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The incorporation of nucleoside analogs by human immunodeficiency virus type 1 reverse transcriptase decreases in the presence of polyamines

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Abstract Nucleoside analogs (NAs) are an important class of anti-retroviral compounds used against human immunodeficiency virus (HIV). We have analyzed the potential effect of polyamines on the incorporation of NAs during DNA synthesis by HIV type-1 (HIV-1) reverse transcriptase (RT). The polyamines exert the ability to decrease the incorporation of various dideoxynucleoside triphosphates (ddATP, ddTTP or ddCTP) with both RNA/DNA and DNA/DNA substrates in the following order: spermine > spermidine > putrescine. The reduction is a sequence-independent effect, taking place at different sequence context. The results suggest that polyamines might affect the inhibition of reverse transcription by nucleoside analogs HIV-1 RT directed.

Keywords: HIV-1; Reverse transcriptase; Nucleoside analogs; DNA synthesis; Polyamines

1. Introduction

The reverse transcriptase (RT), a key enzyme in the life cycle of the human immunodeficiency virus type 1 (HIV-1), was found to be a successful target for the treatment of AIDS [1]. HIV-1 RT is multifunctional enzyme, exhibiting several enzymatic activities by a single polypeptide. The enzyme catalyzes the transcription of the viral genomic single-stranded RNA into the double-stranded DNA in the cytoplasm by two DNA polymerase activities, RNA-dependent DNA polymerase (like cellular enzymes) and RNA-dependent DNA polymerase (unlike cellular enzymes) [2]. The enzyme carries out error-prone DNA synthesis, since it lacks an intrinsic $3' \rightarrow 5'$ proofreading exonuclease activity and copies RNA with low fidelity during both RNA → DNA and DNA → DNA replication steps, thus contributing to mutagenesis [3–7]. The high error rates of HIV-1 RT can be attributed to efficient misinsertion and extension of mispaired nucleotides during the RNA- and DNA-dependent DNA polymerization steps. HIV-1 RT readily utilizes many nucleoside analogs (NA's) of the normal deoxynucleoside triphosphate (dNTP) substrates [8]. NAs, upon entering the cell are converted into their triphosphates by cellular kinases and subsequently incorporated into DNA during replication [9,10]. The antiviral activity of NA is attributed to the preferential incorporation of their triphosphate forms into the 3′-end of viral DNA by RT leading to premature termination of DNA polymerization. The nucleoside RT inhibitors (NRTIs), representing one of the major classes of anti-HIV drugs, are incorporated into the nascent viral DNA by HIV-1 RT, inducing chain termination because they lack a 3′ hydroxyl group [8]. Although NRTIs reduce viral load in HIV-1 infected individuals, the effectiveness of NRTI therapy is limited by the rapid emergence of drug-resistant RT [11]. Mutations that arise in the *pol* gene of HIV (that encodes the RT) lead to the synthesis of RT that retain full catalytic activities, yet is highly resistant to anti-retroviral drugs [8].

The conditions during intracellular proviral dsDNA synthesis in vivo are complex. The accuracy of reverse transcription, its inhibition by NRTIs and resistance to this inhibition might be influenced by the physiological microenvironments and by cellular and/or viral factors/proteins involved in the reverse transcription complex. The accessory proteins/factors that comprise the retrovirus replication complex can interact with the viral nucleic acid, or with HIV-1 RT and/or other accessory proteins. Our recent studies showed that polyamines may affect the efficiency and specificity of mispair formation [12]. Polyamines exert the ability to reduce the misincorporation and mispair extension by HIV-1 RT with both RNA/DNA and DNA/DNA template-primers.

Polyamines are essential for cell growth. The polyamines putrescine, spermidine and spermine, present in all living cells, are low molecular weight aliphatic molecules that participate in many cellular processes by binding and modulating the functions of RNA, DNA dNTPs and proteins [13–15]. Their concentrations in cells are cell-cycle dependent [16]. The polyamines have been demonstrated to potently interact with various nucleic acids [17] and are probably involved in transcription and translation of genetic material. The polyamines have been implicated in the replication of some herpesviruses and retroviruses. The cells infected with a number of viruses (e.g. HSV-1, human cytomegalovirus) have high levels of the polyamines [18,19]. Lymphocytes from patients infected with HIV-1 also have

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Abbreviations: RT, reverse transcriptase; HIV-1, human immunodeficiency virus type-1; NRTI, nucleoside RT inhibitors; NA, nucleoside analogs; DDDP, DNA-dependent DNA polymerization; RDDP, RNA-dependent DNA polymerization

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elevated levels of all three polyamines [20]. Interestingly, spermine and spermidine are integral parts of HIV virions; polyamine incorporation into virions may be important for folding and/or packaging of viral RNA or it may mediate the interaction between viral RNA and the tRNAlys primer.

