Fidelity of the RNA-Dependent DNA Synthesis Exhibited by the Reverse Transcriptases of Human Immunodeficiency Virus Types 1 and 2 and of Murine Leukemia Virus: Mispair Extension Frequencies†

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ABSTRACT: Human immunodeficiency viruses type 1 and 2 (HIV-1 and HIV-2, respectively) exhibit extensive genetic variations. It was postulated that much of this genetic variability stems from the low fidelity of the reverse transcription step. Both HIV reverse transcriptases (RTs) were shown to be particularly error-prone during the in vitro DNA-dependent DNA synthesis relative to other retroviral RTs. Extension of mismatched 3′-termini of the primer DNA was shown to be a major determinant in the infidelity of HIV RTs. However, reverse transcriptases generally exhibit dual template specificities. Therefore, we determined in the current study the fidelity of RNA-dependent DNA synthesis catalyzed in vitro by the RTs of HIV-1 and HIV-2 in comparison with that of murine leukemia virus (MLV) RT. Consequently, we examined the ability of these enzymes to extend preformed 3′-terminal A·A, A·C, and A·G mispairs by quantitating the amount and length of extended primers in a primer extension assay using ribosomal RNA as a template. The results demonstrate that the three RTs studied exhibit efficient extensions from 3′-terminal mispairs with a specificity of A·C > A·A > A·G. Nevertheless, the HIV RTs are qualitatively as well as quantitatively more error-prone than MLV RT. The mispair extension efficiency appears to be affected mainly by the increase of apparent K_m values, rather than by the change in V_{max} values. In all, it is apparent that polymerization errors produced during the RNA-dependent as well as DNA-dependent DNA synthesis by HIV-1 and HIV-2 RTs might be a major cause for the high mutation frequencies observed by these AIDS-causing viruses.

Human immunodeficiency viruses type 1 and type 2 (HIV-1[1] and HIV-2, respectively) are the causative agents of acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983; Clavel et al., 1986; Gallo et al., 1983). These retroviruses were found to exhibit extensive genetic heterogeneity, thus affecting viral pathogenesis and their resistance to drug therapy. The ability of HIV to evade host immune defences appears to be related to the high mutation frequency in the viral genome, particularly within the envelope gene (Hahn et al., 1986; Myers et al., 1991). Thus, the genome of HIV evolves at a rate of about a million times as great as that of eukaryotic DNA genomes and possibly 10 times greater than other retroviruses (Nowak, 1990). Moreover, it was recently suggested that the progression of AIDS in patients can be viewed as a fast evolutionary process due to this exceptionally high mutation rate (Palca, 1991). Sequence changes observed in serial virus isolates taken from a single patient were almost exclusively nucleotide base substitution. Hence, substitutions caused by HIV-1 RT have been assumed to be the primary mechanism for genomic changes in this virus (Hahn et al., 1986).

The enzyme reverse transcriptase (RT) of HIV and other retroviruses is responsible for copying the single-stranded viral RNA genome into a double-stranded proviral DNA (Weiss et al., 1985; Varmus & Brown, 1989). This enzyme is multifunctional, exhibiting several enzymatic activities by a single polypeptide. The three RT-associated activities that catalyze the necessary steps of proviral DNA synthesis are as follows: (a) transcription of viral RNA into the minus DNA strand by the RNA-dependent DNA polymerase (RDDP) function; (b) the hydrolysis of RNA by the ribonuclease H (RNase H) activity from RNA–DNA heteroduplex; (c) copying of the minus strand DNA into the second plus strand DNA by DNA-dependent DNA polymerase (DDDP) function (Goff, 1990; Hizi et al., 1991b). Unlike cellular DNA polymerases, retroviral RTs have no 3′→5′ exonuclease proofreading activity. However, the relatively low fidelity of the DNA-dependent DNA polymerization step detected with HIV-1 RT is even 10-fold higher than that seen with other proofreading-deficient RTs [i.e., RTs of avian myeloblastosis virus (AMV) and murine leukemia virus (MLV)] (Preston et al., 1988; Roberts et al., 1988; Takeuchi et al., 1988). Efficient extension of mismatched 3′-termini of DNA was shown to be a major determinant of the low fidelity of HIV-1 RT (Perrino et al., 1989). Our recent studies have found that HIV-2 RT, similar to HIV-1 RT, extends preformed mispaired termini efficiently during the DNA-dependent DNA polymerization process. The relative mispair extension efficiencies obtained with both HIV RTs [(1/2000)–(1/35000)] were 6- to 8-fold higher than the corresponding ones obtained by MLV RT [(1/12000)–(1/200000)] (Bakhanashvili & Hizi, 1992b). We have recently suggested the possibility that this low fidelity of DNA synthesis might be associated with the low cysteine content of the two HIV RTs (Bakhanashvili & Hizi, 1992a). Thus, HIV-1 and HIV-2 RTs apparently belong to a less accurate subclass of RTs. Since RTs exhibit a dual template specificity, there is a possibility that polymerization errors

