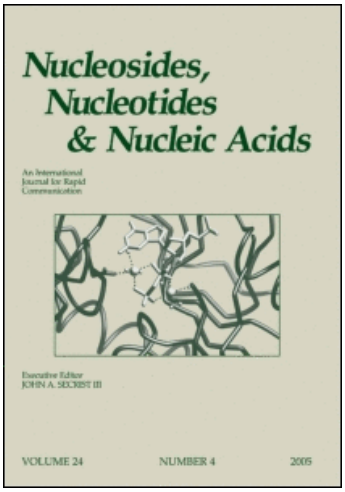


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# Inhibition of Reverse Transcriptase-Mediated cDNA Synthesis by Antisense Oligonucleotides

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# INHIBITION OF REVERSE TRANSCRIPTASE-MEDIATED cDNA SYNTHESIS BY ANTISENSE OLIGONUCLEOTIDES

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**ABSTRACT:** Unmodified and modified (phosphorothioate) oligonucleotides inhibit cDNA synthesis by reverse transcriptase (RT). Antisense oligonucleotide/RNA hybrids specifically arrest primer extension. However, phosphorothioate oligomer, inhibit polymerization by binding to the AMV RT or MMLV RT, rather than to the template RNA. There was no competitive binding of the phosphorothioate oligomer to the HIV RT during reverse transcription.



## INTRODUCTION

Antisense oligonucleotides can be designed to bind (hybridize) to specific mRNA molecules and suppress translation, thus inhibiting protein synthesis.[1-6] However, the mechanism by which the antisense oligonucleotide inhibits retroviral protein synthesis, syncytium formation, or reverse transcriptase activity, has not been fully elucidated. In particular, phosphorothioate oligonucleotides (S-ODNs) have both sequence-dependent and -independent activities. We present here a detailed analysis of the effects of unmodified or modified (phosphorothioate) antisense- and homo-oligonucleotides on cDNA synthesis by the reverse transcriptases (RTs) of Avian myeloblastosis virus (AMV), Moloney murine leukemia virus (MMLV), and Human immunodeficiency virus (HIV).

## EXPERIMENTAL

The unmodified oligonucleotide derivatives, 5'-d[TTGTGTCAAAAGCAAGT] [17 cap (n)], 5'-d[CACCA-ACTTCTTCCACA] [17 sc(n)] , dT20(n), dA20(n), dG20(n), and dC20(n), and the modified (phosphoro-thioate) oligodeoxyribonucleotide derivatives, 5'-d[TsTsGsTsGsTsCsAsAsAsAsGsCsAsAsGsT] [17 cap(s)], dT20(s), dA20(s), dG20(s), and dC20(s) were synthesized on a Applied Biosystems DNA synthesizer Model 392. The oligonucleotide derivatives were purified by reverse phase HPLC on an oligo-DNA column.

*Enzymes and RNA template.*

*Escherichia coli* RNase H and RNasin were from Promega. T4 polynucleotide kinase was purchased from Toyo Boseki. AMV and MMLV with RNase H, as well as rabbit ß-globin mRNA were purchased from

GIBCO/BRL and were used without further modifications . HIV RT with RNase H was from Seikagaku Kogyo. A fragment , about 150 nucleotides long, was obtained by directed cleavage of rabbit ß-globin mRNA by *E.coli* RNase H.[7]

*Analysis of the template RNA*

Globin RNA (50 ng, containing 0.3 pmol of the intact ß-globin), primer (50 pmol), and the desired amount of antisense oligonucleotides were preincubated for 30 min at 39℃. After adding 1 $\mu$l of 10 X RT buffer (1M Tris/HCl, pH 8.3, 720 mM KCl, 100 mM MgCl2, 100 mM dithiothreitol) containing 8 units of RNasin, 2 pmol of [$\alpha$-$^{32}$P]dCTP (3000Ci/mmol; Ci= 37GBq; NEN), 5 nmol of the three dNTPs, and 2.5 nmol of dCTP, the volume of the mixture was adjusted to 10 $\mu$l with sterile water. AMV RT (1-10units-ie., 0.13-1.3 pmol) or MMLV (50-200 units) was then added. The reaction with HIV RT was allowed to proceed with 1 unit, and was incubated for 1 h at 39℃. The cDNA was chloroform extracted according to standard procedures, electrophoresed on a 10% polyacrylamide gel. The results obtained for cDNA synthesis were corrected for the amount of label incorporated into each fragment.

