Studies of drug-resistant reverse transcriptases (RTs) reveal the roles of specific structural elements and amino acids in polymerase function. To characterize better the effects of RT/template interactions on dNTP substrate recognize, we examined the sensitivity of human immunodeficiency virus type 1 (HIV-1) RT containing a new mutation in a “template grip” residue (P157S) to the 5′-triphosphates of (−)β,2′,3′-dideoxy-3′-thiacytidine (3TC), (−)β,2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC), and 3′-azido-3′-deoxythymidine (AZT). A primer extension assay was used to monitor quantitatively drug monophosphate incorporation opposite each of multiple target sites. Wild-type and P157S RTs had similar catalytic activities and processivities on heteropolymeric RNA and DNA templates. When averaged over multiple template sites, P157S RT was 2–7-fold resistant to the 5′-triphosphates of 3TC, FTC, and AZT. Each drug triphosphate inhibited polymerization more efficiently on the DNA template compared with an RNA template of identical sequence. Moreover, chain termination by 3TC and FTC was strongly influenced by template sequence context. Incorporation of FTC and 3TC monophosphate varied up to 10-fold opposite 7 different G residues in the DNA template, and the P157S mutation altered this site specificity. In summary, these data identify Pro157 as an important residue affecting nucleoside analog resistance and suggest that interactions between RT and the template strand influence dNTP substrate recognition at the RT active site. Our findings are discussed within the context of the HIV-1 RT structure.

Reverse transcriptase (RT)1 converts the human immunodeficiency virus type 1 (HIV-1) plus-stranded RNA genome into double-strand DNA through the complex process of reverse transcription (1). Common HIV-1 therapies employ nucleoside analogs that are metabolized to their active 5′-triphosphates in vivo and are incorporated into viral DNA by RT, terminating DNA synthesis (2, 3). However, the efficacy of nucleoside-based chemotherapy is significantly reduced by the emergence of drug-resistant HIV-1 variants containing mutations in RT that confer reduced susceptibility to nucleoside analogs (2, 3).

Studies of drug-resistant RTs provide valuable information about the contributions of specific amino acids and subdomains to the biochemical mechanisms of RT. For example, viruses resistant to (−)β,2′,3′-dideoxy-3′-thiacytidine (3TC (lamivudine or Epivir)) and (−)β,2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC (emtricitabine or Coviracil)) are the result of substitutions of valine, isoleucine, or threonine for methionine at position 184 (4). Met184, the Met residue in the YMDD active site motif in the palm subdomain of RT, interacts with the 3′-end of the primer and influences polymerase fidelity in cell-free systems (5–11). Thus, a residue that influences normal dNTP discrimination at the RT active site is also involved in nucleoside analog resistance. Other mutations conferring nucleoside analog resistance also cluster around the dNTP binding pocket (9, 12).

Feline immunodeficiency virus (FIV) has been developed as a model for studying HIV-1 pathogenesis (13) and drug resistance (14). FIV RT is similar to HIV-1 RT in amino acid sequence, physical properties, catalytic activities, and nucleoside analog susceptibility (15–18). Moreover, a valine or threonine substitution at Met183 of FIV RT, the residue analogous to Met184 (19, 20), confers resistance to 3TC (18). Recently, a new variant of FIV resistant to 3TC and 3′-azido-3′-deoxythymidine (AZT) was identified (21). Resistance was attributed to a novel proline to serine mutation at position 156 in FIV RT. The analogous position in HIV-1 RT, Pro157, is one of several residues that compose the template grip, a DNA polymerase structural motif that interacts with the template strand (7–9). Hence, the FIV P156S mutant identified a new region of RT that affects active site substrate discrimination. This is interesting because it implies that template interactions away from...
The active site influence dNTP substrate recognition.

