Mode of Action of 5′-Linked Cholesteryl Phosphorothioate Oligodeoxynucleotides in Inhibiting Syncytia Formation and Infection by HIV-1 and HIV-2 in Vitro†

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ABSTRACT: A phosphorothioate homocytidine 10-mer containing a cholesteryl moiety covalently linked to the 5′-end (Chol-SdC10) inhibited syncytium formation in susceptible T cells induced by HIV-1 and HIV-2. The syncytium inhibition effect was minimal with unmodified cytidine homopolymer of the same net charge. Chol-SdC10 was shown to protect CEM cells against infection by cell-free HIV-1 particles without any apparent toxicity to the growth of CD4+ T cells. The DNA polymerase activity of the purified reverse transcriptase (RT) of HIV-1 was markedly inhibited by Chol-SdC10 but the effect on the RNase H activity of RT was minimal. Analysis of the kinetics of reverse transcriptase inhibition mediated by the drug revealed that the inhibition at a higher concentration was competitive with respect to template primer binding and noncompetitive at lower concentrations. Chol-SdC10 also partially blocked the binding of gp120 to CD4 in a solid-phase ELISA. These results confirm that the anti-HIV activity of phosphorothioate cytidine homopolymers increases markedly by covalent modification with the cholesteryl moiety at the 5′-end and demonstrates that the cytoprotective effect is manifested at multiple steps in the virus life cycle. These steps include inhibition of retroviral replication activity as well as the binding and fusion of HIV with CD4+ T cells.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) (Popovic et al., 1984; Gallo et al., 1984; Barre-Sinoussi et al., 1983; Sarngadharan & Markham, 1987). The virus is tropic and cytopathic toward macrophages and T cells bearing the CD4 surface antigen (Klatzmann et al., 1984). The urgent need for chemotherapy of AIDS has directed considerble research interest toward effective anti-HIV agents (Mitsuya & Broder, 1987). One well-characterized compound 3′-azido-3′-deoxythymidine (AZT) has proven to be useful in improving the clinical and immunoogical status of ARC and AIDS patients (Mitsuya & Broder, 1987). This compound has been reported to be a potent inhibitor of the viral reverse transcriptase (RT). Unfortunately, AZT is not without side effects; in addition, an AZT-resistant strain of HIV-1 has now been characterized (Larder et al., 1989). Therefore, the need remains for the development of additional compounds that can interfere with the virus life cycle.

Phosphorothioate oligodeoxynucleotides are a class of compound in which one of the nonbridging phosphodiester oxygen atoms is replaced by a sulfur atom (Stec et al., 1984; Stein et al., 1988). These water-soluble compounds have recently been shown to be sequence-specific (antisense) inhibitors of in vitro HIV-1 replication (Matsukura et al., 1989; Agrawal et al., 1988). It has been also demonstrated that phosphorothioate oligodeoxynucleotides can, in addition, be potent non-sequence-specific inhibitors of HIV-1- (Matsukura et al., 1987; Stein et al., 1989) and HIV-2-induced syncytia formation (R. Pal et al., unpublished observations) and can protect uninfected cells from the cytopathic effect of the HIV-1 Majumdar et al. (1989) have shown that the cytidine homopolymer, SdC28, is a potent inhibitor ofHIV-1 reverse transcriptase with respect to template primer binding. Other workers (Stein et al., submitted for publication) have proposed that SdC28 is also capable of interferring with the gp120–CD4 interaction.

Other workers (Letsinger et al., 1989) have synthesized a series of phosphorothioate oligomers with a cholesteryl moiety covalently linked at the 3′-end. The oligomer structures were complementary to the HIV-1 splice acceptor site at nucleotides 5349–5368, but a thymidine homopolymer 5′-SdT-15-cholesteryl-3′ was also examined. The most active compound in this series was a 3′-cholesteryl 20-mer phosphorothioate. Compared to the unmodified phosphorothioate oligomer, the cholesterol-linked compound was a more potent inhibitor of syncytia formation, reverse transcriptase activity, and p24 production. Interestingly, the relative (unmodified versus modified oligomer) inhibitory effect was most dramatic for the shorter oligomers. The authors also concluded that the inhibition of HIV-1 replication by the shorter modified oligomers was not strongly dependent on oligomer sequence.

In this paper we describe the mode of action of non-sequence-specific phosphorothioate oligomers in inhibiting HIV replication. Compounds with a cholesteryl moiety covalently linked to the 5′-end of the phosphorothiate oligomer were synthesized and assayed for inhibition of multiple steps in the life cycle of HIV. These results were compared with the inhibition observed by using an unmodified phosphorothioate oligomer of the same net charge. Our results show that the covalent linking of cholesterol to the 5′-end of the phospho-

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rothioate oligomer markedly enhances its non-sequence-specific anti-HIV activity.