## RESULTS AND DISCUSSION

We have previously shown that an antisense phosphorothioate oligonucleotide inhibits polymerization by binding to AMV RT, rather than to the template RNA[7]. There is no competitive binding of the phosphorothioate oligonucleotide to the HIV RT during reverse transcription.[7] However, in the case of the antisense phosphodiester oligonucleotide, no interaction was observed with either the AMV RT or the HIV RT. Furthermore, we tested the ability of an antisense oligonucleotide to block, the reverse transcription of rabbit ß-globin mRNA by MMLV RT. The reverse transcription of rabbit ß-globin mRNA was carried out by MMLV RT in the presence of 17sc as the primer and the antisense oligonucleotides (17cap(s) or 17cap(n)), respectively. Fig. 1 shows that the oligomer 17cap(n) induced the characteristic shortened cDNA fragment, whereas 17cap(s) reduced the synthesis of the 110 nucleotide transcript; at a concentration of 1 $\mu$M, the percentage of cDNA synthesis was significantly decreased.

We tested the ability of the homooligonucleotides (dA20 (n or s), dG20 (n or s), dC20 (n or s), dT20 (n or s), and dT15 (n or s) to block the reverse transcription of ß-globin mRNA by the AMV RT and HIV RT. We incubated homooligonucleotide analogues (phosphodiester (n) or phosphorothioate (s)) (0.5-10 $\mu$M) with either AMV RT or HIV RT (1 unit) under the same conditions as described above. Fig.2 shows that the phosphodiester (n) homooligonucleotides did not inhibit cDNA synthesis *via* an antisense mechanism: only the full-length cDNA fragment was obtained (90%). In contrast, cDNA synthesis by the phosphorothioate oligonucleotides could not be carried out, because of a failure of polymerization, due to the competitive binding of phosphorothioate homooligonucleotides to the AMV RT and HIV RT enzymes. However, dA20(s) did not affect the synthesis of the full-length cDNA product any better than the other homooligomers

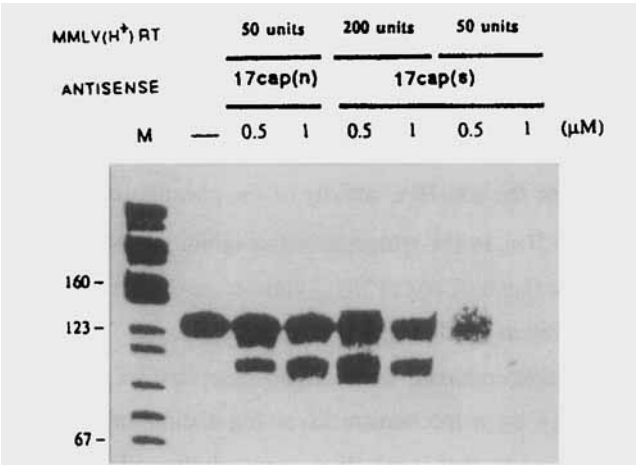


Figure 1. Effect of antisense oligonucleotides on cDNA synthesis using MMLV RT. Reverse transcription proceeded as indicated using 50-200 units of MMLV RT, primed by a 17 mer [17 sc(n) (5 $\mu$M)] complementary to the 113-129 oligonucleotide, without (-) or with various amounts ($\mu$M) of antisense oligonucleotides, 17 cap(n) (left) and 17 cap(s) (right).