In this work, the sensitivities of purified HIV-1 RTs (wild-type (WT), P157S, and M184V for each RT subunit) to nucleoside analogs were examined as a means to address the effects of RT/primer-template interactions on substrate selection. Primer extension assays were used to detect quantitatively drug monophosphate incorporation opposite each of multiple sites on heteropolymeric DNA and RNA templates. We found that P157S confers moderate resistance to 3TC-5'-triphosphate (3TCTP) and FTC-5'-triphosphate (FTCTP) and low resistance to AZT-5'-triphosphate (AZTTP). We also found that the levels of 3T and FTC monophosphate incorporation by HIV-1 RT vary at different template sites and that this site specificity is altered by mutation of Pro157 in the template grip. These findings are discussed in the context of the recently published structure of an HIV-1 RT catalytic complex (9) and suggest that interactions between the RT template grip and the template affect dNTP substrate recognition at the polymerase active site.

EXPERIMENTAL PROCEDURES

Materials—HIV-1 5'-32P-labeled primer-templates were prepared as described (25). Synthetic oligonucleotides were from Operon Technologies, Inc. (Alameda, CA). Restriction enzymes were purchased from New England Biolabs. Ultrapure dNTPs and pKK223-3 were from Amersham Pharmacia Biotech. T7 RNA polymerase, RNasin, and Taq polymerase were from Promega. FTCTP and 3TCTP were synthesized as described (24, 25), and AZTTP was purchased from Moravek Biochemicals (La Brea, CA).

Cloning, Mutagenesis, and Purification of Wild-type and Mutant HIV-1 RT Heterodimers—The coding region of each WT RT subunit (nucleotides (nt) 2551–4229 for p66 and nt 2551–3869 for p51) was amplified from the infectious HIV-1 clone pNL4-3 (Ref. 26; a kind gift of Dr. Arnold Rabson, New Jersey Center for Biotechnology and Medicine, Piscataway, NJ) using polymerase chain reaction (PCR; Ref. 27). The following oligonucleotide primers were used: 5' end of p66 and p51, 5'-ACTATGGAATTCTGAAGACTCGATTCTGAGAC-3', 3' end of p66, 5'-CTGGGAGACCTTCACTATAGAATCCTGAGAC-3'; 3' end of p51, 5'-CTGGGAGACCTTCACTATAGAATCCTGAGAC-3'. Reaction sites for EcoRI and HindIII are underlined; bold nucleotides are the start codon (5'-oligonucleotide) and stop codons (3'-oligonucleotides). PCR conditions were 30 cycles at 94 °C, 1 min; 60 °C, 1.5 min; and 72 °C, 1 min and were carried out in 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 mM MgCl2, 200 μM each dNTP, 200 pmoles each primer, 75 ng of double-strand template, and 2.5 units of Taq DNA polymerase. PCR products were purified through 2% low melting temperature agarose gels, digested with EcoRI and HindIII, and ligated into the corresponding sites in pKK223-3 using standard protocols (27). The entire coding region for each subunit was sequenced at the University of Utah Sequencing Core Facility to ensure no errors were introduced during PCR. The p66 coding region DNA was then excised at the EcoRI and HindIII sites and subcloned into M13mp19, which was used to generate uracilated DNA for site-directed mutagenesis (25). The following mutant oligonucleotides were used: 5'-phosphate-ATCTTATAAGTGGATGGATGTTG-3', to change methionine 184 to valine (M184V) and 5'-phosphate-AAGATCCATCAAGGATATGATT-3', to change proline 157 to serine (P157S; mutagenic nts are italicized). After mutagenesis, a 493-nt EcoRI/AgeI fragment was removed and used to replace the corresponding WT fragment in the plasmids pKK223-3 p66 and p51 expression clones to generate RT expression clones of WT, P157S, and M184V for each RT subunit.