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[1] Abbreviations: HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; RT, reverse transcriptase; MLV, murine leukemia virus; AMV, avian myeloblastosis virus; AIDS, acquired immunodeficiency syndrome; RNase H, ribonuclease H; RDDP, RNA-dependent DNA polymerase; DDDP, DNA-dependent DNA polymerase; rRNA, ribosomal RNA.

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would occur during the RNA-dependent DNA polymerization of the viral RNA genome as well. Until recently, all studies on the fidelity of RTs copying RNA in vitro have used synthetic ribohomopolymers rather than native RNA as templates (Battula & Loeb, 1974; Takeuchi et al., 1988). However, it was suggested very recently that HIV-1 RT is also hightly error-prone in copying native RNA (Ji & Loeb, 1992; Hubner et al., 1992).

In this study we present a primer extension assay, using rRNA as a natural template, employed for measuring the mispair extension efficiencies by both HIV-1 and HIV-2 RTs as well as by MLV RT (as representing relatively low- and high-fidelity RTs, respectively). We find that all three RTs exhibit a relatively high efficiency of mispair extension during RNA-dependent DNA polymerization. Nevertheless, the relative extension frequencies from all mispairs were higher with HIV RTs than with MLV RT. Therefore, these observations indicate that the high error rate of HIV-1 and HIV-2 RTs can be attributed to efficient extension of mispaired nucleotides during the RNA-dependent DNA polymerization as well.

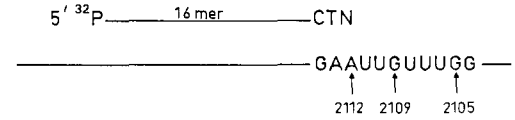
MATERIALS AND METHODS

Enzymes. All reverse transcriptases used in this study were recombinant enzymes expressed by us in *Escherichia coli* and purified from the bacterial extracts. HIV-1 RT was expressed from the BH-10 proviral clone of HIV-1 (Hizi et al., 1988). HIV-2 RT was expressed from the Rod proviral clone of HIV-2 (Hizi et al., 1991a), and MLV RT was expressed from the K clone of MLV (Miller & Verma, 1984; & Hughes, 1988). The enzymes were purified to homogeneity, yielding polypeptides with apparent molecular mass values of approximately 66, 68, and 70 kDa for HIV-1, HIV-2, and MLV RTs, respectively. The enzymes were purified according to Clark et al. (1990). The specific activities of the different RTs used were 4000–5000 units/μg. One unit is defined as the amount of enzyme that catalyzes the incorporation of 1 pmol of dTMP into DNA in the poly(rA)$_n$·oligo(dT)$_{12-18}$-directed reaction in 30 min at 37 °C.

Template-Primers. The template was a mixture of 16S and 23S *E. coli* ribosomal RNA (from Boehringer Mannheim). It was primed with a 2-fold molar excess of the 16mer oligonucleotide that hybridizes to nucleotides at positions 2112–2127 of the 16S rRNA using the *E. coli* 16S rRNA nucleotide numbering system (Brosius et al., 1981). Four versions of the 16-base primers were synthesized separately. All are identical except for the 3′-terminal base (N) which contains either an A, C, G, or T. The sequence of these primers is as follows: 5′ATTTCACATCTGACTN3′. The primers were 5′ end labeled with T4 polynucleotide kinase (from U.S. Biochemicals) and [γ-^{32}P]ATP (from Amersham) and mixed with a mixture of *E. coli* 16S and 23S rRNA in 10 mM NaCl and 20 mM Tris·HCl (pH 8.0). This mixture was placed in boiling water and allowed to cool slowly at room temperature.

