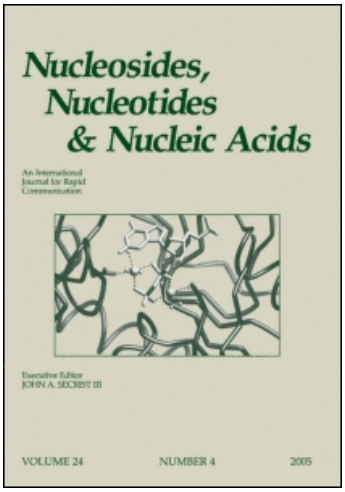

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Phosphoramidate, Phosphorothioate, and Methylphosphonate Analogs of Oligodeoxynucleotide : Inhibitors of Replication of Human Immunodeficiency Virus

Sudhir Agrawal[a]; John Goodchild[a]; Maria Civeira[b]; Prem S. Sarin[b]; Paul C. Zamecnik[a]
[a] Worcester Foundation for Experimental Biology, Shrewsbury, MA [b] Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD

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PHOSPHORAMIDATE, PHOSPHOROTHIOATE, AND METHYLPHOSPHONATE ANALOGS OF OLIGODEOXYNUCLEOTIDE : INHIBITORS OF REPLICATION OF HUMAN IMMUNODEFICIENCY VIRUS

Sudhir Agrawal, John Goodchild, Maria Civeira[+], Prem S. Sarin[+], and Paul C. Zamecnik*

Worcester Foundation for Experimental Biology, Maple Avenue, Shrewsbury, MA 01545; [+]Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892.

ABSTRACT: Modified oligodeoxynucleotides complementary to RNA of human immunodeficiency virus (HIV-1) were tested for their ability to inhibit virally induced syncytium formation and expression of viral p24 protein. The modification of oligomers include replacement of phophodiester backbone with phosphorothioate, methylphosphonate and various phosphoramidates. Cells infected for four days, then treated with the antisense oligomers also showed inhibition of viral expression.

Oligodeoxynucleotides which are complementary to certain messenger or viral RNAs, reffered to as 'antisense' compounds, have been reported to have inhibitory effects against Rous sarcoma virus[1,2] and HTLV-III[3-7] now called HIV-1. However, the susceptibility of the phosphodiester linkage in oligodeoxynucleotides to degradation by nucleases[8] would be expected to reduce their potency and in vivo persistance as antiviral agents. Phosphate backbone modified analogs of oligodeoxynucleotides are resistant to nucleases and have generally increased hydrophobicity which should give longer survival time in vivo and may increase cell membrane permeability.[9,10]

We have investigated three classes of phosphate backbone modified oligodeoxynucleotides viz. methylphosphonate, phosphorothioate and various phosphoramidates for their antiviral activity against HIV-1.

Oligonucleotides were synthesized by using an automate DNA sythesizer (Biosearch 8600). Methylphosphonate analogs were assembled from nucleoside methylphosphonamidites as reported earlier[11]. Phosphorothioate and phosphoramidates were synthesized by oxidizing

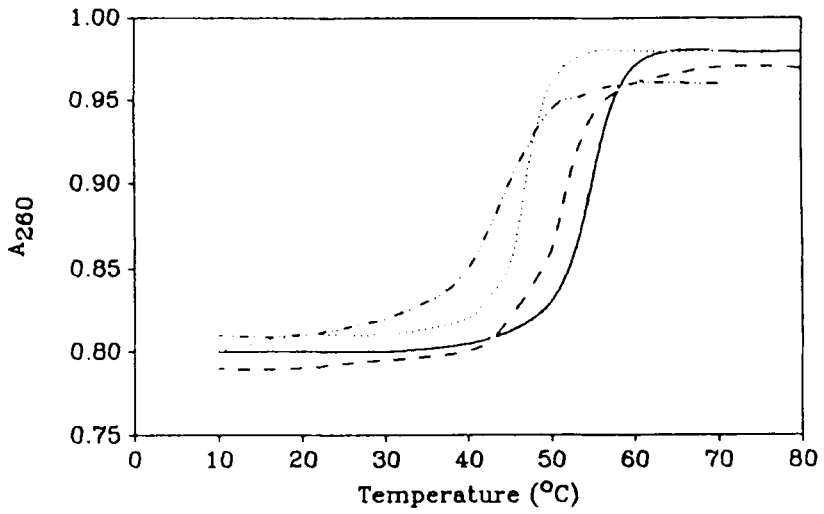

FIGURE 1 : Melting curve of oligonucleotide Sequence 1 with four different phosphate backbones was hybridized to diester comple mentary 20 mer. (——) phosphodiester, (----) methylphosphonate, (.....) phophorothioate and (-·-·-) phosphor-N-butylamidate.

polymer bound oligonucleoside H-phosphonates with appropriate reagent[6]. Purification and characterization of these oligonucleotides were carried out in similar way as reported previously[6].

