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# Guanine-rich oligonucleotide modified at the 5′ terminal by dimethoxytrityl residue inhibits HIV-1 replication by specific interaction with the envelope glycoprotein

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**Abstract**

Previous studies have shown that a guanine-rich oligonucleotide SA-1042, DmTr-TGGGAGGTGGGTCTG, neutralizes HIV-1 infectivity, blocks syncytium formation and inhibits the binding of recombinant gp120 to immobilized soluble CD4 in vitro (Furukawa et al., 1994). We have now investigated the precise mode of action of SA-1042. We show here that SA-1042 specifically antagonizes the binding of anti-V3 loop antibodies or anti-CD4 binding-site antibodies to recombinant gp120, and also blocks the binding of an anti-V3 loop antibody to the V3 peptide ($gp120_{IIIB}$: aa302–324). In contrast, SA-1042 does not inhibit gp120 binding of monoclonal antibodies directed to other regions of gp120, such as the conserved N-terminal regions ($gp120_{IIIB}$: aa35–108 or $gp120_{IIIB}$: aa72–130) or the C-terminal region ($gp120_{IIIB}$: aa481–496). Furthermore, SA-1042 does not interfere with the binding of monoclonal antibodies directed to other molecules, gp41, CD4, CD11a, CD18, CD26, CD44 or CD54. These data suggest that SA-1042 exerts its antiviral effects by targeting the V3 loop as well as the CD4 binding site on gp120.

*Keywords:* HIV-1; Oligonucleotide; Dimethoxytrityl-oligomer; Gp120; V3 loop

## 1. Introduction

Infection of human immunodeficiency virus type-1 (HIV-1) to susceptible cell is initiated by the binding of the viral envelope glycoprotein, gp120, to the cellular receptor, CD4, which is then followed by fusion of the virus particle with the cell membrane (Dalgleish et al., 1984). Although the precise mechanism of virus entry is unclear, it is believed that specific regions of gp120 and the transmembrane glycoprotein gp41 are involved in this process. Therefore, the interaction is an attractive target for antiviral therapeutic intervention (Linsley et al., 1988; Skinner et al., 1988; Kang et al., 1991; Steimer et al., 1991; Matthew et al., 1994). Antibodies to the CD4 binding site of gp120 neutralize virus-to-cell binding and cell fusion (Kang et al., 1991; Steimer et al., 1991). Moreover, antibodies to epitopes on gp41

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(Buchacher et al., 1992; Muster et al., 1993), and in particular, antibodies to the third variable (V3) region of gp120, which is a disulfide-linked loop consisting putatively of 35 amino acids, block HIV-1 infection without interfering with the binding of gp120 to CD4 (Linsley et al., 1988; Steimer et al., 1991).

Oligonucleotide derivatives designed as antisense molecules were originally believed to inhibit specific HIV-1 gene expression in vitro by inhibiting the translation process (Dash et al., 1987). Furthermore, a variety of possible mechanisms by which oligonucleotides inhibit viral replication also exist. For example, oligodeoxycytidine, S-dC28, which has a phosphorothioate backbone, was established as a potent inhibitor of HIV-1 (Matsukura et al., 1987; Marshall et al., 1992). S-dC28 appears to bind to CD4 and block the gp120/CD4 interaction (Stein et al., 1991). Moreover, this oligonucleotide inhibits the binding of anti-V3 loop antibodies (Stein et al., 1993) and also blocks reverse transcriptase (RT) activity (Majumdar et al., 1989). Alternatively, another oligonucleotide derivative ISIS5320 composed of guanine-rich sequence of $T_2G_4T_2$ with a phosphorothioate internucleoside linkage, which is folded into an intramolecular guanosine (G)-tetrad, exerts anti-HIV-1 activity by binding to the V3 loop of gp120 (Wyatt et al., 1994; Buckheit et al., 1994). The G-quartet structure and the phosphorothioate backbone of this molecule was reported to be essential for preventing virus-to-cell interaction (Wyatt et al., 1994). In addition, 3′-end modified phosphodiester oligonucleotides, composed entirely of deoxyguanine and deoxythymidine inhibit HIV-1 replication by inhibiting HIV-1 integrase activity and/or by blocking the interaction between gp120 and CD4 (Ojwang et al., 1994, 1995).

We have previously reported that the guanine-rich phosphodiester oligonucleotide, TGGGAG-GTGGGTCTG, containing a natural phosphodiester backbone, exhibits potent anti-HIV-1 activity upon covalent linking of the 5′-end with a dimethoxytrityl (DmTr) group (Furukawa et al., 1994) or another trityl-type substituent (Hotoda et al., 1994). The DmTr-linked pentadecaoligonucleotide, SA-1042, prevented the virus entry stage of HIV-1 infection, most likely by inhibiting the interaction of gp120 with CD4, and not by the original antisense mechanism or by blocking the HIV-1 RT activity (Furukawa et al., 1994).