Each of the 6 clones was expressed in Escherichia coli DH5α Life Technologies, Inc., and RT p66/p51 heterodimers were purified as described previously (22) with several modifications. The concentration of isopropyl-β-D-thiogalactopyranoside used to promote RT expression was raised to 30 μM, but the conditions for cell growth and harvesting cells were unchanged. A total of 10 g of E. coli cell paste consisting of 3 g of p66 paste and 7 g of p51 paste was used for purification. The resulting lysate had a 2-fold molar excess of p51 relative to p66 to facilitate the purification of p66/p51 heterodimers rather than p66/p66 homodimers (29). The lysate was centrifuged as described (22), and the supernatant was desalted by dialysis against buffer M (50 mM Tris-HCl, pH 7.0, 1 mM EDTA, 1 mM dithiothreitol, 0.1% Nonidet P-40, and 10% glycerol) at 4 °C (22). The desalted, cleared lysate was loaded onto a 400-ml DEAE-cellulose column (Whatman) as described (22). RT activity eluted in the void volume in buffer M, and peak protein-containing fractions were pooled and loaded onto a buffer M-equilibrated heparin column (POROS 20 HE1, 10 × 0.46 cm) at 5 ml/min using a BioCAD SPRINT perfusion chromatography system (Perceptive Biosystems, Framingham, MA). The column was washed with 4 column volumes of buffer M, and RT was eluted with a 12-column volume linear gradient of 50 mM NaCl in buffer M. Excess p51 subunit eluted first at 210 mM NaCl followed by p66/p51 eluted near the start of the gradient at approximately 35 mM NaCl. Purity was confirmed by PAGE and stained SDS-polyacrylamide gel electrophoresis (PAGE; data not shown). The expression levels, chromatographic behavior, and yields of WT and mutant RTs were very similar, suggesting that the mutant RTs were properly folded. All three RT preparations were free of detectable 3'-5'-exonuclease activity (data not shown). RT active sites were determined as described previously (23); each p66/p51 preparation was nearly 100% active (data not shown).

Processivity Assay—HIV-1 oligonucleotide primers (primer 4737 and primer 4765) were used in a 50-nt DNA template, respectively, minor-strand pHIV-pol DNA and plus-strand pol RNA transcripts were created as described previously (22). 5'-32P-End-labeling of the oligonucleotide primer, annealing of primer-templates, and processivity reactions were carried out essentially as described (22, 23, 30) in 15-μl volumes except the primer-template concentration was 10 nm, and concentrations of each RT were varied between 0 and 30 nM. From product resolution by 7% urea, 8% PAGE, products were visualized with a Molecular Dynamics PhosphorImager and quantitated using IMAGEQUANT software. Values of k, were calculated from steady-state reactions where product formation was linearly proportional to RT concentration.

Drug Susceptibility Assay—For DNA-template polymerization, a synthetic DNA oligonucleotide 20 nt long (20-mer; Ref. 31) was 5'-end-labeled with [γ-32P]ATP and hybridized to a 46-nt DNA oligonucleotide (46-mer; Ref. 31) as described previously (22). Primer extension reactions (15-μl volume) contained 10 nm primer-template and 50 nM RT in buffer with 25 μM Tris-HCl, pH 8.0, 30 mM KCl, and 2 mM dithiothreitol. After a 5-min preincubation at 25 °C, reactions were started by the addition of 10 μM MgCl2, 2 or 4 dNTPs, and 0–200 μM FTCTP, 3TCTP, or AZTTP (see figure legends for specific concentrations). After 10 min at 37 °C, the reactions were stopped by the addition of EDTA to 50 mM final concentration. A portion of each reaction was mixed with formamide loading dye (27), resolved by 7% urea, 16% PAGE, and visualized and quantitated as described for the processivity reactions.

