Enzymatic Characterization of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Resistant to Multiple 2',3'-Dideoxynucleoside 5'-Triphosphates*

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A set of five mutations (A62V, V75I, F77L, F116Y, and Q151M) in the polymerase domain of reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1), which confers on the virus a reduced sensitivity to multiple therapeutic dideoxynucleosides (ddNs), has been identified. In this study, we defined the biochemical properties of RT with such mutations by using site-directed mutagenesis, overproduction of recombinant RTs, and steady-state kinetic analyses. A single mutation, Q151M, which developed first among the five mutations in patients receiving therapy, most profoundly reduced the sensitivity of RT to multiple ddN 5'-triphosphates (ddNTPs). Addition of other mutations to Q151M further reduced the sensitivity of RT to ddNTPs. RT with the five mutations proved to be resistant by 65-fold to 3'-azido-2',3'-dideoxythymidine 5'-triphosphate (AZTTP), 12-fold to ddCTP, 8.8-fold to ddATP, and 3.3-fold to 2',3'-dideoxyinosine 5'-triphosphate (ddGTP), compared with wild-type RT (RTwt). Steady-state kinetic studies revealed comparable catalytic efficiency (Kcat/Km) of RTs carrying combined mutations as compared with that of RTwt (<3-fold), although a marked difference was noted in inhibition constants (Ki) (e.g. Ki of a mutant RT carrying the five mutations was 62-fold higher for AZTTP than that of RTwt). Thus, we conclude that the alteration of RT's substrate recognition, caused by these mutations, accounts for the observed multi-ddN resistance of HIV-1. The features of multi-ddNTP-resistant RTs should provide insights into the molecular mechanism of RT discriminating ddNTPs from natural substrates.

The accumulating data suggest that the development of HIV-1 variants with reduced susceptibility to reverse transcriptase (RT) inhibitors is related to clinical deterioration in patients receiving RT inhibitors (1–6). It has been shown that the high error rate of HIV-1 RT, approximately 1–10 misincorporations/HIV-1 genome/round of replication (7–9), suggests that misincorporation by HIV-1 RT is responsible for the hypermutability of HIV-1, enabling HIV-1 to rapidly acquire drug resistance. This notable diversity of HIV-1 genome resulting from error-prone RT is likely to be associated with "natural" drug resistance seen in HIV-1 from patients receiving no prior antiretroviral therapy (10). In this regard, the combined use of multiple antiretroviral agents has been postulated to block or retard the emergence of HIV-1 less susceptible to therapeutic agents. However, several recent reports have demonstrated that HIV-1 can acquire resistance to multiple drugs in vitro and in vivo (11–16). These data may suggest that even combination therapy may ultimately fail to suppress the replication of this hypermutable virus, although it is possible that combination chemotherapy continues to be efficacious if HIV-1 variants have a substantial replication disadvantage due to the altered enzymatic conformation/function as compared with wild type HIV-1; thus, the progression of the disease may be significantly delayed (17).

We and others have recently demonstrated that HIV-1 can develop a novel set of five mutations (A62V, V75I, F77L, F116Y, and Q151M) during combination chemotherapy with 3'-azido-2',3'-dideoxythymidine (AZT; zidovudine) plus 2',3'-dideoxyinosine (ddI; didanosine) or 2',3'-dideoxyxytidine (ddC; zalcitabine) and that these mutations confer resistance to various antiretroviral 2',3'-dideoxynucleoside analogs (ddNs) on HIV-1 (11, 13, 18). In this study, we attempted to define the biochemical properties of RT carrying all or a subset of the five mutations. We constructed a cartridge mutagenesis system, which enabled us to introduce the desired mutations into relatively short fragments to generate both infectious HIV-1 clones (18) and recombinant RTs. We then examined the susceptibility of mutant RTs to multiple ddNTPs and determined steady-state kinetic constants. We also discuss the relationships of the observed HIV-1 resistance to multiple ddNs and altered RT functions caused by all or a subset of the five mutations.