In this report, we further characterize the mechanism of action of SA-1042 and provide evidence to show that SA-1042 specifically interferes with the V3 loop as well as the CD4 binding site on viral gp120. SA-1042 inhibits the binding of several antibodies recognizing these two functional domains of gp120, whereas this oligonucleotide is ineffective in blocking the binding of antibodies directed to other sites of gp120. In addition, SA-1042 does not inhibit the binding of antibodies directed to human CD4, CD11a, CD18, CD26, CD44, or CD54 on T-cells. The mechanism of action of SA-1042 relative to that of other virus entry inhibitors is discussed.

## 2. Materials and methods

### 2.1. Cells and virus

The human T lymphoblastoid cell line C8166 and human cutaneous T-cell lymphoma cell line H9 were obtained from the MRC AIDS Reagent Project [NIBSC, Potters Bar, UK]. The human T-cell transformed cell line MT4 was kindly provided by Dr. N. Kobayashi and Dr. N. Yamamoto (Department of Virology and Parasitology, Yamaguchi University, Yamaguchi, Japan). H9 cells chronically infected with HIV-$1_{HXB2}$ (H9/HXB2 cells) were obtained after transfection with the infectious molecular clone, pHXB2 (MRC AIDS Reagent Project). All cells were maintained in RPMI 1640 (Gibco BRL) supplemented with 10% fetal calf serum (FCS) (Gibco BRL), 1 mM glutamine, 100 U/ml of penicillin and 100 $\mu$g/ml of streptomycin.

### 2.2. Antibodies and compounds

Monoclonal antibody $\alpha$70 (anti-gp120: aa306–324 of IIIB; mouse IgG1) was obtained from Intracel (London, UK). ABi13–106 (anti-gp120: aa35–108 of IIIB; rat IgG1) and ABi13–107

(anti-gp120: aa72–130 of IIIB; rat IgG2a) were from Advanced Biotechnologies (Maryland, USA). 1C1 (anti-gp120: aa481–496 of IIIB; mouse IgG1) was from Atlas Bioscan (West Sussex, UK). 11/75a (anti-gp120: V3 loop conformation of IIIB; IgG2a) (McKeating et al., 1992a, 1993), 10/540w (anti-gp120: aa300–315 of IIIB; rat IgG1), 8/38 (anti-gp120: aa311–321 of IIIB; rat IgG2a) (McKeating et al., 1992a), 39.3b (anti-gp120: CD4 binding site conformation of IIIB/RF/MN; rat IgG2a) and 38.1a (anti-gp120: aa427–436 of IIIB; rat IgG2b) (Cordell et al., 1991; McKeating et al., 1992b) were from the MRC AIDS Reagent Project. 2F5 (anti-gp41: aa662–667 of BH10; human IgG3) (Buchacher et al., 1992; Muster et al., 1993) was from Waldheim Pharmazeutika (Vienna, Austria). The following antibodies were provided by Becton-Dickinson (California, USA): Leu3a (anti-human CD4; mouse IgG1), LB2 (anti-human CD54; mouse IgG2b), G25-2 (anti-human CD11a; mouse IgG2a), L130 (anti-human CD18; mouse IgG1) and L178 (anti-human CD44; mouse IgG1). BA5 (anti-human CD26; mouse IgG2a) was from Binding Site Ltd. (Birmingham, UK). Each isotype control was from Pharmingen (California, USA). Peroxidase-conjugated secondary antibodies (goat anti-mouse IgG and goat anti-rat IgG) and dichlorotriazinyl amino fluorescein (DTAF)-conjugated secondary antibodies (goat anti-mouse IgG, goat anti-rat IgG and goat anti-human IgG) were from Bio-Rad Laboratories (California, USA) and Pierce Chemical Company (Illinois, USA), respectively.

Aurintricarboxylic acid (ATA) and dextran sulfate (MW8000) were obtained from Sigma (Dorset, UK).

### 2.3. Oligonucleotides

The structure of a DmTr-modified oligonucleotide is illustrated in Fig. 1 (Furukawa et al., 1994). All oligomers used in this study were synthesized on an automated DNA synthesizer 380B (Applied Biosystems) and purified by $C_{18}$ reverse phase high pressure liquid chromatography (HPLC) by an acetonitrile gradient (20–45%) in 0.1 M triethyl ammonium acetate buffer (pH 7.2). The compounds were detritylated in diluted trifluoroacetic acid, when necessary.