For RNA-templated polymerizations, a 49-nt RNA was generated by in vitro transcription. DNA-oligonucleotides were first polymerized to form a 46-nt complementary chain of the appropriate polarity and hybridized with a 3'-OH terminating primer to form a heteroduplex RNA-DNA molecule that was 66 nt long containing a T7 RNA polymerase promoter. The sequence of the + strand oligonucleotide was 5'-TAATACGACTACTATAGGGTATTGAGC-3'. The oligonucleotides were hybridized at a concentration of 0.1 μg/ml in 40 μM Tris-HCl, pH 8.0, and 100 mM KCl at 95 °C for 5 min followed by cooling to room temperature. The resultant duplex DNA was precipitated with sodium acetate and ethanol (27), dried, resuspended in water to a concentration of 1.5 μg/ml, and used in vitro transcription reactions according to instructions from Promega. This generated a 49-nt RNA with the same sequence as the 46-nt DNA template described above plus 3 additional nts at the 5'-end (5'-GGG . . . 3'). The RNA was annealed to the 20-mer primer, and drug susceptibility reactions were carried out as described above except that RNasin was included in the reactions (1 unit/ml).

Calculation of Chain Termination Probabilities—Incorporation of a nucleotide analog lacking a 3' OH terminates primer extension. Therefore, the proportion of primers that terminate at a given site relative to the total amount of primer elongated up to and beyond that site defines the probability of incorporating a chain terminating nucleotide. We call this chain termination probability. A similar parameter was previously used to describe pauses in DNA synthesis in steady-state reactions (22, 32). Chain termination probabilities were calculated from band intensities quantified using IMAGEQUANT software after visualization by a Molecular Dynamics PhosphorImager.
HIV-1 RT/Template Interactions Influence Drug Incorporation

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RESULTS

Recent studies of the model AIDS virus, FIV, identified P156S as a novel RT mutation that confers low level resistance to both 3TC and AZT (21). Alignment of FIV Pro156 with the sequence and crystal structure of HIV-1 RT indicates that this residue is part of the template grip region of the enzyme (7–9, 21). This suggests that dNTP analog discrimination in FIV is influenced by residues involved in template binding that do not directly contribute to the RT active site.

To determine whether the analogous residue (Pro157) plays a similar role in HIV-1 RT and to characterize better the effect of template interactions on active site substrate discrimination, we examined the catalytic activity, processivity, and drug triphosphate susceptibility of purified recombinant P157S HIV-1 RT. A primer extension assay was used to measure polymerization and drug monophosphate incorporation at multiple sites within DNA and RNA templates. The biochemical properties of this template grip mutant were compared with those of WT HIV-1 RT and M184V RT, an active site mutant that exhibits both resistance to 3TC and AZT (21). This suggests that HIV-1 RT mutants, we employed a primer extension assay that detects the incorporation of chain terminating nucleotide analogs opposite multiple sites on heteropolymeric RNA and DNA templates (Fig. 2).

To address these issues in our study of HIV-1 RT mutants, we employed a primer extension assay that detects the incorporation of chain terminating nucleotide analogs opposite multiple sites on heteropolymeric RNA and DNA templates (Fig. 2).

Susceptibility to FTCTP and 3TCTP—To evaluate whether P157S RT confers FTCTP resistance, primer extension reactions on DNA and RNA templates were carried out in the presence of fixed amounts of dATP and dCTP and increasing concentrations of FTCTP (Fig. 2). In the reactions lacking FTCTP, the primer was extended the expected 6 nts plus an additional 1 to 2 nts (Fig. 2, B and C, lanes 1; Note: addition of these extra nts is likely due to nucleotide misincorporations and/or trace contaminating dNTPs which are not expected to affect drug incorporation at the G positions in the template). As the concentration of FTCTP was increased in each reaction with WT RT, chain termination also increased 1 and 3 nts from the primer, presumably due to incorporation of FTC monophosphate (FTCMP; Fig. 2, B, WT lanes 2–8, bands labeled G1 and G2). As expected for a chain terminator, FTCP incorporation was also accompanied by a corresponding decrease in the amount of full-length products formed. P157S RT appeared moderately resistant to FTCTP as evidenced by a shift in the distribution of products (lower yields of termination products at G1 and G2 and concomitant higher yields of full-length products; Fig. 2B, P157S lanes 2–8). This shift is most evident at the lower FTCTP concentrations (lanes 2–5). Similar results were seen on the RNA template (Fig. 2C).
HIV-1 RT/Template Interactions Influence Drug Incorporation

