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Mechanism of inhibition of HIV-1 infection *in vitro* by guanine-rich oligonucleotides modified at the 5′ terminal by dimethoxytrityl residue

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ABSTRACT

Oligodeoxyribonucleotides (ODN) linked at their 5′-end with dimethoxytrityl (DmTr) residue were examined for antiviral activities against human immunodeficiency virus type 1 (HIV-1). We found that guanine-rich oligonucleotides exhibit anti-HIV activity upon 5′-end modification with DmTr. One oligonucleotide, DmTr-TGGGAGGTGGGTCTG (SA-1042), showed potent anti-HIV activity *in vitro*. A greater than 95% reduction of infectivity was observed if the cells were treated with 10 μg/ml of SA-1042 at the time of viral infection. PCR analysis confirmed that there was a significant reduction of provirus in the cells exposed to virus in the presence of SA-1042. By contrast, no inhibition was observed if the cells were treated with the oligomer 1 h after virus adsorption. SA-1042 prevented syncytium formation between chronically infected cells and CD4 positive uninfected cells. Furthermore, the oligomer interfered the interaction of purified gp120 to the CD4 receptor. By contrast, SA-1042 had no inhibitory effect on chronically HIV-infected cells. These results strongly suggest that the DmTr-ODNs with appropriate base sequences antagonize HIV-1 infection during the stage of virus–cell interaction.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-l), the etiologic agent of acquired immunodeficiency syndrome (AIDS) , is tropic and cytopathic toward T cells and macrophages bearing the CD4 surface antigen (1–3). HIV-1 enters target cells after binding of its outer envelope glycoprotein gp120 to the CD4 receptor (4–6).

This interaction is the initial event in the viral replicative cycle and consequently is an important target for therapeutic intervention. A number of approaches to this drug target have been described and these include strategies using CD4 analogues, neutralizing antibodies and polyionic compounds (7–10).

It has been recently demonstrated that the ODN analogue, phosphorothioate cytidine homopolymer is capable of interfering with the gp120–CD4 interactions, and is also a potent inhibitor of HIV-1 reverse transcriptase (11,12). Other workers have shown that guanine-rich phosphorothioate oligonucleotides exhibit anti-HIV activity by blocking the interaction of the V3 domain of gp120 with cellular receptor (13).

We tested the anti-HIV activity of phosphodiester ODNs in which the 5′-end was covalently linked to a DmTr residue.We found that one ODN complementary to the second splicing acceptor site of HIV-1 inhibited virus-induced cytopathic effect (in preparation). In the present study, we investigated the sequence specificity and mode of action of DmTr-modified ODNs. We demonstrate here that guanine-rich DmTr-ODN in which the base sequence is not complementary to HIV-1 RNA also exhibites potent anti-HIV activity. By contrast, an unmodified phosphodiester ODN with the same base sequence was not found to exhibit any anti-HIV activity. Furthermore, we show that the DmTr-ODN blocks the early stage of HIV-1 infection, including attachment and fusion of HIV-1 virions to susceptible cells.

MATERIALS AND METHODS

Cells and viruses

The cell lines used were the the human T-lymphoblastoid cell line C8166 cell line (ADP 013), the human cutaneous T-cell lymphoma line H9 (ADP 001), H938 cells (14) and 8E5/LAV cells (ADP 110) (15) and the human T-cell transformed line MT-4 (kindly provided by Drs N.Kobayashi and N.Yamamoto, Department of Virology and Parasitology, Yamaguchi University, Yamaguchi, Japan). Cells were maintained in RPM1-1640 medium containing 10% fetal calf serum and antibiotics. The stock of HIV-1 (IIIB) was prepared from the culture supernatants of acutely HIV-1 infected MT-4 cells. The virus stocks were stored in small aliquots at −70°C until use.

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Synthesis and purification of oligodeoxynucleotides

ODNs with DmTr covalently linked at the 5'-end and corresponding unmodified oligomers were synthesized on an automated DNA synthesizer (Applied Biosystems; model 380-B). All oligomers were purified by C18 reverse phase HPLC by an acetonitrile gradient (20−45%) in an 0.1 M triethyl ammonium acetate (pH 7.2) buffer.