### 2.4. Inhibition of the binding of monoclonal antibodies

#### 2.4.1. Solid-phase enzyme-linked immunosorbent assay (ELISA)

50 ng/well of recombinant $gp120_{IIIB}$ and 500 ng/well of a V3 peptide ($gp120_{IIIB}$: aa302–324 of IIIB; TRPNNNTRKSIRIQRGPGRAFVT) (Intracel) were immobilized on microtitre plates (Corning 25860 and Sterilin 612U96, respectively) by incubating 100 $\mu$l of the antigen solutions in phosphate-buffered saline (PBS) at room temperature for 2 h. The wells were thoroughly washed with 0.05% Tween 20-PBS and blocked with 200

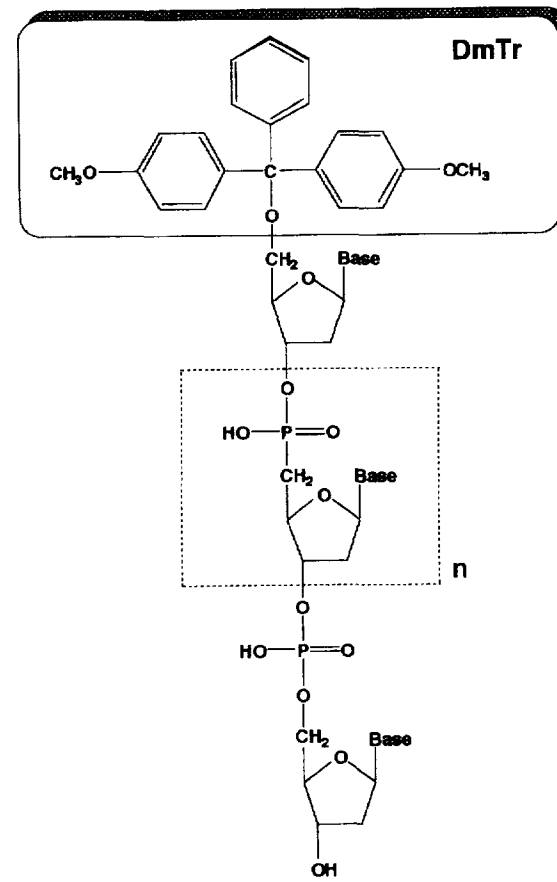


Fig. 1. Molecular structure of a DmTr-linked oligonucleotide.

$\mu$l of 1% bovine serum albumin (BSA)-PBS overnight at 4°C. The wells were then thoroughly washed and 100 $\mu$l of monoclonal antibody solutions in the presence of inhibitors at various concentrations were added to the wells in duplicate in 1% BSA-PBS (or 10% FCS-PBS). After 30 min of incubation at room temperature, the wells were washed and the bound antibodies were detected by incubation with appropriate secondary antibodies conjugated with horseradish peroxidase at room temperature for 45 min. This was followed by the addition of 200 $\mu$l of 3, 3', 5, 5'-tetramethylbenzidine solution, TMB-ELISA (Gibco BRL). The enzyme reaction was allowed to progress at 37°C for 30 min and terminated with 50 $\mu$l of 1 N $H_2SO_4$. The concentration of the first antibody was such to give an $OD_{450}$ of 1.0–1.5 determined by titration. Isotype control antibodies confirmed that the binding of the antibodies used in this study was specific for gp120 or V3 peptide.

*2.4.2. Flow cytometric analysis*

H9/HXB2 cells, H9 cells or MT4 cells ($1 \times 10^6$) were treated with various concentrations of SA-1042 and 1 $\mu$g of purified monoclonal antibodies or 10 $\mu$l of hybridoma supernatant (if 39.3b or 11/75a was used) in a total volume of 100 $\mu$l of RPMI 1640 containing 10% fetal calf serum in a 5 ml tube 2051 (Costar). The reaction mixture was then incubated at room temperature for 30 min. As a control, cells were incubated with the same monoclonal antibody in the absence of SA-1042, washed with 0.1% BSA-PBS, and then incubated with 100 $\mu$l of appropriate DTAF-conjugated secondary antibody for 1 h at 4°C. Cells were washed with 0.1% BSA-PBS prior to being fixed overnight in an equal volume of isotonic 8% formalin-PBS. The fluorescence was logarithmically acquired on a FACScan flow cytometer (Becton-Dickinson), and a minimum of 5000 lymphocyte equivalent events were analyzed with Lysis 2 V1.1 software. Nonspecific fluorescence was quantified through staining with isotype control antibody. Data were expressed as a percent of the mean fluorescence intensity of the control.

## 3. Results

*3.1. Inhibition of binding of anti-V3 antibodies by oligonucleotide derivatives*

In order to assess whether the DmTr-modified oligonucleotide (SA-1042) interacts with the V3 loop of gp120, we examined the binding of anti-V3 monoclonal antibodies (mAbs) to recombinant $gp120_{IIIB}$ in a solid-phase ELISA. gp120 was reacted with an anti-V3 loop antibody and the bound ligand was detected using a secondary antibody conjugated to horseradish peroxidase. The binding of mAb $\alpha$70, which recognizes aa306–324, and 11/75a, which recognizes a V3 loop conformational epitope of gp120, were markedly inhibited by SA-1042 (Fig. 2). The inhibition was dose-dependent, with 50% inhibitory concentrations ($IC_{50}$) of 50 and 25 $\mu$g/ml, respectively. We also tested the binding of two other antibodies to the V3 region, 10/540w and 8/38, and similar inhibition was observed (the $IC_{50}$ values were less than 200 $\mu$g/ml) (data not shown). In contrast, no inhibition of anti-V3 antibody binding was observed when an unmodified oligonucleotide with the same nucleotide sequence, SA-1043 was used (Fig. 2). This unmodified oligonucleotide also lacks anti-HIV-1 activity (Furukawa et al., 1994).

