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Molecular mechanism of dioxolane nucleosides against 3TC resistant M184V mutant HIV

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Abstract

The mutation and resultant adaptability of HIV-1 reverse transcriptase (RT) present a major challenge to the design of the effective antiviral strategies because many initially potent drugs lose efficacy over time. Even though there is an urgent need for a comprehensive understanding of the molecular mechanism of anti-HIV drug resistance by mutant RTs, the unavailability of the structural information of the mutant RTs has prevented detailed investigations. In this study, the active site of the 3TC-resistant (M184V) RT is constructed by a computational method, which clearly shows that the side chain of Val184 occupies the binding site for the nucleoside triphosphates. Therefore, the distance between the side chain of Val184 and the sugar moiety of the nucleoside triphosphate must be closely related to the cross-resistance by M184V RT. The natural substrates, 2′-deoxyribo nucleoside triphosphates, escape from the steric stress from the bulky side chain of Val184 by virtue of the D-sugar conformation as well as the interaction of its 3′-OH group with Tyr115, which locates the nucleoside triphosphate out of the clashing distance from Val184. Similarly, the energy-minimized structures of various D-dioxolane nucleoside triphosphates (TP)/RT complexes indicate that the D-dioxolane sugar moiety acquires enough distance from Val184 due to the specific interaction of its 3′-oxygen atom with the nearby enzyme residues such as Tyr115 and Arg72.

Keywords: HIV; 3TC-resistance; Dioxolane; Nucleoside; Molecular modeling

1. Introduction

Concerns about the development of viral resistance to 3TC (Kavlick et al., 1995; Tisdale et al., 1993) have prompted the discovery of structurally related nucleosides with antiviral activity against HIV isolates containing the 3TC resistance mutation. Since the discovery of D-dioxolane guanosine and its prodrug, D-dioxolane 2,6-diaminopurine (DAPD or amdoxovir), which are active against AZT- as well as 3TC-resistant mutants (Gu et al., 1999; Furman et al., 2001), nucleosides which have a dioxolane sugar moiety have been studied in the pursuit of new drug candidates for anti-HIV therapy. Among the series of the nucleosides, thymidine, 5-fluorocytidine and guanosine analogues (Fig. 1) showed interesting and potent anti-HIV activity against 3TC-resistant mutant RT (Table 1). In this context, it is of great interest to understand the characteristic role of the dioxolane sugar moiety as well as the heterocyclic bases for the anti-HIV activity of these D-dioxolane nucleosides against 3TC-resistant mutant RT. For this purpose, various D-dioxolane nucleoside triphosphates (thymine, 5-fluorocytosine and guanosine derivatives), which are active against M184V RT were docked into the active site of the computer-generated M184V RT, and the resulting complexes were energy-minimized (Chong et al., 2002a,b). Molecular modeling studies indicate that, unlike other nucleosides cross-resistant to M184V RT, the D-dioxolane nucleosides are able to escape the stress of Val184 by adjusting their conformations with no energy costs. Also, the 3′-oxygen atom of the D-dioxolane sugar moiety is critical in locating the nucleoside triphosphate away from Val184 by interacting with the active site residues of RT.

2. Materials and methods

All molecular modeling of the enzyme–substrate complexes was carried out using SYBYL 6.6 (Tripos Inc., St. Louis, MO), and the Monte Carlo conformational search (Chong and Chu, 2002) of the D-dioxolane thymine was performed by Macromodel 7.0 (Schrödinger, Inc. Portland, OR). The enzyme site of the enzyme–ligand complex was constructed as previously reported (Chong et al., 2002a,b).

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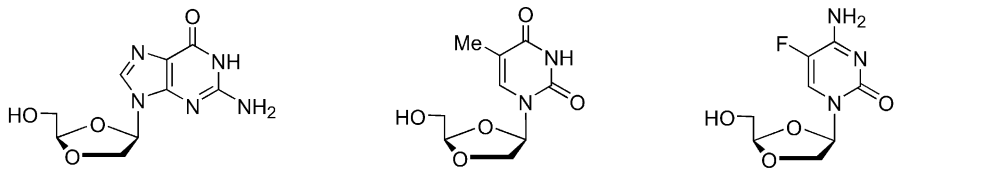

D-dioxolane guanine (DXG) D-dioxolane thymine D-dioxolane 5-fluorocytosine (D-FDOC)

Fig. 1. D-Dioxolane nucleosides active against M184V HIV-1 RT.