Cytopathic effect inhibition assay

The procedure for measuring anti-HIV-1 activity in MT-4 cells was performed as described previously (16). Briefly, exponentially growing MT-4 cells were centrifuged for 5 min at 140×*g*. The cell pellet was suspended in a small quantity of RPMI-1640 medium and infected with 100 $TCID_{50}$ HIV-1 for 1 h. The cells were then washed with RPMI-1640 medium, resuspended in the same medium, and distributed in 96-well plates containing serial dilution of oligomers. Each well contained 2.5×10^4 cells in 200 μl medium. At day 6, cell viability was assessed by the MTT method. The concentrations of the compounds giving 50% inhibition of HIV-induced cytopathic effect (IC_{50}) were determined from the dose−response curves.

Western blot analysis of HIV-1 protein

MT-4 cells were infected with HIV-1 for 1 h as described in a previous section. The cells were then washed in RPMI-1640 medium and transferred to 24-well plates and cultured for 5 days in the presence of various concentrations of ODNs. The cells were collected, washed with PBS and lysed in SDS sample-loading buffer (17). The lysates were treated at 100°C for 5 min and fractionated on a 12.5% (w/v) SDS polyacrylamide gel (17), then transferred onto nitrocellulose filter. The extent of viral antigen was assessed with a HIV-1 antiserum from an infected patient and anti-human/horseradish peroxidase conjugate.

Short-term experiment for inhibition of HIV-1 replication

MT-4 cells (2×10^6) were infected with HIV-1 (IIIB) at 400 $TCID_{50}$ in RPMI-1640 medium in the presence or absence of 10 μg/ml of SA-1042 for 1 h at 37°C. After incubation, the cells were washed three times and cultured for 20 h in the absence or presence of SA-1042, respectively. After cultivation the cells were collected, washed and lysed in a buffer containing 1% of Triton X-100. The resulting lysates were incubated at 65°C for 30 min, and finally clarified by centrifugation (10 000×*g*, 10 min). The level of HIV-1 p24 core antigen in the clarified supernatants was determined by p24 antigen enzyme-linked immuno sorbent assay (Abbot).

Polymerase chain reaction analysis of HIV-1 proviral DNA

Cells were pelleted by low-speed centrifugation (3000×*g*, 10 min) and washed three times with PBS. The total cellular DNA was extracted from the cells using a genomic DNA isolation kit (QIAGEN) according to the manufacturer's instructions. The presence of HIV-1 provirus was assessed following 35 cycles of amplification with a Perkin-Elmer Cetus thermal cycler and primer pairs corresponding to HIV-1 gag sequences 901−927 and 1483−1509 and human β-actin sequences 2111−2140 and 2481−2510, respectively. The amplified samples were subjected to 0.8% agarose gel electrophoresis, stained with ethidium bromide and photographed.

Syncytia formation assay

Syncytia were measured by mixing 2×10^5 8E5/LAV cells with 2×10^5 C8166 cells in the presence or absence of 12.5 μg/ml of SA-1042. The culture was incubated at 37°C for 24 h and photographed.

Quantitation of cell fusion

Indicator H938 cells (2×10^5) containing integrated CAT gene under the control of HIV-1 LTR were mixed with 8E5/LAV cells (2×10^5) in the presence or absense of SA-1042, then co-cultivated at 37°C for 24 h. The cells were collected, lysed and amount of CAT protein was determined by enzyme-linked immuno sorbent assay (Boehringer).

Binding assay

Binding of gp120 to CD4 was analyzed using commercial CD4/gp120 capture ELISA kit (American Biotechnologies, Inc.). ODN samples were mixed with 100 ng of gp120 in a total volume of 100 μl. This mixture was added to 96-well plate coated with CD4 and incubated for 1 h. The solutions were aspirated, and the wells were washed several times with PBS. The extent of gp120 binding was assessed with the use of anti-gp120/horseradish peroxidase conjugate.

Polyacrylamide gel electrophoresis

ODNs were incubated at 37°C for 10 min in Phosphate buffered saline at a concentration of 1 mg/ml. Then subjected to 15% non-denaturing polyacrylamide gel electrophoresis, stained with ethidium bromide and photographed.