The observation that HIV-1 RT in the presence of polyamines has an enhanced fidelity of DNA synthesis, prompted us to study the effect of polyamines on the incorporation of nucleoside analogs during DNA synthesis by HIV-1 RT. Our results demonstrate that polyamines exert the ability to reduce the incorporation of various dideoxynucleoside triphosphates tested (e.g. ddATP, ddTTP or ddCTP) with both RNA/DNA and DNA/DNA template-primers. The data suggest that polyamines represent the cellular component that may have an impact on the development of resistance to NRTIs.

2. Materials and methods

2.1. Materials

The HIV-1 RT was the purified recombinant enzyme expressed in *Escherichia coli* from BH-10 proviral clone [21,22]. The specific activity of the enzyme was 4000–5000 U/μg. One unit was 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. T4 polynucleotide kinase was purchased from MBI (Fermentas). The dNTPs and ddNTPs used were of the highest purity available (MBI Fermentas) with no detectable traces of contamination by other dNTPs and ddNTPs.

2.2. Template-primers

The template-primer substrates used for assessing the incorporation of various NA's have been previously described [6,23]. Four template-primer substrates were used for the analysis of the incorporation of various dideoxynucleoside triphosphates during the DNA synthesis by HIV-1 RT. The ribosomal RNA (rRNA) (a mixture of 16S and 23S *E. coli* rRNA), as a native substrate, was primed with 16-mer oligonucleotide primers, that hybridizes to the nucleotides at positions 2012–2027 of the 16S rRNA (the sequence of the primer is 5′-ATTTCACATCTGACTT-3′) or that hybridizes to the nucleotides at positions 2014–2029 of the 16S rRNA (the sequence of the primer is 5′-GGATTTCACATCTGAC-3′). The (N)$_{30}$ synthetic oligonucleotide DNA template identical to nucleotides 2000–2029 of *E. coli* 16S rRNA (5′-AGGCGGTTTGTTAAGTCAGATGTGAAATCC-3′), was primed with an (N)$_{16}$ oligonucleotides, (utilized with rRNA template) that hybridizes to the nucleotides at positions 13–28 of the template DNA (5′-ATTTCACATCTGACTT-3′) or that hybridizes to the nucleotides at positions 15–30 of the template DNA (5′-GGATTTCACATCTGAC-3′). The primers were end labeled at the 5′-end with T4 polynucleotide kinase and [γ^{32}P] ATP. Unincorporated radioactivity was removed by using G-25 microspin columns (Pharmacia Biotech.), according to the instructions of the manufacturer. The end-labeled primers were annealed to the template RNA or DNA as described [6,23].

2.3. DNA polymerization reaction

The incubation mixture (10 μl) contained 50 mM Tris HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml BSA, 5′-end labeled substrates and nucleoside analog. The reaction products of NA incorporation were analyzed by electrophoresis through 16% PAGE, as described [5,6]. Extension of the 5′-end labeled primers was detected by autoradiography. The extension products were quantified by densitometric scanning of gel autoradiographs and the percentage of the total amounts of primers extended was calculated.

2.4. The gel retardation assay

Complex formation between the oligonucleotide DNA and HIV-1 RT was characterized by the electrophoretic retardation of DNA as a result of its association with the enzyme. The binding reaction assays were conducted as described previously [24].

3. Results

The incorporation of NA into the 3′-end of DNA is important for antiviral activity. The reduction in incorporation of the dideoxynucleoside triphosphates in DNA may decrease their potential for chain termination. Reverse transcription involves the synthesis of DNA using RNA and DNA templates to convert the plus strand genomic RNA into dsDNA [2]. Hence, it was of interest to elucidate the role of various polyamines in the incorporation of different NAs by HIV-1 RT during double-stranded DNA synthesis.

3.1. The incorporation of various nucleoside analogs by HIV-1 RT in the presence of polyamines

The incorporation of nucleoside analogs by HIV-1 RT was assessed with defined substrates in the absence or presence of 5 mM polyamines – putrescine, spermidine and spermine, using correctly-paired DNA/DNA and RNA/DNA running-start template-primers at a fixed concentration of 1.0 mM of dATP and ddCTP, allowing the extension of the 16mer primer. The results of the primer extension assays show that HIV-1 RT displays NA incorporation activity incorporating ddCTP opposite the template G at site 10 of template DNA (Fig. 1A, lane 1) or at site 2009 of template RNA (Fig. 1B lane 1) following the initial incorporation of two running-start A's prior to reaching the template target site G. Interestingly, the incorporation of ddCTP was reduced in the presence of the polyamines tested (Fig. 1A and B, lanes 2–4). Indeed, the decrease in the amount of the 19mer product was detected. We observed that the pattern of reduction in the presence of polyamines was different; spermine prevented most efficiently the incorporation of ddCTP by HIV-1 RT (Fig. 1A and B, lane 2).