*3.2. SA-1042 interferes with antibodies which bind specifically to the V3 loop*

To confirm the specificity of the inhibition of antibody binding by SA-1042, we examined the binding of two mAbs to the N-terminal region, ABi13–106 (aa35–108) and ABi13–107 (aa71–130), a mAb to the C-terminal region, 1C1 (aa481–496) and a mAb to the CD4 binding site, 39.3b (a conformational epitope of CD4 binding site) of gp120. As shown in Fig. 3, SA-1042 failed to inhibit the binding of mAbs to the N-terminal or C-terminal regions, while dose-dependent inhibition was observed with the use of mAbs to the V3 loop. The binding of an anti-CD4 binding site mAb was partially inhibited by the addition of 400 $\mu$g/ml of SA-1042. The inhibition of binding was 38%. To evaluate the direct effect of SA-1042

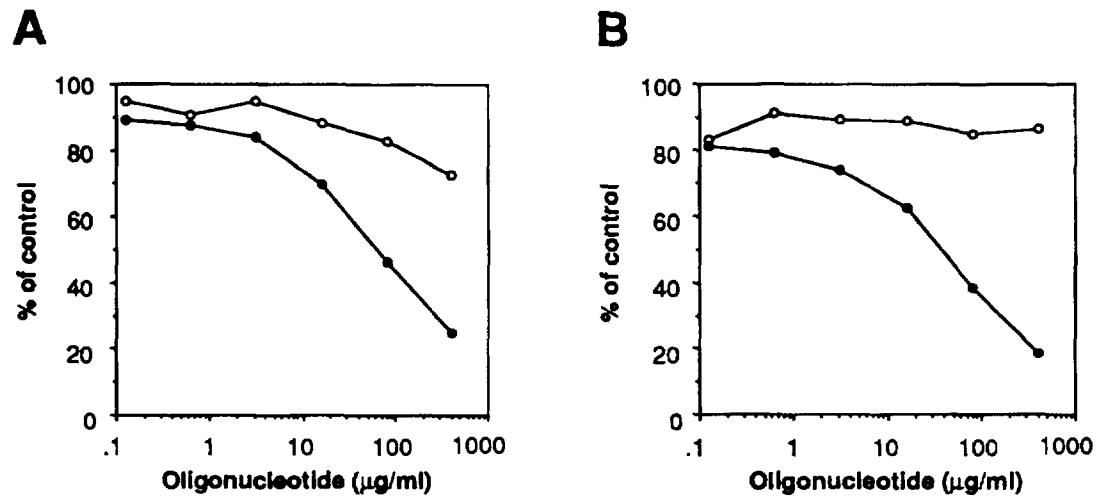


Fig. 2. Inhibition of binding of anti-V3 loop mAbs to recombinant gp120 in the presence of DmTr-modified oligomer, SA-1042 (closed circles) or unmodified oligomer, SA-1043 (open circles), as detected by solid-phase ELISA. The amount of α70 (A) or 11/75a (B) bound to wells coated with gp120 was studied as a function of concentration as described in Section 2. The concentration of each anti-V3 loop mAb was 0.5 μg/ml or 1/500 of hybridoma supernatant, respectively.

on the accessibility of the V3 region, the V3 peptide (aa302–324) was also used as an immobilized antigen. In this case, the binding of α70 mAb was also inhibited by SA-1042 in a concentration-dependent manner with an $IC_{50}$ value of 0.40 μg/ml (Fig. 4). These results suggest that SA-1042 interferes with the V3 loop by interacting directly with the whole region or a subsection of it without anchoring to other regions.

### *3.3. Comparison of SA-1042 and other binding antagonists in their ability to inhibit the anti-V3 antibody binding*

It has been reported that dextran sulfate, aurintricarboxylic acid (ATA) and oligodeoxycytidine with a phosphorothioate internucleoside linkage (S-dC28) are also able to inhibit the binding of antibodies to the V3 loop (Schols et al., 1990; Stein et al., 1993). We therefore compared SA-1042 with these inhibitors for their ability to block binding of antibodies to the V3 peptide or recombinant gp120 by solid-phase ELISA. This was carried out in the presence of 1% BSA or 10% FCS to increase the protein concentration (Table 1). The $IC_{50}$ value for dextran sulfate with V3 peptide-coated plates (550 μg/ml) was 11 times higher than that determined with gp120-coated plates (50 μg/ml) in the presence of 1% BSA. In contrast, the $IC_{50}$ value for SA-1042 with the V3 peptide (0.40 μg/ml) was 125 times lower than the value obtained with the gp120-coated plates (50 μg/ml) in the presence of 1% BSA. In addition, the $IC_{50}$ value for SA-1042 in 10% FCS was similar to that in 1% BSA, with either gp120 or the V3 peptide as the target, while for dextran sulfate the $IC_{50}$ value in 10% FCS was 4–6 times higher in both cases than with 1% BSA. S-dC28 showed consistent $IC_{50}$ values (1.6–1.7 μg/ml) with either gp120 or the V3 peptide in 1% BSA, but the $IC_{50}$ values were increased 3–6-fold if 10% FCS was added to the reaction mixture. ATA showed similar $IC_{50}$ values (5.1–13 μg/ml) irrespective of the conditions used. Thus, SA-1042 was able to recognize the V3 loop without interference from serum proteins, which was not the case for dextran sulfate or S-dC28.

