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INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE BY DEFINED TEMPLATE/PRIMER DNA OLIGONUCLEOTIDES: EFFECT OF TEMPLATE LENGTH AND BINDING CHARACTERISTICS

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The interaction of partially double stranded DNA oligonucleotides with HIV-1 RT was studied by investigating their ability to inhibit the homopolymeric poly(rC) directed (dG) synthesis reaction. A 20/18mer oligonucleotide, with a sequence based on the Lys^3-tRNA primer region, showed stronger inhibition of the homopolymeric RT reaction than a G/C rich oligonucleotide series lacking or possessing a hairpin moiety. Interaction of the enzyme with the G/C rich oligonucleotides, as determined by IC_{50} measurements, was insensitive to the extent of the unpaired template region at the 3' or 5' position. Addition of a hairpin moiety, composed of four thymidine bases, onto G/C rich oligonucleotides increase their inhibitory potency (at least six times) and shifted the mode of inhibition of RT to competitive with respect to poly (rC).(dG), which was otherwise mixed (competitive/noncompetitive) for the linear G/C rich and 20/18mer oligonucleotides. The results indicate that interaction of the enzyme with the primer/template stem, but not with the unpaired template region, is an important step in complex formation.

KEY WORDS: HIV-1, reverse transcriptase, inhibition, oligonucleotides

INTRODUCTION

Proliferation of the Human Immunodeficiency Virus (HIV-1) is dependent on the activity of the enzyme reverse transcriptase (RT), which is crucial for viral genome replication[1]. During this process 18 nucleotides at the 3' end of Lys^3-tRNA are utilized to prime the viral RNA dependent DNA synthesis, by annealing to a complementary site on the viral RNA, termed the Primer Binding Site. The 3' OH of the

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Abbreviations: AIDS, acquired immune deficiency syndrome; HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase, RNase 'H', ribonuclease H; dNTP, deoxynucleoside 5'-triphosphate

Lys^3-tRNA then initiates nascent DNA synthesis through the activity of RT[2,3]. HIV-1 RT, is a heterodimer, composed of two subunits with molecular mass of 66 and 51 kDa. HIV-1 RT catalyses at least three distinct reactions: RNA dependent DNA synthesis (reverse transcription), hydrolysis of the viral RNA template (ribonuclease 'H' activity) and DNA dependent DNA synthesis, to produce pro-viral DNA[4,5].

Earlier kinetic studies have demonstrated that the RNA dependent DNA synthesis proceeds through an ordered mechanism in which template primer combines with the free enzyme to form the first complex in the reaction pathway[6]. This binding is competitively inhibited by free primer and it has been suggested that the free enzyme recognizes the primer rather than the template during enzyme-template/primer complex formation[7,8]. Moreover, previous studies have suggested downregulation of RT activity at high concentration of dNTPs, which have been shown to act both as substrates and as non-competitive inhibitors[9,10].

HIV RT has been the prime target for anti-HIV therapeutic drugs such as Zidovudine (azidothymidine) and dideoxyinosine[11]. However, relative toxicity of these nucleoside analogues and emergence of viral strains resistant to these inhibitors has limited their use in treatment of AIDS. Several classes of non-nucleoside inhibitors have been described to date and these include derivatives of TIBO compounds[12], nevirapine[13], pyridinone[14,15] and bisarylpiperazine[16]. Thus far only few oligonucleotides DNA analogues, such as phosphorothioate oligodeoxynucleotides[17], have been described as inhibitors of HIV-1 RT and hence, as potential anti-viral agents.

Significant interest has been shown in attempting to delineate the mechanism of inhibition of DNA polymerases, including HIV-1 RT by synthetic polynucleotides. Understanding interactions of oligonucleotides with RT will aid in rational, oligonucleotide based, drug design and in structural analysis through the formation of enzyme/substrate complexes suitable for X-ray crystallographic studies. To this end, we have used a number of synthetic, template/primer DNA oligonucleotide analogues to inhibit the poly(rC) directed (dG) synthesis reaction by HIV-1 RT. Particularly, we have examined the primer/template requirement for the enzyme by monitoring the potency of inhibition by the synthetic oligonucleotides, as a function of unpaired template length. Furthermore, the effect of a nucleic acid secondary structure ('hairpin' moiety) on inhibition of the enzyme was also investigated.

MATERIALS AND METHODS

Purification of RT

Recombinant HIV-1 RT (p66) cloned from the coding region of the Pol gene (LAV) and expressed in *Escherichia coli*[18] was purified by immunoaffinity chromatography essentially as described by Tisdale *et al.*[19]. Heterodimeric RT (p66/p51) was generated by chymotryptic digestion and purified by hydrophobic chromatography[20]. RT was concentrated using an amicon (PM-30) and stored at -70°C until used.

Reverse Transcriptase Assay

Reverse transcriptase activity was assayed by following the incorporation of [^{P}3H]-dGTP onto a poly(rC).(dG) template primer (rC.dG), using a conventional filter

binding assay. The enzymic reaction was carried out at 37°C in 50 mM Tris-HCl pH 8.0, 1 mM DTT, 5 mM $MgCl_2$, 0.05% nonidet P-40, 5 μM [^{P}3H]-dGTP (2 Ci/mmole) and 5 μg/ml rC.dG, in a total volume of 50 μl. At the end of each incubation period, an aliquot (40 μl), was pipetted onto a 3MM filter disc which was subsequently washed five times (5 min. each time) in 10% TCA, containing 10 mM sodium pyrophosphate. After allowing to air dry, discs were resuspended each in 10 ml of scintillant and counted to determine the incorporation of dGMP.