The influence of the polyamines on the incorporation of dideoxynucleoside triphosphates by HIV-1 RT was further examined with two different DNA/DNA and RNA/DNA standing-start template-primers (wherein the target template residue immediately follows the 3′-terminal end of the primer), at a fixed concentration of 1 mM of ddATP (with substrate set I) or ddTTP (with substrate set II), in the absence or presence of 5 mM of spermine, spermidine or putrescine. Apparently, RT readily incorporates NAs; the 17-mer product is accumulated following the incorporation of ddATP opposite the template T at site 12 of template DNA (set I) (Fig. 1A, lane 5) or opposite template U at site 2011 of template RNA (set I) (Fig. 1B, lane 5) or following the incorporation of ddTTP opposite the template A at site 14 of template DNA (set II) (Fig. 1A, lane 9) or opposite template A at site 2013 (set II) (Fig. 1B, lane 9) by HIV-1 RT. However, the incorporation of either ddATP or ddTTP was reduced in the presence of the polyamines tested (Fig. 1A and B, lanes 6–8 and 10–12, respectively), during both DDDP and RDDP reactions. The decrease in the amount of 17mer products was detected. As with running-start substrate, the propensity of the polyamines to affect the NA incorporation capacity of HIV-1 RT, was most pronounced for spermine (Fig. 1A and B, lanes 6 and 10).

The experiments described in Fig. 1A and B, depict that HIV-1 RT with both DNA/DNA and RNA/DNA template-primers exerts lower capacity of incorporation of all dideoxynucleoside triphosphates tested in the presence of polyamines, suggesting that there is no difference in the nucleic acid nature (RNA vs DNA) of the template.