The thermal stability of the oligomers complexed with complementary diester sequence was assessed by hyperchromicity at 260 nm from 10° to 80° at a concentration of about 5μM. Figure-1 shows the melting curve of each obtained at 100 mM NaCl. The T_m of diester control was 55°, followed by methylphosphonate 51.5°, phosphorothioate 49° and phosphoramidate (n-butyl) 45°. All samples showed similar hyperchromicity (~20%) except phosphoramidates (~16%).

The inhibition of HIV-1 expression in H-9 or MOLT-3 cells in the presence of antisense oligonucleotides was carried out by infecting $5x10^5$ cells per ml with $2.5-5x10^8$ virus particles of HIV-1 ($HTLV-III_B$ or $HTLV-III_C$). Infection of cells was carried out by simultaneous addition of virus and antisense oligomer to cells in culture. After four days, the cells and supernatant were examined for the

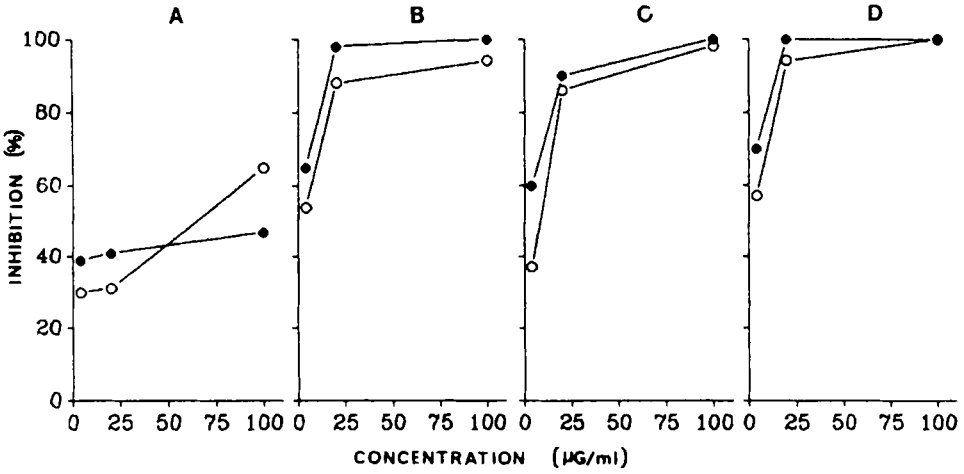

FIGURE 2 : The percentage of inhibition of syncytia formation (o) and p17 expression (●) as a function of concentration for sequence 1, A-diester, B-methylphosphonate, C-phosphorothioate and D-phosphor-N-butylamidate.

level of HIV expression by counting syncytia (MOLT-3 cells) and determining viral antigen (p17 or p24) expression as well as cell viability.

The sequence of oligonucleotides tested were complementary to two sites in HIV RNA previously shown to be good targets for inhibition of HIV replication[3]. These sites were, a splice donor site near the 5'-end of the RNA used to assemble shorter mRNAs and a splice acceptor site used to generate tat mRNA. The complementary sequences prepared were ACACCCAATTCTGAAAATGG (sequence 1) binding to splice acceptor site (5349-5368)[12] and GCGTACTCACCAGTCGCCGC (sequence 2) binding to splice donor site (280-299)[12]. Figure-2 illustrates the result of inhibition of syncytia formation and p17 expression at different concentration of sequence 1, A-phosphodiester, B-methylphosphonate, C-phosphorothioate and D is phosphor-N-butylamidate. The backbone modified oligonucleotides typically are more active than the diester series and 80-100% inhibition of syncytia formation and p17 expression at a concentration of 20μg per ml (~3μM) of antisense oligonucleotide. There was no cytotoxicity observed at 3-5 μM concentration of these oligonucleotides. Similar results were obtained with sequence 2 also.

The random sequence (20 mer) phosphorothioate showed no inhibition at 0.6 μM concentration, at 3 μM concentration it was as active as antisense sequence and showed more cytotoxicity. The random sequence of phosphormorpholidate showed only 15% inhibition of p17 and syncytium at 3 μM concentration.

Sequence 1 also showed inhibition of p24 expression when it was added to cells which were already infected for four days. Although inhibition was somewhat lower than when the compound was given simultaneously with the virus, it was still high. At oligonucleotide concentration of 12.5, 25, 50, 100 and 200 μg/ml, the percentage of inhibition were, respectively, 4, 19, 70, 82, and 86.

Preliminary acute toxicity studies in mice were performed with sequence 1 with three different phosphate backbones, a diester, a phosphorothioate and a phosphormorpholidate. The results show a dose of 40mg/Kg bodyweight of these compounds are toxic.

These studies show that phosphate backbone modified analogs of oligodeoxynucleotides are potent inhibitors of HIV-1 replication in cell culture and could be potentially useful in the treatment of AIDS.

ACKNOWLEDGEMENT

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