Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 4019–4022

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Studies of Molecular Mechanism of Tenofovir against 3TC- and AZT-Resistance Mutant HIV-1 Reverse Transcriptase

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Received 5 August 2003; accepted 26 August 2003

Abstract—Molecular modeling study shows that conformational flexibility of acyclic nature of TFV provides energetically indistinguishable multiple conformations, which do not experience the cross-resistance conferred by mutant RTs. TFV-DP is located far away from the bulky side chain of Val184 in M184V RT and tenofovir is readily translocated without steric hindrance with Asp185 after incorporation into the growing primer chain complexed with AZT-resistant RT.
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Tenofovir disoproxil fumarate (TDF, PMPA, 9-[(*R*)-2-(phosphonomethoxy)propyl]adenine monohydrate) is the oral prodrug of tenofovir (TFV) and it is rapidly converted to TFV following absorption, which is readily metabolized intracellularly to the active diphosphate form, TFV-DP.[1,2] TFV has demonstrated in vitro activity against both wild type and mutant strains of HIV.[3] Thus, HIV expressing M184V alone showed 0.7-fold change sensitivity to TFV and high-level (24-fold) AZT-resistant HIV remained sensitive to tenofovir (2.4 fold increase). 3TC-resistance by M184V HIV-1 reverse transcriptase (RT) is related to alterations in affinity of RT for 3TC with little or no change in affinity for the corresponding dNTP substrate, dCTP. The steric hindrance between the unnatural L-oxathiolane sugar of 3TC and bulky side chain of Val184 is known to be responsible for the reduced binding affinity of 3TCTP to the active site of RT.[4,5] The flexible acyclic nature of TFVDP, however, is not affected by the unfavorable steric interference by Val184. In the presence of physiological concentrations of ATP, wild-type (wt) RT mediated the removal of AZTMP and d4TMP while RT with D67N/K70R/T215Y mutations mediated 3- to 5-fold increased removal of AZTMP and d4TMP.[6] Interestingly, under similar conditions, TFV removal by both wt and mutant RT was 10–30-fold less than AZTMP and d4TMP removal, and the mutant RT mediated a less than 2-fold increased removal of TFV compared with wt RT.[6] Therefore, it would be informative if the differential effects of the AZT-resistant mutations on the AZTMP and TFV incorporation at the 3′-end of the primer DNA chain with respect to the wild type can be assessed. In order to understand the molecular mechanism of tenofovir against drug-resistant mutants, molecular modeling studies of structurally diverse nucleoside RT inhibitors (AZT, 3TC and tenofovir) complexed with the wt and mutant (M184V and D67N/K70R/T215Y) RT were performed.

All molecular modeling of the enzyme-substrate complexes[5] was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) 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).[7] The crystal structure of AZT[8] was used as the initial Cartesian coordinates. The initial conformation of TFV was constructed by builder module in Spartan 5.1.1 (Wavefunctions, Inc. Irvine, CA), which was cleaned up and geometrically optimized through quantum mechanical ab initio calculations using RHF/3-21G* basis. The inhibitor triphosphates were manually docked to the active site of the enzyme and phosphodiester bonds between the terminal residue of the primer chain and AZT or TFV were constructed. 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. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å

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doi:10.1016/j.bmcl.2003.08.052

from the merged inhibitors and the mutated residues 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.

M184V RT discriminates the modified ribose ring of 3TC at the level of incorporation by steric hindrance, but the acyclic nature of tenofovir may help to explain its favorable resistance profile. The minimal and flexible nature of the acyclic linker allows tenofovir to evade the unfavorable steric hindrance. The crystal structure of RT–DNA–tenofovir binary complex[9] revealed that there are at least two conformations of the terminal tenofovir nucleotide. The interactions between tenofovir and the amino acid residues at the polymerase active site, notably Met184 of the YMDD motif, are minimal in comparison to the protein-DNA contacts that have been observed with the sugar ring of other nucleotides in related crystal structures. Thus, the energy-minimized structure of 3TC-resistant RT (M184V) shows that Val184 is located away from the relatively small acyclic phosphonate moiety (Fig. 1).

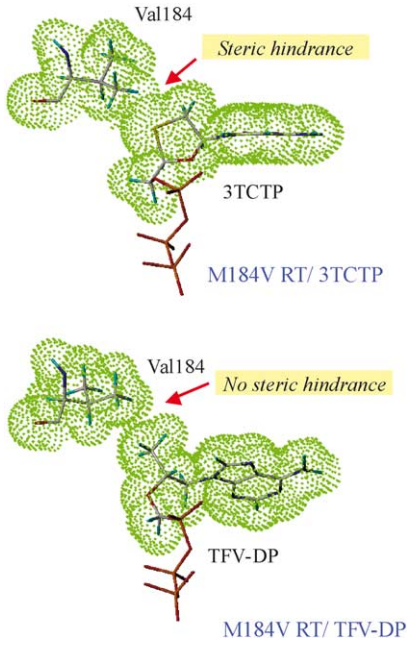

Figure 1. Energy-minimized structure of M184V RT complexed with TFV-DP (below) and 3TCTP (above).

