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Molecular mechanisms of adefovir sensitivity and resistance in HBV polymerase mutants: a molecular dynamics study

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Abstract—Molecular modeling studies of adefovir diphosphate with the wild type and the mutant HBV polymerase–DNA complex demonstrated that the increase in adefovir sensitivity toward HBV polymerase mutants (rtL180M, rtM204V/I, rtL180M-M204V/I) is a result of increased van der Waals interaction and is supplemented by the decreased affinity of natural substrate toward the mutant HBV polymerase. In the case of rtN236T mutant, loss of two hydrogen bonds accompanied by significant decrease in electrostatic interactions is observed, which explains the observed decrease in drug sensitivity and binding affinity of adefovir diphosphate toward the rtN236T mutant HBV polymerase.

1. Introduction

Although effective vaccines have been available for Hepatitis B virus (HBV) for the past 20 years, chronic infection with HBV is still among the top 10 viral infections.[1] Agents currently available for treatment of HBV can be divided into two groups. The immunomodulators, which include interferon-α, thymosin-α1[2] and potential therapeutic vaccines,[3] and nucleoside or nucleotide analogues, among which lamivudine (3TC) and adefovir dipivoxil (Hepsera®) have been approved by US FDA and L-FMAU (clevudine), L-dT (telbivudine) and entecavir are undergoing phase III clinical trials. Nucleoside analogues act by suppressing HBV replication at the level of DNA synthesis. Besides their potent antiviral activity, other advantages include oral administration, excellent tolerance, and the possibility of treatment of several subgroups of chronic hepatitis B patients with high risk of side effects after or with contraindications to IFN-α therapy.

The efficacy of IFN-α against HBeAg-positive HBV has been established in numerous studies.[4] However, the overall response rates are low and treatment with IFN-α is associated with substantial dose-limiting adverse effects[5] (depression and influenza-like symptoms). On the other hand, lamivudine (2′,3′-dideoxy-3′-thiacytidine; [−]-β-L-isomer), a nucleoside analogue, which was developed for the treatment of HIV (approved by US FDA for HIV infection in 1995), but was also shown to be a potent inhibitor of HBV DNA replication[6] (approved by US FDA for chronic HBV infection in 1998), is effective in reducing serum HBV DNA levels by about 4.0 $\log_{10}$ copies/mL.[7] However, long-term lamivudine treatment for chronic hepatitis B virus (HBV) infection induces the emergence of lamivudine resistant HBV mutant strains. The emergence rate of lamivudine-resistant HBV ranges from 17% to 46% at 1 year to as high as 67–75% after 3–4 years of continuous therapy.[8] The predominant lamivudine resistant mutations in HBV-infected patients are rtM204V and rtM204I (C domain), which frequently occur in combination with second mutation, rtL180M (B domain). The clinical frequency of lamivudine-resistant mutants were 18.6% for rtM204I, 1.4% for rtM204V, 11.4% for rtL180M–M204I, and 64.3% for rtL180M–M204V.[9]

The recent addition to NRTI's arsenal against HBV is adefovir dipivoxil. The drug received approval by the US FDA for the use in treatment of chronic HBV infection, in September of 2002. It has demonstrated activity against wild-type and lamivudine resistant strains of HBV.[10] But in contrast to lamivudine therapy, ADV therapy is associated with delayed and infrequent selection of drug resistant viruses.

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Recently, after 96 weeks of therapy with adefovir dipivoxil, a novel mutation from asparagine to threonine at residue rt236 in domain D of the HBV pol. has been reported.[11] In vitro HBV carrying rtN236T mutation displayed reduced susceptibility to ADV, but remained sensitive to lamivudine.[11,12] This prompted us to study the molecular basis of resistance conferred by this novel mutation on the efficacy of ADV-DP. The work presented here demonstrates the molecular basis of mechanism of ADV-DP against lamivudine-resistant mutants and its decrease in susceptibility for rtN236T HBV pol. mutant.