Oligonucleotide Synthesis and Annealing

Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer (model 380 B). After releasing with ammonium hydroxide, oligonucleotides were precipitated with chilled ethanol, and collected by centrifugation. After further ethanol precipitations, the DNA oligonucleotides were dissolved in de-ionised water and their concentration estimated spectrophotometrically by measuring the absorbance at 260 nm. The oligonucleotide concentration was calculated considering that one unit of absorbance at 260 nm is equivalent to 20 μg/ml for single stranded DNA and 50 μg/ml for double stranded DNA.

Equimolar amounts of complimentary oligonucleotide were annealed by boiling for 5 min. in annealing buffer (20 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$ and 5 mM NaCl) and gradually allowing to cool to room temperature. The molar concentration of annealed oligonucleotides was estimated, by taking into consideration the single and double stranded composition of each oligonucleotide, according to the following formula:

$$\text{Conc. } (\mu\text{g/ml}) = [(P \times A_{260} \times 50) + (F \times A_{260} \times 20)]/T$$

where P = number of paired bases, A_{260} = absorbance at 260 nm, F = number of unpaired bases, T = total number of bases.

Molar concentration were estimated by assuming that the molecular weight of each oligonucleotide can be calculated according to the following formula:

$$\text{M.wt.} = (\text{Total number of bases}) \times 310.$$

Three series of partially double stranded DNA oligonucleotides were tested for their ability to inhibit the poly(rC) directed (dG) RT reaction. The sequence of these oligonucleotides is shown in Table 1. The first series consist of a 20mer template hybridized to an 18mer primer whose sequence is analogous to the Lys_{P_3}-tRNA primer binding site sequence. This series consist of two members termed 20/18mer and XX/18mer whose sequence differs only by one nucleotide at the 5' end of the template. The second series is composed of a G/C rich 9mer primer hybridized to a G/C rich template with variable numbers of unpaired adenosines (ranging from 6 to 19) at either the 5' or 3' end. Members of this series shall be refered to as linear

TABLE 1
Sequences of oligonucleotides studied

20/18mer	XX/18mer	Linear G/C rich series n=6-19	Hairpin G/G rich series n=6-19
CG	CG	GC	TT
AT	AT	GC	TT
GC	GC	GC	GC
GC	GC	CG	GC
GC	GC	GC	GC
AT	AT	GC	CG
CG	CG	CG	GC
AT	AT	AT	GC
AT	AT	AT	CG
GC	GC	$(A)_n$	AT
CG	CG		AT
CG	CG		$(A)_n$
CG	CG		
GC	GC		
CG	CG		
GC	GC		
GC	GC		
TA	TA		
A	A		
A	C		

G/C oligonucleotides. Finally, the third series consists of self complementary G/C rich oligonucleotides, identical to the second series, except for the direct linkage of the template/primer stem through a hairpin moiety generated by four thymidine bases. The estimated Tm and Ti for the G/C rich oligonucleotides (assuming NaCl concentration of 50 mM, half of that used for the RT assay in this study) were 63°C and 48°C respectively. Data analysis for the RT assays was performed by taking an average of two independent studies.

RNase 'H' Assay

RNase 'H' activity of RT was assayed at 37°C in 50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT and [^{35}S]-labelled RNA/DNA hybrid substrate. The hybrid substrate was constructed by RNA synthesis on an M13 (mp18) template, primed with universal primer, using RNA polymerase (Type I from *E. coli*- Sigma) in the presence of ATP, GTP, CTP and [^{35}S]-UTP. After hydrolysis, the substrate was acid precipitated on to glass fibre filter discs (GF/C-Whatman), using 5% (w/v) TCA. Each disc was

washed five times prior to scintillation counting. The use of TCA does not cause any noticeable loss of oligonucleotides, as results obtained with TCA are similar to ones obtained using binding on ion exchange filters (our own observation).

RESULTS

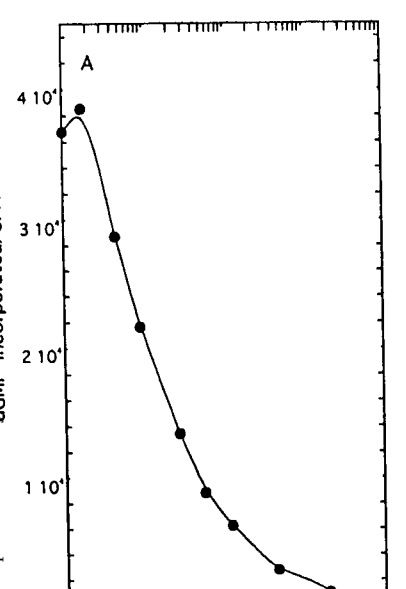
The interaction of synthetic DNA oligonucleotides with HIV-1 RT was investigated by studying their ability to inhibit the poly(rC) directed (dG) synthesis reaction.