DNA Polymerization Reaction. The polymerization reactions for HIV RTs contained 50 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 mM KCl, primed RNA, and various concentrations of dATP. For MLV RT, the reactions were carried out in the presence of 0.5 mM $MnCl_2$ substituting for $MgCl_2$. Reactions were incubated at 37 °C. At different times aliquots (5 μL) were removed into 5 μL of formamide dye mix, denatured at 100 °C for 5 min, cooled on ice, analyzed by electrophoresis through 20% polyacrylamide gels, and quantitated by densitometric scanning of the gel autoradiographs. The incubation times in all experiments were chosen so that products were accumulating linearly with time. The kinetic parameter values K_m and V_{max} were determined from the double-reciprocal plots of V versus dATP concentrations (Lineweaver–Burk curves).

Chart I: Primer-Template Used for Determining the Extension Kinetics for Matched and Mismatched 3′-Terminal Primers[a]



[a] The primer is 16 nucleotides long with 3′-terminal nucleotide N, representing A, C, G, or T, and annealed to a complementary section of 16S rRNA template.

RESULTS

The error rates of DNA polymerases depend in part on the efficiency with which the enzyme extends mismatched primer termini, following a misincorporation event (Echols & Goodman, 1991). Our previous comparative studies on the fidelity of DNA synthesis of HIV-1 RT, HIV-2 RT, and MLV RT were focused on the DDDP activity of these enzymes, using single-stranded $\phi x174am^3$ DNA as a template (Bakhanashvili & Hizi, 1992b). The current study is devoted to the investigation of the fidelity of the RDDP activity of these three RTs. We studied the ability of these RTs to extend DNA primers with preformed mispaired termini, using rRNA as a template and dATP as the only deoxynucleotide triphosphate. We have chosen the same type of preformed mismatches as those employed previously for the fidelity of the DDDP activity. Furthermore, the primer DNA was designed to hybridize the rRNA sequence with the best fit to the $\phi x174am^3$ DNA sequence used in our previous study (Bakhanashvili & Hizi, 1992b). Consequently, we have chosen the sequence of the 16S rRNA residing between nucleotides 2127 and 2105 (see Chart I). Before measuring the efficiency of mispair extension by the RTs, a time course study was performed for each paired and mispaired terminus to find the range of time during which the products accumulate linearly as a function of time. On the basis of these results, the reaction times for both HIV RTs were chosen to be 2–5 min for the paired termini and 15 min for the mispaired termini; the reaction times for MLV RT were 7 and 20 min, respectively (data not shown).

Kinetic Analysis of Mispair Extension by HIV-1 and HIV-2 RTs. To evaluate the mispair extension efficiency by HIV-1 and HIV-2 RTs, we prepared a series of templates with 16mer primers that formed the 3′-terminal mismatches A·A, A·C, or A·G at position 2112 of 16S rRNA. The extension from the preformed mispairs was detected by an increase in the length of the oligonucleotide primers to 17 nucleotides or greater. The ability of HIV-1 RT and HIV-2 to extend each of the three mispairs under steady-state kinetic conditions is illustrated in Figures 1 and 2 for HIV-1 RT and HIV-2 RT, respectively. Extension from the A·A purine–purine mispair is shown by elongation of the 16mer primer producing oligonucleotide products from 17 to 22 nucleotides in length. The 19mer product presumably results from misinsertion of dAMP opposite the template guanidine at position 2109 (see Chart I). Subsequent extension from the newly formed G·A mispair involves incorporation of complementary dAMPs opposite the template uridine at positions 2108–2106, leading to synthesis of 20–22mer products. Extension from the A·G purine–purine mispair results in the production of the 17mer,