The three-dimensional structure of HBV polymerase is not yet available, however, its homology model has been reported.[13,14] Our model was constructed using HIV-1 reverse transcriptase as a template.[14] The two Mg^{2+} ions, thymidine triphosphate and 7/4 (template/primer) duplex were located in the same position as the HIV-1 RT.[15] The heterocyclic moiety of the fifth nucleotide in the template of the 7/4 (template–primer) duplex was modified to the base complementary to the incoming NRTIs. Various mutants of HBV polymerase were generated using mutate command in biopolymer module of SYBYL 6.7 and were subsequently minimized. For energy minimization studies, SYBYL version 6.7 (Tripos Associates, St. Louis, MO) was used. All the minimization calculations were performed on Silicon Graphics Octane2 workstation. The initial conformation of ADV was constructed by builder module in SPARTAN 5.1.1 (Wavefunctions, Inc. Irvine, CA), which was 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. Gästeiger–Hückel charges were 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 the enzyme site from the biopolymer module. In order to eliminate the local strains resulting from merging nucleotides and/or point mutations, residues inside 6 Å from the mutated residues and merged nucleotides were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme–inhibitor complexes were further minimized by using Kollman-all-atom force field until the iteration number reached 5000 or the energy difference from one iteration to the next is less than 0.0001 kcal/mol.[14]

The minimization results were further confirmed by performing molecular dynamics simulations on modeled ternary complex using molecular graphics and simulation program MACROMODEL, version 8.5 (Schrödinger, Inc). Molecular dynamics simulations on HBV pol. (wild type, rt-Leu180Met, rt-Met204Val, rt-Met204Ile, rt-Leu180Met & rt-Met204Val, rt-Leu180Met & rt-Met204Ile, and rt-Asn236Thr)–DNA–nucleotide (dATP, ADV-DP) were performed with MMFFs force field in the presence of GB/SA continuum water model on a Silicon Graphics Tezro workstation running IRIX 6.5 operating system. Dynamics simulations were carried out by heating the system from 0 to 300 K over 5 ps and equilibrating at 300 K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms. For dynamics calculations, the residues further away than 15 Å from the active site were not considered and the residues from 6 to 15 Å were constrained by harmonic constraints. Only residues inside the 6 Å sphere from the nucleotide were allowed to move freely.

2. Results and discussion

In wild type HBV pol., 3TC-TP makes favorable van der Waals contact with Met204. However, rtM204V HBV pol. mutation causes the movement of 3TC-TP toward Val204 in order to maintain the favorable van der Waals contact. Movement of 3TC-TP toward the Val204 results in the disruption of the active site geometry.[16] Thus, the rtM204V HBV pol. mutation discriminates the oxathiolane ring of lamivudine at the level of incorporation by disruption of the active site (coordination geometry of Mg^{2+}), which is well correlated with the observed decrease in efficacy of the 3TC in M204V/I mutant.[16]

In rtL180M HBV pol. mutant, the substitution of leucine with methionine (not shown in Fig. 1 for the sake of clarity) changes the binding mode of ADV-DP slightly by pushing the ADV-DP toward the sugar moiety of the final residue of primer strand (Fig. 1a). This is because methionine side chain is much longer than isoleucine and it extends around the ADV-DP. In order to maintain the proper van der Waals interaction and to avoid any steric clash with the mutated methonine side chain, ADV-DP moves toward the 204 region (Fig. 1a). In the case of natural substrate (dATP), the 4′-oxygen atom of the sugar moiety does not make van der Waals contact with the 2′-hydrogens of sugar moiety of the final residue of the primer strand (Fig. 1b). But, in the case of ADV-DP, the 4′-oxygen atom makes significant van der Waals contact with the 2′-hydrogens of sugar moiety of the final residue of primer strand (Fig. 1a). This results in enhanced binding affinity of ADV-DP toward the rtL180M mutant.

In the wild type HBV pol., Met204 does not make significant van der Waals contact with the acyclic moiety of ADV-DP (Fig. 2b). Mutation rtM204V fills the gap between Val204 and the acyclic moiety of ADV-DP and the sugar moiety of the final residue of the primer strand, thereby causing extensive van der Waals interactions (Fig. 2a). A similar pattern is observed with the rtM204I HBV pol. mutant. Mutation M204I fills this gap better, resulting in increased favorable van der Waals interactions (Fig. 2c).

