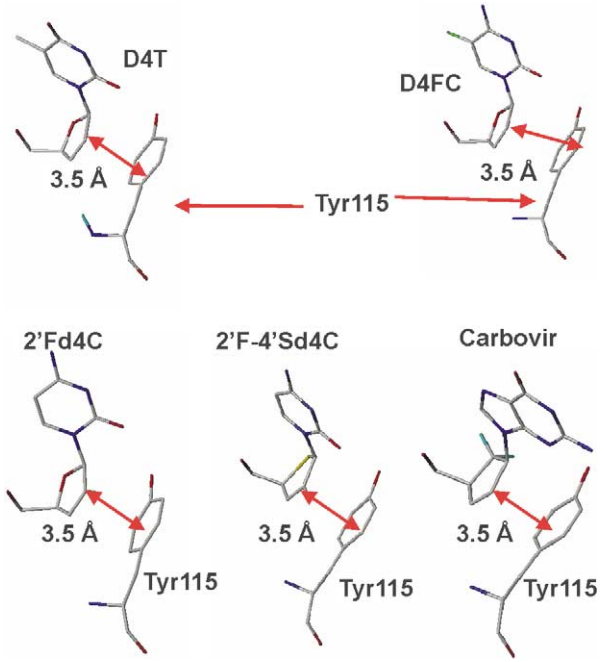

Figure 4. 2′,3′-double bonds of the 2′,3′-unsaturated nucleosides (D4T, D4FC, 2′Fd4C, 2′F-4′Sd4C and carbovir) are separated from the aromatic side chain of Tyr115 by 3.5 Å in parallel by the hydrophobic π–π interaction.

In the structures of ternary complexes (NRTI–DNA–M184V RT), 3TC is located too close to the Val184 resulting in significant steric hindrance (Fig. 5), suggesting that 3TC has difficulty in binding to the active site of M184V RT. As mentioned before, the 2′-fluorine atom rotates the sugar moieties of 2′Fd4C and 2′F-4′Sd4C toward the residue 184. In M184V RT, this conformational difference was manageable when the 4′-substituent was an oxygen atom (2′Fd4C), but the 4′-sulfur atom (2′F-4′Sd4C), which has larger van der Waals radius than oxygen atom, cannot avoid the unfavorable steric hindrance with the side chain of Val184, resulting in high cross-resistance to M184V RT (Fig. 5). However, the 4′-CH_2 group in carbovir is not close enough to Val184 because there is no 2′-fluoro substituent (Fig. 4). Therefore, the steric hindrance between the sugar moiety of carbovir and Val184, if any, should not be significant.

In summary, the π–π interaction can contribute, in part, to the observed strong binding of the 2′,3′-unsaturated nucleosides at the active site of RT, resulting in potent anti-HIV activity including against M184V mutant. Every 2′,3′-unsaturated nucleoside analogue studied showed the same pattern of interaction with the adjacent aromatic side chain of Tyr115 in the hydrophobic π–π interaction. However, the 2′-fluorine substitution rotates the sugar moiety to push the 4′-substituent toward Met184. Therefore, 2′-fluoro 2′,3′-unsaturated

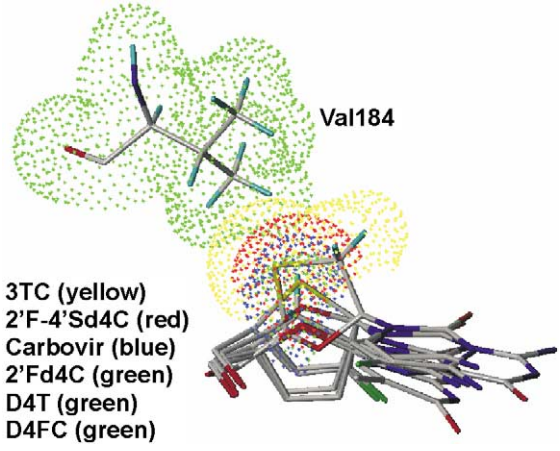

Figure 5. Even though the carbovir has a bulky 4′-CH_2 group (blue), it does not experience serious steric hindrance with Val184. However, 2′F-4′Sd4C with bulky 4′-sulfur (red) experience steric hindrance because of the rotation by 2′-fluoro substituent. L-Configured 3TC (yellow) also has big steric hindrance with Val184.

nucleoside with a bulky substituent at the 4′-position experienced steric hindrance with Val84, and thereby reduced antiviral potency, which was confirmed by the significant cross-resistance of 2′F-4′Sd4C to M184V RT.

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