2.1. M184V HIV-1 RT

The crystal structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer–template duplex (PDB entry 1rtd) (Huang et al., 1998) was used as a template to generate the three-dimensional structure of the M184V RT. The residue Met184 was mutated to Val184 in the biopolymer module in SYBYL 6.7. Hydrogen atoms were added to the mutated residue and the side chain was fixed. Gästeiger–Hückel charges (Gästeiger and Marsili, 1980; Purcell and Singer, 1967) and Kollman-All-Atom charge (Blaney et al., 1982; Wipff et al., 1983) were loaded to the TTP and M184V RT, respectively, and the residues inside 6 Å from TTP were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol (Chong et al., 2002a,b). The annealed M184V RT-TTP complex was fully minimized by using the Kollman-All-Atom force field for 5000 iterations.

2.2. Nucleoside reverse transcriptase inhibitors (NRTIs)

The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates of D-dioxolane cytosine (Kim et al., 1992) or conformational analysis (3TC) (Lee and Chu, 2001). The heterocyclic base was appropriately modified before docking into the enzyme. Also, the heterocyclic moiety of $n + 1^{th}$ nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, in 3TCTP and DXGTP modeling, the adenine moiety which was in the original X-ray structure (1rtd) (Huang et al., 1998) was modified to guanine and cytosine, respectively. The enzyme site and the inhibitor were merged to form a catalytic complex and then the NRTI triphosphate was situated in such an orientation that it could be paired with its complementary base in the template strand by adjusting the torsional angles to those found in the X-ray structure (Huang et al., 1998).

2.3. Method

Gästeiger–Hückel charge (Gästeiger and Marsili, 1980; Purcell and Singer, 1967) was given to the ligand with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges (Blaney et al., 1982; Wipff et al., 1983) were loaded to the enzyme site from the biopolymer option in SYBYL 6.6. Fluorine parameters were obtained from the literature (Cornell et al., 1995; Dunitz and Taylor, 1997) 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 mutated residues were annealed (Chong et al., 2002a,b) until the energy change from one iteration to the next was less than 0.05 kcal/mol (hot region: 6 Å, interesting region: 12 Å). The annealed enzyme–inhibitor complexes were minimized by using Kollman-All-Atom force field (Weiner et al., 1986) until for 5000 iterations. The binding affinities of the examined structures toward HIV-1 RT were estimated by means of the relative binding energy (E_{rel}, Table 1) between the inhibitor triphosphate (3TCTP, D-dioxolane thimidine triphosphate, D-dioxolane 5-fluorocytidine triphosphate, or DXGTP)-RT complex and the corresponding natural substrate (dGTP, TTP or dCTP)-RT complex in the energy-minimized states (Lee and Chu, 2001; Chong et al., 2002a,b).

2.4. Monte Carlo conformational search

The Monte Carlo conformational search of D-dioxolane thymine was performed in 5000-step, in the presence of

Table 1
Antiviral activities of 3TC and D-dioxolane nucleosides against 3TC-resistant RT (M184V)

	3TC		D-Dioxolane T		DXG		D-FDOC	
	EC_{50}[a]	FI	EC_{50}[b]	FI	EC_{50}[a]	FI	EC_{50}[b]	FI
WT	0.041	–	0.200	–	0.21	–	0.045	–
M184V	>50	>1200	0.088	0.44	0.44	2.1	0.030	0.67

[a] CMB cells (16).
[b] PBM cells (Chong et al., 2003).

GB/SA water model using MM3 force field (Macromodel) (Chong and Chu, 2002).