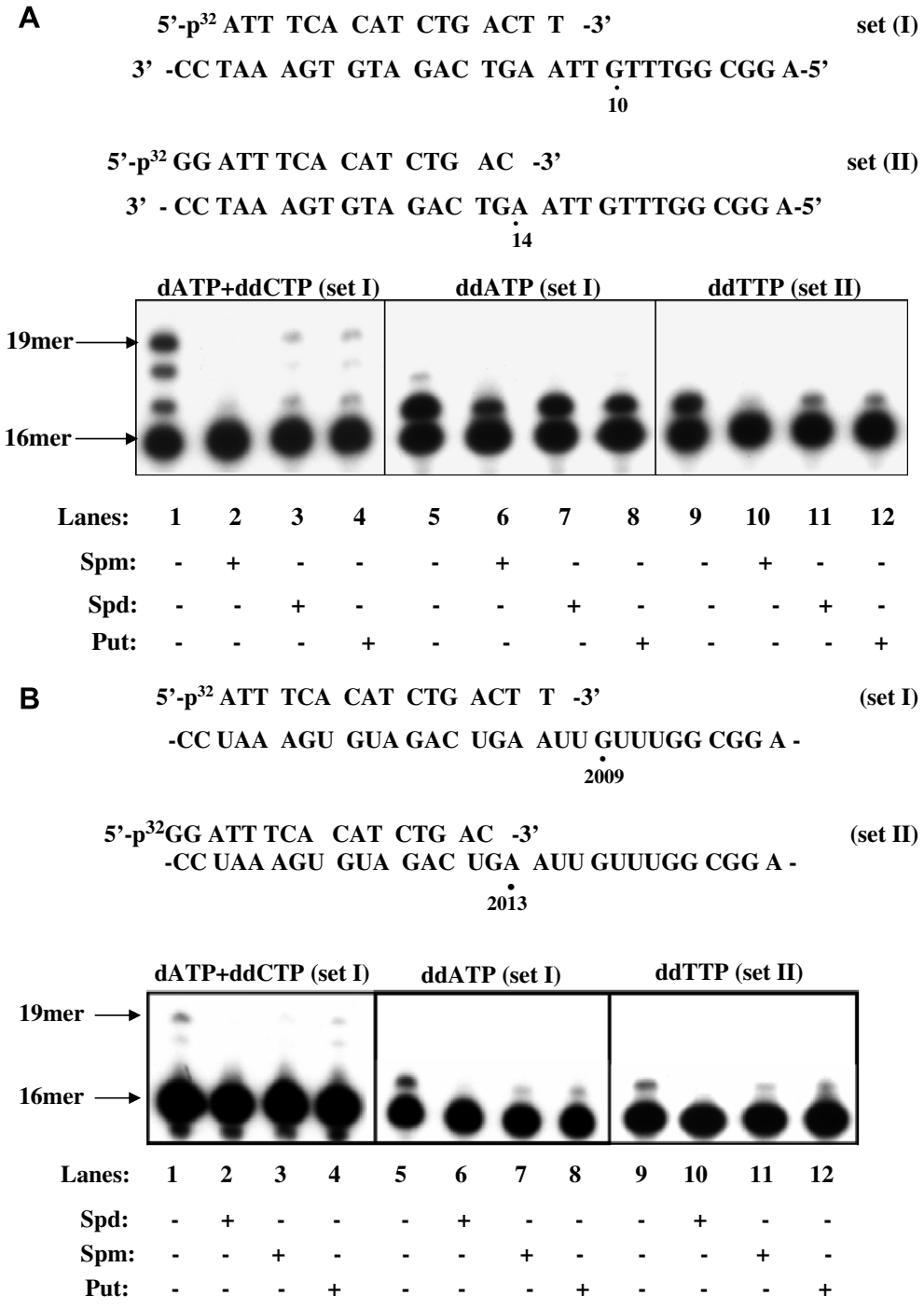

Fig. 1. Incorporation of various nucleoside analogs by HIV-1 RT. (A) The DNA/DNA template-primer (set I) was incubated with HIV-1 RT, 1 mM dATP and ddCTP in the absence (lane 1) or presence of 5 mM of spermine (lane 2), spermidine (lane 3) or putrescine (lane 4). The DNA/DNA template-primer (set I) was incubated with HIV-1 RT and 1 mM ddATP in the absence (lane 5) or presence of 5 mM of spermine (lane 6), spermidine (lane 7) or putrescine (lane 8). The DNA/DNA template-primer (set II) was incubated with HIV-1 RT and 1 mM ddTTP in the absence (lane 9) or presence of 5 mM of spermine (lane 10), spermidine (lane 11) or putrescine (lane 12). (B) The RNA/DNA template-primer (set I) was incubated with HIV-1 RT, dATP and ddCTP in the absence (lane 1) or presence of 5 mM of spermine (lane 2), spermidine (lane 3) or putrescine (lane 4). The RNA/DNA template-primer (set I) was incubated with HIV-1 RT and ddATP in the absence (lane 5) or presence of 5 mM of spermine (lane 6), spermidine (lane 7) or putrescine (lane 8). The RNA/DNA template-primer (set II) was incubated with HIV-1 RT and ddTTP in the absence (lane 9) or presence of 5 mM of spermine (lane 10), spermidine (lane 11) or putrescine (lane 12). The positions of the primers are indicated by arrows.

3.2. The efficiency of incorporation of dideoxynucleoside triphosphates by HIV-1 RT in the presence of polyamines

The above experiments provide a qualitative indication of the incorporation of dideoxynucleoside triphosphates by HIV-1 RT in the absence and presence of polyamines. Since, a comparable pattern of reduction in the incorporation of dideoxynucleoside triphosphates was observed with both DNA and RNA templates used in the presence of all three