EXPERIMENTAL PROCEDURES

Materials—dNTP, ddNTP, poly(rA), poly(rC), poly(rI), (dT)12–18, (dc)12–18, (dg)12–18, and CNBr-activated Sepharose 4B were purchased from Pharmacia Biotech Inc. AZTTP, [3H]NTPs, and [3H]ddATP were purchased from Moravek Biochemicals (Brea, CA). Phage M52 genomic RNA (single-stranded) was purchased from Boehringer Mannheim. Purified anti-RT monoclonal antibody, M33 (19), was purchased from Advanced BioScience Laboratories, Inc. Restriction enzymes and other enzymes used for plasmid constructions were purchased from New England Biolabs, Inc. (Beverly, MA), U.S. Biochemical Corp., or Life Technologies, Inc. Plasmid pKRT2 (20) and p66/p51 heterodimer RT (21) were obtained from R. D’Aquila and C. Debouck, respectively, through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health. Nevirapine (22) was kindly provided by Boehringer Ingelheim Pharmaceuticals.
Plasmid Construction—Various RT expression vectors were generated using a BH10-derived RT overexpression vector, pRT2 (20). First, an excised EcoRI-HindII fragment containing the RT-coding region was subcloned to pTZ19R (Pharmacia), generating pTZRT07, which produced a single-stranded DNA using helper phage M13KO7 (Life Technologies, Inc.). Following single-stranded DNA purification, site-directed mutagenesis was performed as directed in the manufacturer's protocol (T7-Gen In Vitro Mutagenesis, U. S. Biochemical Corp.). Two restriction sites, Xmal and NheI, were introduced to pTZRT07 with deduced amino acid substitution (Fig. 1, column 1) and, after purification, combined, heated to 75°C for 5 min, and cooled slowly to room temperature over 1 h. Annealed template-primers were stored at -20°C until use.

Drug Susceptibility Assays—Buffer compositions of reaction mixtures used for determination of susceptibilities of RT were as follows: buffer A (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 50 μg/ml lysozyme), and then disrupted by osmotic shock through rapid chilling and spotted on a DE81 filter, and the radioactivity in the product was counted as described above. The Km, Vmax, values were determined from initial linear steady-state velocities with Lineweaver-Burk plot analyses, and the values were calculated by dividing Vmax by active enzyme concentrations.

RESULTS

Overproduction and Purification of RT—Wild-type RT (designated RTwt) and all mutant RTs were overproduced at about 1 mg of protein/200 ml culture and were purified using a Sepharose 4B column (1 ml each of the remaining dNTPs). The KCl concentration used (150 mM) in buffer C was chosen as an optimal concentration as assessed by the incorporation activity using the wild-type RT (RTwt). The concentration of RT in each reaction was 0.5–1.5 nM (see “Results” for determining concentrations of active RT molecules). Concentrations of homopolymeric template-primers and MS2/22A were 0.5 and 0.1 μM (expressed as 3′-hydroxyl primer termini), respectively. Reactions were initiated by raising the reaction temperature from 0 to 37°C, and the reaction mixtures were incubated for 30 min at 37°C and quenched by 25 μl of 0.5 M EDTA. Products were analyzed by a DE81 filter binding assay as described above.

steady-state Kinetic Analysis—The buffer used for steady-state kinetic analysis for the homopolymeric template-primers was the same as buffer A or B with the exception of [3H]nTP concentrations and the volume of reaction mixture (100 μl). The buffer composition for conducting a single nucleotide incorporation assay using 0.5 μM MS2/22A (expressed as 3′-hydroxyl primer termini) was the same as that of buffer C except that only [3H]dATP was present as a nucleotide substrate. Concentrations of active RT molecules). Concentrations of homopolymeric template-primers and MS2/22A were 0.5 and 0.1 μM (expressed as 3′-hydroxyl primer termini), respectively. Reactions were initiated by raising the reaction temperature from 0 to 37°C, and the reaction mixtures were incubated for 30 min at 37°C and quenched by 25 μl of 0.5 M EDTA. Products were analyzed by a DE81 filter binding assay as described above.