Materials and Methods

Oligomers. Phosphorothioate oligodeoxynucleotides were prepared on controlled pore glass supports (1 μmol of loaded nucleoside) by a conventional protocol using hydrogen phosphonate chemistry (Froehler et al., 1986) followed by a terminal oxidation with sulfur (Agrawal et al., 1988; Letsinger et al., 1989), cleavage of the DMT group (2.5% dichloroacetic acid in dichloromethane), and deprotection and release of the oligomers from the support by treatment with ammonium hydroxide. For the cholesterol derivatives, the final coupling was carried out with cholesteryl hydrogen phosphonate (Cremlyn et al., 1978) in place of DMT-deoxycytidine hydrogen phosphonate. The ammoniacal solutions were evaporated and the oligomers were isolated by HPLC using an ODS Hypersil C-18 column with a 0.03 M triethylammonium acetate (pH 7.0)/acetonitrile gradient (increasing 1%/min), starting at 30% acetonitrile for the cholesteryl compounds and 1% acetonitrile for the unsubstituted phosphorothioates. The oligomers were then desalted by rechromatographing, using a water/acetonitrile gradient, and were collected as powders by lyophilization. After lyophilization the cholesteryl-linked oligomers of deoxycytidine were insoluble in water, but could be redissolved readily by exposing the aqueous suspension to a puff of ammonia vapor [see Mori et al. (1989) for comparable behavior of long-chain deoxycytidine phosphorothioates and phosphoroselenoates]. Comparative analytical chromatography of the purified products was carried out on a Dionex HPLC unit with the column, buffer, and gradient indicated above, starting at 0% acetonitrile and a flow rate of 1.5 mL/min, and polyacrylamide gel electrophoresis was performed by using a 20% gel [bis(acrylamide)/acrylamide 1/28.4, wt/wt; 8.3 M urea; TBE buffer at pH 8.3] with nucleotide bands visualized by staining with methylene blue. For SdC6, SdC11, Chol-SdC5, and Chol-SdC10, respectively, the elution times (peak maximum) were 14.5, 15.8, 45.9, and 43.7 min and the electrophoretic mobilities (relative to bromophenol blue) 0.93, 0.84, 0.79, and 0.82.

Virus and Cells. Chronically infected Molt3/HIV-1 (HTLV-IIIB isolate) and Molt3/HIV-2 cells (SBL6669 isolate) and uninfected CEM and SupT1 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. 8E5 cells were obtained from the AIDS Research Repository Rockville, MD, and were maintained in the same RPMI-1640 medium as described above. The virus stock for cell-free infection assays were prepared by culturing Molt3/HIV-1 cells in fresh medium. The cell-free supernatant was collected after 24 h, filtered, and stored in aliquots of 1 mL at -70 °C for further use.

Cell-Free Infection. CEM cells (1×10^5) were incubated with cell-free HIV-1 for 60 min at 37 °C. The cells were then washed in RPMI 1640 medium and transferred to six-well plates in 2 mL of medium and an additional 2 mL of RPMI medium was added after 24 h. For detecting viral protein synthesized in infected CEM cells, cells were radiolabeled with [^{35}S]methionine for 7 h 5 days after infection and the proteins were immunoprecipitated by human serum containing antibodies to HIV-1 proteins as described below.

Radiolabeling of Cells and Immunoprecipitation Assay. Infected cells (10^6 cells/mL) were radiolabeled by incubating at 37 °C for 7 h in methionine-free medium containing [^{35}S]methionine (100 μCi/mL). Labeled cells were washed with phosphate-buffered saline (PBS) and disrupted at 4 °C in PBS containing 0.1% sodium dodecyl sulfate, 1% Triton X-100, and 0.5% sodium deoxycholate (PBS-TDS). The lysate was adsorbed for 4 h at 4 °C with 0.2 mL of 10% protein A Sepharose (PAS) and 10 μL of normal human serum and clarified by centrifugation. Immunoprecipitation reactions were performed by the addition of 10 μL of anti-HIV-1 positive human serum and 0.2 mL of 10% PAS to 1 mL of the clarified cell extract. The samples were incubated for 18 h at 4 °C and the immunoprecipitates were collected by centrifugation at 2000*g* for 10 min. The pellet was repeatedly washed in PBS-TDS; resuspended in 75 μL of 0.065 M Tris-HCl (pH 6.7), 1% SDS, 10% glycerol, 2.5% β-mercaptoethanol, and 0.1% bromophenol blue; heated for 3 min at 90 °C; and analyzed by SDS–PAGE.