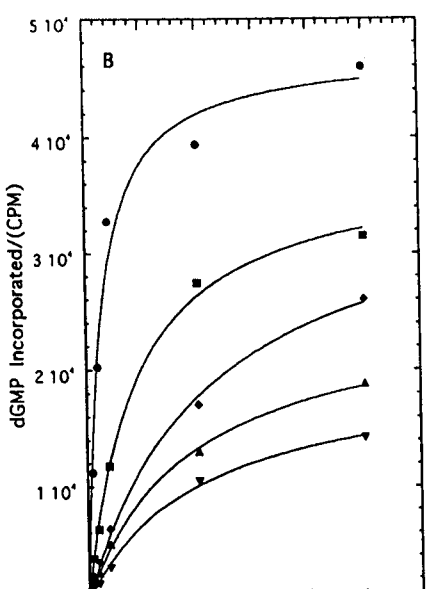
Interactions of 20/18mer and XX/18mer with RT

The 20/18mer an XX/18mer oligonucleotides acted as a substrate for RT with a K_m of 28.9 μM and 44.1 μM respectively (not shown). The variation in K_m between the two oligonucleotides could be due to the one base difference in template sequence at the 5' end. Variations in kinetic constants for HIV-RT with varying base sequence of template/primer have been previously reported. The binding characteristics of one of these oligonucleotides (XX/18mer) with RT was studied by measuring inhibition with respect to the homopolymeric template primer (rC.dG). A typical inhibition curve is shown in Figure 1(a) and this yields an IC_{50} for inhibition of 1.5 μM. In order to determine the mode of inhibition with this oligonucleotide, the velocity of the reverse transcription reaction was measured in the presence of varying concentrations of poly(rC).(dG) and XX/18mer. Typical saturation curves are shown in Figure 1(b) and the replots from these curves are given in Figure 1(c). Such replots whereby the straight lines from plotting [I] vs. K_m/V_{max} and [I] vs. $1/V_{max}$, intersect at the same ordinate are typical of linear mixed type inhibition[22] whereby $\alpha > 1$ and $\beta = 0$. The intercepts of the replot straight lines at the abscissa give an estimated value for K_i and αK_i of 0.2 and 4.8 μM respectively and a value for α of about 21. This suggests that the competitive element in this type of inhibition is stronger than the non-competitive element. It also indicates that the enzyme may have two potential binding sites for the oligonucleotide, one of which is the template/primer binding site and a secondary site where the oligonucleotide binds and non-competitively inhibits the homopolymeric synthesis reaction. Therefore, this oligonucleotide seems to be able to bind to the free enzyme and to the enzyme/template primer complex.

Interaction of linear G/C oligonucleotides with RT and effect of template length

We have also studied the interaction of a set of partially double stranded oligonucleotides that has no sequence specificity, but rather is rich in guanine and cytosine bases. This set of oligonucleotides had a feature in that the unpaired adenine region at the 5' end of the template, varied in length between its members. The inhibitory potency of these oligonucleotides was studied, and only slight variation in the IC_{50} values were observed with increasing template length [Figures 2(a) and 2(c)], suggesting no influence of the length of the template's 5' end on binding strength of these oligonucleotides to HIV-1 RT. The measured IC_{50} values for these oligonucleotides





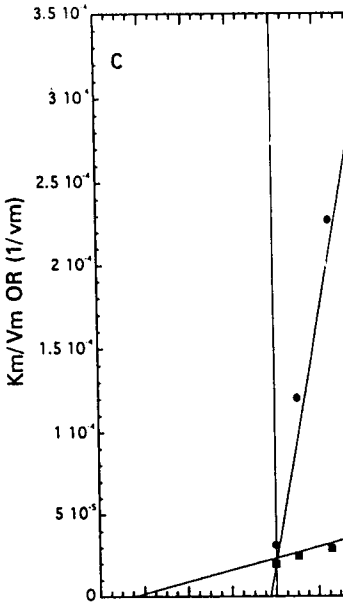


FIGURE 1 Kinetics of Inhibition of RT reaction with XX/18mer. (A) Typical inhibition curve showing the effect of [XX/18mer] on the velocity o reaction. (B) Saturation curves of the RT reaction in the presence of varying concentrations of the inhibitor XX/18mer. Inhibitor concentrations (•), 1.1 (■), 2.6 (♦), 4.6 (▲) and 8.7 μM (▼). Curves were obtained by fitting raw data to the Michaelis-Menten equation. (C) Replots of the da (B) to calculate K_i and αK_i. The figure shows a plot of [I] vs. Km/Vm (•) [I] vs 1/Vm (■).