Enzyme Preparation—Escherichia coli J M109 (Promega, Madison, WI), transformed with a wild-type or mutant RT expression vector, were cultured with 50 μM IPTG at 37°C for 18 h, followed by washings (3 ml with 1 mM EDTA, and 15 ml with a 0.1 M Tris, pH 8.0, 0.01% Triton X-100, 20 mM KCl, 0.05% NaCl) to remove periodically four or five times during the course of the assay and spotted on a DE81 filter, and the radioactivity in the product formed was counted as described above. The Km, Vmax, values were determined from initial linear steady-state velocities with Lineweaver-Burk plot analyses, and the values were calculated by dividing Vmax by active enzyme concentrations.
(p51/p66), have comparable kinetic parameters of enzyme-template/primer interactions, concluding that the homodimeric form of RT can serve as a model for the interactions of heterodimeric form of RT with template-primer.

Sensitivity of RT with Various Mutations to Dideoxynucleotides—We first asked which of the five mutations (A62V, V75I, F77L, F116Y, and Q151M), seen in HIV-1 variants isolated from patients receiving combination chemotherapy (11, 13, 18, 25), had the most profound effect on the sensitivity of RT to ddNTPs tested (Table I). The A62V mutant RT (designated RTA62V) was slightly more sensitive to all ddNTPs examined, relative to RTwt. The V75I mutant RT (RTV75I) was slightly less sensitive to ddNTPs except to ddATP, and both F77L and F116Y mutant RTs (RTF77L and RTF116Y, respectively) had IC50 values to ddNTPs tested comparable with that of RTwt. In contrast, the Q151M mutant RT (RTQ151M) proved to be substantially less sensitive to all ddNTPs tested (Table I).

In order to examine the effects of various combinations of mutations on the sensitivity of RT against ddNTPs, we introduced four mutations (A62V, V75I, F77L, F116Y) in additon to the Q151M mutation. In contrast to inconsiderable changes observed with single substitution of each of these four amino acids, combined mutations brought about marked increases in IC50 values (Table I, Fig. 3). RT with four mutations (RTA62V/75I/77L/116Y) and that with all five mutations (RTA62V/75I/77L/116Y/151) had the most profound effect on the sensitivity of RT to ddNTPs tested (Table I).

We also examined the sensitivity of selected RTs to a non-nucleoside RT inhibitor, nevirapine (22), using [3H]dGTP and poly(rC)-(dG)12-18 as a substrate and a template-primer, respectively. The IC50 values of nevirapine with RTwt and RTQ151M were 0.50 and 0.59 μM, respectively. These results are consistent with our previous observations that the infectious mutant HIV-1 carrying all five mutations was as sensitive to nevirapine as wild-type HIV-1 (18).

Determination of Concentrations of Active RT Molecules—Because of the nature of immunoaffinity chromatography, it was possible that improperly folded and/or degraded proteins were co-purified with active enzyme molecules. We therefore determined the concentration of active RT using the method previously described by Reardon and Miller (26). Since the dissociation of enzyme-template-primer complex (Koff) is slow relative to the rate of polymerization, the amplitude of the burst formation at a pre-steady state under the condition of single nucleotide incorporation is stoichiometric with regard to the number of active enzyme molecules (26–28). In this assay, the incorporation of a single [3H]dAMP to heteropolymeric RNA/DNA template-primer (MS2/22A) mediated by any of five RT preparations tested (RTwt, RTA62V, RTV75I, RTF77L, RTF116Y, RTQ151M) was biphasic with respect to time (as shown in Fig. 4). The burst formation was observed with slow steady-state rates of 0.024, 0.016, 0.012, 0.015, and 0.022.