RESULTS

Inhibition of HIV-1 replication by DmTr-linked ODNs

The structure of DmTr-linked ODN is illustrated in Figure 1. DmTr-linked ODNs were obtained as an intermediate product

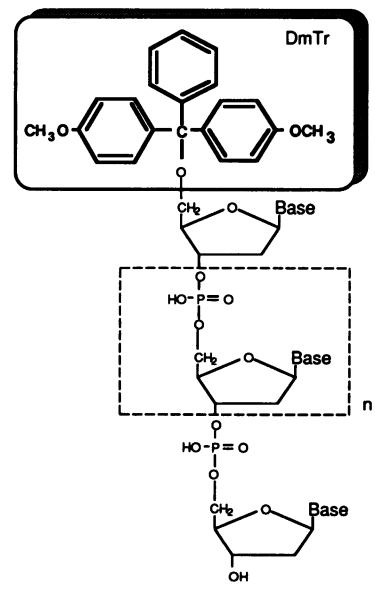

Figure 1. Molecular structure of DmTr-linked ODN.

of automated DNA synthesizer. The nucleotide sequences of DmTr-linked and unmodified ODNs are listed in Table 1. They are complementary to six different sites of HIV-1 RNA (18).

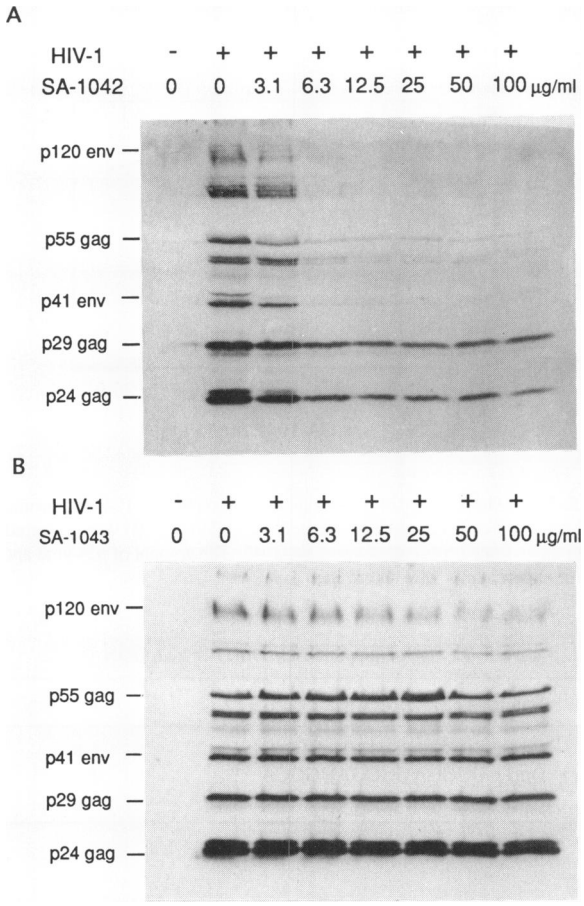

Figure 2. Inhibition of viral antigen synthesis by SA-1042. MT4 cells were infected with HIV-1 and cultured in the presence of indicated concentrations of (**A**) SA-1042 or (**B**) SA-1043. At 5 days post infection cells were lysed, fractionated on a SDS–PAGE and immunoblot with sera from a HIV-1-seropositive patient.

The anti-HIV activity of these ODNs was evaluated by their ability to inhibit HIV-1 induced cytopathic effect (CPE) on MT-4 cells. Among these, ODN 11 (SA-1042) was capable of inhibiting virus-induced CPE. The IC_{50} value of SA-1042 was 6.3 μg/ml (Table 1) and no cytotoxity was found up to 100 μg/ml (data not shown). By comparison, an unmodified ODN with the same sequence, ODN 12 (SA-1043), exhibited no activity up to 100 μg/ml. The rest of the DmTr-modified or unmodified oligomers were all found to be inactive.

A Western blotting assay was performed to evaluate the effect of SA-1042 on viral antigen synthesis in MT-4 cells acutely infected with HIV-1. As shown in Figure 2A, production of viral antigens was significantly decreased in the presence of 6.3 μg/ml of SA-1042. In contrast, no significant inhibition of antigen production was observed with SA-1043 up to 100 μg/ml (Fig. 2B). This suggests that modification of 5′-end of the ODN with DmTr is critical for its activity.

