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Nucleotide Insertion Fidelity of Human Hepatitis B Viral Polymerase

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The hepadnaviruses replicate their nucleic acid through a reverse transcription step. The MBP-fused HBV polymerase was expressed in *E. coli* and purified by using amylose affinity column chromatography. The purified protein represented DNA-dependent DNA polymerase activity. In this report, the MBP-HBV polymerase was shown to lack 3'→5' exonuclease activity, like other retroviral RTs. The ratio of the insertion efficiency for the wrong versus right base pairs indicates the misinsertion frequency (*f*). The nucleotide insertion fidelity (1/*f*), observed with the MBP-HBV polymerase and HIV-1 RT, was between 60 and 54,000, and between 50 and 73,000, respectively, showing that they are in close range. A relatively efficient nucleotide incorporation by the MBP-HBV polymerase was observed with a specificity of three groups: (1) A : T, T : A>C : G, G : C (matched pairs), (2) A : C, C : A>G : T, T : G (purine-pyrimidine and pyrimidine-purine mispairs), and (3) C : C, A : A, G : G, T : T>T : C, C : T>A : G, G : A (purine-purine or pyrimidine-pyrimidine mispairs), and their order is (1)>(2)>(3). The data from the nucleotide insertion fidelity by the MBP-HBV polymerase suggest that the HBV polymerase may be as error-prone as HIV-1 RT.

Keywords: Exonuclease activity, Fidelity, HBV polymerase, Mispairs, Replication.

Introduction

The human hepatitis B virus (HBV) virion DNA is a relaxed circular, partially duplex species of 3.2 kb (Summers *et al.*, 1975). The HBV persistently infected over 300 million people worldwide and caused chronic hepatic insufficiency and hepatocellular carcinoma (Ganem, 1982; Ganem and Varmus, 1987).

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The compact HBV genome contains four open-reading frames (ORF), in which presurface/surface antigen, a core antigen, polymerase, and X protein are encoded (Ganem and Varmus, 1987). The ORF of the polymerase gene occupies 80% of the whole genome.

The hepadnaviruses, including the HBV, are similar to retroviruses in that they replicate through an RNA intermediate (Summers and Mason, 1982; Ganem and Varmus, 1987). The process of the HBV DNA synthesis includes the replication of a minus-strand DNA from a pregenomic RNA (Summers and Mason, 1982; Miller *et al.*, 1984), degradation of pregenomic RNA, and synthesis of plus-strand DNA from the minus-strand DNA template. All of these processes are performed by the virus-encoded polymerase containing DNA-dependent DNA polymerase, RNA-dependent DNA polymerase, RNase H activities (Ganem and Varmus, 1987; Khudyakov and Makhov, 1989), and protein priming activity (Wang and Seeger, 1992; Zoulim and Seeger, 1994; Lanford *et al.*, 1995). Sequence alignments between the coding region of the hepadnavirus polymerase with that of the retroviral reverse transcriptases (Toh *et al.*, 1983), as well as a mutational analysis of retroviral *pol* genes (Tanese and Goff, 1988) allowed the assignment of specific functions to the structural domains of the protein. The P protein is composed of four domains (Toh *et al.*, 1983; Khudyakov and Makhov, 1989): from the amino terminus, terminal protein domain followed by the spacer domain, a reverse transcriptase domain and the C-terminal RNase H domain (Radziwill *et al.*, 1988; Li *et al.*, 1989; Chang *et al.*, 1990). HBV polymerase shares several regions of amino acid homology with other DNA-directed and RNA-directed polymerases (Poch *et al.*, 1989; Delarue *et al.*, 1990; Mendez *et al.*, 1994; Sousa *et al.*, 1996). The reverse transcriptase domain of HBV polymerase has several conserved motifs, and five amino acid residues in the conserved motifs A, B', C, D and E were mutated to show that they are critical in DNA polymerase activity and essential to DNA binding (Kim *et al.*, 1998).