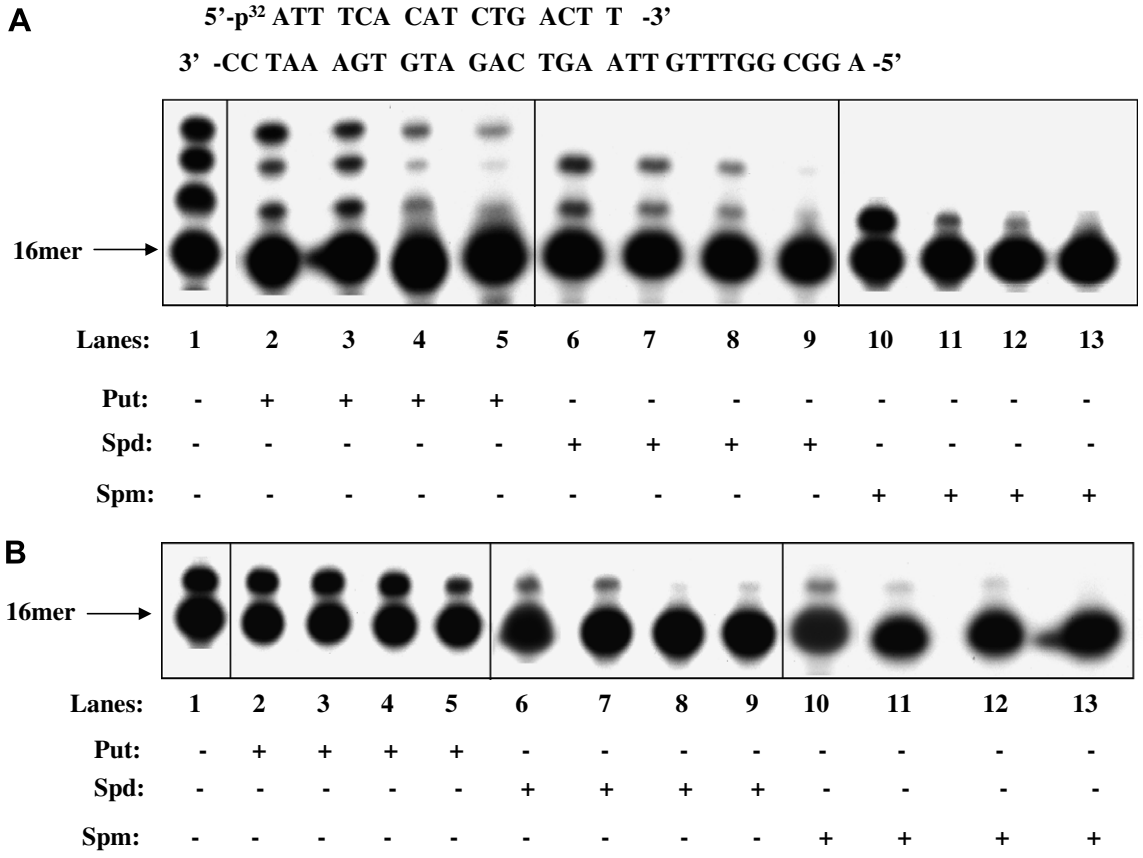

Fig. 2. Incorporation of nucleoside analogs by HIV-1 RT in the presence of various concentrations of polyamines with DNA/DNA template-primers. (A) The running-start substrate was incubated with HIV-1 RT, dATP and ddCTP in the absence (lane 1) or presence of various concentrations of putrescine (lanes 2–5), spermidine (lanes 6–9) or spermine (lanes 10-13). Lanes 2, 6 and 10-with 1 mM polyamines. Lanes 3, 7 and 11-with 2 mM polyamines. Lanes 4, 8 and 12-with 5 mM polyamines. Lanes 5, 9 and 13-with 10 mM polyamines. (B) The standing-start substrate was incubated with HIV-1 RT and ddATP in the absence (lane 1) or presence of various concentrations of putrescine (lanes 2–5), spermidine (lanes 6–9) or spermine (lanes 10–13). Lanes 2, 6 and 10 – with 1 mM polyamines. Lanes 3, 7 and 11– with 2 mM polyamines. Lanes 4, 8 and 12-with 5 mM polyamines. Lanes 5, 9 and 13– with 10 mM polyamines. The position of the 16mer primer is indicated by arrow.

polyamines tested, we have elected to use DNA template for a further quantitative comparisons. The efficiency of incorporation of dideoxynucleoside triphosphates by HIV-1 RT with both running-start and standing-start substrates was examined in the presence of various concentrations of putrescine, spermidine or spermine. The results obtained illustrate that the polyamines tested – putrescine, spermidine and spermine, cause a concentration – dependent decrease in the incorporation capacity of HIV-1 RT of ddCTP (with running-start template-primer) (Fig. 2A, lanes 2–13) and of ddATP (with standing-start template-primer) (Fig. 2B, lanes 2–13); although the pattern of reduction was different. The inhibitory effects were found for concentrations of spermine or spermidine higher than 1 mM and of putrescine higher than 5 mM.

To further characterize the influence of spermine on the incorporation of nucleoside analogs by HIV-1 RT, we have employed an assay that measures site-specific dideoxynucleoside triphosphates incorporation. We carried out a dose response experiments using standing-start template-primers for investigation of the kinetic parameters governing the incorporation frequency by HIV-1 RT in the absence or presence of 5 mM spermine. The rate of insertion of ddATP was examined opposite the template T residue at position 12 of template DNA (Fig. 3) and of ddTTP opposite the template A at site 14 of template DNA (Fig. 4). The quantitation of the primer extension bands resolved by PAGE provides a clear indication of the ability of HIV-1 RT to insert dideoxynucleoside triphosphates. Kinetics of incorporation showed that the intensity of the bands corresponding to ddATP or ddTTP-terminated DNA molecules increases as a function of ddATP or ddTTP concentrations (Figs. 3 and 4, respectively). However, the intensity of the ddATP or ddTTP-terminated products strongly decreased in the presence of spermine. We have measured the ratios of gel band intensities, estimated by densitometry and determined the extension rates (V = percentage of primer extended) as a function of ddATP or ddTTP concentrations. The kinetic parameters are summarized in Table 1. The ratio of dideoxynucleoside triphosphates incorporation efficiency of HIV-1 RT in the presence of spermine relative to that of the enzyme alone, demonstrates 33-fold and 21-fold decrease in the efficiency of ddATP or ddTTP incorporation, respectively.

3.3. *Effect of polyamines on the interaction of HIV-1 RT with DNA substrate*

During DNA synthesis the binding of HIV-1 RT to the template-primer precedes its binding to nucleoside triphosphate substrates. HIV-1 RT binds DNA with no apparent sequence