Syncytium Assay. The syncytium assays were performed in 96-well microtiter plates by mixing 1×10^5 CEM cells with 5×10^3 chronically infected Molt3/HIV-1 or Molt3/HIV-2 cells. The samples were incubated in a CO_2 incubator at 37 °C for 18 h for the HIV-2 isolate and for 40 h for the HIV-1 isolate and the number of giant cells in each well was quantitated by microscopic examination. For assaying syncytia induced by 8E5/HIV-1 cells, 1×10^5 SupT1 cells were mixed with 2.5×10^4 infected cells and the culture was incubated for 18 h at 37 °C in a CO_2 incubator.

Reverse Transcriptase and DNA Polymerase Assays. Reverse transcriptase assays were carried out in a final volume of 50 μL containing 50 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 10 mM dithiothreitol, 10 mM $MgCl_2$, 2.5 μg of $(dT)_{12-15}(rA)$, 12 μM [^{3}H]dTTP (27 300 cpm/pmol), 100 μg/mL bovine serum albumin (BSA), and 5 μg of HIV-1 reverse transcriptase purified from virions by an immunoaffinity procedure described elsewhere (Sarngadharan et al., 1976; Veronese et al., 1986).

DNA polymerase assays were performed in a final volume of 50 μL containing 50 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 10 mM dithiothreitol, 6.7 mM $MgCl_2$, 32 mM dATP, 32 μM dTTP, 25 μg of $(dT)_{12-15}(dA)_n$, 12 mM [^{3}H]dTTP (27 300 cpm/pmol), 100 μg/mL BSA, and 0.005 units of *Escherichia coli* DNA polymerase I (BRL). Incubations were carried out at 37 °C for 60 min and were terminated by the addition of 100 mL of yeast tRNA and about 3 mL of ice-cold 10% trichloroacetic acid containing 0.02% sodium pyrophosphate. The acid-insoluble precipitates were collected on glass fiber filters, washed, and dried and their radioactivity was determined with a Beckman scintillation spectrometer.

Ribonuclease H Assay. For the measurement of RNase H activity, 25 μg of purified HIV-1 RT was incubated for 30 min at 37 °C in 200 μL of a reaction mixture containing 50 mM Tris-HCl, pH 8.0, 1.5 mM $MgCl_2$, 10 mM DTT, 0.1 M NaCl, and 10 000 cpm of φX174 DNA [^{3}H]RNA. The reaction was terminated by the addition of 200 μg of yeast transfer RNA in 100 μL of ice-cold 15% trichloroacetic acid. Following a 15-min incubation at 4 °C, the reaction mixture was centrifuged at 5000*g* for 10 min at 4 °C. The supernatant (400 μL) was withdrawn and mixed with 5 mL of Aquasol (New England Nuclear) and the amount of radioactivity was determined with a Beckman scintillation spectrometer.

Binding of gp120 to CD4 by Solid-Phase ELISA. This assay was performed as described previously (Lederman et al., 1989) with certain modifications. Briefly, sCD4 was attached to the polystyrene plates by incubating 50 μL of protein solution (5 μg/mL) in phosphate-buffered saline (PBS) overnight at 4 °C. The wells were washed with PBS containing 0.05% Tween-20 and blocked by incubating with 0.1 mL of 1% BSA in PBS (BSA–PBS) at 37 °C for 2 h. The wells were then

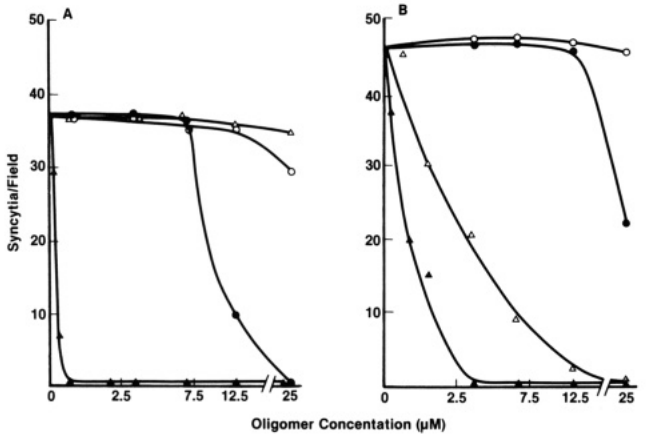

FIGURE 1: Inhibition of HIV-induced syncytium formation by cholesteryl-modified oligomers. CEM cells were cocultured with either Molt3/HIV-1 (A) or Molt3/HIV-2 (B) cells in the presence of indicated concentrations of inhibitors. Syncytia were counted after 18 h in the case of HIV-2 or after 42 h in the case of HIV-1. In both panels HIV-induced syncytium formation was assayed in the presence of SdC11 (△), Chol-SdC10 (▲), SdC6 (○), and Chol-SdC5 (●).