### *3.4. Effect of SA-1042 on the binding of antibodies to native molecules expressed on cells*

In order to examine the effect of SA-1042 on the V3 loop of native gp120, HIV-1-chronically-infected cells (H9/HXB2 cells) were used. As

shown in Fig. 5, the binding of 11/75a to H9/HXB2 cells was markedly inhibited, as measured by flow cytometric analysis, in the presence of SA-1042. The inhibition was dose-dependent with an $IC_{50}$ value of about 5.0 μg/ml (Fig. 5).

The viral transmembrane glycoprotein, gp41, is also considered to play an essential role in virus entry as well as cell-to-cell fusion (Freed et al., 1990; Helseth et al., 1990). Monoclonal antibody 2F5, which recognizes gp41, is an established inhibitor of various strains of HIV-1 (Buchacher et al., 1992; Muster et al., 1993). In addition, the cell surface molecules, CD4, CD26, CD11a, CD18 and CD54, have been reported as putative viral receptors or accessory molecules which are considered to promote cell fusion as well as T-cell activation; antibodies to these ligands are reported to neutralize these activities (Dalgleish et al., 1984; Hildreth and Orentas, 1989; Gruber et al., 1991; Valentin et al., 1990; Callebaut et al., 1993; Fecondo et al., 1993). In our next series of experiments, we examined whether SA-1042 could inhibit the binding of neutralizing antibodies directed against either gp41 or other cellular transmembrane molecules. As shown in Fig. 6, the binding of anti-V3 mAb 11/75a, and mAbs 39.3b and 38.1a to the CD4 binding site was significantly inhibited by SA-1042. However, no inhibition was observed when neutralizing antibodies against the other ligands were used. These observations strongly suggest that SA-1042 specifically interferes with the V3 loop as well as the CD4 binding site of the envelope glycoprotein with no apparent interaction with other cell surface molecules considered to be involved in the virus-cell interaction.

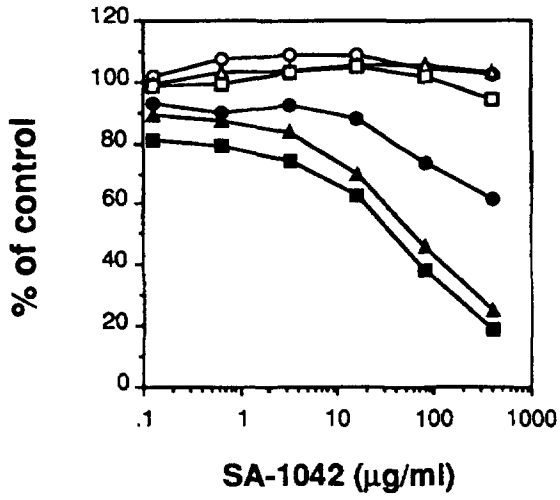


Fig. 3. Effects of SA-1042 on the binding of various antibodies directed to gp120 as determined by solid-phase ELISA. SA-1042 was added to gp120-coated wells with 1C1 (0.07 μg/ml) (open circles), ABi13-106 (3 μg/ml) (open triangles), ABi13-107 (0.2 μg/ml) (open squares), 39.3b (0.1 μg/ml) (closed circles), x70 (0.5 μg/ml) (closed triangles) and 11/75a (1/500) (closed squares) which recognize aa481-496, aa37-108, aa72-130, a conformational epitope of CD4 binding site, aa306-324, and a V3 conformational epitope of gp120, respectively.

## 4. Discussion

In this study, we have demonstrated that SA-1042 is able to prevent the binding of several mAbs to the V3 loop and/or to the CD4 binding site on gp120. Also, in agreement with previous studies (Schols et al., 1990; Stein et al., 1993; Harrop et al., 1994), we confirmed that dextran sulfate, aurintricarboxylic acid (ATA) and phosphorothioate cytidine oligomer (S-dC28) inhibit the binding of mAbs to the V3 loop. It has been reported that dextran sulfate interferes with both the binding of anti-CD4 mAb to the V1 domain of CD4 molecule, which is essential for interaction with gp120, and binding of a mAb to the C-terminal region on gp120 (Lederman et al., 1989; Harrop et al., 1994). S-dC28 and ATA also inhibited the binding of the equivalent anti-CD4 mAb (Schols et al., 1989; Stein et al., 1993). However, SA-1042 did not interfere with the binding of a mAb to the V1 domain of CD4 or to the C-terminal region of gp120. Furthermore, inhibition of the binding of neutralizing antibodies to other cell surface molecules involved in the membrane fusion process and/or normal immunological responses, including gp41, CD11a, CD18, CD26, and CD54, was undetectable. Therefore, SA-1042 is unlikely to affect other functional molecules necessary for cell fusion, virus entry or normal T-cell function, and may possess more selective anti-HIV-1 activity than either of these three other inhibitors (dextran sulfate, ATA and S-dC28). In contrast, an unmodified oligomer SA-