3. Results

3.1. M184V HIV-1 RT versus 3TC-TP

The computer-generated three dimensional structure of M184V RT has almost no difference from the wild-type (WT) RT even at the active site because, by simply rotating its side chain to the open NRTI binding site, the mutated residue, Val184, can assume a stable conformation. As a result, the deoxyribose moiety of TTP is in close proximity to Val184 (3.3 Å) but not enough to be sterically hindered (Fig. 2a). However, even though the natural substrate such as TTP can manage to fit into the active site without unfavorable steric hindrance, it is obvious that the approach of the sterically demanding Val184 into the NRTI binding site significantly limits the conformation of the sugar moiety of the NRTI, which can be confirmed by the binding mode of 3TC triphosphate (Fig. 2b). For the sake of proper base pairing with the complementary base in the template strand, the unnatural L-configuration of 3TC locates its oxathiolane sugar moiety at the upper open space of the NRTI binding site in the WT RT, where Met184 contributes to the binding of 3TCTP by an extended van der Waals contact to the oxathiolane sugar moiety of 3TCTP (Fig. 2b). However, in M184V RT, 3TCTP cannot be accommodated into the binding site, which is already occupied by the side chain of Val184 (Fig. 2b).

3.2. M184V HIV-1 RT versus D-dioxolane nucleoside-TP

Our molecular modeling study indicated that D-dioxolane nucleoside triphosphates-RT complexes maintain the favorable binding modes through the interaction of its 3′-oxygen with active site residues such as Arg72 or Tyr115 depending on the dioxolane sugar conformation (3′-endo or 3′-exo) (Fig. 3). The calculated relative binding energies, based on the minimized energy of enzyme–ligand complex, free enzyme and ligand (Lee and Chu, 2001; Chong et al., 2002a,b), showed that the D-dioxolane nucleoside triphosphates can bind to the WT as well as M184V RT with the same efficiency (Table 2). In WT RT, the energetically more stable 3′-exo conformation of D-dioxolane sugar moiety, which can be found in the crystal structure of D-dioxolane cytosine (Kim et al., 1992), locates its 3′-oxygen atom within hydrogen bonding distance to the amide backbone of Tyr115 (Fig. 3a). On the other hand, the 3′-endo conformation pulls Arg72 into the nucleoside triphosphate binding site by the interaction between the 3′-oxygen and ε-side chain of Arg72 (Fig. 3a). It is interesting that, even though every dioxolane nucleoside triphosphates was constructed based on the same crystal structure of dioxolane cytosine, the purine nucleoside DXG prefers the 3′-exo conformation (Fig. 3a) but the pyrimidines such as dixolane thymine and 5-fluorocytosine (Fig. 3a) prefer to have the 3′-endo conformation. In M184V RT, thymidine and guanosine analogues readily changed their conformations in order to cope with the steric pressure on the active site by the bulky side chain of Val184 (Fig. 3b). However, the 5-fluorocytosine derivative maintained its conformation in M184V RT by virtue of another hydrogen