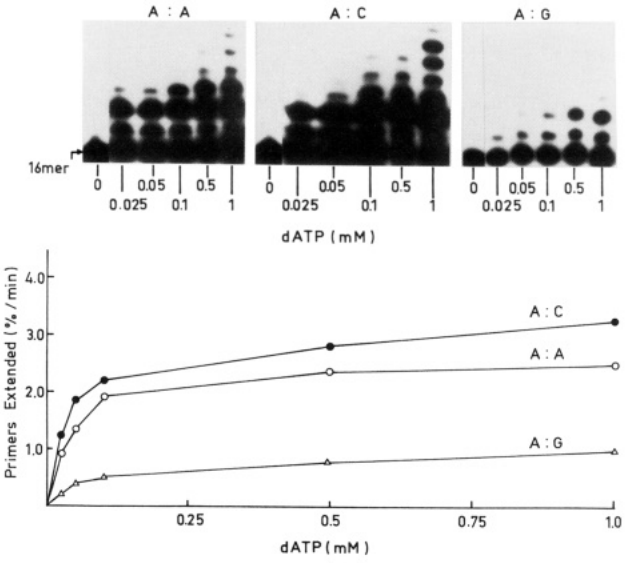


FIGURE 1: Kinetics of mispair extension by HIV-1 RT. The 16mer oligonucleotide primers were hybridized to 16S rRNA to produce the indicated 3′-terminal mispairs at position 2112 and extended for 15 min at 37 °C with HIV-1 RT. Each reaction contained 0, 0.025, 0.05, 0.1, 0.5, or 1.0 mM dATP as the only deoxynucleotide triphosphate substrate. Polyacrylamide gel electrophoretic analyses of the extended primers from A·A (○), A·C (●), and A·G (△) mispairs are shown in the upper panel at left, center, and right, respectively. The primer position is indicated by an arrow. The quantitation of the primers extended was plotted in the lower panel after scanning the autoradiograms.

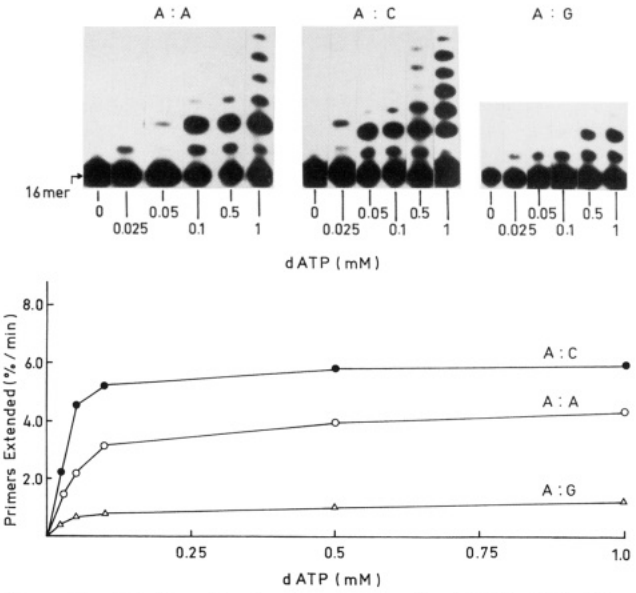


FIGURE 2: Kinetics of mispair extension by HIV-2 RT. The experiments were conducted with HIV-2 RT and analyzed as described in detail for HIV-1 RT in Figure 1.

18mer, and 19mer products. Extension from the A·C purine–pyrimidine mispair results in the production of oligonucleotides from 17 to 23 nucleotides in length. The 23mer product probably results from an additional misinsertion of dAMP opposite the template guanidine at position 2105 (indicating that the extension from an A·C mispair involves multiple misinsertions). Thus, the A·C and A·A mispairs are extended more efficiently than the A·G mispair; i.e., a greater proportion of the mispaired primer is extended and the products are longer.

DNA polymerases are expected to exhibit different binding affinities for each type of mispair, resulting in different efficiencies of mispair extensions. To evaluate the mispair extension efficiency, we measured the ratios of gel band intensities, estimated by densitometry, and determined extension rate (v = percent of primer extended per minute) as a function of dATP concentrations for every given mispair. The apparent values of the kinetic constants, K_m and V_{max}, for the extension of each terminus were calculated from the double-reciprocal plots of the initial velocities versus the substrate concentrations (Lineweaver–Burk curves). It is evident from Table I that both HIV RTs discriminate against extending A·A, A·C, or A·G mispairs, primarily by a large increase (of 1200- to 2500-fold) in apparent K_m values compared to paired A·T termini (with only a slight reduction of about 2- to 5-fold in apparent V_{max} values). Hence, these results indicate that K_m discrimination is predominantly the mechanism determining the fidelity of RNA-dependent DNA polymerization as well as of DNA-dependent DNA polymerization by both HIV RTs. The extension rates from all mispairs were determined under identical conditions, thus allowing the measurement of extension frequencies as the ratios of apparent V_{max}/K_m values obtained with correct and incorrect base pairs at a given sequence on the template. Estimates of substrate efficiencies (i.e., V_{max}/K_m) for both HIV RTs indicate that the A·C mispair was extended 2.5-fold more efficiently than the A·A mispair and 8-fold more efficiently than the A·G mispair. Consequently, the general trend in the order of mispair extension efficiency by both HIV RTs is A·C > A·A > A·G.