Binding mode of ADV-DP in double mutants (rtL180M–rtM204V and rtL180M–rtM204I) is same as that observed in single mutations, rtL180M or rtM204V. Since both these mutations are located in

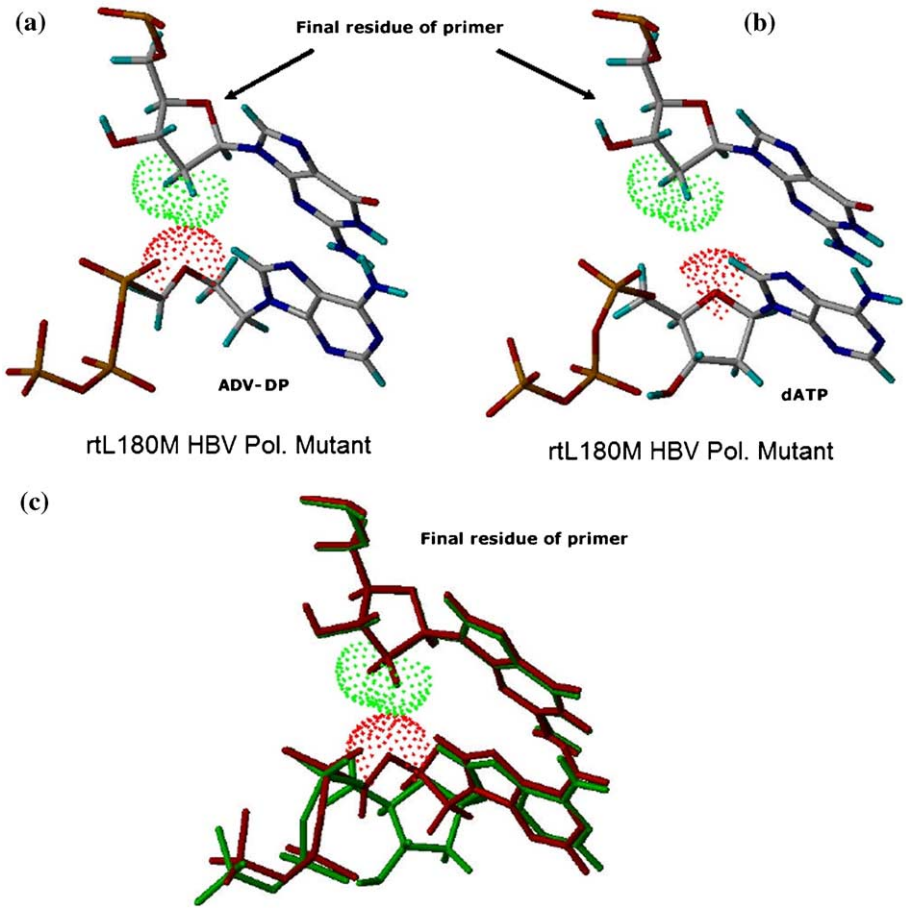

Figure 1. (a) and (b) Binding mode of natural substrate (dATP) and ADV-DP in rtL180M mutant HBV pol. is shown here along with the van der Waals radii of 4′-oxygen and 2′-hydrogens. (c) dATP and ADV-DP are superimposed onto each other to see the difference in their binding mode.

different domains of HBV pol. it was not possible to show these effects in single graphic. Both these mutations result in increase in van der Waals interaction between ADV-DP, the final residue of the primer strand and the surrounding residues (as observed in individual single point mutations).

In literature,[13,17] a range of fold increase (FI) values have been reported for these double mutants. In these studies, ADV-DP was found to be more active against double mutants when compared to single point mutants.[13,17] Our calculations also predict the efficacy of ADV-DP in these double mutants to be better than that in single point mutants. The decreasing order of binding affinity of ADV-DP in these mutants from our studies is: rtL180M–rtM204I > rtL180M–rtM204V > rtM204I > rtM204V ≈ rtL180M > WT > rtN236T (Table 1).

As can be seen in Figure 3a and b, rtN236T mutation does not change the binding mode and binding geometry of natural substrate significantly. It also does not change the net binding affinity of natural substrate (dATP) (Table 1), even though it loses one hydrogen bond with Ser85 (Fig. 3a and b), but it gains another one between the hydroxy group of Thr236 and the amide backbone (Fig. 3a). Hence, no significant change in the binding affinity of dATP for WT and rtN236T mutant HBV pol. is observed (Table 1). However, for ADV-DP, in wild type HBV pol., one hydrogen bond is between the terminal hydrogen of the Asn236 and the oxygen atom of the γ-phosphate of ADV-DP (Fig. 3d). The second hydrogen bond is between the carbonyl of the Asn236 and the hydroxy group of the Ser85 (Fig. 3d). The loss of these two hydrogen bonds (Fig. 3c) accompanied by significant loss of favorable electrostatic interactions (Table 1), results in decreased binding affinity of ADV-DP toward the rtN236T mutant. On comparing the binding mode of dATP and ADV-DP from Figure 3, the role of Asn236 in stabilizing the γ-phosphate of ADV-DP becomes evident. Asn236 plays an important role in stabilizing the γ-phosphate of ADV-DP, which is further confirmed by comparing the relative binding energy of dATP toward WT HBV pol. and rtN236T HBV pol. mutant (Table 1).