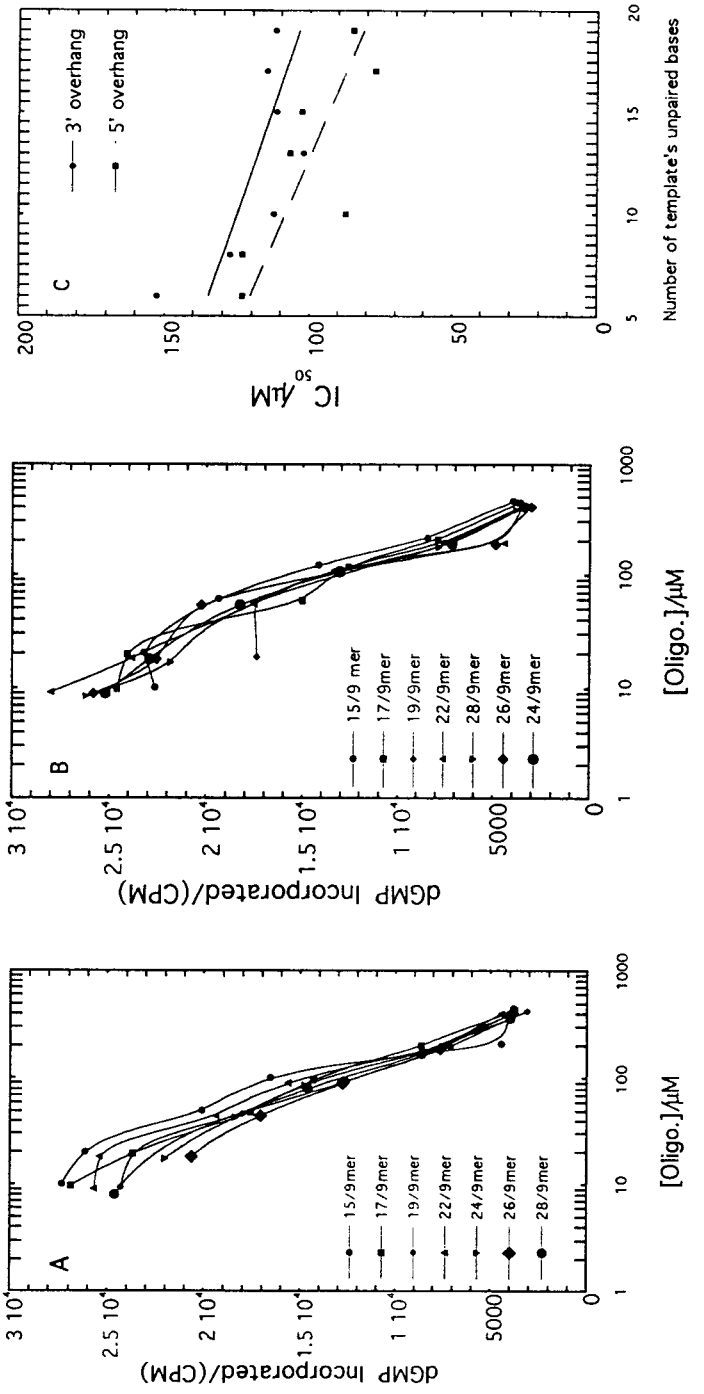


FIGURE 2 Inhibition of RT reaction with linear G/C rich oligonucleotides. (A) Typical inhibition curve showing the effect of G/C rich oligonucleotides, possessing 5' overhang, on the velocity of the RT reaction. (B) Typical inhibition curve showing the effect of G/C rich oligonucleotide, possessing 3' overhang, on the velocity of the RT reaction. (C) Effect of unpaired template length of oligonucleotides in (A) and (B) on potency of inhibition.

were higher than those obtained for the XX/18mer, indicating that either the sequence specific oligonucleotide interacted much stronger than these G/C rich oligonucleotides or that stronger binding was a reflection of the longer double stranded region in the XX/18mer. Similarly, when the same experiment was repeated with 'anti-sense' oligonucleotides that had the template's unpaired adenine region at the 3' end, the calculated IC_{50} values did not vary with template length and were similar to the IC_{50} values of the oligonucleotides with the unpaired adenine region at the 5' end [Figures 2(b) and 2(c)]. This suggests that the length of the template's single stranded region at the 3' or 5' end of the DNA oligonucleotides' stem does not significantly influence their interaction with HIV-1 RT.

The mode of inhibition for a representative oligonucleotide of this series (28/9mer) was determined from the saturation curves shown in Figure 3(a). The replots were again typical of a mixed competitive inhibitor with a K_i and αK_i of 24.5 and 67.5 μM giving a value for α of about 2.8. The K_i and αK_i were higher than those observed for the XX/18mer confirming weaker interaction of these G/C rich oligonucleotides with RT [Figure 3(b)].

Interactions of non-linear G/C oligonucleotides with RT, effect of template length and 'hairpin' moiety:

The above experiments were repeated with identical oligonucleotides, that had a 'hairpin' moiety added to the stem through the addition of four thymidine bases to the sequence[23]. Inhibition with these 'hairpin' oligonucleotides was at least six times (and up to twelve times) stronger than that observed with the equivalent linear series [Figure 4(a)]. As expected, the inhibition potency was not influenced by the unpaired template region [Figure 4(c)]. Similar results were obtained when the same experiments were repeated with oligonucleotides that had the template's unpaired adenine region at the 3' end [Figures 4(b) and 4(c)]. This further confirms the lack of influence of the template's unpaired region on binding to RT.

When the mode of inhibition for a representative of this series (32mer) was determined, the replots from the saturation curves shown in Figure 5(a), did not give, to our surprise, a pattern typical of mixed inhibition, but rather, the mode of inhibition was competitive with respect to poly(rC).(dG) [Figure 5(b)] with an estimated K_i of 16.5 μM. This suggests that the presence of a 'hairpin' moiety both strengthens the inhibition and excludes the binding of the oligonucleotide on RT to a single site, thereby rendering the inhibition competitive.

Effect on RNase 'H' activity

Finally, we studied the inhibition of the RNase 'H' activity of RT by the XX/18mer and the 32mer 'hairpin' oligonucleotides. The results presented in Figure 6 show that both oligonucleotides inhibit this activity with IC_{50} values of 9 and 25 μM respectively. The value are higher than what was observed for the reverse transcriptase activity of RT, suggesting stronger interaction with the reverse transcription active site. However, this difference could also be attributed to the difference in substrates used for assaying the two enzymatic activities (homopolymeric for RT and heteropolymeric for RNase 'H'.