It is essential for all DNA-dependent DNA polymerase to

copy DNA with high fidelity. It is well known that HIV (human immunodeficiency virus) shows high genetic variability (Coffin, 1986; Nowak, 1990) and the virus evades immune pressure by the continuous production of new mutants that are resistant to immunological attack (Nowak, 1992). It has been suggested that the genetic variability is derived from the combined effect of a high error rate of reverse transcriptase (Preston *et al.*, 1988; Roberts *et al.*, 1988; Yu and Goodman, 1992; Hubner *et al.*, 1992), viral genomic recombination, the selection forces of the human immune system, the requirement for growth in multiple cell types during pathogenesis, and persistent immune activation associated with the HIV disease (Drousopoulos *et al.*, 1998). Mutation rates of HIV in a single cycle of reverse transcription are in the range 10^{-4}~10^{-5} mis-incorporations per nucleotide (Pathak and Temin, 1990). Numerous studies have shown that HIV-1 RT, which lacks proofreading exonuclease activity, is particularly error-prone and produces errors about ten times as frequently as MMLV (moloney murine leukemia virus) RT (Preston *et al.*, 1988; Roberts *et al.*, 1988).

The HBV polymerase exhibits similarity to the HIV RTs in that they show a sequence homology and contain reverse transcription steps in their replication. Although the number of isolates genotyped is much smaller than that for HIV, there is considerable evidence that HBV undergoes a significant degree of variation. In the wild type isolates of HBV, the sequence of the genome may vary up to 10% of nucleotide positions, despite the conservation of coding and function (Harrison, 1996). Genes S, C, and P, region X, the precore region, and the pre-S2/pre-S1 regions were ranked in order of increasing variability (Lauder *et al.*, 1993). However, the source of genetic variation is not well known. Thus, it is critical to evaluate the contribution of the HBV polymerase to mutations. In contrast to the enormous amount of information, little is known about the fidelity of DNA synthesis exhibited by the HBV polymerase. Therefore, we attempted to examine the fidelity of the MBP-HBV polymerase in this report.

The MBP-fused HBV polymerase was expressed in *E. coli* and purified by using amylose column chromatography and the MBP-HBV polymerase showed similar catalytic activity against several polymerase inhibitors, as the reported data of HBV polymerase from viral particles (Jeong *et al.*, 1996). In this study, the purified enzyme was analyzed for 3'→5' exonuclease activity. Moreover, kinetic assay was applied to measure the nucleotide insertion fidelity, and it was compared with that of HIV-1 and MMLV RTs, representing relatively low and high fidelity, respectively. Our data show that the MBP-HBV polymerase lacks 3'→5' exonuclease activity. Also the nucleotide insertion fidelity observed with the polymerase and HIV-1 RT was in close range, suggesting that the HBV polymerase may be as error-prone as HIV-1 RT.

Materials and Methods

Enzymes HBV sequences (subtype *adr*) (Rho *et al.*, 1989), spanning nucleotide position 2305 to 1617 that contain the entire polymerase gene (*pol*), were cloned in frame (pMPLX), as previously described (Lee *et al.*, 1993). The MBP-fused HBV polymerase was expressed in *E. coli* strain NM522 and purified. The purification of the MBP-HBV polymerase was performed by using an amylose affinity column chromatography, according to the manufacturer's recommendation (New England Biolabs, Beverly, USA), but with some modifications (Jeong *et al.*, 1996) and the MBP-HBV polymerase was more than 90% of the purified protein. HIV-1 and MMLV RTs were purchased from Boehringer Manheim Biochemicals (Mannheim, Germany) and Promega (Madison, USA), respectively.

Table 1. Template and primers used for analysis of terminal mismatch excision (A) and site-specific nucleotide misincorporation (B).

(A)

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5'CCC CTA GAA GAA GAA A3'--------------primer strand
3'GGG GAT CTT CTT CTT AGGGGAGCG5'----template strand
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(B)

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5'CCC CTA GAA GAA GAA A3'--------------primer strand
3'GGG GAT CTT CTT CTT TGGGGAGCG5'--template-strand G
3'GGG GAT CTT CTT CTT TAGGGAGCG5'--template-strand A
3'GGG GAT CTT CTT CTT TTGGGAGCG5'--template-strand T
3'GGG GAT CTT CTT CTT TCGGGAGCG5'--template-strand C
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Nucleotides and template-primers Unlabeled nucleotides and [γ-^{32}P]TTP (3,000 Ci/mmol) were purchased from Pharmacia (Uppsala, Sweden) and Amersham International (Buckinghamshire, UK), respectively. Oligonucleotides were synthesized in Integrated DNA Technology Inc (Coralvillo, USA). The different template-primer substrates were used for measuring the fidelity of the MBP-HBV polymerase. For measuring terminal nucleotide excision and site-specific nucleotide misincorporation, hybrid molecules between 16-mer oligonucleotide and 24-mer template containing 8 nucleotides protruding 5'-end were used as substrates (Table 1). Primers were 5' end-labeled with [γ-^{32}P]TTP and T4 polynucleotide kinase, and further purified by electrophoresis through a 20% PAGE containing 8 M urea. Each template was primed with the 16-mer oligonucleotide that hybridizes to the template. Partially double-stranded template-primer structures were created by hybridizing labeled oligonucleotides to the non-labeled template-strand in the presence of 0.2 M NaCl and 60 mM Tris-HCl (pH 7.4). The mixture was heated at 70°C and allowed to cool down slowly to room temperature. An excess of template was used to ensure that most primers were annealed to a template and the annealed template-primers were purified by Sephadex G-50 column chromatography.