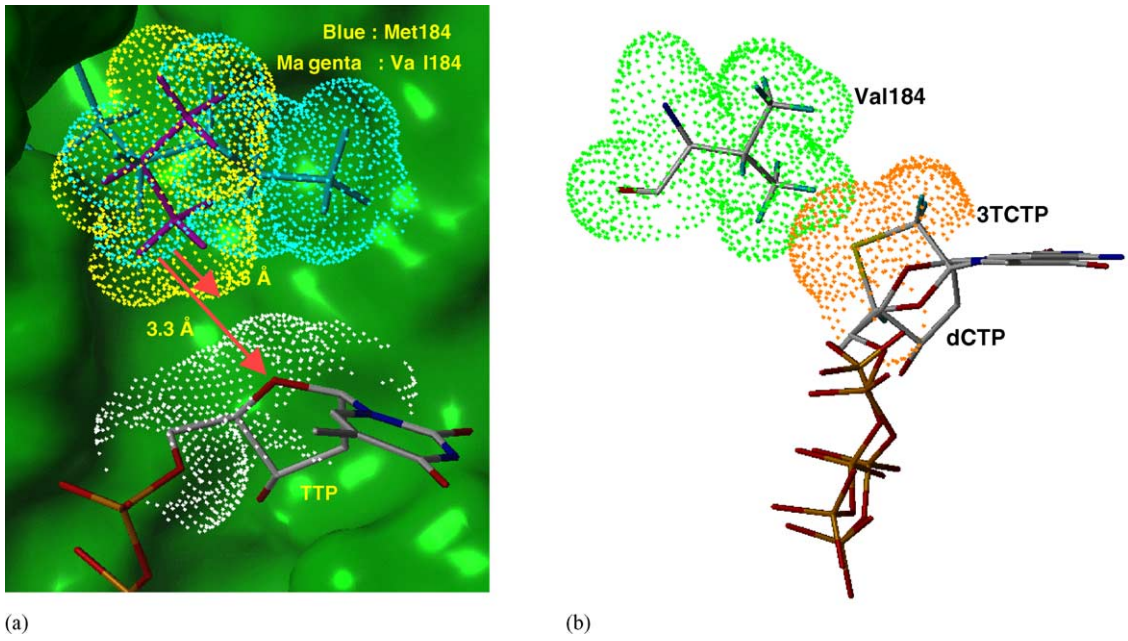

Fig. 2. (a) Val184 protrudes (∼1.5 Å) its bulky side chain into the binding site of the nucleoside triphosphate of HIV-1 RT. Natural substrates, such as TTP, manage to bind to the active site of M184V RT. (b) L-Nucleoside, such as 3TC, locates its sugar moiety at the upper open space of the NRTI binding site, which is already occupied by Val184. Therefore, in M184V RT, 3TCTP cannot be accommodated into the binding site.

