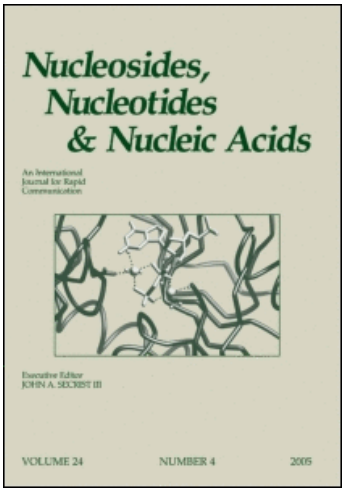

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ALPHA-OLIGODEOXYNUCLEOTIDES AS INHIBITORS OF HIV REVERSE TRANSCRIPTASE

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Abstract : A number of oligodeoxynucleotides containing thymine bases linked to the deoxyribose in the α-configuration have been synthesized. In view of the possible activity of α-oligomers as antisense compounds against HIV replication, the effects of α-thymidylate oligomers (α-dT_n, n=1-16) in a poly(A)-directed HIV reverse transcriptase (RT) reaction were investigated. No priming activity could be detected with the α-dT oligomers at concentrations up to 100 μM. However, in the presence of the natural ß-dT_{12-18} primer, 50% inhibition of RT activity was achieved at a concentration of about 1 μM with the α-dT oligomers containing ≥ 10 monomer units. The kinetics of RT inhibition by the α-decamer was found to be competitive with respect to the natural primer, ß-dT_{10}.

INTRODUCTION

Oligodeoxynucleotides, which are complementary to vital segments of the HIV genome, referred to as "antisense" compounds, offer a valuable approach in the search for highly specific drugs against HIV replication. In natural oligodeoxynucleotides, the purine and pyrimidine bases are attached to the D-2-deoxyribose by a glycosyl bond in the ß-configuration. These deoxyribonucleosides are then joined by 5'- 3' phosphodiester bonds. The use of these ß-oligodeoxynucleotides as antisense molecules is compounded by poor cellular uptake and premature degradation by nucleases which hydrolyse the internucleoside P-O

linkages (1). Ideally, antisense molecules should be resistant towards degradation by nucleases, be taken up by cells and exhibit a strong affinity for their complementary target sequences.

In attempts to achieve these goals, an intercalating agent has been attached to the oligodeoxynucleotide chain (2,3) and the phosphodiester linkage has been modified (4-6). An alternative approach is based on the use of oligomers containing α-2'-deoxynucleotides. These compounds are resistant to nucleases (7,8), form stable parallel duplex structures with their ß-anomeric complements (9-12) and can be automatically assembled by using DNA synthesizers (13). In this study, we determined the ability of α-thymidylate oligomers to interact with the ß-poly(A)-directed HIV reverse transcriptase reaction. This system may provide useful information on the potential of antisense α-oligomers as anti-HIV agents.

RESULTS AND DISCUSSION

A number of oligodeoxynucleotides were synthesized, all of which contained thymine bases linked to deoxyribose in the α-configuration (13). The number of monomers in the α-oligodeoxynucleotides varied from 1 to 16 : α-dT, α-dT_2, α-dT_4, α-dT_6, α-dT_8, α-dT_{10}, α-dT_{12}, α-dT_{14} and α-dT_{16}. These compounds were evaluated against HIV-associated reverse transcriptase using ß-poly(A) as a template. This reverse transcriptase reaction is usually primed by a ß-thymidylate oligomer containing 12 to 18 monomers.

The RT-assay conditions were chosen such that immediate mixing of template [poly(A)], ß-primer (ß-dT_{12-18} or ß-dT_{10}) and/or α-oligomers occurred at 4 °C prior to the addition of the RT enzyme preparation, after which the temperature was raised to 37 °C. Whereas ß-dT_{12-18} efficiently primed the HIV RT reaction at a concentration of 7.5 μM, no such priming activity was seen when α-dT_8, α-dT_{10}, α-dT_{12}, α-dT_{14} and α-dT_{16} were incubated at concentrations up to 100 μM in the absence of the natural primer (Table 1). These observations indicate that the α-dT oligomers did not function as primers for the RT reaction.

When varying concentrations of the α-thymidylate oligomers were incubated in the presence of 7.5 μM ß-dT_{12-18}, 50% inhibition of [methyl-^{3}H]dTTP incorporation was achieved at concentrations of 1-5 μM

TABLE 1. Inhibitory effects of various α-oligodeoxynucleotides on HIV-associated reverse transcriptase.

Compound	Priming activity[a] (μM)	ID-50[b] (μM)
α-dT	-	> 100
α-dT_2	-	> 100
α-dT_4	-	> 100
α-dT_6	-	45
α-dT_8	> 100	12
α-dT_{10}	> 100	4.4
α-dT_{12}	> 100	2.1
α-dT_{14}	> 100	1.4
α-dT_{16}	> 100	1.2

[a]Dose required to obtain a 50% incorporation of [methyl-^{3}H]dTTP in the absence of normal primer [ß-dT_{12-18}].
[b]Dose required to inhibit the HIV-RT reaction by 50% in the presence of 7.5 μM of ß-primer [ß-dT_{12-18}].

with α-dT_{10}, α-dT_{12}, α-dT_{14} and α-dT_{16} (Table 1). RT-inhibitory activity was not observed for the α-oligodeoxynucleotides containing 1 to 4 thymidylate monomers but gradually increased when the chain length of the oligodeoxynucleotide increased from 6 to 14 units, whereafter it levelled off (Table 1). The α-oligomers, α-dT_{12} and α-dT_{14} were found not to be inhibitory to the RT reaction when poly(I) instead of poly(A) was used as exogenous template and ß-dC_{12-18} in stead of ß-dT_{12-18} as the primer (data not shown). These observations indicate that the α-dT_n oligomers owe their inhibitory effect on RT activity to interference with the template function of the ß-poly(A).