Kinetic Analysis of Mispair Extension by MLV RT. The fidelity of the RNA-dependent DNA polymerization by MLV RT was evaluated with the same template-primers given in Chart I. The extension of A·A, A·C, and A·G mispairs was carried out under similar conditions, and the results are shown in Figure 3. Extension from all preformed mispairs is shown by the elongation of the 16mer primers, producing oligonucleotide products from 17 to 20 nucleotides in length. However, a higher proportion of the A·C mispaired primer was extended relative to the primers with A·A and A·G mispairs. The kinetic parameters of mispair extension catalyzed by MLV RT are summarized in Table I. The apparent K_m values for mispair extension are 1000- to 2000-fold greater than those calculated for the correct A·T pairs, while V_{max} values are 2.5- to 9-fold lower. The relative extension frequencies by MLV RT from all three mispairs were 2- to 4-fold lower than that of HIV-1 RT or HIV-2 RT.

DISCUSSION

Recent evidence have suggested that both HIV-1 and HIV-2 RTs belong to a subclass of retroviral RTs with a diminished fidelity of DNA synthesis (Preston et al., 1988; Roberts et al., 1988; Takeuchi et al., 1988, Weber & Grosse, 1989; Bakhanashvili & Hizi, 1992b). These findings were evident for the DNA-dependent DNA polymerase activities of the studied RTs. Interestingly, a major factor in introducing mutations by HIV RTs is apparently their ability to extend efficiently mispair termini (Perrino et al., 1989; Bakhanashvili & Hizi, 1992b), since several studies have suggested that HIV-1 RT is not more error-prone for the rates of misincorporation than MLV RT, AMV RT, or DNA polymerase α (Ricchetti & Buc, 1990; Perrino et al., 1989). In general, retroviral RTs exhibit a dual template specificity, copying both RNA and DNA templates into their complementary DNA. Consequently, there is a possibility that base substitution mutations by RTs could arise during RNA-dependent DNA polymerization as well. Indeed a very recent report has suggested that HIV-1 RT is less accurate than MLV RT in reverse transcribing the lacZα RNA template by exhibiting a 4-fold

Table I: Kinetics of Mispair Extension by HIV-1, HIV-2, and MLV Reverse Transcriptases

primer-template	HIV-1 RT			HIV-2 RT			MLV RT		
	K_m (μM)	V_{max} (%/min)	relative extension frequency	K_m (μM)	V_{max} (%/min)	relative extension frequency	K_m (μM)	V_{max} (%/min)	relative extension frequency
A	50 ± 4.2	4.8 ± 0.14	1/3600	66 ± 7.2	2.7 ± 0.9	1/2800	200 ± 39	0.7 ± 0.099	1/9000
C	24 ± 2.1	6.2 ± 0.21	1/1300	46 ± 5.3	3.5 ± 0.1	1/1400	130 ± 31	1.4 ± 0.061	1/3000
G	41 ± 5.1	1.4 ± 0.061	1/10000	125 ± 40	1.2 ± 0.042	1/12000	235 ± 45	0.37 ± 0.042	1/19000
T	0.021 ± 0.0018	7.2 ± 0.25	1	0.053 ± 0.006	5.9 ± 0.22	1	0.12 ± 0.011	3.6 ± 0.12	1

[a] Oligonucleotide primers (16mer) were hybridized to 16S rRNA to generate the indicated 3′-termini. Extension reactions performed for 5 min with either HIV-1 RT or HIV-2 RT at 37 °C contained 0, 0.025, 0.05, 0.1, 0.5, or 1.0 mM dATP (see Figures 1 and 2). Extension reactions performed for 20 min with MLV RT contained 0, 0.05, 0.1, 0.5, 1.0, or 2.0 mM dATP (see Figure 3). The percent of 16mer extended was quantitated as described. The apparent K_m and V_{max} values ± SE were determined by the method of Wilkinson (1961). Relative extension frequencies are ratios of the rate constant (V_{max}/K_m) for the mispair divided by the corresponding rate constant for the paired A·T terminus.