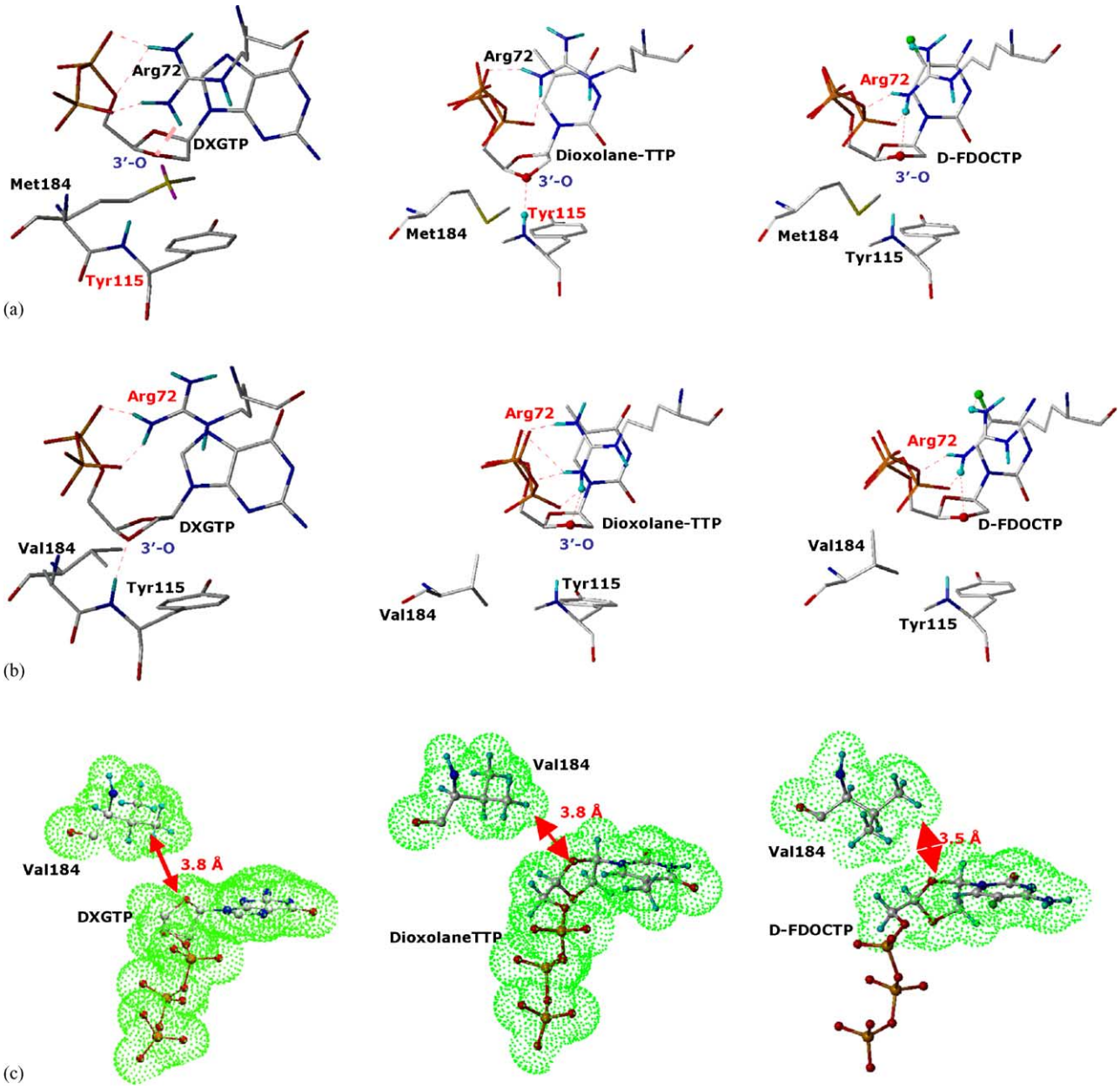

Fig. 3. D-Dioxolane nucleoside triphosphates-RT complexes maintain the favorable binding modes through the interaction of its 3′-oxygen with active site residues such as Arg72 or Tyr115 depending on the dioxolane sugar conformation (3′-endo or 3′-exo). (a) WT RT, (b) M184V RT. (c) The contribution of 3′-oxygen atom by interaction with the enzyme residues (Tyr115 and Arg72) enables the dioxolane sugar moiety to stay away from Val184.

Table 2
Fold increases (FI) and the calculated relative binding energies (E_{rel}) of 3TC and D-dioxolane nucleosides against 3TC-resistant RT (M184V)

	3TC		D-Dioxolane T		DXG		D-FDOC	
	FI[a]	E_{rel}[b] (kcal/mol)	FI[a]	E_{rel}[b] (kcal/mol)	FI[a]	E_{rel}[b] (kcal/mol)	FI[a]	E_{rel}[b] (kcal/mol)
WT	–	76.4	–	16.91	–	12.05	–	10.83
M184V	>1200	−28.8	0.44	20.20	2.1	5.67	0.67	35.56

[a] Fold increase.
[b] Relative binding energy: binding energy of NRTI triphosphate to RT − binding energy of dNTP to RT.

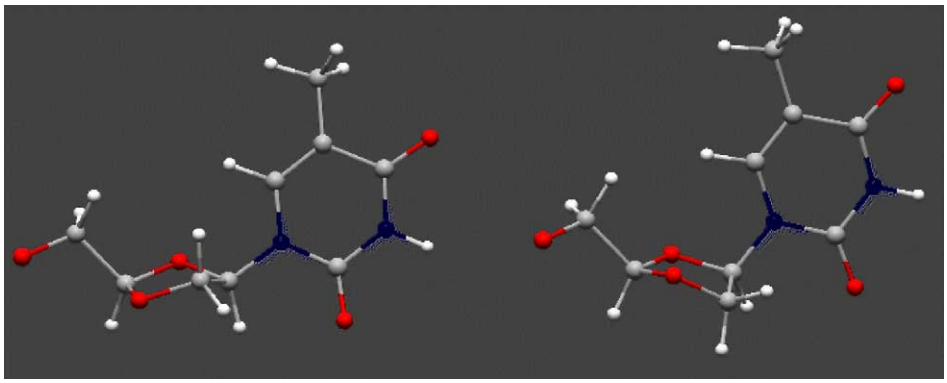

Fig. 4. Conformational search of D-dioxolane thymine by Monte Carlo method in 5000 steps found the global minimum as 3′-endo sugar conformation (a), but the 3′-exo conformation (b) was only 0.4 kcal/mol less stable than the global minimum.