Fig. 2. Effect of FTCTP on primer extension by WT, P157S, and M184V RTs. A, primer-template. The sequences of the 20-mer primer and 46-mer DNA template are shown. The highlighted template Gs and As are the target sites for FTCP/MTP and AZTMP incorporation, respectively. The long primer extension products expected in reactions containing only dCTP and dATP or all 4 normal dNTPs are indicated as “Full-length.” B, DNA template; C, RNA template. Reactions were as described under “Experimental Procedures” using 10 nM primer-template, 50 nM RT, 600 nM dCTP, and 20 μM dATP. The concentrations of FTCTP were 0, 0.4, 1, 5, 6, 10, 20, and 40 μM, respectively. Products were resolved by 7 M urea, 16% PAGE, and a typical PhosphorImage is shown. Arrows on the left of each panel indicate incorporation sites of FTCPMP (G1 and G2). The 5'-32P-labeled 20-mer primer and full-length products expected in these reactions are indicated on the right. In reactions lacking RT, only a single band corresponding to the 20-mer was seen (data not shown). Note: the origin of the high molecular weight bands in C, lane 4, middle, is not known. These were observed rarely in reactions on the RNA template. D, inhibition of full-length product formation by FTCTP. The amount of full-length product synthesized at each FTCTP concentration on the DNA template was quantitated and expressed relative to control reactions lacking drug triphosphate. Circles, WT RT; squares, P157S RT; triangles, M184V RT. As except as noted below, each point represents the average ± S.D. of 2–5 independent determinations. Error bars less than 0.02 are too small to be visible. The following data points are from single determinations: 0.05 μM FTCTP and 0.1 μM FTCTP (for P157S RT and M184V RTs). The line connecting each point was drawn by the smooth curve fit function in KaleidaGraph version 3.0.8.

and in reactions containing 3TCTP in place of FTCTP, indicating that P157S RT may be broadly resistant to oxathiolane nucleoside analogs (data not shown). M184V RT formed almost no detectable chain-terminated products at all FTCTP concentrations tested (Fig. 2, B and C, M184V lanes 2–8).

To quantitate the degree of resistance conferred by the P157S mutation, the fraction of full-length product formed in each reaction was determined and plotted as a function of drug concentration (Fig. 2D). This dose-response curve clearly shows the resistance of P157S RT to FTCTP. For example, at 1 μM FTCTP on the DNA template (Fig. 2D), only 55% of the extended primer was polymerized to full-length product by WT RT (relative to control reactions lacking FTCTP). At this same FTCTP concentration, P157S RT incorporated less FTCMP than WT RT; as a result, nearly 85% of the extended primer was polymerized to full length. M184V RT was strongly resistant to FTCTP and polymerized 100% of the extended primers to full length at this FTCTP concentration. All data sets on the DNA and RNA templates demonstrated that the relative sensitivities of the RTs to FTCTP and 3TCTP were WT > P157S > M184V RT (Fig. 2D and data not shown).

IC50 values calculated from Fig. 2D and other dose-response curves not shown are summarized in Table I (IC50 is defined as the concentration of drug that inhibits full-length product formation by 50%). The IC50 values of FTCTP for P157S RT were 6.7 and 5.5 μM on the RNA and DNA templates, respectively. Thus P157S RT was 2–3-fold resistant to FTCTP compared with WT RT. M184V RT, on the other hand, was approximately 50-fold resistant to FTCTP on these templates (data not shown). All three RTs were more sensitive to FTCTP than 3TCTP. This is consistent with a previous report (33) and shows that HIV-1 RT can distinguish between dNTPs differing only in an electronegative fluorine substituent at position 5 of the pyrimidine ring. Interestingly, this discrimination was slightly exaggerated by the P157S mutation. With WT RT the IC50 values for FTCTP (5–6 μM) were about 3 times higher than those for FTCTP (2–3 μM). Introduction of the P157S mutation increased this difference making the IC50 values for 3TCTP (30–50 μM) about 7 times higher than for FTCTP (6–7 μM). The IC50 values of FTCTP and 3TCTP were consistently higher for both WT and P157S RTs on the RNA template compared with DNA.