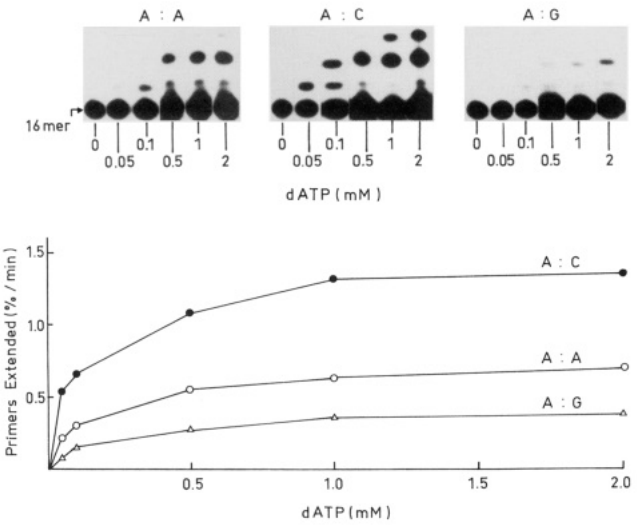


FIGURE 3: Kinetics of mispair extension by MLV RT. Oligonucleotide primers (16mer) were hybridized to 16S rRNA as described in detail in Figure 1 and extended for 20 min at 37 °C with MLV RT. Each reaction contained 0, 0.05, 0.1, 0.5, 1.0, or 2.0 mM dATP as the only deoxynucleoside triphosphate substrate. The experiments were analyzed as described in Figure 1 for HIV-1 RT.

difference in the frequency of misincorporation (Ji & Loeb, 1992). The main focus of our present comparative study was to assess the ability of mispair extension displayed by HIV-1, HIV-2, and MLV RTs. Our study complements the study of Ji and Loeb, by demonstrating that the error rates exhibited during the RDDP reaction result from a higher frequency of mispair extension in addition to enhanced rates of misinsertion.

The experiments reported herein demonstrate that HIV-1 and HIV-2 RTs extend efficiently all three mispaired DNA primers during RNA-dependent DNA polymerization (Figures 1 and 2). The primer extension assay has been applied to estimate quantitatively the efficiency of extending mismatched 3′-termini compared to correctly matched primer termini. The kinetic studies indicate that the K_m values observed for the extension of mispaired primer termini were by 1200- to 2500-fold higher than those for the correctly-paired termini, whereas the V_{max} values exhibited only about a 5-fold difference (Table I). Taken together, changes in dATP affinities detected between correct and incorrect pairs is the predominant mechanism for determining the mispair extension. Since extension rates from all three mispairs were determined under identical conditions, the extension frequencies were defined as the ratios of apparent V_{max}/K_m values obtained with correct and incorrect base pairs at a given sequence of the template (Mendelman et al., 1990). Thus, both HIV RTs appear to catalyze extension of A·C mismatch most easily, i.e., about twice more efficiently than from A·A mispair and about 8-fold more efficiently than from A·G mispair (Table I). Comparison of the mispair extension efficiencies detected with both HIV RTs and MLV RT revealed qualitative as well as quantitative dissimilarities. MLV RT displays a different pattern of extension (Figure 3) and catalyzes the mispair extension less efficiently than both HIV RTs (Table I). The general trend of mispair extension efficiency with all three enzymes tested was A·C > A·A > A·G; i.e., a purine–pyrimidine transition mispair is extended more efficiently than purine–purine transversion mispairs. Several factors may be responsible for the preferential extension of the A·C mispair. Discrimination may be determined by hydrogen bonding between substrate and template bases and nearest neighbour base-stacking interactions. Indeed, according to the geometric selection principle the most stable misinsertions will involve base pairs that are closest to Watson–Crick geometry (e.g., A·C) (Echols & Goodman, 1991). Other base pairs that deviate markedly from Watson–Crick geometry are discriminated against more efficiently.

It is evident that both HIV RTs extend mispaired termini with an efficiency 2- to 3.5-fold higher than that of MLV RT. This fact implies that both HIV-1 and HIV-2 RTs are more error-prone than MLV RT during the RNA-dependent DNA polymerization. Consequently, the relative ease by which both HIV RTs can extend mismatched base pairs during the RNA-dependent DNA polymerization is very likely to contribute to the high frequency of base substitutions during the overall reverse transcription process.