bonding between its 5-fluorine atom and Arg72 (Fig. 3b). Therefore, dioxolane 5-fluorocytosine derivative is strongly biased to 3′-endo conformer. In either way, the contribution of 3′-oxygen atom by interaction with the enzyme residues (Tyr115 and Arg72) enables the dioxolane moiety to stay away from Val184 (Fig. 3c). The conformational search of D-dioxolane thymine by the Monte Carlo method in 5000 steps (Chong and Chu, 2002) found the global minimum as the 3′-endo sugar conformation with 28 hits, but the 3′-exo conformation (26 hits) was only 0.4 kcal/mol less stable than the global minimum (Fig. 4). Therefore, the dioxolane sugar moiety is flexible enough to change its conformation depending on its environment to cope with the steric pressure conferred by bulky side chain of Val184, which can explain the maintenance of its anti-HIV activity against the M184V mutant.

4. Discussion

Among enzymes involved in the synthesis of nucleotides as well as DNA, some exceptions have been found to the rule that only one of the enantiomeric forms of chiral molecules may bind effectively at the catalytic sites of enzymes, displaying biological activity. These exceptions include: herpes virus-1 TKs (HSV-1 TK) (Bennett et al., 1993; Spadari et al., 1995), cellular deoxycytidine kinase (dCK) (Fu et al., 1999), mitochondrial deoxyguanosine kinases (dGK), and HIV-1 reverse transcriptase (Yamaguchi et al., 1994; Focher et al., 1995; Van Draanen et al., 1992). Recent findings have indicated that several β-L-nucleoside analogues exhibit potent antiviral activity against both human HIV and HBV replication, accompanied by low host cellular toxicity when compared to their corresponding natural β-D-counterparts (Coates et al., 1992; Furman et al., 1992, 1995). In order for L-nucleosides to be active against HIV-1 RT, their L-ribose moieties should bind to the enzyme active site in an opposite orientation with respect to the D-enantiomer (Lee and Chu, 2001), and the three dimensional structure of the catalytically competent HIV-1 RT shows an open space at the active site enough to accommodate the L-configured sugar moiety (Huang et al., 1998). The computer-generated, energy-minimized model of HIV-1 RT complexed with 3TCTP clearly shows that the 3TCTP and TTP nucleoside ring configurations are enantiomeric, so that alignment of the triphosphates and bases would cause the carbohydrate ring of the β-L-inhibitor to project 1.5–2.0 Å further toward residue 184 than would the ribose ring of TTP. As a result, in WT RT, Met184 and the β-L-oxathiolane moiety of 3TC are in a very stable extended van der Waals contact. Therefore, the most significant difference between the interaction of wild-type HIV-1 RT and the 3TC-resistant mutant HIV-1 RT (M184V RT) with deoxyribose and β-L-oxathiolane nucleoside inhibitors would be a very close contact between the Cγ2 methyl of Val and the protruding oxathiolane ring. This unfavorably close contact (1.8 Å in M184V versus 3.3 Å in wild-type, Fig. 2a) would result in severe steric hindrance. In this context, it is noteworthy that to date no L-configured nucleoside has demonstrated antiviral activity against the M184V RT.