In summary, from our molecular dynamics studies, we have demonstrated that rtN236T HBV pol. mutation is the true ADV-DP mutation, in a sense that, this mutation does not affect the binding affinity of natural substrate (dATP) significantly, but it decreases the binding affinity of ADV-DP toward rtN236T HBV pol. drastically. Lamivudine resistant mutations (rtM204V and rtM204I) results in increased van der Waals contacts

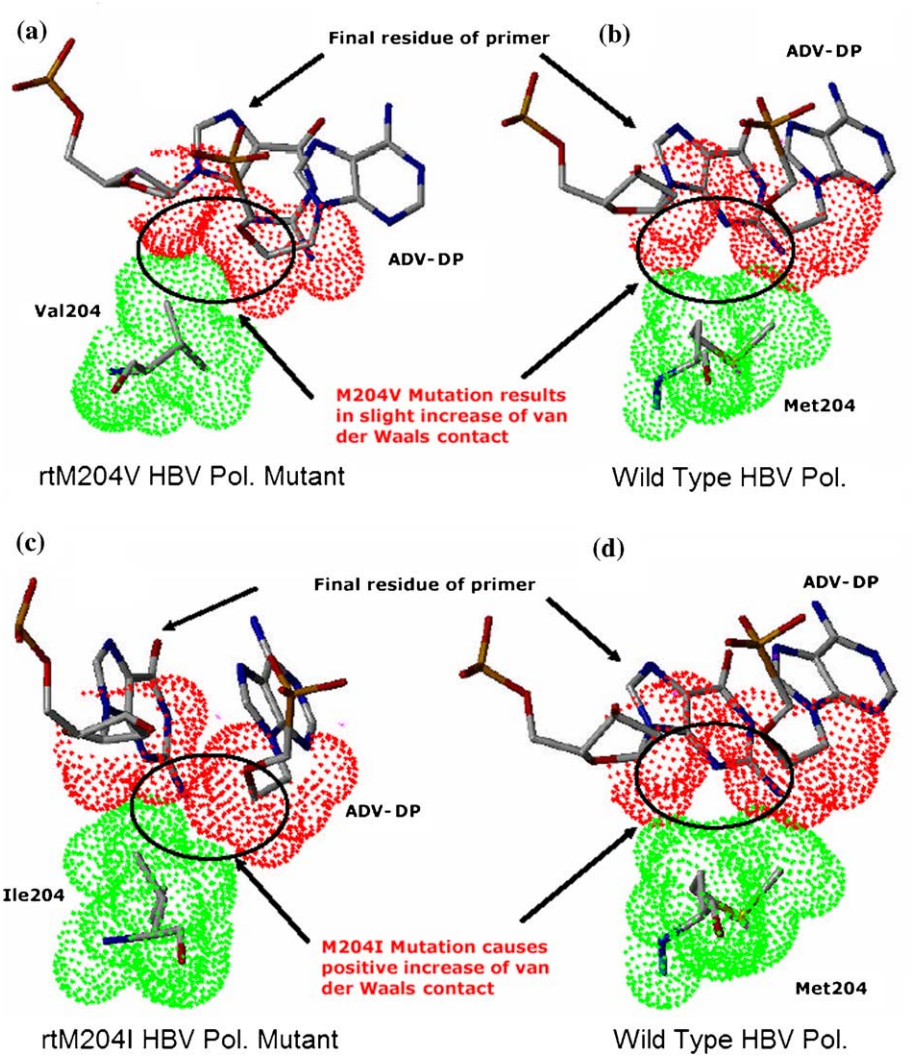

Figure 2. (a) and (b) Binding mode of ADV-DP in rtM204V mutant and wild type HBV pol. (c) and (d) Binding mode of ADV-DP in rtM204I and wild type HBV pol. is shown.