SA-1042 inhibits an early stage of HIV-1 infection

In order to assess the mode of action of SA-1042, we investigated whether the antiviral effect was observed when the oligomer was present during the virus entry phase of infection or at the post exposure phase. MT-4 cells were infected with HIV-1 by simultaneous addition of virus and SA-1042 at 10 μg/ml. After 1 h incubation, the cells were collected by centrifugation, extensively washed to remove free virus and the oligomer, and cultured for 20 h in the absence of the oligomer. Alternatively, cells were infected with HIV-1 for 1 h in the absence of the oligomer, free virus removed by washing, and then cultured for 20 h with 10 μg/ml of SA-1042. Presence of SA-1042 during the period of virus exposure completely suppressed p24 production (Fig. 3, lane 3). By contrast, no inhibition was observed with control cells when the cells were infected with HIV-1 in the absence of the oligomer (Fig. 3, lane 4).

No viral signal was detected with PCR analysis of the DNA that had been extracted from the cells exposed to SA-1042 at the time of virus exposure (Fig. 4A, lane 3). On the other hand, no inhibition of viral signal was observed with the DNA that had been extracted from the cells treated with SA-1042 after virus exposure (Fig. 4A, lane 4). Since the same level of cellular β-

Table 1. ODN number, sequence, position of target site in HIV genome and anti-HIV activity

No.	Sequence (5'-3')	Complementary Site*	Function	IC50 (μg/ml) for Anti-CPE§
1	DmTr-GAGTCACACAACGAG	118-124	Primer Binding	>100
2	GAGTCACACAACGAG			>100
3	DmTr-GCGTACTCACCAGTC	285-299	1'st Splice Donor	>100
4	GCGTACTCACCAGTC			>100
5	DmTr-CACCCAATTCTGAAA	5355-5369	1'st Splice Acceptor	>100
6	CACCCAATTCTGAAA			>100
7	DmTr-CTGGCTCCATTTCTT	5407-5421	Tat Initation	>100
8	CTGGCTCCATTTCTT			>100
9	DmTr-TACTTACTGCTTTGA	5619-5633	2'nd Splice Donor	>100
10	TACTTACTGCTTTGA			>100
11 (SA-1042)	DmTr-TGGGAGGTGGGTCTG	7954-7978	2'nd Splice Acceptor	6.3
12 (SA-1043)	TGGGAGGTGGGTCTG			>100

§Assays are described in 'Materials and Methods'. Values are means of duplicate determinations.

actin signal was detected from all the preparations, it was suggested that the DNA preparations were intact (Fig. 4B, lanes 1–4). In conclusion, simultaneous addition of SA-1042 and virus resulted in no apparent viral replication, suggesting that inhibition occured at the stage of viral entry, RT, or integration. This also strongly suggested that SA-1042 did not act as an antisense molecule which might prevent splicing or translation of viral RNA. In support of this view, SA-1042 was not able to inhibit replication of virus in chronically infected cells (Fig. 5).

Base composition specific inhibition of HIV-1 replication by DmTr-ODNs

One characteristic feature of SA-1042 is its high guanine content; the oligomer contains 9 guanine residues out of 15 residues. By contrast, oligonucleotides which showed no antiviral activity containing less than 4 guanine residues (Table 1). We then asked if any other guanine-rich DmTr-ODNs, with sequences not complementary to HIV-1, could exhibit anti-HIV activity. Table 2 shows that three different DmTr-modified guanine-rich sequences could inhibit CPE in MTT assay with IC_{50} values equal to or lower than 2 μg/ml . These findings strongly suggest that the mode of action of the DmTr-linked ODN is not based on an antisense mechanism. It has been reported that cytidine phosphorothioate homo-oligomer exhibited anti-HIV activity (19). In another series of experiments, we tested the antiviral activity

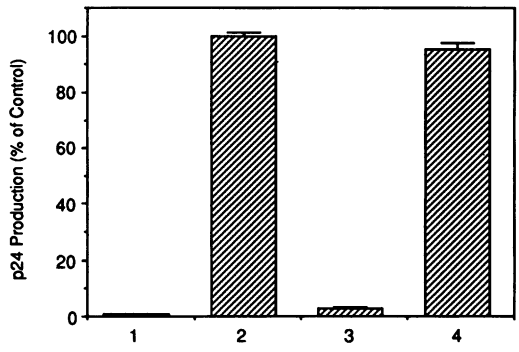

Figure 3. Effect of time of addition of SA-1042 relative to HIV-1 infection on viral replication in MT-4 cells. (1) Mock-infected control, (2) HIV-1 infected control, (3) MT-4 cells were infected with HIV-1 for 1 h in the presence of 10 μg/ml of SA-1042, then washed three times and cultivated for 20 h in the absence of the oligomer, (4) MT-4 cells were infected with HIV-1 for 1 h, then washed three times and cultivated for 20 h in the presence of 10 μg/ml of SA-1042. After the cultivation, cell extracts were prepared and the amount of viral p24 antigen was determined.