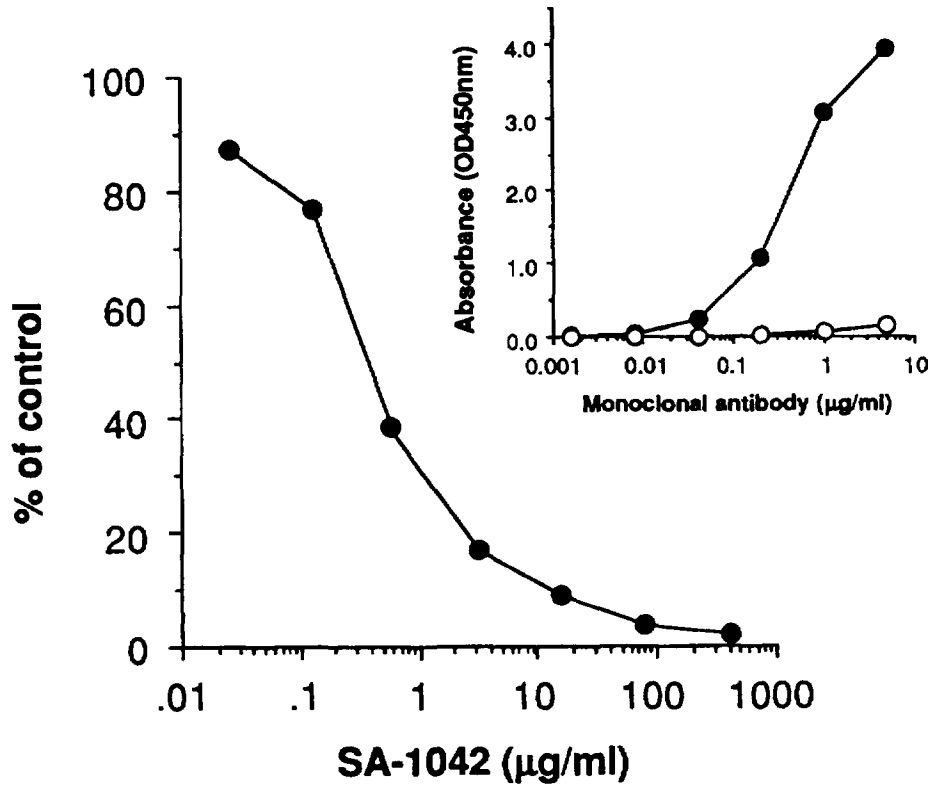


Fig. 4. Concentration-dependent inhibition of the binding of anti-V3 loop mAb (α70) to V3 peptide (TRPNNNTRKSIRIQRGP-GRAFVT) by SA-1042 as detected by solid-phase ELISA. The inset shows the titration curve of α70 (closed circles) and an isotype control (open circles). A concentration of 0.2 μg/ml of α70 was used.

1043 and a DmTr-modified oligomer which has no guanine cluster in the nucleotide sequence, did not inhibit either the gp120/CD4 interaction or the anti-V3 mAbs binding (data not shown). These results suggest that both the 5′-end modification and the nucleotide sequence are essential not only for anti-HIV-1 activity but also for interaction with these two domains of gp120.

CD4 evokes a conformational change of gp120, with the V3 loop exposed on the outside (Sattentau and Moore, 1991). This region then becomes more accessible for proteolytic cleavage (Clements et al., 1992) or binding of heparin, also a putative V3 inhibitor (Harrop et al., 1994). It may be assumed that the synthetic V3 peptide used in this study might mimic the V3 loop exposed on gp120 after CD4 binding. The results obtained in this study also demonstrate that SA-1042, in contrast to dextran sulfate, ATA and S-dC28, is a more potent inhibitor in the blocking of a mAb to the V3 peptide than gp120 in a solid-phase ELISA. This suggests that the mode of action of SA-1042 in blocking the V3 loop is different from that of other putative inhibitors and SA-1042 may preferentially interact with the V3 loop on CD4-activated gp120 rather than on normally configured gp120.