Terminal mismatch excision The 3'→5' exonuclease activity was measured as the removal of mismatched 3'-terminal nucleotides from the 5'-[γ-^{32}P] end-labeled oligonucleotide, determined by the increase in the mobility during the gel electrophoresis. The reactions were carried out in 25 μl containing 300 ng of mismatched template-primer, 50 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.5 mM $MnCl_2$, 50 mM KCl, and 0.01% Nonidet P-40. Reaction was started by the addition of 0.1 μg the MBP-HBV polymerase or 1 unit of the Klenow fragment of *E.*

coli polymerase I as a positive control, or 50 ng HIV-1 RT as a negative control. After incubation for 40 min at 37°C, the reactions were stopped by the addition of equal volumes of formamide dye mix. Electrophoretic analyses were performed in 20% polyacrylamide sequencing gels followed by autoradiography (Roberts *et al.*, 1988).

Site-specific nucleotide mis-incorporation The template-primer substrates containing 8 nucleotides protruding 5'-end was shown in Table 1(B) and used to measure the rates of dNTP incorporation opposite the G, A, T or C. Reactions for kinetic analysis contained 50 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.5 mM $MnCl_2$, 50 mM KCl, 0.01% Nonidet P-40, [γ-^{32}P]-end-labeled template-primer and increasing concentrations of single dNTP. Reactions were incubated at 37°C for 1 min for correct insertion and 2 min for misinsertion.

Gel electrophoresis and autoradiography Reaction products were analyzed by electrophoresis through 20% polyacrylamide sequencing gels containing 8 M urea followed by autoradiography.

Analyzing gel band intensities A gel-based steady-state kinetic assay (Boosalis *et al.*, 1987; Mendelman *et al*, 1989; Mendelman *et al*, 1990) was used to determine misinsertion efficiency (f_{ins}) for all the mispairs. The bands in each lane of a gel autoradiograph were scanned on a phosphoimager (Imaging plate autoradiography) system (BAS FLA2000, Fuji, Japan). The exposure time for the autoradiograms used for the kinetic analyses was between 20 min and 60 min, and in the linear response range of the film used so that the values are quantitative. The velocity of extending a primer by one (band T°) or two nucleotides (band T*) is expressed as $v = I^*/(I^\circ+I^*)t$, where I° and I* are band intensities measured by the phosphoimager system after t min of reaction time. The sensitivity of the phosphoimager system is great enough to read the intensities of the bands, although band T* is hardly detectable by the naked eye in some of the gels.

Results

Analysis of the 3'→5' exonuclease activity of the MBP-HBV polymerase After incorporating a wrong nucleotide, DNA polymerase can either excise mispaired terminus if the polymerase is associated with 3'→5' exonuclease proofreading activity, and continue chain elongation beyond the mismatch, or extend the mispaired nucleotides, resulting in transition or transversion mutations (Bakhanashvili and Hizi, 1992). All of the RTs studied thus far lack the 3'→5' exonuclease proofreading activity. In order to analyze the fidelity of the MBP-HBV polymerase, we examined whether the MBP-HBV polymerase has 3'→5' exonuclease activity or not. The exonuclease activity was analyzed with template-primer depicted in Table 1(A). As shown in Fig. 1, efficient excision of the terminal nucleotide was found to occur within 3 min when the Klenow fragment of *E. coli* polymerase I was used as a positive control (lane 4). In contrast, there was no