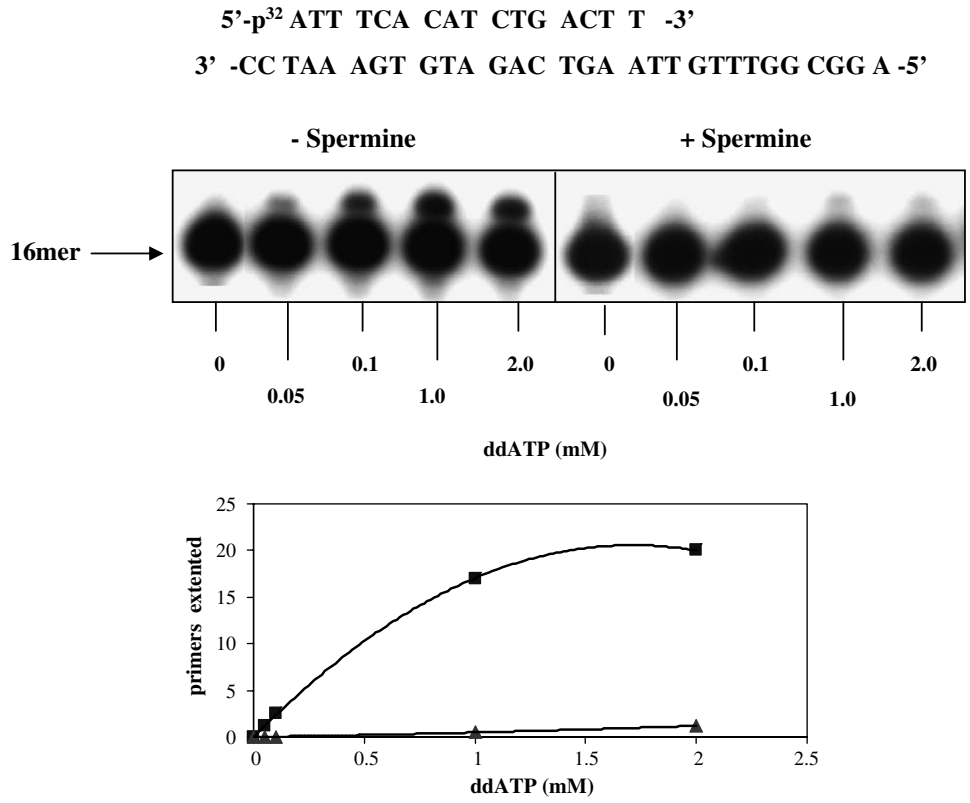

Fig. 3. Kinetics of incorporation of ddATP by HIV-1 RT in the presence of spermine. The standing-start substrate (set I) was incubated with HIV-1 RT and increasing concentrations of ddATP in the absence or presence of spermine. Each reaction contained 0, 0.05, 0.1, 1.0 or 2 mM ddATP, as the only dideoxynucleoside triphosphate substrate. PAGE analysis of the extended primers are shown in the upper panel. The quantitation of the primers extended was plotted in the lower panel after scanning the autoradiograms.

specificity [24]. The nucleoside analogs generally do not influence the binding of the RT to the template-primer. The addition of nucleoside analogs, e.g. AZT-TP or ddTTP had no apparent effect on the stability of the complex. We have investigated the interaction of HIV-1 RT with dsDNA in the presence of polyamines. The gel retardation assay was utilized to examine the effect of polyamines (putrescine, spermidine or spermine) on the enzyme–DNA interaction. The results of the experiment depicted in Fig. 5, demonstrates complex formation between HIV-1 RT and DNA by the electrophoretic retardation of the DNA, as a result of its association with the enzyme (lane 2). When the reactions were performed in the presence of 5 mM putrescine (lane 3), spemidine (lane 4) or spermine (lane 5), there was no significant change in the intensity of the bands corresponding RT–DNA complex.

4. Discussion

The effectiveness of the NRTI therapy depends on (i) the uptake of the NA into the cell; (ii) the conversion of the NA to the triphosphate form; (iii) the incorporation of the NA into the DNA; and (iv) the removal of the incorporated chain terminator [8]. The focus of our present comparative study was to assess the incorporation of various dideoxynucleoside triphosphates by HIV-1 RT in the presence of polyamines using an in vitro reconstituted system. The results obtained suggest that polyamines possess the potential to reduce the number of various dideoxynucleoside triphosphates incorporated at the 3′-termini in DNA that block elongation by HIV-1 RT during both RNA → DNA and DNA → DNA polymerization steps. HIV-1 RT could be sensitive to changes in the polyamines concentration. Indeed polyamines exert a concentration-dependent reduction effect. The degree of reduction correlates with cationic charge; divalent putrescine is least effective, whereas tetravalent spermine is more potent than trivalent spermidine. Remarkably, spermine was detected as a major natural intracellular compound protecting DNA from free radical attack [25]. The current study shows that the polyamines display the ability to reduce the incorporation of nucleoside analogs by HIV-1 RT in the following order: spermine > spermidine > putrescine. An important biochemical factor is the degree by which accessory factors affect the NA incorporation process during DNA synthesis. The comparative data presented in Table 1 indicate 33-fold for ddATP and 21-fold for ddTTP reduction in the efficiency of incorporation by the enzyme in the presence of spermine. Notably, the incorporation of ddATP (purine NA) seems to be better suppressed by spermine than the incorporation of ddTTP (pyrimidine NA). Additional studies are needed to further evaluate whether the extent of influence of the polyamines on the incorporation of NA depends on the nature of NA (purine vs pyrimidine).