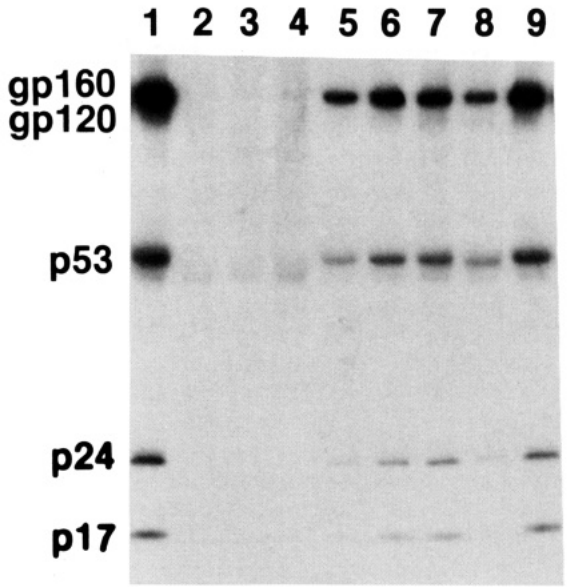

FIGURE 2: Cell-free infection of CEM cells by HIV-1 in the presence of the oligomers. CEM cells (1×10^5) were infected with HIV-1 in the absence or presence of different concentrations of oligomers. The viral proteins expressed in the infected cells after 5 days were labeled with [^{35}S]methionine for 7 h and immunoprecipitated with human serum containing antibodies to HIV-1 proteins and analyzed by SDS–PAGE. Proteins were from cells infected with no added oligomer (lane 1) and in the presence of 20 μM Chol-SdC10 (lane 2), 10 μM Chol-SdC10 (lane 3), 5 μM Chol-SdC10 (lane 4), 20 μM SdC11 (lane 5), 10 μM SdC11 (lane 6), 5 μM SdC11 (lane 7), 20 μM Chol-SdC5 (lane 8), and 20 μM SdC6 (lane 9).

thoroughly washed and 50 μL of purified gp120 (0.5 μg/mL) in the absence or presence of inhibitor was added to the well in PBS–BSA solution. After 1 h of incubation at room temperature, the wells were washed and the bound ligand after washing was detected by incubation with anti-gp120 monoclonal antibody conjugated with horseradish peroxidase (1:600 dilution in PBS–BSA solution containing 0.2% Triton X-100) for 60 min. This was followed by the addition of 50 μL of tetramethylbenzidine solution. The enzyme reaction was allowed to progress at room temperature for 30 min and the reaction was terminated by addition of 50 μL of 4 N H_2SO_4. The optical density was measured at 450 nm in an Organon Tecknica ELISA reader.

Results

Inhibition of HIV-1- and HIV-2-Induced Syncytia Formation by Phosphorothioate Derivatives. The specific interaction of the surface protein gp120 of the HIV-1 virion with CD4 antigen has been shown to be the initial step in HIV-1 infection. The interaction between gp120 and CD4 at the cell surface frequently results in the formation of multinucleated giant cells (Sarngadharan & Markham, 1987). We have examined the ability of phosphorothioate oligomers to inhibit syncytia formation by both HIV-1- and HIV-2-infected cells upon incubation with susceptible CEM cells. Experiments using Chol-SdC5 and Chol-SdC10 were performed and compared with results obtained with the unmodified phosphorothioate oligomers of the same net charge.

The specificity of syncytium formation induced by HIV-1- and HIV-2-infected Molt 3 cells used in our assay was determined by using sCD4 and OKT4a monoclonal antibody as inhibitors. Both sCD4 and the OKT4a monoclonal antibody completely blocked the syncytium formation induced by HIV-1-infected cells (data not shown). Although OKT4a inhibited syncytium formation induced by HIV-2-infected cells, sCD4 was less effective in this process (data not shown). Similar observations have been reported elsewhere (Clapham et al., 1989). Figure 1 shows the results on syncytium inhibition in the presence of cholesteryl-modified phosphorothioate cytidine homopolymer. For HIV-1 (Figure 1A), Chol-SdC5 was only effective at 12.5 μM. With the HIV-2 isolate (Figure 1B), both Chol-SdC10 and SdC11 were inhibitory, but the ID_{50} for the former (about 0.8 μM) was still somewhat less than for the latter (about 1.5 μM). Chol-SdC5 was not inhibitory except at a concentration of 25 μM, and SdC6 was entirely ineffective up to 25 μM.