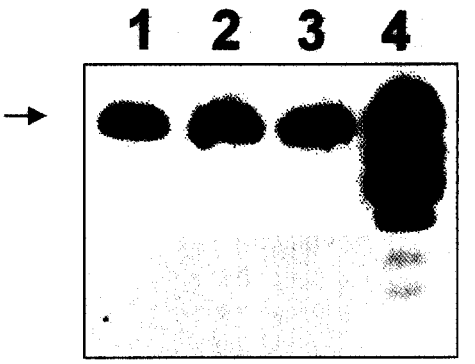

Fig. 1. Electrophoretic analysis of terminal mismatch excision. Reactions for terminal mismatched (A : A) excision by no enzyme (lane 1), the MBP-HBV polymerase (lane 2), HIV-1 reverse transcriptase (lane 3) and Klenow fragment of *E. coli* polymerase I (lane 4) were performed as described in Materials and Methods. Incubations were performed for 40 min at 37°C with the MBP-HBV polymerase, HIV-1 RT as a negative control and for 3 min with the Klenow fragment of *E. coli* polymerase I as a positive control. The position of the 16-mer primer is indicated by an arrow.

change in the length of the oligonucleotide primer after an incubation of up to 40 min when the MBP-HBV polymerase (lane 2) was used. The same result was obtained with HIV-1 RT used as a negative control (lane 3). The result shows that the MBP-HBV polymerase is devoid of 3'→5' exonuclease proofreading activity like other RTs.

Site-specific nucleotide mis-incorporation The lack of proofreading activity enables the analysis of the fidelity of DNA polymerization activity without the interference of exonuclease activities. To obtain the steady-state kinetic parameters, K_m and V_{max} for incorrect dNTP incorporation were measured by using a gel-based assay (Boosalis *et al.*, 1987; Mendelman *et al.*, 1989 and 1990; Creighton *et al.*, 1995). The rates of nucleotide insertion (dNTP) were examined opposite the template A, G, T or C residue at template-strand A, G, T or C primed with 5'-[^{32}P] end-labeled nucleotide primer as shown in Table 1(B). For each template-primer, four separate reactions were carried out, each using a single dNTP to measure the rate of synthesis of the correct pair and three possible mispairs. Before measuring the kinetic constants for mis-incorporation of a nucleotide, a time course study was done for each template-primer to find the range of time during which products accumulate linearly with time. To evaluate the nucleotide insertion fidelity, we measured the ratios of gel band intensities, estimated by a phosphoimager system, and determined the extension rates as a function of dNTP concentrations for the MBP-HBV polymerase. As described above, the rates of nucleotide insertion (dNTP) were examined opposite the template A, G, T or C residue at template-strand A, G, T or C primed with 5'-[^{32}P] end-labeled nucleotide primer, and four separate reactions were carried out for each template-primer. However, the only data of template-