AZT resistance correlates with multiple mutations in HIV-1 reverse transcriptase (RT), including M41L, D67N, K70R, L210W, T215Y/F and Q219K.[10] The extent of AZT resistance is related to the combination of mutations present. In general, two or more of these mutations are needed in RT to show high-level AZT resistance. The efforts to elucidate the biochemical phenotype for HIV-1 resistance to AZT have resulted in the recent report that the AZT resistance phenotype is related to increased rates of phosphorolytic removal of chain-terminating AZT from the 3′-terminus of the primer.[11,12] Even though it is not clear how the AZT resistance mutations enhance excision specifically for AZTMP, this removal may be accomplished by two mechanisms that use different substrates to carry out the phosphorolysis reaction (PPi[11] or ATP[12]). A great degree of torsional freedom of tenofovir may also provide multiple conformations at the RT active site likely inducing an unfavorable environment for excision of tenofovir. Thus, the energy-minimized structures of the wild type and AZT-resistant RT complexed with AZTMP-terminated primer as well as TFV-terminated primer were compared. In viral DNA synthesis, after incorporation of dNTP, primer chain is translocated by one base-pair for the next round of chain elongation (Fig. 2).

As the translocation is immediately followed by recruitment of the next dNTP by RT, in order for the excision reaction to occur, the incorporated dNTP or NRTI-TP should stay at the dNTP binding site before translocation. In this regard, the prevention of the translocation of the AZTMP-terminated primer by AZT-resistant RT may be a plausible explanation for the increased excision rate of AZTMP.[13] The energy-minimized structure

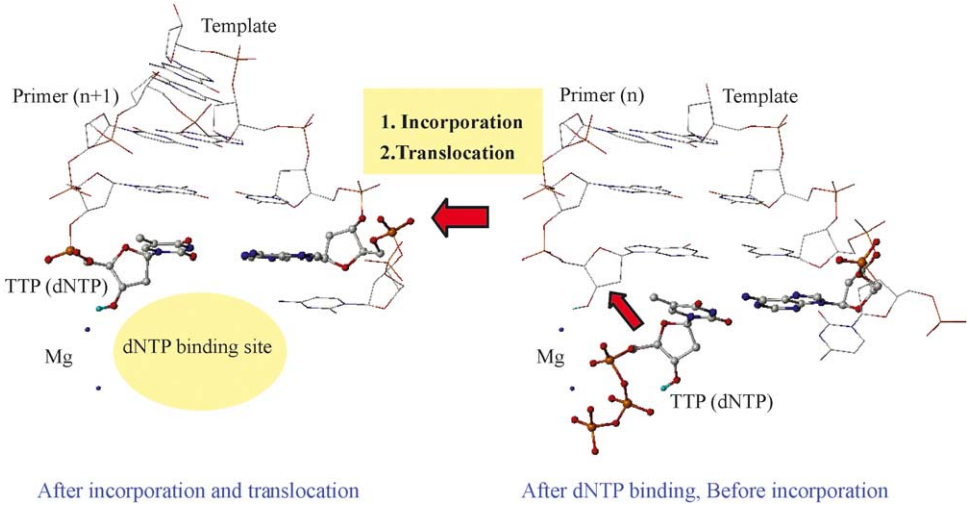

Figure 2. Translocation of primer after incorporation of dNMP.

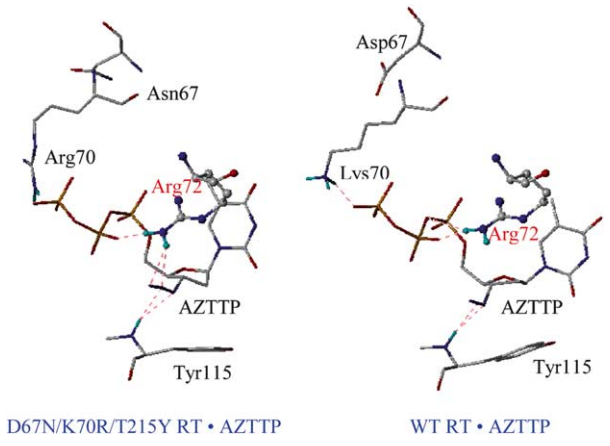

Figure 3. Binding modes of AZTTP complexed with AZT-resistant RT (left) and wt RT (right).