Finally, the kinetics of RT inhibition by the α-thymidylate decamer was studied with respect to the natural primer, ß-dT_{10}. In these experiments the concentrations of template [poly(A)] and substrate ([methyl-^{3}H]dTTP) were kept constant while the RT-inhibitory effects of different concentrations of α-decamer were determined in the presence of varying concentrations of ß-dT_{10}. The Lineweaver Burk plot depicted in Fig. 1 shows a competitive type of inhibition by α-dT_{10} with respect to

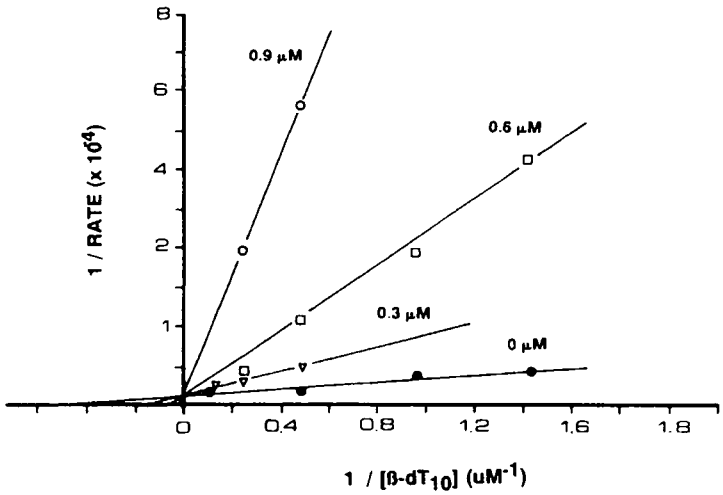

FIG. 1 Lineweaver Burk plot for the kinetics of inhibition of HIV reverse transcriptase by the α-thymidylate decamer. Poly(A) was used as the template and ß-dT_{10} as the primer of which the concentration varied.

ß-dT_{10}. The Km value for the natural substrate was 2.7 µM, whereas the concentration of α-decamer that doubled the slope in this plot (Ki value) was 0.08 µM.

Based on these findings, we postulate that antisense α-oligodeoxynucleotides consisting of at least 10 monomeric units may have considerable potential as anti-HIV agents. The α-thymidylate oligomers are not cytotoxic to MT-4 cells at concentrations up to 100 µM (data not shown). Whether these or other α-oligodeoxynucleosides with defined sequences are also able to block HIV replication remains subject of further study.

EXPERIMENTAL SECTION

Compounds. The α-oligodeoxynucleotides α-dT, α-dT_2, α-dT_4, α-dT_6, α-dT_8, α-dT_{10}, α-dT_{12}, α-dT_{14} and α-dT_{16} were dissolved in sterile water. Concentrations were determined spectrophotometrically (molar absorption coefficients : 8880, 17760, 35520, 61800, 70000, 83300, 88800, 109800 and 138400, respectively). All stock solutions were stored at -20 °C until used.

Reverse transcription assay. Reverse transcriptase (RT) assays were carried out with partially purified human immunodeficiency virus (HIV). The culture fluids from a HUT-78/HTLV-III_B producing cell line were clarified by low speed centrifugation. Virus particles were subsequently sedimented by centrifugation at 100,000 g for 60 min. The viral pellet was incubated on ice for 30 min with a solubilization buffer containing 0.1 % Triton X-100, 1 mM Dithiothreitol and 50 % glycerol and stored in aliquots at -70 °C until used. The inhibitory effects of the α-oligodeoxynucleotides on RT activity were performed with exogenous poly(A) as template and ß-dT_{12-18} as primer. The reaction mixture (50 µl) contained 50 mM Tris-HCl (pH 7.8), 5 mM dithiothreitol, 300 µM glutathione, 500 µM EGTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 µg bovine serum albumin, 1 µM [methyl-^{3}H]dTTP (specific radioactivity : 30 Ci/mmol) (5 µCi), 4.4 µg/ml poly(A), 7.5 µM ß-dT_{12-18}, 0.03 % Triton X-100, 10 µl solution containing varying concentrations of the compounds and 10 µl of the reverse transcriptase preparation. The poly(I).oligo(dC) reaction mixture contained the same reagents as the poly(A).oligo(dT) reaction mixture, except for that the concentrations of $MgCl_2$ and KCl were adjusted to 2.5 mM and 200 µM, respectively, whereas [methyl-^{3}H]dTTP was substituted by [methyl-^{3}H]dCTP (specific radioactivity : 19.4 Ci/mmol) (5 µCi). The reaction mixtures were incubated at 37 °C for 60 min, whereafter 100 µl calf thymus DNA (150 µg/ml), 2 ml $Na_4P_2O_7$ (0.1 M in 1 N HCl) and 2 ml TCA (10 % v/v) were added. The solutions were kept on ice for at least 30 min, after which the acid-insoluble material was washed and analyzed for radioactivity.

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