To exclude the possibility that the inhibition of syncytium formation observed in infected cells were due to inhibition in the synthesis or processing of cell-surface glycoprotein by modified or unmodified phosphorothioate oligomers, we performed the following experiment: Molt3/HIV-1 cells were treated with the oligomer at the appropriate concentration for 48 h and then radiolabeled with [^{35}S]methionine in the presence of the oligomer for 7 h. The newly synthesized proteins in the cells were analyzed by immunoprecipitation with HIV-1 antibody positive human serum (Sarngadharan & Markham, 1987). Chol-SdC10 had no effect on the synthesis or processing of viral glycoprotein when the cells were treated with the oligomer at a concentration capable of inhibiting syncytia formation (data not shown).

Cell-Free Infection. To determine whether these oligomers could block infection of CD4+ cells with cell-free virions, CEM cells were infected with HIV-1 and treated with different concentrations of the phosphorothioate oligomers. The expression of viral proteins in the infected cells was determined by radiolabeling the cells with [^{35}S]methionine and by immunoprecipitating with human serum containing anti-HIV antibodies. The results are shown in Figure 2. SdC6 (20 μM) had no cytoprotective effects on the infection of CEM cells as revealed from the expression of viral proteins, while Chol-SdC5 was minimally effective at that concentration. SdC11 demonstrated a dose-dependent inhibition of viral replication, but Chol-SdC10 gave conplete cytoprotection at a concentration as low as 5 μM.

Mode of Action of Chol-SdC10 in Inhibiting HIV Replication. Sequence-nonspecific phosphorothioate oligodeoxynucleotides have been shown to be potent inhibitors of HIV reverse transcriptase in vitro (Majumdar et al., 1989). Al-

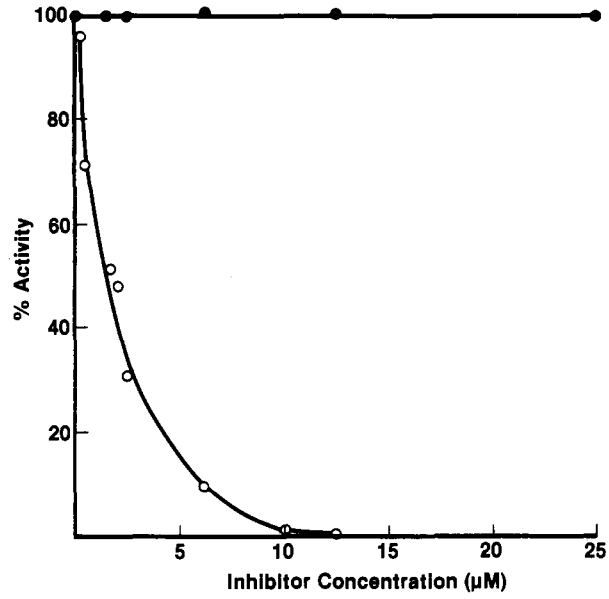

FIGURE 3: Inhibition of reverse transcriptase activity of HIV-1 by oligomers. Purified reverse transcriptase, 1.5 ng, was incubated with the indicated concentration of either Chol-SdC10 (○) or SdC11 (●) for 30 min at room temperature. The rest of the reaction components were then added to the mixture and the assay was performed at 37 °C for 60 min. The total concentration of oligomer in the experiment with Chol-SdC10 was maintained at 12.5 μM by the addition of SdC11 to the Chol-SdC10 solution.

though these compounds inhibit the reverse transcriptase enzyme, some recent studies have demonstrated that they may interfere in the binding and fusion of HIV with susceptible T cells (Stein et al., submitted for publication). Both these properties of HIV-1 were studied in the presence of Chol-SdC10.

