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SULFATED ALKYL OLIGOSACCHARIDES WITH POTENT INHIBITORY EFFECTS ON HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Abstract—Compounds with medium relative molecular masses active against human immunodeficiency virus (HIV) were synthesized. Sulfated alkyl oligosaccharides such as sulfated octadecyl maltohexaoside, sulfated dodecyl laminaripentaoside and sulfated dodecyl laminari-oligomer caused 50% inhibition of virus infection in the EC_{50} range of 0.4–0.7 μg/mL *in vitro* using the MT-4 cell line and HIV-$1_{HTLV-IIIB}$ virus isolate, though sulfated oligosaccharides without alkyl groups showed low anti-HIV activities. This anti-HIV activity was close to the EC_{50} of 0.43 μg/mL for a highly active sulfated polysaccharide curdlan sulfate which was reported to inhibit completely the HIV infection at a concentration as low as 3.3 μg/mL. These compounds were also active against HIV-2 and a clinically isolated HIV-1 with reduced AZT sensitivity. For such sulfated alkyl oligosaccharides, the mechanism of inhibition of HIV infection was assumed to be the inhibition of HIV binding to the cell and to some extent the interaction of the alkyl portion with the lipid bilayer of the virus.

Acquired immunodeficiency syndrome (AIDS§) drugs have been eagerly sought for since AIDS was found to be caused by human immunodeficiency virus (HIV) [1]. Although many attempts have been made to develop agents that may be useful in the prevention and therapy of AIDS, only azidothymidine (AZT) is a commercially available, approved drug. Recently, dideoxyinosine [2] has been approved as a new AIDS drug. However, the long-term use of dideoxynucleotide analogues is often limited by serious side-effects such as bone marrow suppressions [3]. Moreover, clinical isolates have been obtained which show reduced sensitivity to AZT [4]. Thus, anti-HIV agents attacking the virus with different modes of action should be examined.

Following the suggestion that sulfated polysaccharides might have inhibitor effects on the infection of human immunodeficiency virus (HIV) [5–7], we investigated the anti-HIV activity of a number of sulfated synthetic and natural polysaccharides [8–11], as we had previous experience in the synthesis of sulfated polysaccharides with high anticoagulant activity [12].

Lentinan sulfate [10], which was obtained by sulfation of a branched 1,3-β-D-glucan lentinan, and curdlan sulfate [11], which was prepared by sulfation of a linear 1,3-β-D-glucan curdlan, showed high anti-HIV activity and low anticoagulant activity. Curdlan sulfate with an average M_r of approximately 79×10^3 and with a sulfur content of 15.2% inhibited completely the HIV infection of MT-4 cells at a concentration as low as 3.3 μg/mL [11]. On the other hand, it is reported that the anti-HIV activity of dextran sulfate was increased with M_r increasing from 1000 to 10×10^3 and remained virtually constant when the M_r was increased further to 500×10^3 [13]. These observations indicate a potent influence of the relative molecular mass of sulfated polysaccharides on the biological activity.

Ionic interactions between charged biological macromolecules have been well analysed in the interaction of heparin with antithrombin III [14]. In this case, a negatively charged pentasaccharide portion of heparin is assumed to react with a positively charged portion of antithrombin III, i.e. several amino acid residues ranging from the residue No. 282 to 289 (Lys^+-Ser-Leu-Ala-Lys^+-Val-Glu-Lys^+) [15]. This helical region in antithrombin III contains three positively charged lysine residues in appropriate spacial positions for the interaction with sulfamide and sulfate groups in the heparin pentasaccharide both of which are negatively charged.