Table 1. Correlation between E_{rel} and IC_{50} (molecular dynamics studies)

HBV polymerase	dATP	ADV-DP		
	Enzyme–DNA–dATP complex[a]	Enzyme–DNA–ADV–DP complex [a]	ΔE_{rel} [a]	Fold increase[b] (FI)
WT	−8521	−8662	−141	1.0
rtL180M	−8467	−8645	−178 (−124)	0.7
rtM204V	−8458	−8638	−180 (−117)	0.7
rtM204I	−8449	−8657	−208 (−136)	0.5
rtL180M–rtM204V	−8396	−8623	−227 (−102)	0.2–0.8
rtL180M–rtM204I	−8389	−8633	−244 (−112)	0.2–1.8
rtN236T	−8516	−8548	−32 (−27)	4.4[c]

[a] ΔE_{rel} = (enzyme–DNA–ADV-DP complex) − (mutant–HBV–DNA–dATP complex), in kcal/mol, values in parenthesis are (enzyme–DNA–ADV-DP complex) − (WT–HBV–DNA–dATP complex).
[b] Refs. 13,17a.
[c] Ref. 17b.

between ADV-DP and mutated residues, which accounts for the better binding affinity of ADV-DP toward these mutants. The second lamivudine associated mutation (rtL180M) also results in increase in van der Waals contacts between ADV-DP and the final residue of the primer strand, which accounts for the better

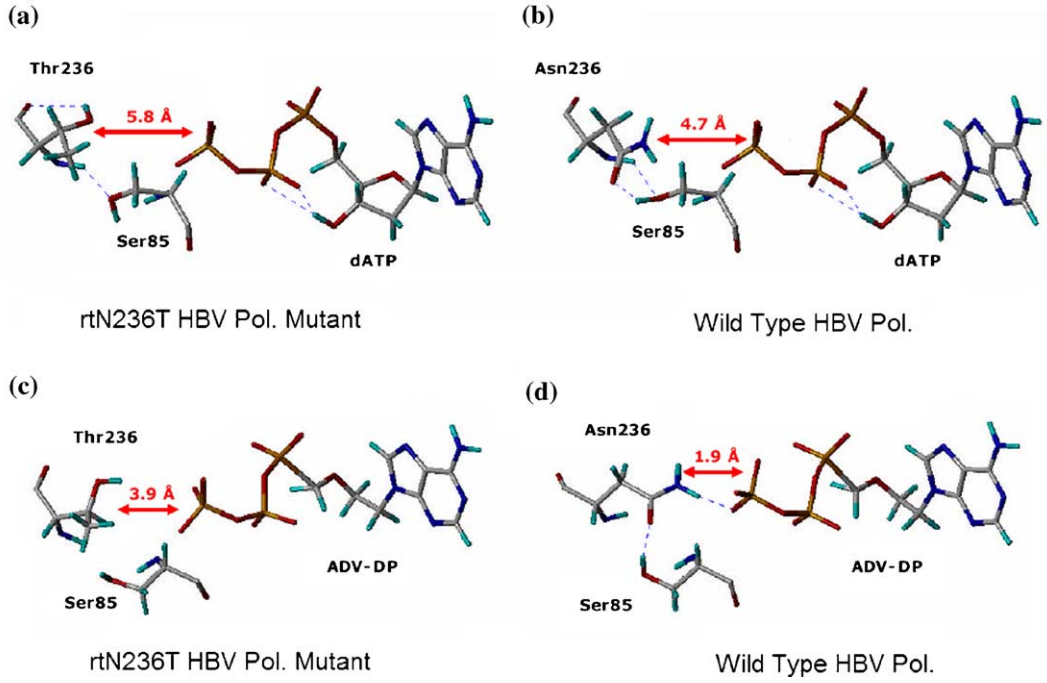

Figure 3. (a) and (b) Binding mode of dATP in rtN236T mutant and wild type is shown here. Notice the distance between the γ-phosphate and residue at rt236 position. (c) and (d) Binding mode of ADV-DP in rtN236T mutant and wild type is shown here.

binding affinity of ADV-DP in these mutants. The observed increase in efficacy of ADV-DP is not only because of the enhanced binding affinity of ADV-DP toward these mutants (rtM204V, rtM204I, rtL180M, rtL180M–M204V/I180M), but also is supplemented by the decreased affinity of the natural substrate (dATP) towards these lamivudine-resistant mutants.

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