The steric pressure on the active site of RT conferred by M184V mutation not only blocks the space where the sugar moiety of the L-nucleoside resides, but significantly limits the conformation of D-nucleoside. The D-2′-fluoro-4′-thio-2′,3′-unsaturated nucleoside is a good example in this category because the cytidine nucleoside, potent against WT RT, shows high cross-resistance to M184V RT (Chong et al., 2002a,b). As its 4′-oxa derivative, D-2′-fluoro-2′,3′-unsaturated nucleoside, is known to maintain its antiviral activity against M184V RT, the characteristic mechanism of resistance of D-2′-fluoro-4′-thio-2′,3′-unsaturated nucleoside is quite interesting. The energy-minimized structure of the D-2′-fluoro-4′-thio cytidine/RT complex shows that, in spite of the same conformation of D-2′-fluoro-4′-thio-2′,3′-unsaturated cytidine as that of its 4′-oxa derivative, the steric hindrance with Val184 caused by the longer C–S bond length as well as the larger van der

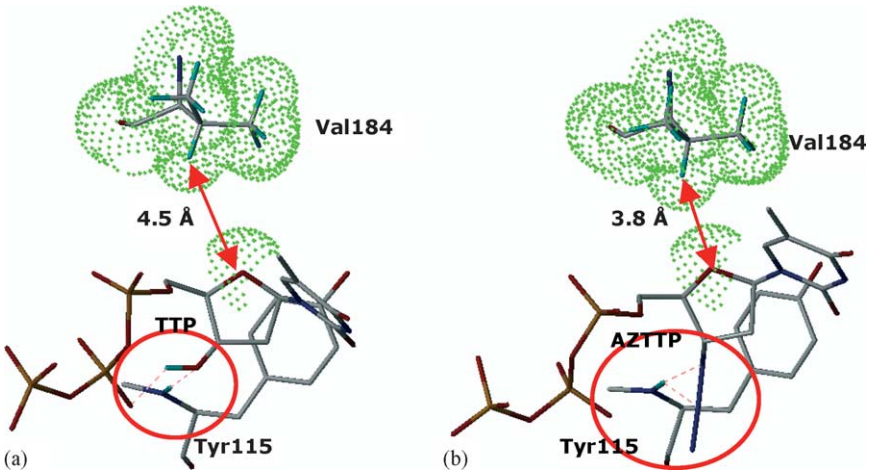

Fig. 5. AZT (b), which has the same conformation as TTP (a), does not experience any cross-resistance to M184V RT. The 3′-azido group of AZT is in the right position to interact with Tyr115 to hold the nucleoside triphosphate away from Val184.

Waals radius of the 4′-sulfur atom result in a deformation of the active site (Chong et al., 2002a,b). Therefore, of great structural interest was how the D-dioxolane nucleosides escape from the steric hindrance with the bulky side chain of Val184 in M184V RT. In every minimized structure of D-dioxolane nucleoside triphosphate/RT complexes, the 3′-oxygen atom of D-dioxolane moiety was always found to be in specific interaction with the nearby enzyme residues regardless of its conformation (Fig. 3), and the conformational analysis showed that the interconversion of the dioxolane conformations can take place without significant loss of energy (Fig. 4). The specific interaction between the dioxolane moiety and the enzyme residues effectively holds the nucleoside triphosphate out of the clashing distance with Val184 (Fig. 3c). In this context, it is interesting that, in the crystal structure of TTP bound to the WT RT, the 3′-OH of TTP is in specific interaction with its own triphosphate moiety as well as the nearby enzyme residue Tyr115 (Fig. 2a) (Huang et al., 1998). As Tyr115 is located at the other side of the active site of RT from Val184, it is possible that the 2′-deoxy sugar moiety of TTP can stay away from Val184 by virtue of the interaction between 3′-OH and Tyr115. Also, AZT, which has the same conformation as TTP, does not experience any cross-resistance to M184V RT (Larder et al., 1995). In our previous study, we have demonstrated that the 3′-azido group of AZT is in the right position to interact with Tyr115 (Fig. 5) (Chong et al., 2002a,b). On the other hand, moderate cross-resistance of ddI and ddC, which do not have any functionality on their sugar rings to interact with the enzyme residues in a specific manner to M184V RT, supports our claim.

In summary, our molecular modeling study unequivocally indicates that the functional 3′-oxygen atom of the dioxolane moiety mimics the 3′-OH group of the natural substrate to hold the nucleoside triphosphate away from Val184.

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