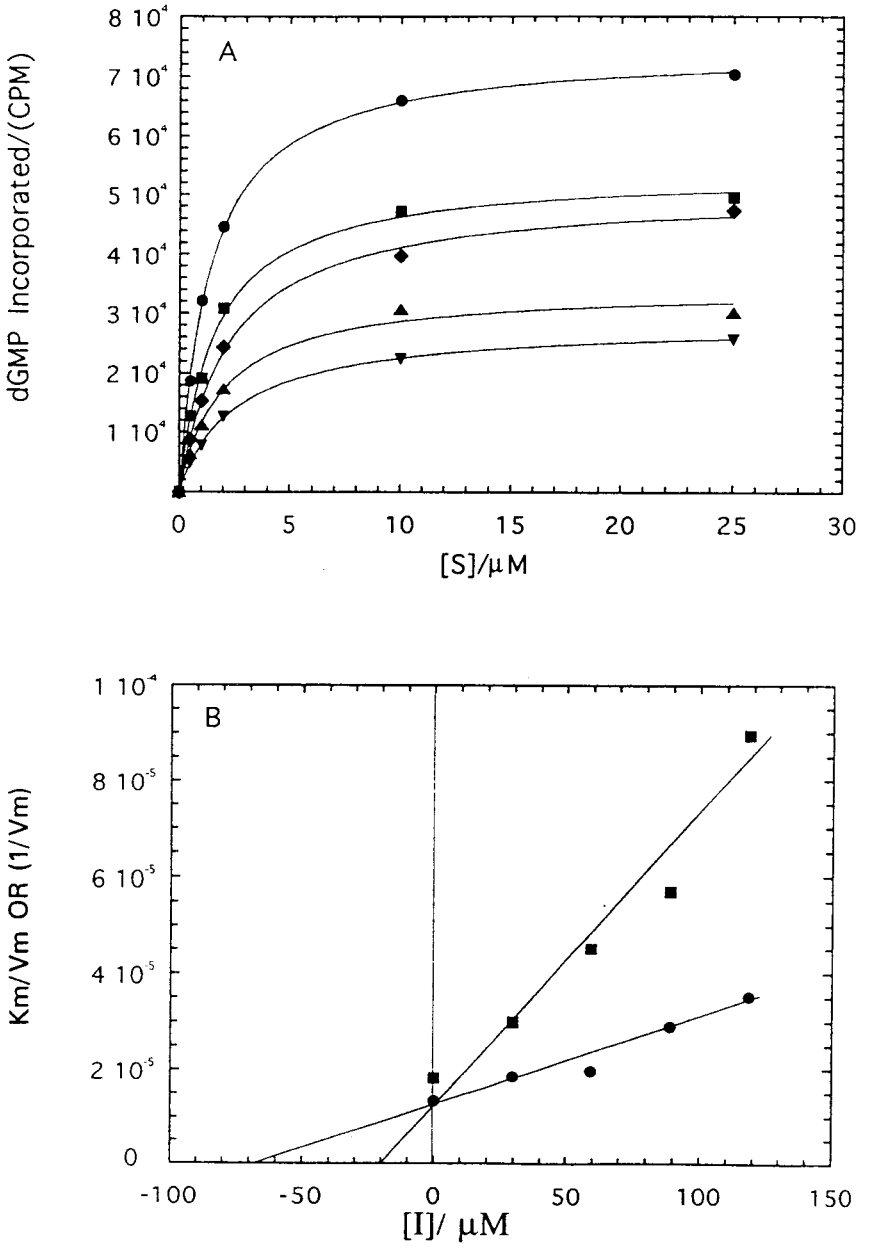


FIGURE 3 Kinetics of Inhibition of RT reaction with linear G/C rich oligonucleotides. (A) Saturation curves of the RT reaction in the presence of varying concentrations of the inhibitor 28/9mer. Curves were obtained by fitting raw data to the Michaelis-Menten equation. I=0 (●), I=29.6 μM (■) I=59.2 μM (♦), I=88.7 μM (▲), I=118 μM (▼). (B) Replots of the data from (A) to calculate K_i and αK_i. The figure shows a plot of [I] vs. Km/Vm (●) and [I] vs 1/Vm (■).