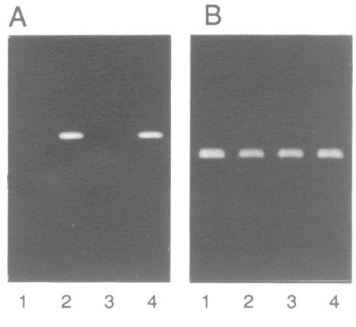

Figure 4. PCR analysis of HIV-proviral DNA (**A**) or cellular β-actin DNA (**B**) in MT-4 cells exposed to the SA-1042 during or after virus infection. The protocols for virus infection and treatment are identical to those described in the legend to Figure 3.

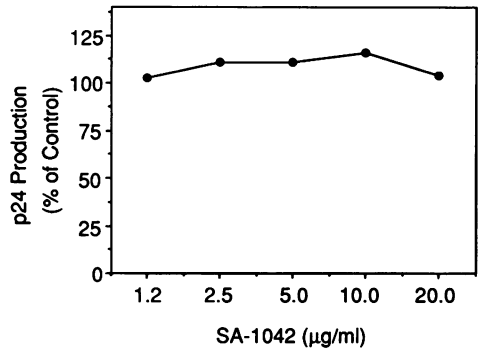

Figure 5. Failure to reduce p24 gag antigen synthesis by SA-1042 in chronically infected cells. H9 cells chronically infected with HIV-1 (IIIB) were cultured for 72 h with the indicated concentrations of SA-1042. The amount of p24 viral antigen in culture supernatants was determined.

Table 2. Antiviral activity of guanine-rich DmTr-linked ODN

No.	Sequence (5′–3′)	IC_{50} (μg/ml)
1	DmTr-TGGGTGGGTGGGTGGG	1.8
2	DmTr-UGGGAUGGGAUGGGA	2.0
3	DmTr-UGGGUGGGUGGGUGGG	1.8

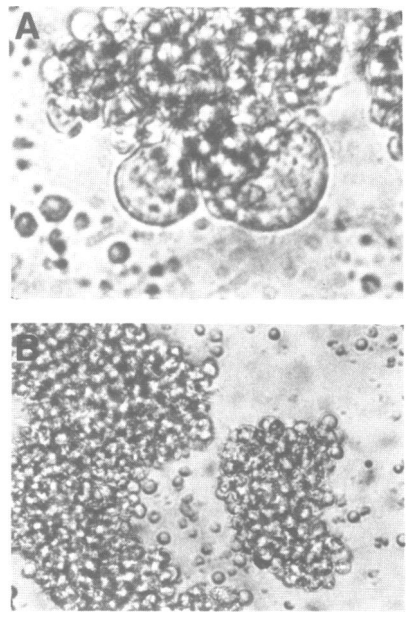

Figure 6. Inhibition of syncytium formation induced by 8E5/LAV cells in the presence of SA-1042. (**A**) C8166 cells co-cultured with 8E5/LAV cells for 24 h. (**B**) C8166 cells co-cultured with 8E5/LAV cells for 24 h in the presence of 12.5 μg/ml of SA-1042.

of DmTr-linked cytidine or thymidine homopolymer. However, no activity was observed with these oligomers (data not shown).

SA-1042 inhibits cell fusion between CD4 positive cells and chronically HIV-1 infected cells

Interaction of gp120, expressed on the membranes of infected cells, with a CD4 receptor on the susceptible cells has been shown to be the initial step of HIV-1 infection and results in the formation of syncytia (4,20). We examined the effect of SA-1042 on the gp120−CD4 dependent cell fusion. For this assay we used 8E5/LAV cells (15). The 8E5/LAV cells carry a single integrated copy of the entire HIV-1 genome but produce non-infectious virus particles because of a point mutation in the reverse transcriptase gene (21,15). The cells express all of the viral antigens except reverse transcriptase and endonuclease and when mixed with $CD4^+$ C8166 cells induce cell fusion identical to that observed with cultured T cells infected with wild-type virus (Fig. 6A).