The first step in DNA synthesis involves physical binding of the enzyme to the DNA substrate, followed by binding of the appropriate dNTP to the RT–DNA complex and subsequent incorporation of the nucleotide. We have shown by gel retardation assay, that polyamines have no substantial influence on the formation or stability of the RT–DNA complex, which

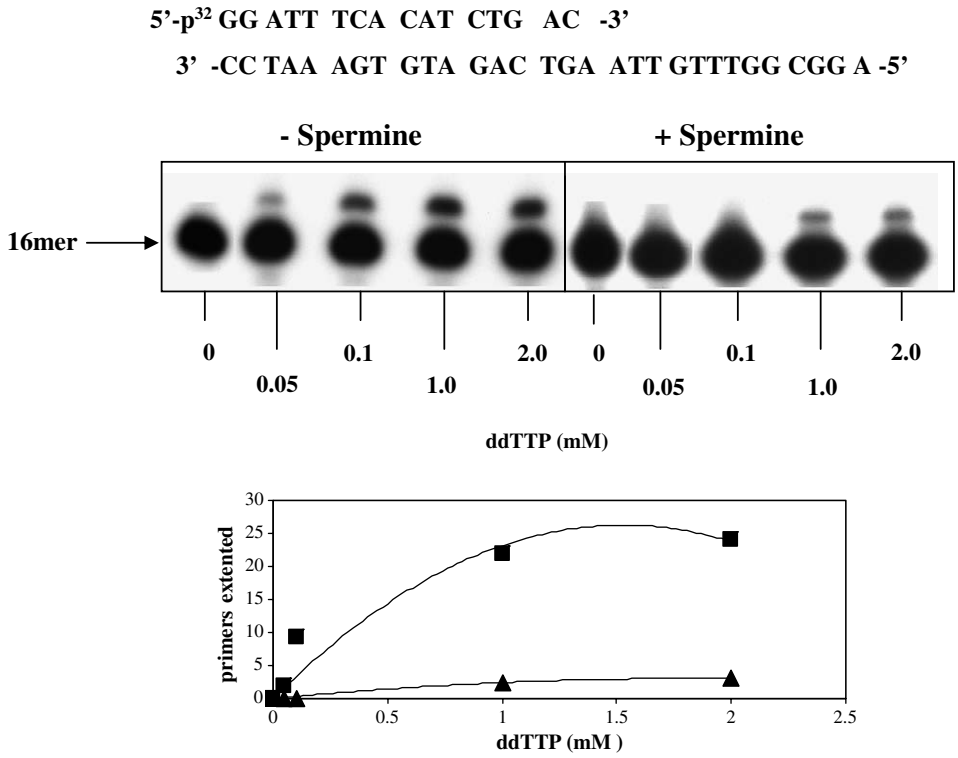

Fig. 4. Kinetics of incorporation of ddTTP by HIV-1 RT in the presence of spermine. The standing-start substrate (set II) was incubated with HIV-1 RT and increasing concentrations of ddTTP in the absence or presence of spermine. Each reaction contained 0, 0.05, 0.1, 1.0 or 2 mM ddTTP, as the only dideoxynucleoside triphosphate substrate. PAGE analysis of the extended primers are shown in the upper panel. The quantitation of the primers extended was plotted in the lower panel after scanning the autoradiograms. The primer position is indicated by arrow.

Table 1
Kinetics of incorporation of ddATP and ddTTP by HIV-1 RT in the absence and presence of spermine

Nucleoside analog incorporated	Wild-type HIV-1 RT			Wild-type HIV-1 RT in the presence of 5 mM spermine		
	K_m (mM)	V_{max} (%/min)	V_{max}/K_m	K_m (mM)	V_{max} (%/min)	V_{max}/K_m
ddATP	0.4 ± 0.03	2.0 ± 0.17	5	0.78 ± 0.072	0.12 ± 0.09	0.15
ddTTP	0.21 ± 0.03	2.5 ± 0.3	12	0.52 ± 0.06	0.3 ± 0.04	0.57

The kinetic K_m and V_{max} values were determined from Figs. 3 and 4, as described in Materials and Methods. The relative nucleoside analog incorporation efficiency are ratios of rate constant (V_{max}/K_m) for the incorporation of nucleoside analog by the HIV-1 RT in the absence of spermine, divided by the corresponding rate constant for the incorporation of nucleoside analog by the enzyme in the presence of spermine.

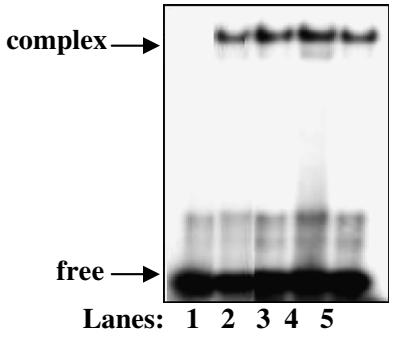

Fig. 5. Effect of polyamines on the complex formation between HIV-1 RT and DNA. Incubation mixtures contained 5′-end labeled DNA/DNA substrate (set I) (lane 1). The binding activity of HIV-1 RT was analyzed in the absence (lane 2) and presence of 5 mM of putrescine (lane 3), spermidine (lane 4) or spermine (lane 5). Arrows to the left of the figure indicate the migration positions of the enzyme-DNA complex (complex) and the DNA substrate alone (free).

is the first step in DNA polymerization (Fig. 5). Direct binding of polyamines to DNA and their ability to modulate DNA–protein interactions appear to be important in the molecular mechanisms of polyamine action [14]. Apparently, polyamines inhibit the incorporation of nucleoside analogs during subsequent steps in DNA polymerization.