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**Table I**

| RT         | Amino acid | IC50 μM  |
|------------|------------|----------|
| RTwt       | Ala        | 4.3 ± 0.4, 0.43 ± 0.07, 0.033 ± 0.004, 0.023 ± 0.001 |
| RTV75I     | Val        | 3.9 ± 0.4, 0.21 ± 0.01, 0.029 ± 0.004, 0.014 ± 0.001 |
| RTF77L     | Ile        | 4.6 ± 1.3, 1.4 ± 0.2, 0.071 ± 0.005, 0.072 ± 0.019 |
| RTF116Y    | Leu        | 4.5 ± 1.5, 0.42 ± 0.07, 0.028 ± 0.009, 0.044 ± 0.007 |
| RT151M     | Met        | 5.5 ± 1.1, 0.42 ± 0.06, 0.027 ± 0.001, 0.022 ± 0.002 |
| RT75/77/116/151 | Leu-Met | 11 ± 3, 2.3 ± 0.6, 0.075 ± 0.007, 0.19 ± 0.02 |
| RT77/116/151 | Leu-Met   | 20 ± 4, 2.6 ± 0.5, 0.045 ± 0.006, 0.18 ± 0.03 |
| RT62/75/77/116/151 | Leu-Met | 37 ± 3, 3.9 ± 0.2, 0.054 ± 0.001, 0.08 ± 0.13 |
| RT62/75/77/116/151 | Leu-Met | 39 ± 5, 5.0 ± 0.3, 0.11 ± 0.01, 1.5 ± 0.2 |
S: for RTwt, RT151, RT75/77/116/151, RT62/75/77/116/151, and RT151/215, respectively. These rates were in agreement with $k_{\text{cat}}$ values determined by Lineweaver-Burk analyses (Tables II and V), and also agreed with previously reported values ranging 0.0065–0.06 s$^{-1}$ for RTwt (26–29). From the replot of the burst amplitudes (extrapolated y intercept) versus RT amounts added in the reactions, the concentration of active RT was determined (as shown in Fig. 4). These data indicated that approximately 40% of the total protein in the RT preparations represented active RT molecules.

Substrate Analysis of RTs with Various Mutations—We also determined steady-state kinetic constants of RTwt and mutant RT preparations in processive (using poly(rA)$_{12-18}$ or poly(rC)$_{12-18}$ as a template-primer) and distributive (using poly(rI)$_{12-18}$ or MS2/22A as a template-primer) modes. It was found that steady-state constants of RTwt to natural substrates were in the range previously reported by other groups (12, 26–30) and that there were only up to 3-fold differences in $K_m$, $k_{\text{cat}}$, and $k_{\text{cat}}/K_m$ values of RTwt and mutant RT preparations (Table II), suggesting that, like other ddN-associated RT mutations such as L74V, L74V/T215Y, T215Y (29), or K65R (30) the mutations examined in this study caused no significant detectable changes in the catalytic efficiency of RT. It should be noted, however, that a small difference in the enzymatic activity of RT may produce a considerable difference in the replication rate of HIV-1 in vivo (17).

Inhibitor Analysis of RT with Various Mutations—We also conducted inhibitor analyses of RTwt, RT151, RT75/77/116/151, and RT62/75/77/116/151, with respect to selected ddNTPs (ddATP, ddCTP, ddGTP, ddTTP, and AZTTP). As shown in Fig. 5, linear competitive inhibitions were observed for RTwt and all mutant RTs with all ddNTPs examined. It was noted that the $K_i$ values of three mutant RTs (RT151, RT75/77/116/151, and RT62/75/77/116/151) to each ddNTP were all significantly greater than those of RTwt (Table III), a finding in agreement with the elevated $IC_{50}$ values with combined mutations (Table I). The $K_i$ value to AZTTP inhibition with RTwt reported here (10 nM) is comparable with the previously reported values ranging from 2–35 nM determined under similar processive conditions (26, 29–31). It should be noted that, with combined mutations, the most sig-