Susceptibility to AZTTP—P156S RT from FIV is cross-resistant to AZTTP (21). To determine if the corresponding HIV-1 RT mutant is also cross-resistant to AZTTP, primer extension reactions containing fixed amounts of all 4 normal dNTPs and increasing concentrations of AZTTP were carried out on the RNA and DNA templates (Fig. 3). In the absence of AZTTP, each RT extended the primer to the template end plus one additional nt (Fig. 3, lanes 1). This extra dNMP was likely added through a non-templated mechanism (34) that is not expected to affect drug incorporation. As increasing concentrations of AZTTP were added to the reactions, increasing amounts of chain termination occurred due to AZTMP monophosphate (AZTMP) incorporation opposite the template A residues 8, 15, and 20 nts from the primer (Fig. 3, lanes 2–8). Quantitation of the full-length products revealed that P157S RT synthesized slightly more full-length DNA relative to WT RT at all AZTTP concentrations below 5 μM on the DNA template but not the RNA template (data not shown). M184V and WT RTs were

| TABLE I |
|---|
| The resistance of P157S RT to FTCTP, 3TCTP, and AZTTP |
| dNTP analog | Template | WT RT | P157S RT |
|---|---|---|---|
| FTCTP | RNA | 3.3 μM | 6.7 (2μM) |
| | DNA | 1.9 | 5.5 (3μM) |
| 3TCTP | RNA | 8.4 | 46 (6μM) |
| | DNA | 5.0 | 34 (7μM) |
| AZTTP | RNA | 0.27 | 0.34 (10μM) |
| | DNA | 0.17 | 0.33 (20μM) |

Values in parentheses represent resistance relative to WT RT.
equally sensitive to AZTTP in these reactions, in agreement with the results of others (35, 36).

IC₅₀ values from these data are shown in Table I. For each RT, the AZTTP IC₅₀ values were 10–100 times lower than those for FTCTP and 3TCTP. P157S RT was 2-fold resistant to AZTTP on the DNA template but not the RNA template. Thus it appears that, like FIV RT containing a serine at position 156, HIV-1 P157S RT has a low level of cross-resistance to AZTTP.

**Site Specificity of FTCMP/3TCMP Incorporation**—In the experiments measuring sensitivity to FTCTP (Fig. 2) and 3TCTP (data not shown), drug monophosphate incorporation by WT RT at DNA template sites G₁ and G₂ did not appear equal (Fig. 2B, WT lanes 2–4). To quantify this difference, the probability of chain termination at each of these sites was determined as a function of FTCTP concentration (Fig. 4). This revealed a strong preference for drug monophosphate incorporation at template site G₂. While ~2 μM FTCTP was sufficient to cause 50% chain termination at G₂, as much as 25 μM was required to achieve a similar level of termination at G₁. Based on these EC₅₀ values (concentration of drug triphosphate that results in 50% chain termination probability), we estimate that FTCMP incorporation by WT RT occurred approximately 10 times more readily at G₂ than at G₁.