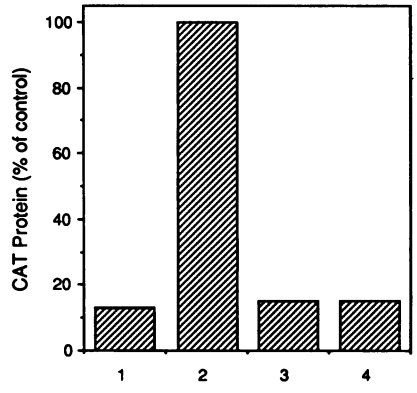

Figure 7. Inhibition of cell fusion-dependent CAT production by SA-1042. (1) H938 cells, (2) H938 cells were mixed with 8E5/LAV cells, (3) H938 cells were mixed with 8E5/LAV cells in the presence of 12.5 μg/ml of SA-1042, (4) H938 cells were mixed with conditioned medium of 8E5/LAV cells. After 24 h, cell lysates were prepared and amount of CAT protein was determined.

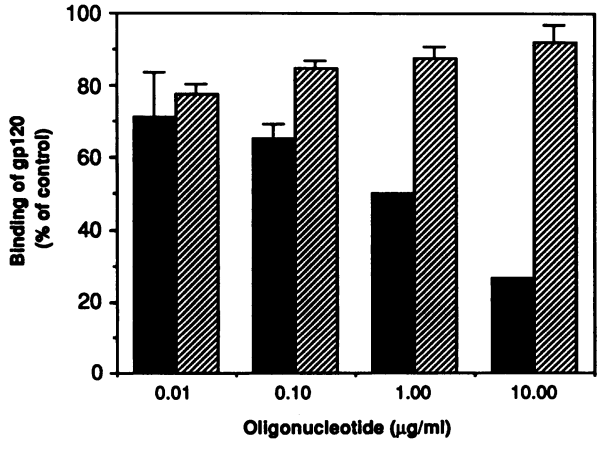

Figure 8. Inhibition of gp120-CD4 binding. SA-1042 (solid bars) and SA-1043 (hatched bars) as determined by solid-phase ELISA. The amount of gp120 bound to wells coated with sCD4 was studied as a function of concentration as described in the 'Materials and Methods'.

Treatment of the cells with SA-1042 (12.5 μg/ml) at the time of cell mixing completely inhibited the fusion (Fig. 6B). The inhibition of syncytium formation was further confirmed by the CAT-reporter system (Fig. 7). In this assay we used H938 cells as an indicator cell line, which carry the HIV-1 long terminal repeat ligated to the chloramphenicol acetyltransferase (CAT) gene (14). Since 8E5/LAV cells produce non-infectious virus, no stimulation of CAT production was observed with the 48-h conditioned medium of 8E5/LAV cells (Fig. 7, lane 4). Co-cultivation of H938 cells with 8E5/LAV cells resulted in 9-fold activation of CAT production (Fig. 7, lanes 1 and 2). Addition of 12.5 μg/ml of SA-1042 reduced production of CAT protein to the basal level (Fig. 7, lane 3). In a control experiment, the presence of SA-1042 did not inhibit CAT production of chronically HIV-1 infected H938 cells (data not shown).

Inhibition of gp120 attachment to CD4 by SA-1042

To evaluate the possibility that SA-1042 might prevent binding of gp120 to the CD4 receptor, the binding of gp120 to sCD4

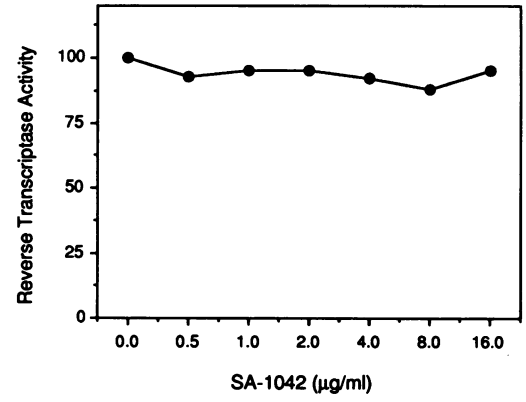

Figure 9. Effect of SA-1042 on HIV-1 reverse transcriptase activity. Purified reverse transcriptase and reaction component suppslied by manufacturer (Boehringer) were mixed in the presence of indicated concentrations of SA-1042, and the assay was performed at 37°C for 1 h.