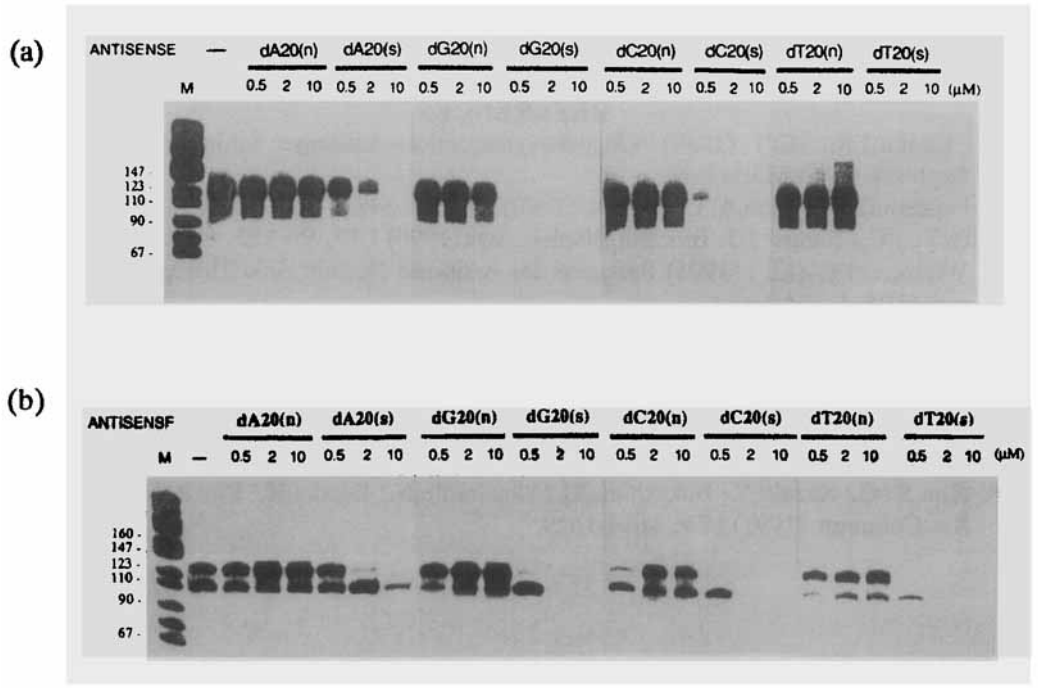


Figure 2. Effect of the homooligonucleotides on DNA synthesis. (a) Reverse transcription proceeded as indicated using 1 unit of AMV RT primed by 5 $\mu$M oligomer 17 sc(n), without (-) or with various amounts ($\mu$M) of the unmodified homooligomers (n) and modified homooligomers (s). (b) cDNA analysis of HIV RT, used in place of AMV RT.

(dG20(s), dT20(s), and dC20(s)) at the concentration of 0.5 $\mu$M. We examined how to prevent cDNA elongation by dC15(s) instead of dC20(s), using the same conditions described above. The homooligomer dC15(s) allowed the HIV RT-mediated allowed synthesis of a full-length cDNA fragment of 97% at high concentrations (2-10 $\mu$M). We found similar results for the anti-HIV activity of the phosphorothioate homooligonucleotides, dC28(s) and dC15(s), in the cytopathic effect inhibition assay using MOLT-4 cells.[8] It is worthwhile to note that 0.02 $\mu$M dC28(s) showed more activity than 0.1 $\mu$M dC15(s), and a 0.5 $\mu$M concentration of dC15(s) was relatively toxic. These results support the proposal that the phosphorothioate homooligonucleotides act as inhibitors of reverse transcription, essentially by a mechanism involving their binding to the reverse transcriptase enzyme, in a process that is mostly sequence-independent. On the other hand, in the case of HIV-1, the inhibition by antisense phosphorothioate oligonucleotides is length- and sequence-dependent: oligonucleotides complementary to different regions of HIV-1 mRNA block viral replication following binding to their target sequences.



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