The mispair extension efficiencies of the three RTs were studied previously for the DDDP activity (Perrino et al., 1989; Bakhanashvili & Hizi, 1992b) and in the present study for RDDP activity, using sequences that are similar but not identical. As can be seen from Chart II, the variances in the extension spectrum generated by the enzymes are different for these two reactions. The extended products detected during the RNA-dependent DNA polymerization are longer than during the DNA-dependent DNA polymerization. Further-

Chart II: Spectrum of the Primer Extension Products from the Three Different Mispairs As Accomplished by HIV-1, HIV-2, and MLV RTs during RNA-Dependent and DNA-Dependent DNA Polymerization Reactions[a]

Type of mismatch	Template – primer	
	RNA · DNA 5′-CTN — GAAUUGUUUG –	DNA · DNA 5′-CCN — GGATGTTTC –
A:A		
A:C		
A:G		

[a] The results for the RDDP activity summarize the data presented in the current study, whereas the results for the DDDP activity of the RTs are taken from our previous study (Bakhanashvili & Hizi, 1992b). The conditions under which the fidelity of the DNA-dependent DNA synthesis was analyzed, using *φX174am*[3] DNA as a template, were identical to those described in detail in Materials and Methods for the analysis of the fidelity of the RDDP reaction with rRNA as a template (e.g., divalent cations, dATP concentrations). The nucleotide sequences of the template-primer systems used for both DNA polymerizing reactions are given in the upper panel. The horizontal arrows indicate the direction and length of the extension products from the mispair up to the position of the last detectable dAMP incorporated. Solid arrows refer to the results obtained with MLV RT and the dotted arrows to those observed with HIV-1 and HIV-2 RTs. The positions of the mispairs produced during the primer extensions are indicated by triangles.

more, the extended long products involve additional misinsertions.

The comparative analysis reveals that, under the experimental conditions employed, the relative extension frequencies obtained with all three mispairs during the RNA-dependent DNA polymerization process are higher than those observed for the DNA-dependent DNA polymerization (Chart II). The largest difference of about 4- to 18-fold was observed with MLV RT while with both HIV RTs it appeared to be about 2- to 8-fold higher. Interestingly, our results are supported by the recent findings of Hubner et al. who found, using the *φXam16* reversion assay of HIV-1 RT, that the fidelity of the RNA-dependent DNA synthesis is substantially lower than that of the DNA-dependent DNA synthesis (Hubner et al., 1992). These observations are not in line with those of Ji and Loeb, who found (using the M13 lacZα forward mutation assay) similar frequencies of misinsertion for both RDDP and DDDP activities (Ji & Loeb, 1992). This dissimilarity probably stems from the difference in methods employed or from the differences between the rates of misinsertions and the rates of mispair extensions.

As to the dissimilarities observed in our primer extension studies between the DDDP and RDDP reactions, several possible reasons can account for this disparity. First, the dissimilarity may stem from the nature of the substrate transcribed (RNA versus DNA). Second, the flanking sequences might have an influence on the mispair extension efficiencies (see Chart II). Third, the catalytic properties of reverse transcriptases might affect the error-proneness observed during RNA-dependent and DNA-dependent DNA polymerization processes. Since all RTs possess an RNase H activity in addition to the DNA polymerase functions, it is possible that the hydrolysis of the RNA template from RNA·DNA heteroduplex can affect selectively the mispair primer extension during the RNA-dependent (and not the DNA-dependent) DNA polymerization. Recent computer analysis of the nucleotide sequences of HIV-1 isolates revealed that the nonrandom distribution of base substitution in the genome of this virus is influenced by local nucleotide sequences (6 base pairs long). It was suggested that the error spectra of these cyclic sets of 6mers differ from each other (Doi, 1991). Hence, there is a possibility that the difference in the error spectrum generated during RNA-dependent and DNA-dependent RNA polymerization reactions stems from the difference in the local nucleotide sequences upstream of the preformed mispairs between RNA and DNA templates. We are currently studying this specific issue.

In conclusion, the data presented here, combined with results from our previous studies, strongly suggest that the relatively high error-proneness of both the RNA-dependent and DNA-dependent DNA polymerase activities of HIV-1 and HIV-2 RTs might be the main cause for the high mutation frequencies observed with both human immunodeficiency viruses. A better understanding of the mechanism of this unique property may provide information that could be exploited for the development of novel specific anti-HIV drugs.

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