### Table II

| Substrate | RTwt | RT151 | RT75/77/116/151 | RT62/75/77/116/151 | $K_m$ | $K_i$ |
|-----------|------|-------|----------------|-------------------|------|------|
| dATP      | 0.080 | 0.029  | 0.024          | 0.019             | 0.009 | 0.089 |
| dCTP      | 0.043 | 0.091  | 0.032          | 0.025             | 0.006 | 0.068 |
| dGTP      | 0.081 | 0.214  | 0.068          | 0.045             | 0.011 | 0.098 |
| dTTP      | 0.082 | 0.224  | 0.073          | 0.050             | 0.012 | 0.096 |
| AZTTP     | 0.080 | 0.088  | 0.071          | 0.065             | 0.013 | 0.085 |

**Fig. 3.** Sensitivities of various RT preparations against selected dideoxynucleotides. Each plot represents the mean percentage of activity of quadruplicate determinations of RTwt (●), RT151 (●), RT77/116/151 (○), RT75/77/116/151 (△), and RT62/75/77/116/151 (□) in the presence of ddATP (A), ddCTP (B), ddGTP (C), or AZTTP (D). Substrate concentrations used are described under “Experimental Procedures.” The IC$_{50}$ values determined are summarized in Table I.
significant difference was observed in AZTTP inhibition profiles; the Ki value increased 3.5-fold with RT151, 50-fold with RT75/77/116/151, and 62-fold with RT62/75/77/116/151 (Table III). The Ki values for the three mutant RTs were also found to be substantially high to all ddNTPs examined (Table III).

We also determined steady-state kinetic constants using [3H]ddATP as a nucleotide substrate. The Km(ddATP) value of RTwt and mutant RTs (Table IV) was virtually identical to its Ki value with ddATP (Table III), consistent with a previous report demonstrating that the Ki value of ddNTP against dNMP incorporation into a heteropolymeric template-primer was almost equal to Km(ddNTP) (27), indicating that ddNTP and dNTP behave as classical competitive substrates to each other under single nucleotide incorporation assay conditions (27).

RT Carrying Q151M and T215Y Mutations—Most of the HIV-1 variants carrying all or several of the five mutations, isolated from patients receiving combination chemotherapy with AZT plus ddC or AZT plus ddI, had the previously reported AZT, ddC, or ddI-associated pol gene mutations except for K219Q and K219E, and none had the most potent AZT-associated mutation, T215Y (11, 13, 18). In this regard, it is possible that one or more of the five mutations are incompatible with AZT, ddC, or ddI-associated mutations. In order to test this possibility, we produced a recombinant RT (RT151/215) carrying the Q151M mutation and T215Y (32). We found, however, that RT151/215 had an enzymatic activity comparable with that of RTwt and was less susceptible to all four ddNTPs tested, ddATP, ddCTP, ddGTP, and AZTTP (Table V).

DISCUSSION

The mutations responsible for resistance of HIV-1 against nucleoside RT inhibitors have been mapped to the RT-encoding region of the pol gene, and accumulated data suggest that altered substrate recognition by RT is associated with drug resistance (29, 30, 33). However, the changes in the sensitivity of RT to the triphosphate of a ddN appear to account partly for in vitro resistance of HIV-1 to the ddN (29, 34). Indeed, several enzymatic studies have demonstrated that the sensitivities of RT to ddNTPs differ from the in vitro viral sensitivities to ddN (31, 34, 35). For instance, a recombinant infectious HIV-1 carrying four AZT-associated amino acid substitutions (D67N, K70R, T215Y, and K219Q) is about 100-fold less sensitive to AZT than the wild-type HIV-1 strain in vitro (34); however, an RT with the same four amino acid substitutions is as sensitive to the inhibition of AZTTP as is the wild-type RT (RTwt) (31).
to ddG in a cell culture system. These observations do not appear to be easily explained under one unifying theory at present. In the present study, we demonstrate that a unique set of five mutations (A62V, V75I, F77L, F116Y, and Q151M) alters RT’s substrate recognition and confers resistance to various ddNTPs on RT, which may fully account for the observed in vitro resistance of HIV-1 to multiple ddNs (18).