To determine whether FTCMP or 3TC monophosphate (3TCTP) incorporation varies at other G residues, we conducted primer extension assays on the same 46-mer DNA template (Fig. 2A) but now in the presence of all four normal dNTPs. (Note: only dATP and dCTP were included in the assays summarized in Fig. 2 and Table I.) As expected, all three RTs efficiently extended the primer to the end of the template in the absence of drug triphosphate (Fig. 5, lanes 1). In reactions containing FTCTP or 3TCTP, the amount of chain termination at the seven template G sites increased in proportion to the amount of drug added, and the yields of full-length product correspondingly decreased (Fig. 5, lanes 2–8; termination sites labeled G₁, G₂, G₃, etc.). A comparison of chain termination probabilities at each of the G residues in the DNA template showed that the levels of FTCTP and 3TCTP incorporation were site-specific. WT RT preferentially incorporated FTCTP opposite G₂, G₃, and G₆ with lower incorporation occurring at the other sites (Fig. 6A). P157S RT also exhibited site-specific preferences for drug monophosphate incorporation; however, these preferences differed somewhat from those of the WT RT (compare Fig. 6, A and B). Thus, the resistance of P157S RT to FTCMP is due largely to reduced incorporation at sites G₂ and G₆; EC₅₀ values at these sites were ~2 μM for WT RT and ~14 μM for P157S RT (data not shown). Resistance at sites G₄ and
probabilities were calculated as described under “Experimental Proce-
do” for WT RT (A) and P157S RT (B) at FTCTP concentrations of 3 
rtRNA from the data in Fig. 5A and additional experiments not shown. 
Each bar represents the FTCMP termination probability at the indi-
cated template site and is the average ± S.D. of 2–3 independent 

G<sup>5</sup> was relatively modest (1.5–2-fold), whereas at G<sup>1</sup>, G<sup>6</sup>, and 
G<sup>7</sup> both the P157S and WT RTs incorporated FTCMP almost equally (Fig. 6).

This experiment leads to two important conclusions. First, the 
efficiency of drug monophosphate incorporation by HIV-1 RT is 
dependent on template site. Second, a single amino acid 
change in the RT template grip (P157S) changes this site de-
pendence. Taken together, these data indicate that interactions 
between RT and the template strand influence substrate rec-
ognition. The proline at position 157 in HIV-1 RT is highly conserved 
in retroviruses, retrotransposable elements, retrons, and hepatitis B virus (5, 6), suggesting that it is structurally and/or 
functionally important. In HIV-1 RT crystal structures, Pro<sup>157</sup> 
lies near the N terminus of helix E (residues 155–174) in a 
region of the RT template grip that is proximal to but not 
directly part of the catalytic active site (7–9). Pro<sup>157</sup> is directly 
involved in template binding through the minor groove and 
makes van der Waals contacts with the sugar and base of the 
template strand two base pairs “behind” the incoming dNTP 
(Fig. 7). Pro<sup>157</sup> does not appear to contact directly the incoming 
dNTP, indicating that the resistance of P157S RT to drug 
triphosphates does not result from direct interactions between 
the 157 position and the incoming dNTP substrate analog. 
Therefore, the effects of P157S on FTC/3TC monophosphate 
icorporation are likely indirect and may involve one or more 
alternative mechanisms. One possibility is that the P157S mutation 
mediates subtle structural rearrangements of other impor-
tant amino acid residues due to the increased conforma-
tional flexibility of the peptide backbone imparted by the Ser 
replacement (42). Pro<sup>157</sup> is within 3–4 Å of Met<sup>184</sup> and Tyr<sup>115</sup>, 
residues that contact the 3’ nucleotide of the primer and the 
incoming dNTP, respectively (9). Positional changes in these 
residues induced by the Pro to Ser substitution at 157 could 
alter the susceptibility of RT to nucleoside analogs. Alter-
natively, the serine substitution may alter the nature of the 
contact between the 157 position of RT and the template 
strand, changing the relative positioning of protein and nucleic 
acid components at the polymerase active site (12). Changes in 
the positions of catalytically relevant amino acids and/or the 
template strand may alter the active site geometry, resulting in 
decreased utilization of drug triphosphates as substrates.