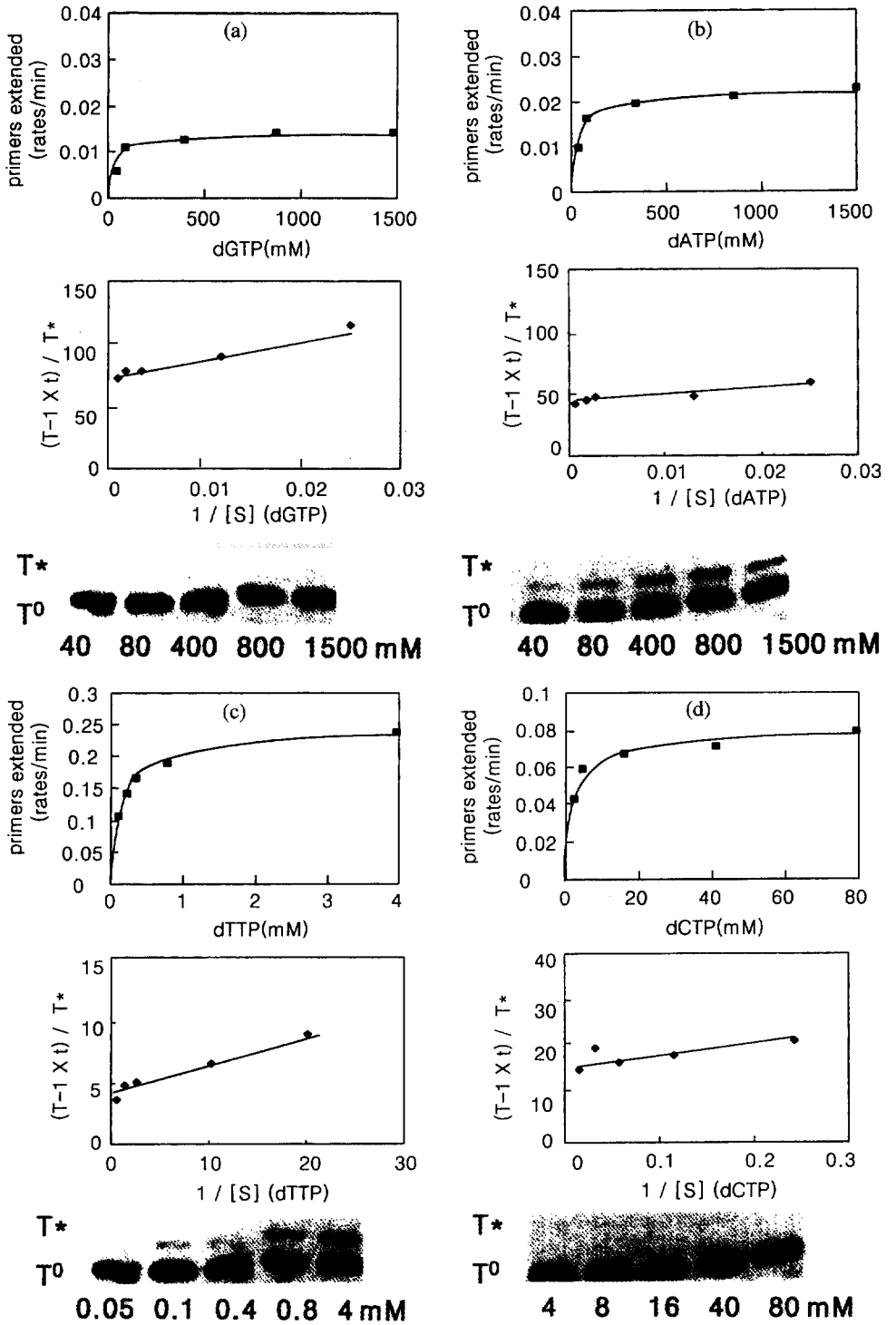

Fig. 2. Kinetic assay for site-specific nucleotide mis-incorporation to template. The 5-[^{32}P] labeled 16-mer oligonucleotide primer was annealed to template-strand A, G, T or C to produce the 3-terminal mispairs, and incubated at 37°C (for 2 and 10 min for correct and incorrect nucleotides, respectively) in the presence of increasing concentrations of single dNTP with the MBP-HBV polymerase. The only data with template-strand A are shown. Each reaction contained (a) dGTP, (b) dATP, (c) dTTP or (d) dCTP as the only deoxynucleotide triphosphate substrate as indicated. The velocity of extending a primer (band T°) by one or two nucleotides (band T*) is expressed as $v = I^*/(I^\circ+I^*)t$, where I° and I^* are band intensities measured by a phosphoimager system after t min of reaction time.

strand A are shown (Fig. 2) to avoid the overlapping of the data. Some of the reactions were inefficient so that the band T* could hardly be seen by the naked eye. However, the sensitivity of the scanner that we used was good enough to read the band. It is apparent that the incorporation with incorrect nucleotides was obtained at much higher nucleotide

Table 2. Kinetic parameters for site-specific misincorporation by the MBP-HBV polymerase, HIV-1 or MMLV RT.
The 5'-[^{32}P] labeled 16-mer oligonucleotide primer was annealed to template-strand G, A, T or C to produce the 3'-terminal mispairs, and incubated at 37°C (for 2 and 10 min for correct and incorrect nucleotides, respectively) with the MBP-HBV polymerase (see Fig. 2), HIV-1 or MMVL RT. The percent of 16-mer incorporated was quantitated. Data shown represent the average values±standard deviations obtained from a non-least square fit of the kinetics data to the Michaelis-Menten equation. Each of the experiments were performed four times for the MBP-HBV polymerase, and twice for HIV-1 and MMLV RT, respectively.
The ratio of the insertion efficiency for wrong (W) versus right (R) base pairs indicates the misinsertion frequency (*f*). The nucleotide insertion fidelity (1/*f*) of polymerase is defined as the reciprocal value of *f*. $f = (V_{max}/K_m)^W / (V_{max}/K_m)^R$ [Boosalis *et al.*, 1987]
ND; not determined