It is evident that Gin$^{151}$ plays a critical role in the enzymatic function of RT and is linked to the development of multi-ddN resistance of HIV-1 (18). The amino acid Gin$^{151}$ consists of the highly conserved sequence, Leu-Pro-Gln-Gly, in the corresponding region within motif B of RT from various animal and human retroviruses (36); however, the substituted amino acid, methionine, is found in the corresponding region of hepatitis B viruses (HBVs), suggesting that Q151M is not overly detrimental to the function of RT. We found in this study that the Q151M substitution decreases the polymerase activity by 30% (as assessed with $K_{	ext{cat}}/K_m$, Table II) with several dNTPs and significantly reduces the sensitivity of RT to ddNTPs. In this regard, various groups have examined the effect of amino acid substitution of Gin$^{151}$ (37–39). A Q151N substitution has shown no significant alteration in the polymerase or RNase H activities of RT (38). A Q151H substitution, however, has been shown to reduce the polymerase activity by 30% and the sensitivity of RT to AZTTP by 4–50-fold (39), and a Q151E substitution has been reported to reduce the polymerase activity by 30% (37) as compared with RT$^{wt}$.

The amino acid Phe$^{116}$, which is located close to the proposed dNTP-binding site of HIV-1 RT (40), is also part of a highly conserved region (motif A) in RTs from various retroviruses, but the corresponding amino acid in RT in hepatitis B viruses has been found to be tyrosine (36), which may explain why the RT with such a substitution at a highly conserved amino acid remains functional. In fact, Boyer et al. (38) have recently reported that F116Y alone does not affect the polymerase and RNase H activity of RT. In our study, RT$^{116}$ carrying F116Y was also functional and behaved similarly to RT$^{wt}$ against ddNTPs (Table I). It is not clear how F116Y affects the function of RT when other mutations are added. The addition of the V75I mutation to RT$^{75/77/116}$, generating RT$^{75/77/116/215}$, further reduced the sensitivity to ddNTPs, although V75I alone had little effect on the sensitivity (Table I). Codon 75 mutation with a different amino acid, V75T, has been identified in HIV-1 variants less susceptible to 2',3'-didehydro-2',3'-dideoxythymidine, ddC, and ddi in vitro (41). These data suggest that the amino acid at codon 75 of HIV-1 RT plays a crucial role in substrate recognition.

Detailed steady-state kinetic analyses revealed that the altered nucleotide substrate recognition of RT is evidently caused by all or a subset of the five mutations, suggesting that these amino acids interact with an incoming nucleotide substrate, although presently it is not possible to distinguish direct effects caused by amino acid substitutions from those mediated via template-primer interactions (40, 42). It has been reported that Gin$^{151}$ is located close to the first single-stranded base of the template and that three amino acids (Val$^{75}$, Phe$^{77}$, and Gin$^{151}$) form part of the “template grip” (43). This may lead to repositioning or conformational change of the template-primer, causing a distortion of the geometry of the polymerase active site, enabling RT to discriminate natural substrates from multiple ddNTPs (40, 43, 44). In this regard, we attempted to define the difference between RT$^{wt}$ and mutant RTs with respect to the RT’s interaction with the template-primer. We then found that all RT$^{wt}$ and mutant RTs examined had a virtually identical profile in the time course of dAMP incorporation into a distorted template-primer for all RTs tested (Table V; $K_{	ext{cat}} = K_m$).