Inhibition of Reverse Transcriptase Activity of HIV-1 by Chol-SdC10. The inhibition of polymerase and RNase H activity of HIV-1 RT by Chol-SdC10 was studied as a function of oligomer concentration by using purified enzyme. As shown in Figure 3, reverse transcriptase activity was markedly inhibited by Chol-SdC10 with nearly 98% inhibition observed at 12 μM concentration. In contrast SdC11 was not inhibitory at any concentration used in this experiment (up to 25 μM). Unlike the polymerase activity, the RNase H activity of HIV-1 RT was not affected by Chol-SdC10 (data not shown). The mechanism of Chol-SdC10-mediated inhibition of reverse transcriptase activity was examined in greater detail by using steady-state kinetics. Since it was previously demonstrated that oligodeoxynucleotides can act as competitive inhibitors of template primer binding by reverse transcriptase (Majumdar et al., 1989), the extent of inhibition was measured at various concentrations of the template primer $(dT)_{15}(rA)$. A double-reciprocal plot of dTMP incorporation versus template primer concentration in the presence of a Chol-SdC10 concentration of less than 5 μM formed a pattern of noncompetitive inhibition. However, at higher concentrations of Chol-SdC10 (6.0, 8.0, 10.0, 12.0 μM) the double-reciprocal plot obtained was linear and formed a pattern of simple competitive inhibition. This results suggests that Chol-SdC10 can combine with more than one form of the enzyme and/or interact with more than one binding site.

Inhibition of Binding and Fusion of HIV-1 with CEM Cells in the Presence of Chol-SdC10. Since Chol-SdC10 is a potent inhibitor of HIV-1 reverse transcriptase, we wanted to examine its effect on virus-induced fusion of susceptible T cells in the absence of any reactive reverse transcriptase activity. For this study we selected the CD4(−) cell line 8E5, a clonal derivative of CEM cells (Folks et al., 1986; Gendelman et al., 1987) carrying a single copy of the entire HIV-1 genome. It produces noninfectious virus particles because of a point mutation in the reverse transcriptase domain of the *pol* gene. The 8E5 cells express viral envelope glycoprotein on the cell surface and when cocultured with CD4+ cells induce cytopathic effects identical with those observed with cultured T cells infected with the wild-type virus. As shown in Figure 4A, cocultivation of SupT1 cells with 8E5 cells resulted in the formation of typical syncytial cells that was blocked in the presence of Chol-SdC10 (10 μM). Chol-SdC10 strongly inhibited the syncytium formation with an ID_{50} of 7.5 μM (Figure 4B). However, it was not as potent an inhibitor as SdC28 (ID_{50} of 0.2 μM). SdC11 was only slightly inhibitory at a concentration of 40 μM (Figure 4B).

The inhibition of syncytium formation of Chol-SdC10 could be due to the interference in the binding of gp120 with CD4 or due to the inhibition of postbinding fusion events. The binding of gp120 to sCD4 attached on a polystyrene plate was measured by solid-phase binding assay (Lederman et al., 1989). The sCD4 coated on a plate was reacted with gp120 (0.5 μg/mL) and the bound ligand was detected with anti-gp120 antibody conjugated to horseradish peroxidase. The binding was inhibited markedly (20 μg/mL, 80% versus control) by the OKT4a antibody but not by OKT4, thus demonstrating the specificity of this interaction. The effect of Chol-SdC10 on the binding of gp120 to CD4 was also examined. Chol-SdC10 showed partial inhibition (50% ± 10%) of the binding of gp120 with CD4, whereas SdC11 was not inhibitory in this binding process.

Discussion

In the present study we have addressed the non-sequence-specific mode of action of phosphorothioate oligodeoxynucleotide in-inhibiting HIV replication. We have demonstrated that covalent modification of a cytidine homopolymer by the addition of a 5′-cholesteryl fragment makes that oligomer a much more potent inhibitor of HIV infectivity than the corresponding unmodified oligomer of the same net charge. This is particularly apparent in the syncytium formation assay when the chronically infected HIV-1 and HIV-2 cells were used for syncytium induction. Oligomers of shorter length lacking cholesterol were not effective at all in blocking syncytia formation of either isolate; this is not surprising given reports (Matsukura et al., 1987; Stein et al., 1989) of the oligomer length dependence of the nonsequence specific effects of phosphorothioate oligomers. Even so, the placement of a cholesteryl fragment at the 5′-end of the 11-mer phosphorothioate oligomer increased its potency in this assay system to a value comparable with SdC28, the most potent oligomer tested. The inhibition of HIV-1-induced cell fusion by Chol-SdC10 was further demonstrated by using a cell line 8E5, which carries an HIV-1 genome mutated in the coding region of the reverse transcriptase gene. As shown here, Chol-SdC10 inhibited the syncytium formation induced by 8E5 cells when cocultured with SupT1 cells. However, it was not nearly as potent an inhibitor (ID_{50} about 7.5 μM) as SdC28 (ID_{50} about 0.2 μM), suggesting that while the two compounds are effective at the level of the cell surface, their exact mode of action at the cell surface may be somewhat different.