A variety of different biological responses have been ascribed to oligonucleotides containing a guanine-rich nucleotide sequence, including antiproliferative and antiviral activities as well as inhibition of enzymatic activities (Fennewald et al., 1995; Ojwang et al., 1994; Yaswen et al., 1993; Burgess et al., 1995). Some of these oligonucleotides were reported to be able to form a G-quartet structure (Wyatt et al., 1993; Rando et al., 1995). For example, anti-HIV-1 oligonucleotide derivatives such as the guanine-rich phosphoroth-

Table 1
Effects of different compounds on the binding of α70 to recombinant gp120 or V3 peptide in the presence 1% BSA or 10% BSA

| Coated antigen | Compound | $IC_{50}$ (μg ml) | |
|---|---|---|---|
| | | 1% BSA | 10% FCS |
| gp120 | SA-1042 | 50 | 46 |
| gp120 | S-dC28 | 1.6 | 5.0 |
| gp120 | Dextran sulfate | 50 | 280 |
| gp120 | ATA | 13 | 7.7 |
| V3 peptide | SA-1042 | 0.40 | 0.70 |
| V3 peptide | S-dC28 | 1.7 | 12 |
| V3 peptide | Dextran sulfate | 550 | 1900 |
| V3 peptide | ATA | 7.7 | 5.1 |

ioate oligomer ISIS5320 (Wyatt et al., 1994) and the 3′-end modified phosphodiester oligomers composed entirely of deoxyguanosine and deoxythymidine (Ojwang et al., 1994; Ojwang et al., 1995) were reported to be able to inhibit HIV-1 replication. Tetramer formation of the guanine clusters of these oligomers appears to be necessary for anti-HIV-1 activity (Wyatt et al., 1993; Rando et al., 1995). Therefore, SA-1042 is also likely to form a G-quartet structure because of the guanine cluster in the nucleotide sequence. Previously, data were presented suggesting that G-rich oligomers which can inhibit phospholipase A2 activity exist as dimers with the structure stabilized by formation of a G-quartet (Bennett et al., 1994), and also that $G_3T_4G_3$ oligonucleotides form an asymmetric diagonally looped dimeric quadruplex structure (Smith et al., 1994). These speculations might also be applicable to SA-1042.

5′-End modification of SA-1042 is believed to be essential for the anti-HIV-1 activity, since the modification enhances the stability of the oligonucleotide in the presence of serum (Furukawa et al., 1994). In this study, we showed that 5′-end modification is necessary for the ability to interfere with the binding of anti-V3 loop mAbs in serum-free conditions. These suggest that the modification could contribute to both the stability and activity of the oligonucleotide.

Taken together, the current data indicate that SA-1042 affects virus–cell interaction by interfering with the CD4 binding site and the V3 loop on

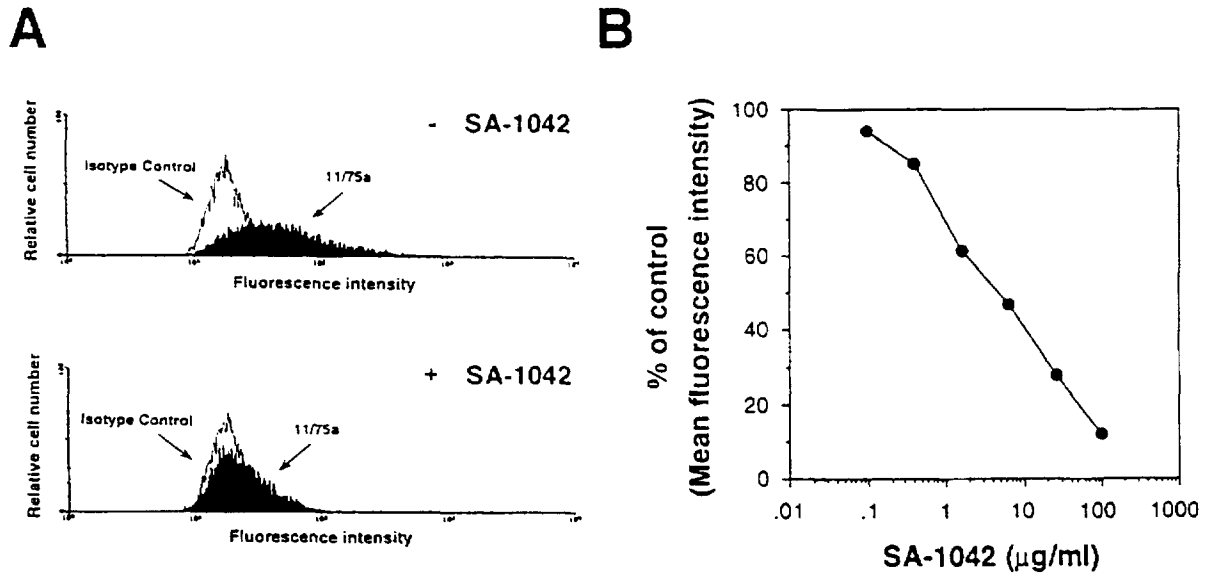


Fig. 5. Inhibition by SA-1042 of anti-V3 loop mAb (11 75a) binding to H9/HXB2 cells. Cells were analyzed by FACScan as described in Section 2. (A) FACS patterns in the presence (lower panel) and absence (upper panel) of 100 μg/ml of SA-1042. (B) Effects of different concentrations of SA-1042 on the binding of anti-V3 loop to native gp120 on the cell surface. Mean fluorescence intensity is determined as a percent of control and plotted as a function of SA-1042 concentration.