### Table III

| Inhibitor | $K_i$ | $K_m$ | $K_{cat}/K_m$ |
|-----------|-------|-------|--------------|
| ddATP     | 0.24  | 1.3   | 4.6          |
| ddCTP     | 3.3   | 22.2  | 42.4         |
| ddGTP     | 0.0067| 0.0003| 0.027         |
| ddITTP    | 0.025 | 0.0011| 0.075         |
| AZTTP     | 0.010 | 0.0005| 0.035         |

### Table IV

| RT          | $K_{cat}(ddATP)$ | $K_m(ddATP)$ | $K_{cat}/K_m$ |
|-------------|-----------------|--------------|--------------|
| RT$^{wt}$   | 0.31 ± 0.02     | 0.027 ± 0.0007| 0.087        |
| RT$^{151}$  | 1.3 ± 0.2       | 0.020 ± 0.0008| 0.015        |
| RT$^{75/77/116}$ | 5.3 ± 0.4     | 0.024 ± 0.0004| 0.0045       |
| RT$^{62/75/77/116/151}$ | 3.9 ± 0.2 | 0.023 ± 0.0004| 0.0059       |

### Table V

| Substrate | $K_m$ | $K_{cat}$ | $K_{cat}/K_m$ |
|-----------|-------|-----------|--------------|
| ddATP     | 0.048 ± 0.010 | 0.021 ± 0.0004 | 0.44        |
| ddCTP     | 4.8 ± 0.9    | 2.1 ± 0.3    | 0.044        |
| ddGTP     | 5.6 ± 0.4    | 1.3 ± 0.03   | 0.23         |
| ddITTP    | 6.0 ± 0.5    | 2.1 ± 0.07   | 0.35         |

Inhibitor analysis of RT$^{wt}$ and mutant RTs

All assays were performed as described under “Experimental Procedures.” $K_i$ values were determined by Lineweaver-Burk analysis, where $K_i$ reported is $K_{cat}$ for a competitive inhibitor. Lineweaver-Burk plots for ddATP inhibition are given in Fig. 5.

Steady-state kinetic constants of RTs with [H]$^3$ddATP as a substrate

The assay condition used for [H]$^3$ddATP substrate analysis is the same as that for [H]$^3$ddATP substrate analysis as described under “Experimental Procedures.”

Substrate analysis of RT$^{151/215}$

The IC$_{50}$ values of ddATP, ddCTP, ddGTP, and AZTTP to RT$^{151/215}$ were 45 ± 5, 50 ± 0.5, 0.11 ± 0.004, and 0.30 ± 0.03 μM, respectively. The assay conditions used were the same as those described in the legends to Tables I and II.
RT62/75/77/116/151, and RT151/215, respectively. Moreover, we have found no significant changes in the processivity of RTs as measured by dTMP incorporation into poly(A) (data not shown). It is worth noting that in the present work, bacterially expressed p66 homodimer RTs were employed; thus, the observed changes caused by amino acid substitutions might differ from those that may occur in the p51/p66 heterodimer RTs. In this regard, Becerra et al. have reported a difference in association constants upon dimerization of p66p66 and p51/p66 subunits of RT (46), which may produce changes in enzyme-substrate interactions differently between heterodimer and homodimer RTs when amino acid substitutions occur. Further experiments using proper heterodimer RTs and pre-steady kinetic analysis are needed to define the structures and functions of HIV-1 RT when these mutations develop.

It is noteworthy that HIV-1 strains carrying Q151M often do not bear amino acid mutations that have been associated with viral resistance to AZT, although HIV-1 variants were isolated from patients receiving long-term therapy including AZT (11, 13, 18). It is possible that certain nucleotide or amino acid sequence(s) inherent to HIV-1 strains in certain patients predispose these viruses not to acquire any AZT-associated mutations but to develop the Q151M substitution and ultimately the rest of the five mutations. Comparative nucleotide sequence analysis of pretherapy HIV-1 that later acquired Q151M and pretherapy strains that later developed the AZT-associated mutations may define such predisposing nucleotide or amino acid sequence(s). It remains to be determined whether HIV-1 carrying Q151M can subsequently acquire the AZT-associated amino acid substitutions. It is also of interest to study the mechanisms of substrate recognition or discrimination of ddNTPs by HIV-1 RT Resistant to Multiple ddNTPs.