Template	dNTP	MBP-HBV polymerase			HIV-1 RT			MMLV RT		
		K_m (μM)	V_{max} (%/min)	1/*f*	K_m (μM)	V_{max} (%/min)	1/*f*	K_m (μM)	V_{max} (%/min)	1/*f*
G	G	290±100	0.007±0.001	51000	290±80	0.016±0.001	68000	ND	ND	ND
	A	350±110	0.008±0.003	54000	310±200	0.016±0.001	73000	ND	ND	ND
	T	7.9±2.5	0.048±0.002	200	3.0±0.7	0.019±0.001	600	47±10	0.060±0.002	1000
	C	0.17±0.06	0.21±0.08	1	0.01±0.003	0.038±0.001	1	0.10±0.03	0.130±0.003	1
A	G	260±120	0.014±0.002	35000	420±96	0.049±0.003	33000	ND	ND	ND
	A	140±20	0.023±0.006	11000	160±100	0.058±0.005	10000	ND	ND	ND
	T	0.12±0.05	0.230±0.016	1	0.020±0.003	0.078±0.005	1	0.30±0.03	0.025±0.001	1
	C	2.0±0.5	0.060±0.001	60	1.5±0.3	0.067±0.002	87	3.0±0.5	0.067±0.005	3
T	G	17±8	0.054±0.015	480	18.0±0.1	0.040±0.001	670	23±11	0.042±0.005	350
	A	0.13±0.05	0.20±0.02	1	0.030±0.003	0.045±0.001	1	0.10±0.05	0.065±0.010	1
	T	320±150	0.010±0.002	49000	190±13	0.038±0.0010	7500	ND	ND	ND
	C	240±100	0.009±0.001	41000	420±200	.040±0.001	15000	ND	ND	ND
C	G	0.14±0.05	0.143±0.021	1	0.03±0.02	0.044±0.001	1	0.200±0.003	0.050±0.001	1
	A	2.5±1.0	0.024±0.010	100	1.5±0.6	0.042±0.001	50	20±5	0.034±0.010	140
	T	97±5	0.007±0.001	14000	150±50	0.042±0.002	5200	ND	ND	ND
	C	86±7	0.006±0.001	14000	200±110	0.041±0.008	7100	ND	ND	ND

concentrations than the correct insertion. The apparent K_m and V_{max} kinetic values for each dNTP, calculated from the Lineweaver-Burk plots of the initial catalytic velocities versus the substrate concentrations, are summarized in Table 2. With respect to the data on HIV-1 RT, there are several reports about f_{ins} values for all 12 misinsertions (Yu and Goodman, 1992; Drosopoulos and Prasad, 1996; Wainberg *et al.*, 1996). However, we used the data of f_{ins} for HIV-1 RT obtained with the same assay condition (Materials and Methods) to compare the fidelity of MBP-HBV polymerase with that of HIV-1 RT.

The K_m value, calculated for incorrect insertion of dNTP opposite the G at template-strand G (Table 2), is about 46 to 2,000-fold higher with the MBP-HBV polymerase and about 300 to 31,000-fold higher with HIV-1 RT, compared to those values obtained for the insertion of correct dCTPs with both enzymes. In the other three sets of experiments, the ratios of K_m value for incorrect insertion versus K_m for correct insertion were compared. The MBP-HBV polymerase represented a lower ratio than HIV-1 RT (data not shown). In contrast, the apparent ratios of V_{max} values, calculated for the insertion of correct to incorrect nucleotides, are 4 to 30-fold for the MBP-HBV polymerase and about 2-fold for HIV-1 RT.

The ratio of the insertion efficiency for wrong versus right base pairs indicates the misinsertion frequency (*f*) : $f = (V_{max}/K_m)^W/(V_{max}/K_m)^R$. The nucleotide insertion fidelity of polymerase is defined as the reciprocal value of *f* (Boosalis *et al.*, 1987). The nucleotide insertion fidelity (1/*f*) of each polymerase is calculated, as shown in Table 2. It is evident from Table 2 that the MBP-HBV polymerase forms the G : T mispair with 270-fold greater efficiency than G : A mispair and with 250-fold higher efficiency than the G : G mispair. Consequently, the specificity of mismatch formation by the MBP-HBV polymerase opposite the template G, A, T or C residue is G : C>G : T>G : G>G : A. The nucleotide insertion fidelity was compared with data from the other three sets of experiments, and the following results was obtained: A : T>A : C>A : A>A : G, T : A>T : G>T : C>T : T, and C : G >C : A>C : C>C : T. Similarly, HIV-1 RT forms the G : T mispair 120-fold more efficiently than the G : A mispair and 110-fold more efficiently than the G : G mispair. Conclusively, all the results show that the transversion (purine : pyrimidine) mismatch is more efficient than the transition (purine : purine or pyrimidine : pyrimidine) mismatch. The same rule applies to HIV-1 RT. In parallel, the same sets of experiments were carried out with MMLV RT to compare the fidelity of MBP-HBV polymerase with that of MMLV RT. However, MMLV RT showed a slow rate of incorporation for all the mispairs making it difficult to