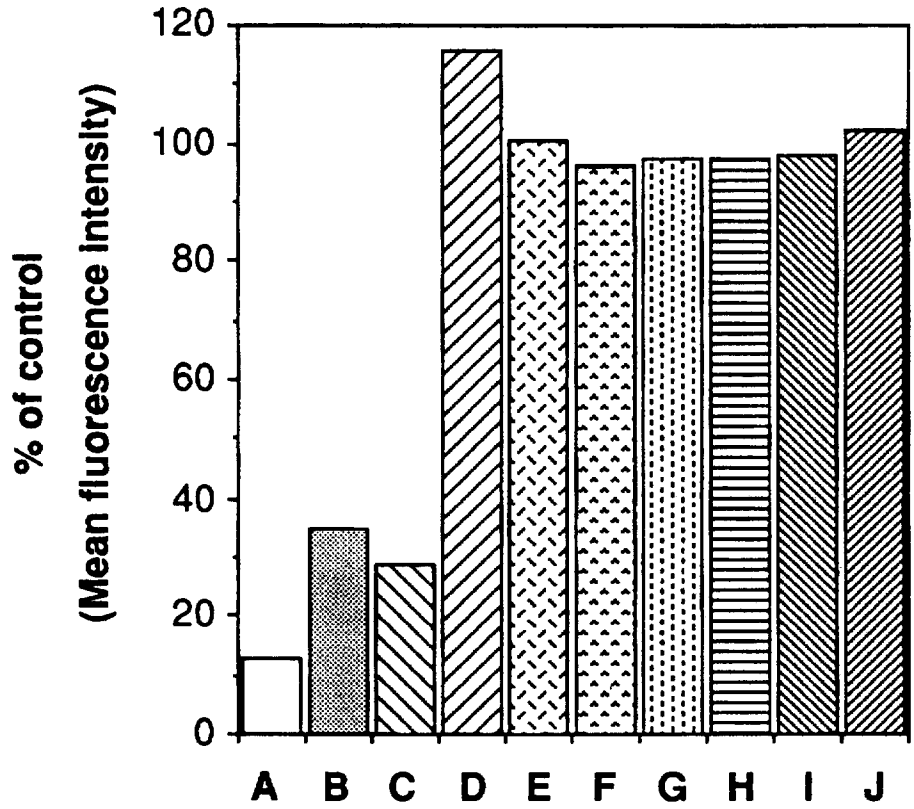


Fig. 6. Effect of SA-1042 on the binding of antibodies against various viral receptors and cell adhesion molecules to CD4-positive cells. Fluorescence intensity is plotted as a function of SA-1042 at a constant concentration (100 μg/ml). The antibody and cell line used in each study are as follows: A, 11 75a (anti-V3 loop: H9/HXB2); B, 39.3b (anti-CD4 binding site: H9/HXB2); C, 38.1a (anti-CD4 binding site: H9 HXB2); D, 2F5 (anti-gp41: H9 HXB2); E, Leu3a (anti-CD4: MT4); F, BA5 (anti-CD26: H9); G, G25-2 (anti-CD11a: H9); H, L130 (anti-CD18: H9); I, L178 (anti-CD44: H9); J, LB2 (anti-CD54: H9).

gp120. Also, the $IC_{50}$ values for SA-1042, in blocking antibodies binding to HIV-$1_{HXB2}$-chronically-infected H9 cells in FACScan data (5.0 μg/ml: 11/75a; 10 μg/ml: 38.1a), were slightly higher than those determined for inhibiting the growth of HIV-$1_{HXB2}$ in MT4 cells after acute infection (1.5 μg/ml) (data not shown). This suggests that the anti-HIV-1 activity of SA-1042 might consist of at least two different mechanisms. It has been previously shown that the application of both anti-CD4 mAb and anti-V3 loop mAb caused a synergistic inhibition of virus replication (McKeating et al., 1992a). It is likely, therefore, that the potent anti-HIV-1 activity of SA-1042 may result from a synergistic interference between the CD4 binding site and the V3 loop.

The V3 loop region of each strain of HIV-1 has a highly variable amino acid sequence. The GPGRAF sequence within the V3 loop, however, is relatively conserved (LaRosa et al., 1990), and a number of anti-V3 mAbs which recognize this conserved feature of the domain were reported to exhibit a broad neutralization profile (Javaherian et al., 1990; Ohno et al., 1991; Gorny et al., 1992). The crystal structure of the complex between a peptide from V3 loop and an anti-V3 mAb suggests that the conformation of the V3 loop of many isolates may be biologically relevant (Ghiara et al., 1994). Therefore, therapeutics that target the V3 loop provide an attractive approach to the chemotherapy of AIDS.

On the basis of the results obtained in this study, SA-1042 was able to specifically block two functional domains on gp120. In addition, the recognition of the V3 loop by SA-1042 was not influenced by an increase in the protein content, by which the effects of dextran sulfate and S-dC28 were markedly reduced. In view of these beneficial activities, SA-1042 may represent a potential candidate for the therapy of AIDS.

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