Experiments on the binding of gp120 with CD4 attached to the polystyrene surface showed partial inhibition of binding of glycoprotein in the presence of Chol-SdC10. Similar inhibition of binding of gp120 to CD4 was also observed in the presence of SdC28 (Pal et al., unpublished results). However,

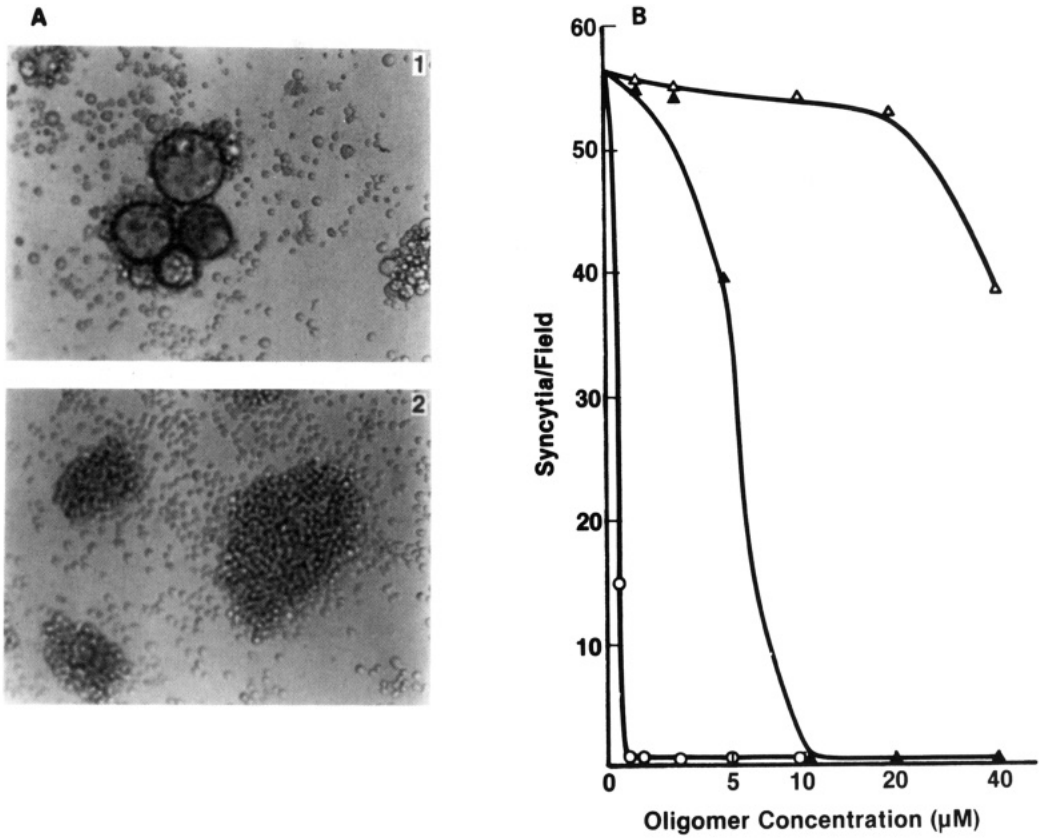

FIGURE 4: Inhibition of syncytium formation induced by 8E5 cells in the presence of the oligomers. Panel A: Frame 1, SupT1 cells cocultured with 8E5 cells; frame 2, SupT1 cells cocultured with 8E5 cells in the presence of 10 μM Chol-SdC10. The cells were photographed 18 h after coculturing. Panel B: SupT1 cells were cocultured with 8E5 cells in the presence of indicated concentrations of Chol-SdC10 (▲), SdC11 (△), and SdC28 (○).

we have also observed that at a concentration of 100 μM, Chol-SdC10 showed no consistent ability to block the binding of Leu3a antibody to CD4+ cells, whereas SdC28, at a concentration of 75 μM, decreased the binding of Leu3a to CD4+ cells by about 50% (Stein et al., submitted for publication). It is thus conceivable that the two oligomers may interfere with HIV binding to CD4 by interacting with two different sites on the CD4 molecule. Alternatively the presence of cholesterol on the oligomer may facilitate its interaction with the lipid bilayer, which may alter membrane fluidity and reorganize surface proteins on the bilayer. The inhibition of cell fusion activity observed with Chol-SdC10 could therefore be an indirect effect.