quantitate the K_m and V_{max} values. Therefore, some of the data are missing (Table 2).

Misinsertion itself is not sufficient to create mutation by a DNA polymerase without the ability to extend the preformed mispairs. Further study about the mispair extension is needed in order to evaluate the fidelity of HBV polymerase exactly.

Discussion

Errors can be generated either by direct insertion of an incorrect nucleotide or by a transient primer slippage (Perrino *et al.*, 1989; Ricchetti and Buc, 1990; Yu and Goodman, 1992). However, the molecular mechanisms governing fidelity of DNA synthesis are largely unknown. Generally, the fidelity of DNA polymerases is an outcome of the combination of the nucleotide insertion, extension, and exonucleolytic editing step (Randall *et al.*, 1987; Boosalis *et al.*, 1987; Mendelman *et al.*, 1989). Thus, retroviral RTs, which lack 3'→5' exonucleolytic activity, will incorporate noncomplementary nucleotide to generate a mispair at the 3'-terminus of the nascent strand and extend the terminal mispair. In this report, the MBP-HBV polymerase appeared to be deficient in 3'→5' exonuclease activity like all RTs studied so far. Hence, the lack of exonuclease activity in the HBV polymerase enables direct measurements of the fidelity of the DNA polymerization activity without interfering with the proofreading activity. The ratio of the insertion efficiency for wrong versus right base pairs indicates the misinsertion frequency (*f*). The nucleotide insertion fidelity (1/*f*) observed with the MBP-HBV polymerase and HIV-1 RT was between 60 and 54,000, and between 50 and 73,000, respectively, showing that they are in close range (Table 2). The nucleotide insertion fidelity of MMLV shows higher or lower value than that of the HBV polymerase and HIV-1 RT. According to Table 2, the specificity of mismatch formation by the MBP-HBV polymerase and HIV-1 RT can be divided into three groups as follows: (1) A : T, T : A>C : G, and G : C (matched pairs), (2) A : C, C : A>G : T, T : G (purine-pyrimidine mispairs), and (3) C : C, A : A, G : G, T : T>T : C, C : T> A : G and G : A (purine-purine and pyrimidine-pyrimidine mispairs). In addition, their order is (1)>(2)>(3). The general rules are: (a) purine-pyrimidine mispairs are easily inserted and extended, (b) pyrimidine-pyrimidine and purine-purine mispairs are more difficult to be extended than to be inserted, for the mispair extension reactions with DNA templates (Mendelman *et al.*, 1990). Therefore, the data on mismatch incorporation obtained with the MBP-HBV polymerase demonstrate that the enzyme shares common patterns with other polymerases examined.

As reported out earlier, misinsertion itself is not sufficient to create mutation by a DNA polymerase without the ability to extend the preformed mispairs. Further study about mispair extension will evaluate the fidelity of the HBV polymerase clearly.

HIV-1 is characterized by the high genetic variability, which may be important in the pathogenesis of HIV and in the resistance of the virus to drug therapy. In the present study, the MBP-HBV polymerase showed that it is devoid of 3'→5' exonuclease proofreading activity like HIV-RT, and has similar values of specificity and the nucleotide insertion fidelity to HIV-RT. The ORF of the polymerase gene occupies 80% of the whole genome, and the ORF of the presurface/ surface antigen is in polymerase ORF with a different reading frame. Moreover, the ORF of X gene is partially overlapped with the polymerase ORF. Even though the information on the mispair extension is not available, the MBP-HBV polymerase may show low fidelity on DNA polymerization like HIV-1 RT. However, HBV might not tolerate mutations, since one mutation will affect several amino acids due to the overlapped genes, in contrast to HIV where 10% of the HIV ORFs are overlapped.

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