The incorporation sites of various dideoxynucleoside triphosphates tested were located at different sequence contexts. Our data indicate that decreased dideoxynucleoside triphosphates incorporation in the presence of polyamines was a general phenomenon, taking place at all sites. Thus, the decrease in efficiency of nucleoside analogs incorporation in the presence of polyamines is probably sequence-independent effect.

Polymerization errors produced during DNA synthesis by RT, which lacks a proofreading activity, are responsible for generation of genetic variations. There might be a potential correlation between the fidelity of DNA synthesis, which in the case of base substitution is an outcome of the ability to incorporate a wrong nucleotide and to elongate the 3′ mis-

pairs, and resistance of HIV-1 RT to nucleoside analogs (the ability of the enzyme to incorporate or reject an incoming deoxynucleoside analog). Interestingly, the mutation rate of HIV-1 in cells is about 15–20-fold less than that predicted by the measured fidelity of purified HIV-1 RT with the same target sequence, suggesting that the reverse transcription process in vivo is less error-prone than in cell-free studies [26]. Furthermore, NRTIs are less potent inhibitors of HIV replication in vivo than might be expected. It is conceivable that discrepancies between the two systems may be due to several endogenous external factors, including the association of viral and/or cellular accessory proteins/factors that can affect the reverse transcription process, its inhibition by NRTIs and resistance to this inhibition in the cytoplasm of infected cells. The HIV-1 Vpr protein was proven to be an example of viral accessory protein that influences the in vivo mutation rate (decreases up to 5-fold) [27]. Cellular tumor suppressor protein p53 in cytoplasm may functionally interact with HIV-1 RT, acting as an external proofreader for mispairs (thus increasing the fidelity of DNA synthesis by HIV-1 RT) [28] and chain terminators incorporated during proviral DNA synthesis (thus reducing the efficiency of anti-HIV drugs) [29]. p53 by its intrinsic exonuclease activity, probably represents the cellular component that may have an impact on the development of resistance to anti-retroviral compounds. Recent studies suggested that the differences in the intracellular free Mg^{2+} concentrations between different cell types or during the cell cycle might strongly affect HIV-1 replication and its inhibition [30]. In mammalian cells polyamines together with magnesium ions account for the majority of the intracellular cationic charges. Our recent studies revealed that polyamines could improve the replication fidelity of HIV-1 RT [12]. The fact that polyamines may enhance the accuracy of HIV-1 RT and may decrease the amount of incorporated NAs, suggests that the fidelity of DNA synthesis by the enzyme and the inhibition by NAs may be affected by alterations in composition of the replication complex. The viral or cellular factors/proteins might have a relatively transient interaction with the reverse transcription complex during various steps in the replication process (interaction with the viral nucleic acid or with RT). The natural polyamines are found in every living cell in micromolar to millimolar quantities. Cellular polyamines are regulated by a complex circuitry of synthesis, degradation as well as cellular uptake and efflux [14]. The increased intracellular polyamine levels have been observed in patients infected with HIV-1 [20]. It is not clear whether the increase in polyamines is a direct or indirect result of HIV-1 infection. The difference in polyamine intake as well as changes in the environment for polyamine synthesis affects polyamine levels. We suggest that alterations in polyamines levels within the cells may affect the accuracy of the reverse transcription process and its inhibition by nucleoside analogs in viral target cells.

The development of viral resistance to anti-retroviral drugs used in the treatment of HIV infection is a significant cause of therapy failure [31–34]. The low fidelity of reverse transcription can lead to changes resulting in the synthesis of new RT molecules resistant to antiviral drugs. It is conceivable that mutants are capable of developing diminished susceptibility to NRTIs by several mechanisms [8]. Some of these mechanisms are associated with an elevation in the accuracy of DNA synthesis by HIV-1 RT. A possible non-viral mechanism that achieves resistance to NRTI would be the reduction in the incorporation of NRTI or the removal of nucleoside analog once it has been incorporated at the 3′ end of the primer by external cellular protein/factor, e.g. proofreading by $3' \rightarrow 5'$ exonuclease activity of cytoplasmic p53 [28,29]. The failure of the HIV-1 RT to incorporate NA in the presence of polyamines may reduce the sensitivity to treatment in vivo. Thus, the increase in the accuracy of DNA synthesis by HIV-1 RT in the presence of polyamines [12] and the reduction in the amount of incorporated NAs at the 3′ end of the primer, suggest that polyamines could confer a cellular resistance mechanism to the antiviral compounds. In view of the significance of mutations of HIV-1 RT to resistance to therapeutic agents, the possible effects of polyamines on incorporation of wrong nucleotides and nucleoside analogs could be of particular importance. Further studies are important for the understanding of the resistance developed with wild-type and mutant HIV-1 RTs, which is an important issue for the successful treatment of HIV treatment.

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