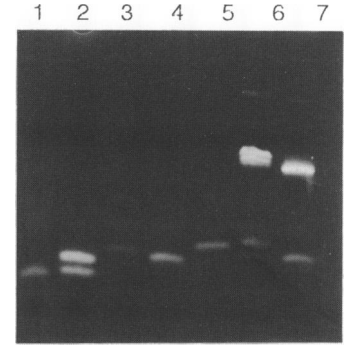

Figure 10. Effect of base-composition and DmTr-modification of ODNs on a mobility on PAGE. ODNs in Table 1 were analysed on a 15% polyacrylamide gel electrophoresis, stained with ethidium bromide and photographed. Lane 1, ODN 1; lane 2, ODN 3; lane 3, ODN 5; lane 4, ODN 7; lane 5, ODN 9; lane 6, SA-1042 (ODN 11); lane 7, SA-1043 (ODN 12) in Table 1.

attached on a polystyrene plate was measured by a solid-phase binding assay. The sCD4 coated on a polystyrene plate was reacted with gp120 (100 ng/ml) and the bound ligand was detected with anti-gp120 antibody conjugated to horseradish peroxidase. The binding was markedly inhibited by SA-1042 in a dose-dependent manner: 75% binding inhibition was achieved at a concentration of 10 μg/ml (Fig. 8). On the other hand, no inhibition was observed with SA-1043.

Failure to inhibit viral reverse transcriptase by SA-1042

We also tested the effect of SA-1042 on HIV-1 reverse transcriptase activity by using purified enzyme. However, SA-1042 was not inhibitory up to 100 μg/ml (Fig. 9). Therefore, it seems unlikely that inhibition of reverse transcriptase activity is responsible for the antiviral action of SA-1042.

Biological property of SA-1042

It has been reported that guanine-rich ODNs can form a non-Watson–Crick base-paired structure (22–24). It was of interest, therefore, to investigate whether 5′-end modification of ODNs might affect their ability to form the aggregation. We compared a mobility of ODNs in Table 1 on a non-denaturing polyacrylamide gel electrophoresis. As shown in Figure 10, SA-1042 and SA-1043 exhibited quite a slower mobility on the gel than other DmTr-linked ODNs, suggesting both ODNs exist as an aggregated form. Mobility of SA-1042 is slightly slower than that of SA-1043. This may reflect molecular weight difference of both ODNs. This result suggest that DmTr-modification does not affect aggregate formation of guanine-rich ODNs. Heat denaturation of aggregated form of SA-1042 before addition to the antiviral assay did not resulted in loss of the activity (data not shown). These results suggest that the aggregate formation is, at least, not a sufficient condition for the antiviral activity of the DmTr-modified ODNs.

In order to further examine a possible mechanism of DmT-modification, we next tested the effect of the modification on a stability of oligonucleotides in a serum containing medium. SA-1042 and SA-1043 were 3′-end-labled with [α-^{32}P]dideoxyadenosine, incubated for 19 h in the presence or absence of 10% calf serum, then analyzed on a polyacrylamide gel electrophoresis. In the absence of the serum, both oligonucleotides were stable after 19-h incubation (Fig. 11, lanes 3 and 5). After the incubation SA-1042 preparation contains fast-mobility species which co-migrate with SA-1043, suggesting partial unmodification might occurred during the incubation (Fig. 11, lane 5). When the ODNs were incubated with the serum, a signal intensity for SA-1043 was greatly declined after incubation with the serum (Fig. 11, lane 4). However, little reduction of signal intensity was observed with SA-1042 (Fig. 11, lane 6). It is important to note that fast-mobility signal in SA-1042 preparation was also disappeared after the incubation, suggesting that the unmodified ODN was preferentially degradated. These result suggest that the DmTr-modification of ODNs increase the stability against nuclease degradation.