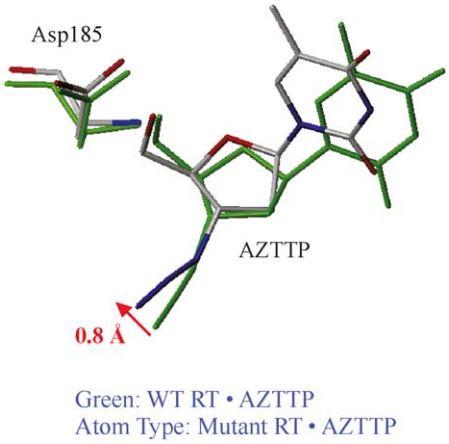

Figure 4. Combination of mutations induce rotation of the 3′-azido group of AZTTP toward Asp185.

of D67N/K70R/T215Y mutant RT complexed with AZTTP shows no significant difference from wt complex in binding mode (Fig. 3). However, small conformational changes resulting from mutation D67N and K70R force a conformational change in Arg72, which induces rotation of 3′-azido group of AZTTP toward Asp185 (Fig. 4).

As Asp185 is located at the trajectory of the primer translocation, the decreased distance between Asp185 and the 3′-azido group of AZTTP results in a huge steric hindrance during the translocation (Fig. 5). Therefore, our energy minimization study shows that the combination of mutations does not affect the binding event of AZTTP but it affects the translocation of the primer chain which has AZTMP at its 3′-end. Thus, resistance by the combination of the mutations seems to be selective to NRTIs which have bulky side chains such as 3′-azido group in AZT. The inability of the primer chain to translocate allows prolonged stay of the AZTMP at the active site and thereby more chance for phosphorolytic removal by the attack of either PPi or ATP.

However, after incorporation into the growing primer chain, tenofovir is readily translocated without any

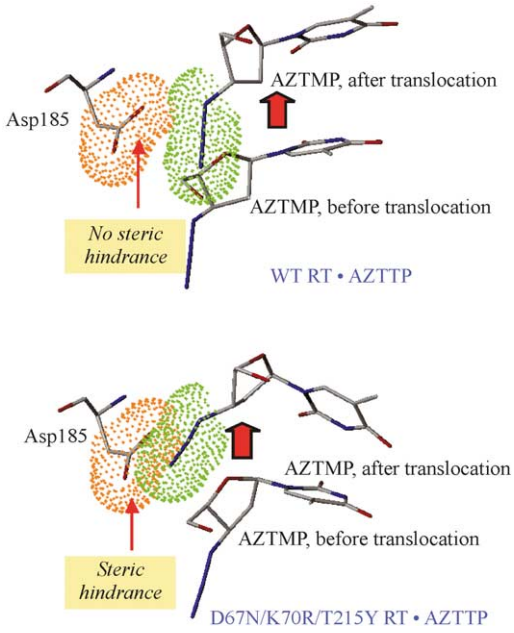

Figure 5. Simulation of the translocation of AZTMP-terminated primer complexed with AZT-resistant RT (below) and wt RT (above).

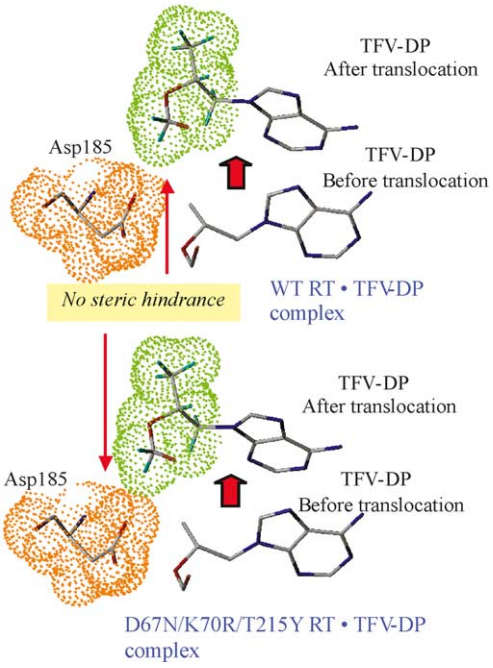

Figure 6. Simulation of the translocation of TFV-terminated primer complexed with AZT-resistant RT (below) and wt RT (above).

unfavorable steric interaction with Asp185 by virtue of its minimal acyclic sugar moiety (Fig. 6).

In summary, it is obvious that the conformational flexibility of acyclic nature of TFV provides energetically indistinguishable multiple conformations, which do not experience the cross-resistance conferred by mutant RTs. M184V RT, which exerts the steric pressure on the nucleoside binding site, cannot discriminate the TFV-DP because the minimal acyclic sugar moiety of TFV-DP is located far away from the bulky side chain of Val184. Increased phosphorolytic removal of AZTMP

from the 3′-end of the primer chain, which has been proposed as the mechanism of AZT-resistance, was visualized in this study. The mutations D67N and K70R push the bulky 3′-azido group of AZT to the vicinity of Asp185. The steric hindrance between those two moieties hampers the translocation of the primer chain containing the AZTMP at the 3′-end, which provides increased chance for AZTMP to be phosphorolytically cleaved.

Acknowledgements

This research was supported by the U.S. Public Health Service Grant (AI32351) from the National Institutes of Health.

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