Evidence for the involvement of RT/template interactions in 
dNTP substrate selectivity is provided by comparing the inhibi-
tion of RNA-directed versus DNA-directed polymerization by 

FIG. 6. Site specificity of FTCMP incorporation. Termination 
probabilities were calculated as described under “Experimental Proce-
do” for WT RT (A) and P157S RT (B) at FTCTP concentrations of 3 
μM from the data in Fig. 5A and additional experiments not shown. 
Each bar represents the FTCMP termination probability at the indi-
cated template site and is the average ± S.D. of 2–3 independent 

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Evidence for the involvement of RT/template interactions in dNTP substrate selectivity is provided by comparing the inhibition of RNA-directed versus DNA-directed polymerization by
dNTP analogs. Each drug triphosphate inhibited polymerization more efficiently on the DNA template relative to an RNA template of identical sequence (Fig. 2 and Table I). This is consistent with the observation of Wilson et al. (33) that $K_v$ values for the inhibition of WT and M184V HIV-1 RTs by 3TCTP and FTCTP are 30–50% higher on RNA templates. Thus, the nature of the template (RNA versus DNA) influences the ability of HIV-1 RT to incorporate nucleoside analogs. It is not known if this is due to global structural differences between RNA and DNA templates and/or subtle differences imparted by the ribose 2′-OH at the polymerase active site, nor is it known if replicating virus shows a similar template bias for drug sensitivity.

Additional evidence that RT/template interactions affect nucleotide selection comes from the experiment showing that both WT and P157S RTs exhibit site specificity in the levels of FTCMP/3TCMP incorporation (Figs. 4–6). Site-specific differences in the incorporation levels of other nucleoside analogs by WT and P157S RTs exhibit site specificity in the levels of FTCMP/3TCMP incorporation are 1 and 2 bases in “front” of the active site (i.e. 5′ on the template strand), while template residues labeled +1 and +2 are 1 and 2 bases in “front” of the active site (i.e. 5′ on the template strand). For clarity, only p66 RT residues Thr58–His96 and Arg109 and DNA residues −4 through +2 are shown. Pro157 (red) contacts the −2 residue of the template strand. Other residues that interact with the template are indicated in gray (Phe61, Leu74, Asp76, Arg78, Asn81, Gln91, Leu92, Gly93, Ile94, Gln151, Gly152, and Lys154; see Refs. 3 and 4). The observation that template grip mutations impart resistance to both nucleoside and pyrophosphate analogs suggests that the template grip influences the organization and selectivity of the active site/dNTP binding pocket. Additional experiments are required to understand fully how the HIV-1 template grip contributes to active site discrimination.

Studies of other DNA polymerases suggest that their template grips also influence dNTP substrate recognition. Protein structure alignments of HIV-1 RT, PolI family polymerases (Klenow, Taq, T7, and Bst), and a Pol-α family polymerase (RB69) show remarkable conservation of structure in the palm subdomains of these proteins including their template grips (49–55). Moreover, amino acid residues known to affect fidelity and/or nucleoside drug susceptibility in HIV-1, E. coli, T4 phage, herpes simplex virus, and hepatitis B virus polymerases map to this region (56–63). Hence, the template grip contributes to dNTP discrimination in evolutionarily diverse polymerases.

In summary, our studies identify Pro157 as an important HIV-1 RT template grip residue that influences 3TC/FTCTP recognition. Three lines of evidence show that RT/template interactions influence active site discrimination as follows: 1) drug monophosphate incorporation is not equal on RNA and DNA templates of identical sequence; 2) drug monophosphate
incorporation is template sequence-dependent; and 3) mutation of a residue known to interact with the template (Pro157) changes the site specificity of drug monophosphate incorporation. These findings, together with recent polymerase fidelity studies (64), imply that the geometry of the RT active site responds to differences in template sequence and/or structure. The underlying mechanisms for this are not known. Specific interactions among amino acid side groups and template atoms may contribute. Changes in active site geometry propagated through subtle structural changes of the template grip may also be involved. Additional biochemical and structural studies are required to address these and other possible mechanisms.

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