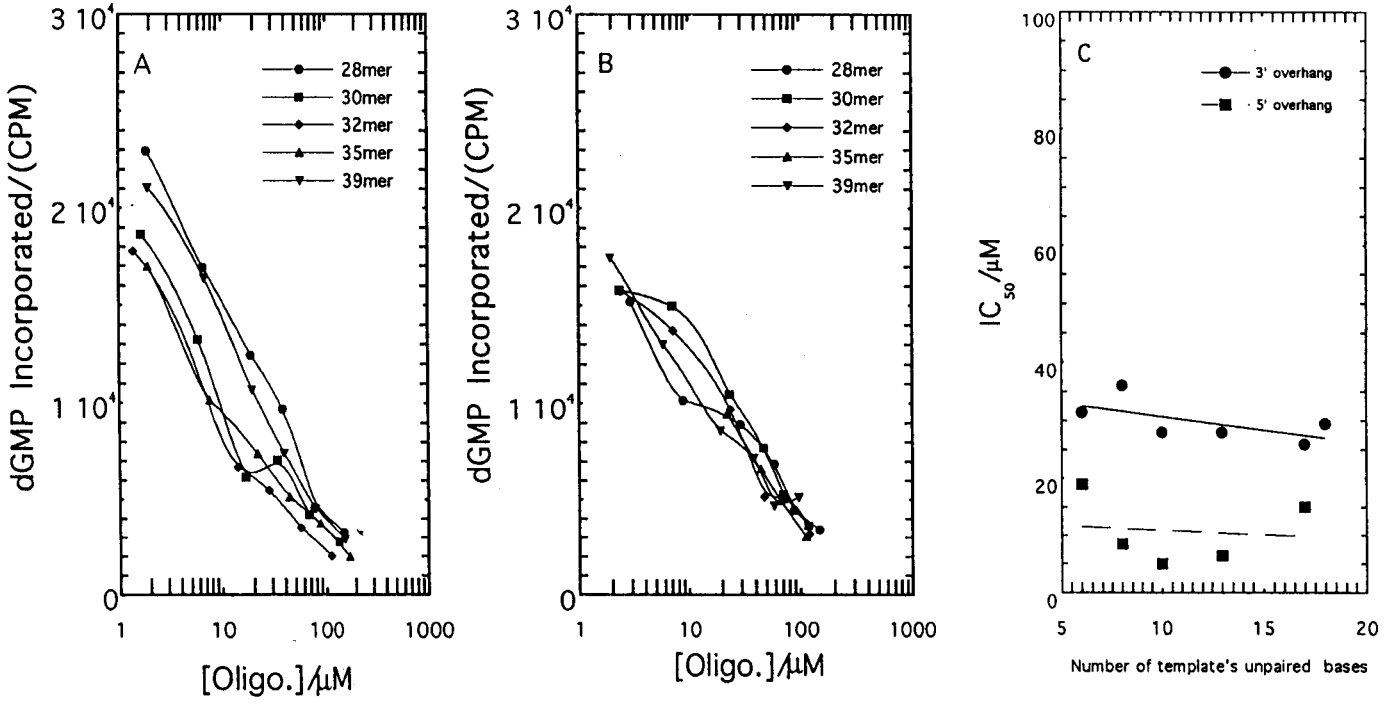


FIGURE 4 Inhibition of RT reaction with 'hairpined' G/C rich oligonucleotides. (A) Typical inhibition curve showing the effect of G/C rich oligonucleotides, possessing 5' overhang, on the velocity of the RT reaction. (B) Typical inhibition curve showing the effect of G/C rich oligonucleotides, possessing 3' overhang, on the velocity of the RT reaction. (C) Effect of unpaired template length of oligonucleotides in (A) and (B) on potency of inhibition.

DISCUSSION

Synthetic oligonucleotide are important as potential inhibitors of HIV RT, for co-crystallization with this target protein and as a tool for deciphering the structure/function relationship of the enzyme. Understanding the mechanism of interaction of oligonucleotides with HIV RT is therefore important for rational, oligonucleotide based, drug design. In this paper we studied the interaction of a number of partially double stranded oligonucleotides with HIV-1 RT. Several novel observations were featured by our study. Firstly, an oligonucleotide (XX/18mer) based on a specific sequence of the Lys^3-tRNA primer binding site was a much stronger inhibitor than oligonucleotides having a non-specific, G/C rich sequence. This is not surprising as the enzyme specifically recognizes the ribonucleotide version of the 18mer primer during reverse transcription of viral RNA *in vivo* [2]. Therefore, the stronger inhibition by the XX/18mer, probably reflects a relationship between oligonucleotide sequence specificity and strength of binding to RT. However, the possibility that the observed variation in inhibitory potency may be a consequence of longer double stranded stem in the XX/18mer as compared to the linear G/C rich series, cannot be discarded. Both explanations are viable as it is well established that homopolymeric and heteropolymeric oligonucleotide substrates are utilized by RT with varying kinetics, indicating that the base composition of an oligonucleotide affects its binding characteristics to the enzyme [21,24,25]. Similarly, other studies have demonstrated an influence of primer length on the kinetics of RT/nucleic acid complex formation during catalysis [24,25].

Moreover, the presence of a 'hairpin' moiety strengthened the inhibitory potency of G/C rich oligonucleotide. This possibly suggests stronger binding of the oligonucleotides to RT through additional interaction(s) facilitated by the 'hairpin' moiety (see later). Alternatively, there could be stabilization of the double stranded region by this 'hairpin' secondary structure, leading to stronger binding and thereby, augmenting inhibition of the RT reaction. This feature is worthy of further investigation as it can be taken advantage of when designing potential antiviral, oligonucleotide based, drugs to enhance their potency of inhibition.

The lack of significant variation of IC_{50} with the length of the template's 3' single stranded region of the G/C rich oligonucleotides, suggests that interaction of the enzyme with the oligonucleotide is not affected by the template length. This is in agreement with earlier findings, using phosphorthioate oligodeoxynucleotides [7], which suggested that the free enzyme recognizes the primer rather than the RNA template and that the template/primer recognition step by RT involves the formation of an initial enzyme primer complex. The same argument could be applied to the unpaired template region at the 5' end, whereby no effect on IC_{50} was noticed with varying unpaired template's length. Therefore, it seems that enzyme-primer interaction is the crucial step for complex formation, irrespective of whether the template is composed of ribo- or deoxyribonucleotides or whether the single stranded template region is at the 3' or 5' end.