As Letsinger et al. (1989) have demonstrated, cholesteryl-liked phosphorothioate oligomers are potent inhibitors of infection of CD4+ Molt3 cells by cell-free virions. We demonstrate here that 5′-cholesteryl-linked phosphorothioate homoligomers are potent direct inhibitors of the HIV-1 reverse transcriptase DNA polymerase activity but not the RNase H activity. The kinetic analysis demonstrates that at high concentrations of Chol-SdC10 the mechanism of this inhibition is competitive with respect to template primer binding. Our observation that Chol-SdC10 mediates inhibition of template primer binding to reverse transcriptase in a competitive manner suggests that this compound also functions as a primer analogue. However, this function requires the addition of cholesterol, since the unmodified SdC11 had no effect on reverse transcriptase activity. Interestingly, even when the concentration of Chol-SdC10 is low relative to that of template primer, inhibition of HIV-1 reverse transcriptase is still observed, but the mechanism of this inhibition is noncompetitive. This indicates that Chol-SdC10 can inhibit HIV-1 reverse transcriptase activity through interactions with the enzyme other than at the template primer binding site. The structural basis for this dramatic augmentation of RT inhibition by the addition of the cholesteryl moiety is not clearly understood. The significance of the ability of this cholesteryl-modified oligomer to inhibit RT looms larger in light of recent observations by Boutorin et al. (1990). This group synthesized phosphodiester homopolymers of thymidine containing 5′-alkylating substituents and 3′-cholesteryl moieties. They reported that cellular uptake of the alkylating oligomer and DNA alkylation was increased dramatically for the 3′-cholesteryl-modified as compared to 3′-unmodified oligomer. Similar types of results using a 5′-undecyl-modified phosphodiester oligomer have also been recently reported (Kabanov et al., 1990). 5′-Modification of an oligomer by distearoylglycerol 3-phosphate (Shea et al., 1990) has also led to a dramatic increase, relative to the unmodified oligomer, in its ability to behave as an antisense inhibitor of vescicular stomatitis virus protein synthesis in infected L929 cells. The mechanism of this effect remains uncertain.

Our results suggest that Chol-SdC10 is capable of inhibiting HIV replication by at least two separate mechanisms. These include prevention of cell-to-cell infection by interfering with the binding and fusion of HIV-infected cells with susceptible CD4+ cells and by direct inhibition of viral reverse transcriptase activity. Although the precise mode of action of Chol-SdC10 on the cell surface is not clearly understood, it appears to interfere partly in the binding of gp120 with CD4. However, subsequent membrane fusion events following binding of the virus to the cell may also be inhibited in the presence of the oligomer. The possibility that Chol-SdC10 is capable of inhibiting multiple steps in the virus life cycle may make it the first of a novel series of anti-HIV agents with potentially useful clinical applications.

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8E5 cells were obtained from AIDS Research Repository, Rockville, MD.

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Bleomycin-Dependent Damage to the Bases in DNA Is a Minor Side Reaction†

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ABSTRACT: The antitumor antibiotic bleomycin degrades DNA in the presence of ferric ions and H_2O_2 or in the presence of ferric ions, oxygen, and ascorbic acid. When DNA degradation is measured as formation of base propenals by the thiobarbituric acid assay, it is not inhibited by superoxide dismutase and scavengers of the hydroxyl radical or by catalase (except that catalase inhibits in the bleomycin/ferric ion/H_2O_2 system by removing H_2O_2). Using the technique of gas chromatography/mass spectrometry with selected-ion monitoring, we show that DNA degradation is accompanied by formation of small amounts of modified DNA bases. The products formed are identical with those generated when hydroxyl radicals react with DNA bases. Base modification is significantly inhibited by catalase and partially inhibited by scavengers of the hydroxyl radical and by superoxide dismutase. We suggest that the bleomycin–oxo–iron ion complex that cleaves the DNA to form base propenals can decompose in a minor side reaction to generate hydroxyl radical, which accounts for the base modification in DNA. However, hydroxyl radical makes no detectable contribution to the base propenal formation.

The bleomycins are a group of glycopeptide antitumor antibiotics that are used effectively in the treatment of several human cancers (Umezawa, 1978). Two features of the bleomycin molecule are related to its biological action: an ability to bind to DNA (largely determined by the bithiazole and terminal amine residues of the molecule) and an ability to bind metal ions (determined by the β-aminoalanine–pyrimidine–β-hydroxyhistidine part) (Umezawa, 1978; Dabrowiak, 1980; Burger et al., 1981). When ferrous ions are complexed to bleomycin in the presence of oxygen, the complex can cleave

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