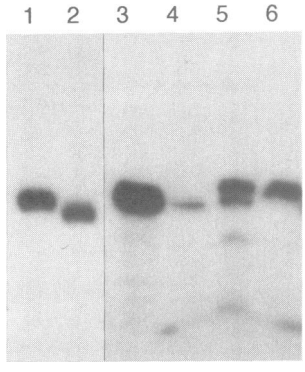

Figure 11. Effect of DmTr-modification on the stability of ODNs. ODNs 3′-end labled with [α-^{32}P]dideoxyadenosine were incubated for 19 h in a RPMI medium either containing 10% calf serum (unimmobilized) or not, then analyzed on a 15% polyacrylamide gel electrophoresis and autoradiographed. Lane 1, SA-1042 before the incubation; lane 2, SA-1043 before the incubation; lane 3, SA-1043 incubated in the absence of the serum; lane 4, SA-1043 incubated in the presence of the serum; lane 5, SA-1043 incubated in the absence of the serum; lane 6, SA-1042 incubated in the presence of the serum.

DISCUSSION

In this study we demonstrated that covalent 5′-end modification of guanine-rich phosphodiester ODNs with a dimethoxytrityl residue produces a potent inhibitor of HIV-1 infection. This modification was essential for anti-HIV activity, since no activity was observed with an unmodified ODN with the same sequence (Table 1 and Fig. 2). A DmTr-modified SA-1042 exhibited the ability to inhibit the gp120–CD4 interaction, whereas unmodified counterpart, SA-1043 did not show any activity (Fig. 8). Since the assay was performed in absence of any nuclease, it seems likely that the modification is mechanistically required for the activity. A second potential mechanism might involves increased resistance to nuclease degradation of DmTr-ODN. In a presence of calf serum, SA-1042 was more stable than SA-1043 (Fig. 11).

The nucleotide composition rather than nucleotide sequence of ODNs appeared to be important for its antiviral activity. Anti-HIV activity was observed with ODN with a high guanine content (SA-1042 contains 9 guanine out of 15 nucleotides). Furthermore, guanine-rich DmTr-ODNs were capable of inhibiting replication of HIV-1 even though they were not complementary to the HIV-1 sequence (Table 2). These observations strongly suggest that the DmTr-ODNs do not act as an antisense molecule. In support of this hypothesis, SA-1042 can only inhibit HIV-1 infectivity when added to susceptible cells simultaneously with virus (Figs 3 and 4). Furthermore the oligomer was not able to inhibit virus production from chronically infected cells with HIV-1 integrated in the host genome (Fig. 5), nor to inhibit replication of a molecular clone of HIV-1 DNA transfected into host cells (data not shown). These observations strongly support the notion that the antiviral effect of the DmTr-ODN could depend on blocking virus production at an early stage of the viral replication cycle.

Syncytium formation is known to be mediated by interaction of viral gp120, expressed on the cell surface of infected cells, with the CD4 receptor on neighboring uninfected cells (4,20). SA-1042 was able to prevent cell fusion between chronically infected 8E5/LAV cells and uninfected C8166 cells or H938 cells (Figs 6 and 7). 8E5/LAV cells contain an HIV-1 genome mutated in the coding region of the RT gene (4,20). This suggests that a non-RT-dependent stage in the HIV replication process may be inhibited by the ODN. It has been reported that 5′-cholesteryl-linked phosphorothioate cytosine homo-oligomer was a potent inhibitor of the HIV-1 reverse transcriptase (25). It has been also reported that 3′-end modified oligonucleotides composed entirely of deoxyguanosine and deoxythymidine inhibit HIV-RT (26).

However, in the present study, DmTr-linked ODN showed no inhibitory activity on the reverse transcriptase *in vitro* assay, which suggests that inhibition of the enzyme may not be responsible for the antiviral activity (Fig. 9). As shown in Figure 8, SA-1042 was able to prevent the binding of purified gp120 to immobilized sCD4 in a dose-dependent manner. The IC_{50} values for the compound in blocking gp120 binding to the receptor are similar to that for inhibiting HIV-1 growth in acutely infected MT-4 cells (Table 1).

Another mechanism to be considered is prevention of the integration process. However, it seems unlikely that the oligomer inhibits integration of proviral DNA since no reduction in the level of provirus was identified when SA-1042 was added 1 h after initial exposure of the cells to virus (Fig. 4B, lane 4) If the oligomer inhibited at the stage of integration, then the functional consequence of this inhibition should result in an obvious reduction in provirus.

Taken together the results presented here indicate that the antiviral activity of the DmTr-modified oligonucleotides is principally mediated by inhibition of virus attachment or the subsequent events involved with virus entry of the host cell. On the basis of the results to-date, one candidate event appears to be the fusion of the virus envelope to the plasma membrane. The precise mode of action of SA-1042 is currently under investigation.

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