Analysis of the mode of inhibition by representative oligonucleotides used in this study indicated that linear oligonucleotides show mixed type inhibition of the poly(rC) directed (dG) synthesis reaction, whilst oligonucleotides with a secondary

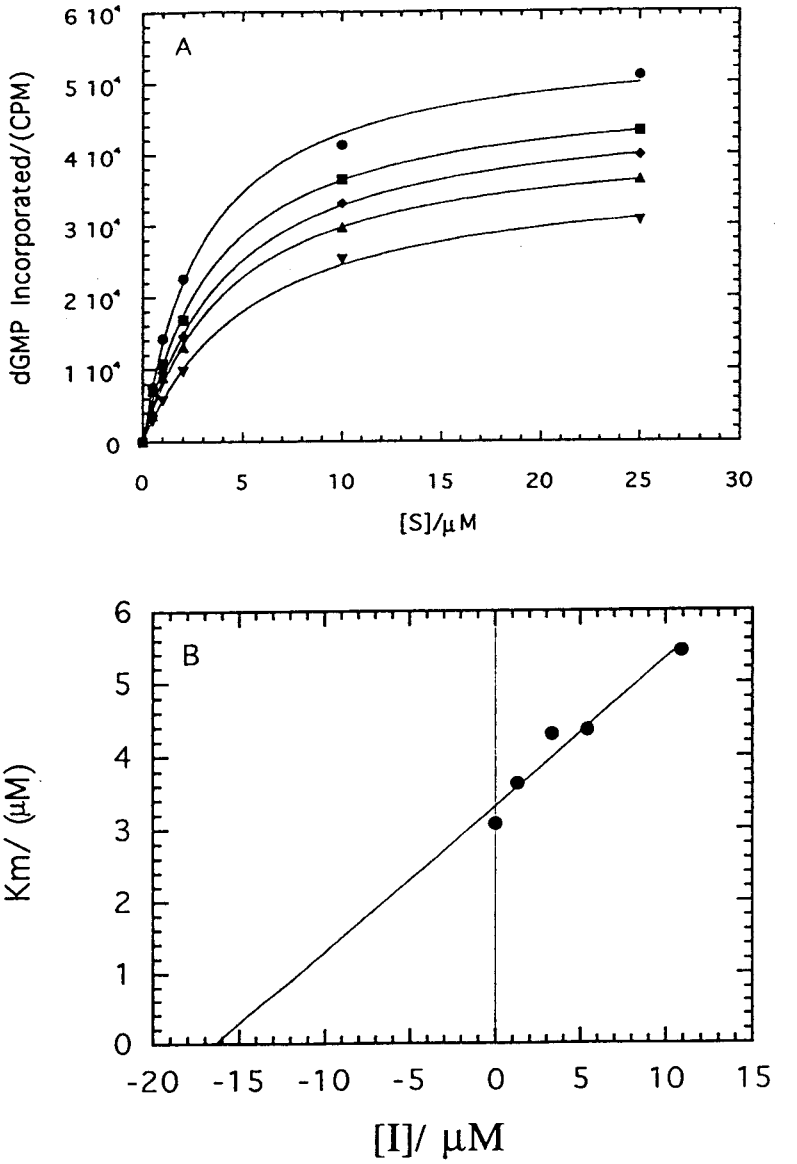


FIGURE 5 Kinetics of Inhibition of RT reaction with 'hairpined' G/C rich oligonucleotides. (A) Saturation curves of the RT reaction in the presence of varying concentrations of the inhibitor 28/9mer. Curves were obtained by fitting raw data to the Michaelis-Menten equation. I=0 (●), I=1.3 μM (■), I=3.3 μM (♦), I=5.4 μM (▲), I=10.9 μM (▼). (B) Replots of the data from (A) to calculate K_i. The figure shows a plot of [I] vs. Km.

structure ('hairpin' moiety) are predominantly competitive. This may suggest that linear oligonucleotides bind to two different sites on the enzyme, one of which is the catalytic primer binding region, and the other, a distinct region (possibly where non-nucleoside inhibitors bind, on the p51 subunit or on the RNase 'H' domain of the p66 subunit), different than the one involved in template/primer stem binding. Binding to these regions would then result in competitive and non-competitive inhibition respectively. Interestingly, the competitive inhibition character of the XX/18mer was stronger than that of the G/C rich 28/9mer (K_i = 0.3 and 67.5 μM respectively), again probably reflecting the effect of oligonucleotide sequence specificity on interaction with the enzyme. Our results for the linear oligonucleotides differ from what has been recently observed for a 58/19mer template-primer which competitively inhibited the poly(rC) directed d(G) synthesis[29]. However, it is interesting that when the 58/19mer was used as the substrate, poly (rA).(dT) was a strong mixed competitive inhibitor of the synthesis reaction[29]. Earlier studies documented evidence[9,10] for the regulation of RT activity by dNTPs. These studies suggested that at high nucleotide concentration, dNTPs act as substrates and as non-competitive inhibitors of the enzyme[10]. This has been hypothesized to occur through allosteric regulation (negative cooperativity)[10] or through the generation of a secondary dNTP site in or near the active site, by isomerization of the enzyme/template-primer/dNTP complex[9]. Similar arguments could be applied for inhibition of RT by the template primer analogues used in this study, and this would offer a further mechanism for regulating RT activity. It is interesting to note that a number of drugs inhibit RT through a non-competitive mechanism, whilst the RT inhibitor nevirapine, shows mixed type inhibition with respect to template/primer, similar to what we observed with the linear oligonucleotide series.

