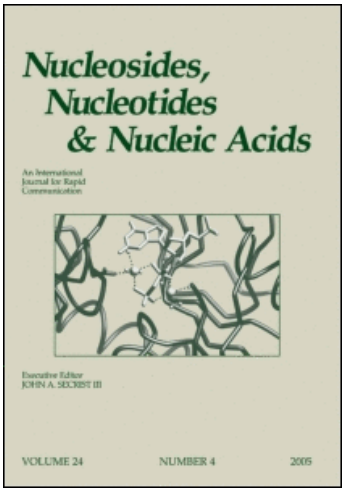

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HIV INHIBITION BY ANTISENSE OLIGODEOXYNUCLEOTIDES

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ABSTRACT

A series of oligodeoxynucleotides containing phosphorothioates and normal internucleotide linkages were synthesized and tested for their antiviral activity against HIV 1.

RESULTS AND DISCUSSION

One promising approach for treatment of HIV infected patients with AIDS is to use antisense oligodeoxynucleotides (ODN) to selectively block virus replication by competitive hybridization.[1] These ODN have to fulfill some special prerequisites such as nuclease resistance, duplex stability and cell membrane permeability. For this purpose a series of ODN containing phosphorothioates and normal internucleotide linkages were synthesized according to the phosphoramidite procedure. These include normal ODN and such which are end-capped at the 3', 5' or both 3' and 5' position.[2]

TABLE: Synthesized oligodeoxynucleotides; Chol.: cholesterylphosphoryl, S: phosphorothioate linkage

1	3'-	T G T G G G T T A A G A C T T T T A C C-5'
2	5'-	A C A C C C A A T T C T G A A A A T G G-3'
3	5'-	A C A ·····A Ts GsG-3'
4	5'-	AsCsA ·····A Ts GsG-3'
5	5'-	AsCsA ·····A T G G-3'
6	5'-	A C A ·····AsTs GsG-3'
7	5'-Chol.-	A C A ·····A T G G-3'
8	5'-Chol.-	A C A ·····A Ts GsG-3'

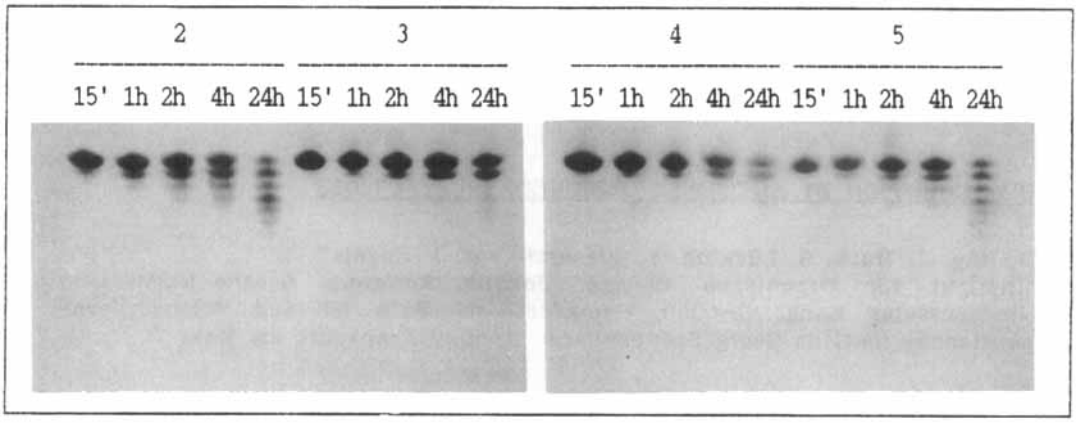

FIGURE: Degradation of the oligonucleotides 2-5 in RPMI 1640 medium containing 15% fetal calf serum. Silver stained 16% PAA/7M urea gel.

The ODN were purified by HPLC and analyzed by ^{31}P-NMR spectroscopy (121.5MHz. D_2O). The spectra of 3-5 are showing a peak at 56,6ppm downfield from 85% H_3PO_4 (PS-resonance) and a peak at -0,4ppm (PO-resonance). Only the 5' end-capped oligomer 5 gave the correct integrated PS/PO peak ratio whereas the spectra of 3 and 4 have shown a reduced sulfur content.[2] This indicates that the PS-linkage is not stable under the synthesis conditions.

The end-capped ODN were tested for their resistance against fetal calf serum (15% in RPMI 1640 medium, 37°C) which always contains exonucleases. Only the 3' (3) and 3',5' (4) ODN are stable under these conditions whereas the normal (2) and 5' (5) end-capped ODN were degraded within 4-6 hours.

The melting temperatures of these end-capped ODN are nearly the same as their natural congeners (delta $T_m \approx 2°C$). To enhance the cell membrane permeability we have synthesized some ODN conjugated at the 5'-terminus to cholesterol[3] via a cholesterylphosphoramidite. The lipophilicity of these conjugates was extremely enhanced as observed by HPLC. Finally the antisense ODN were tested for their ability to inhibit virally induced syncytia formation in human activated lymphocytes in concentations from 1-50µg/ml. As target site within the HIV 1 genome we used the splice acceptor site (5'-CCA UUU UCA GAA UUG GGU GU-3') for the *tat* gene.[4]

It was found that the introduction of thioates enhances the antiviral activity (greater than 85% inhibition in each case). A cholesteryl group at the 5'-terminus causes complete inhibition of syncytia formation at a concentration of 20μg ml. At low concentrations these conjugates were less active compared with the other oligonucleotides tested.

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