The observation that the 'hairpin' moiety increases inhibitory potency and shifts the mode of inhibition to complete competitive, suggested to us that this secondary structure precludes and strengthens binding of the oligonucleotide to one of the two potential sites, probably restricting it to the catalytic active site. It is interesting to note however, that the anti-codon loop of the Lys^3-tRNA is implicated in binding to RT and in the heterodimer, this site is probably p51 subunit[26]. Therefore, it is possible that the site on the enzyme which binds the U-U-U sequence of the Lys^3-tRNA anticodon loop, could be binding T-T-T sequence on the 'hairpin' motif of the G/C rich oligonucleotide, thereby strengthening its interaction with the enzyme. This would result in stronger inhibition than that observed with the linear series. In such a scenario, competitive inhibition by the 'hairpin' oligonucleotides results from binding of the 'hairpin' moiety to the anticodon binding site and sterically hindering proper binding of the poly(rC).(dG) substrate to the polymerase catalytic site. Binding of poly(rC).(dG) and the 'hairpin' oligonucleotide would then be mutually exclusive.

Inhibition of the RNase'H' activity by the XX/18mer is in agreement with earlier biochemical estimates that the distance between the RT and RNase 'H' catalytic sites is filled by a stretch of about 7-19 nucleotides[26,27] and hence should be covered by the XX/18mer. It is interesting that the 32mer, which only has 9 paired bases, also inhibits RNase 'H' activity. One possibility is that this stretch of nucleotides is indeed sufficient to cover the region between the polymerase and RNase 'H' sites. The observed results however, might also indicate allosteric interdependence between the two active sites,

in the sense that binding of the 32mer oligonucleotide to the polymerase active site affects that of the RNase 'H'. Irrespectively, our observations suggest that double stranded DNA oligonucleotides bind to RT in a manner that inhibits RNase 'H' activity, probably by preventing proper access of the DNA/RNA substrate to the RNase 'H' catalytic site. Interestingly, the free p15 RNase 'H' fragment does not show any activity and is only active when associated with the p51 subunit[28], suggesting 'cross talk' between the two domains. It is worth noting that all classes of RT inhibitors previously reported do not impair the RNAse 'H' activity, but only affect the enzyme's polymerase function. A drug which inhibits both enzymic activities has the advantage of greater potency than single target drugs and lesser susceptibility for resistance through mutations in the polymerase region of the RT genome.

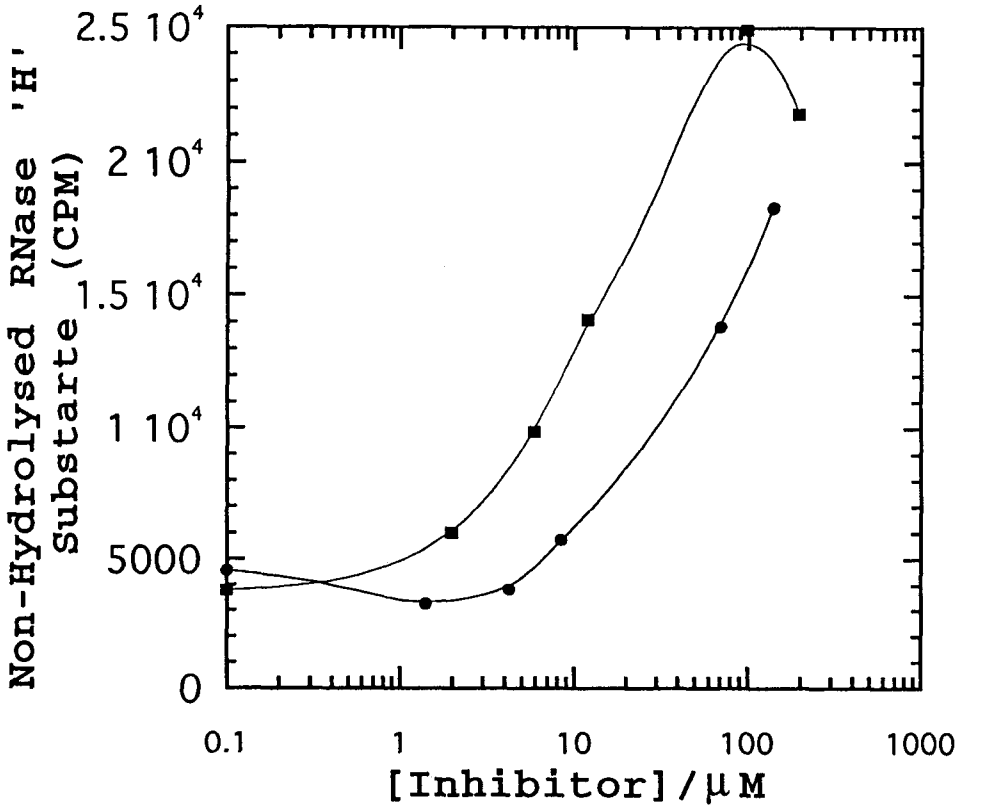


FIGURE 6 Inhibition of RNase 'H' with linear and 'hairpin' oligonucleotides. Typical inhibition curve showing the effect of XX/18mer (■) and 32mer (•) on the velocity of the RT reaction.

The results presented in this paper documented some interesting observations about interactions of synthetic DNA oligonucleotides with HIV-1 RT. These results warrant further characterizations of this system in view of its importance for development of anti-AIDS therapies. To date the only anti-HIV therapeutic drugs are based on nucleoside analogues such as AZT or ddI. It will be interesting to study the effect of combination therapies utilizing both nucleoside and oligonucleotide analogues (once available) to inhibit both the primer and nucleotide binding steps involved in RT reaction(s).

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