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§ Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; AZT, azidothymidine; PBL, peripheral blood lymphocyte; PHA, phytohemagglutinin; CPE, cytopathic effects; MOI, multiplicity of infection; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; FITC, fluorescein isothiocyanate; GPC, gas permeation chromatography; M5S0, sulfated maltopentaoside; M5S10, sulfated decyl maltopentaoside; M6S0, sulfated maltohexaoside; M6S12, sulfated dodecyl maltohexaoside; M6S18, sulfated octadecyl maltohexaoside; L5S0, sulfated laminaripentaoside; L5S12, sulfated dodecyl laminaripentaoside; L5S16, sulfated hexadecyl laminaripentaoside; LOS12, sulfated dodecyl laminari-oligosaccharide; LOS12E, sulfated dodecanoyl laminari-oligosaccharide.

Considering the case of antithrombin III and heparin it seemed that negatively charged portions containing several glucosidic units in sulfated polysaccharides might have strong interactions with positively charged helical regions in the HIV envelope glycoprotein gp120, for example, the amino acid residues of no. 506 through 518 of gp120 (Thr-Lys$^+$-Ala-Lys$^+$-Arg$^+$-Arg$^+$-Val-Val-Gln-Arg$^+$-Glu-Lys$^+$-Arg$^+$). The RNA sequence of HIV gene was analysed by Ratner *et al.* [16]. The strong inhibitory effects of the curdlan sulfate on HIV infection may be caused mainly by its adsorption to gp120 to prevent the virus glycoprotein from binding with CD4 of helper T lymphocytes.

The high relative molecular mass curdlan sulfate intravenously administered showed low toxicities in tests using mice and rats [11]. Consequently, curdlan sulfate might become an anti-HIV drug which inhibits the binding of HIV to helper T lymphocytes in the early stage of infection.

Since it is known that the active pentasaccharide of heparin exhibited high affinity for antithrombin III [17], it might be possible to synthesize a medium relative molecular mass oligosaccharide which interacts potently with the negatively charged portion of the gp120 of HIV. In this study, we attempted to synthesize relatively low relative molecular mass compounds with high anti-HIV activity. Because surface-active agents have been used to destroy lipid bilayers of cells, sulfated oligosaccharides having long alkyl chains are expected to interact to some extent with the lipid bilayer of HIV as well as interacting potently with the envelope glycoprotein gp120, as mentioned above. We report the synthesis of sulfated alkyl oligosaccharides with medium relative molecular masses active against HIV.

MATERIALS AND METHODS

Sulfated alkyl oligosaccharides. This is the first study reported of the glycosidation of oligosaccharides with long chain aliphatic alcohols, although the glycosidation of mono- to tri-saccharides has been reported recently [18]. To the reducing end of peracetylated laminaripentaoside, peracetylated maltopentaoside and maltohexaoside, and peracetylated laminari-oligomers, long chain aliphatic alcohols with carbon numbers of 10 (=decyl alcohol)–18 (=octadecyl alcohol) were bound by catalysis with such Lewis acids as ferric chloride and stannic tetrachloride, or with phosphotungstic acid. As an example, synthesis of sulfated *n*-dodecyl maltopentaoside is described. Peracetylated maltopentaoside with β content of approximately 80% was obtained by acetylating maltopentaoside with acetic anhydride and sodium acetate. It was revealed that in the next glycosidation, peracetyl β- but not α-maltopentaoside reacts with long chain alcohols. Thus, the β anomer was separated from the α one by column chromatography (column: E. Merck Kieselgel 60 silica gel; eluent: ethyl acetate/*n*-hexane, 6/4). Then, peracetyl β-maltopentaoside (0.5 g) was reacted with *n*-dodecyl alcohol (one equivalent to sugar) using stannic tetrachloride (0.3 equivalent) as a glycosidation catalyst. Deacetylation of the peracetylated *n*-dodecyl β-maltopentaoside was carried out with saturated ammonia solution in methanol to produce *n*-dodecyl β-maltopentaoside. *n*-Dodecyl β-maltopentaoside (79 mg) was sulfated with piperidine *N*-sulfonic acid (three equivalents to hydroxyl content of the glycoside) to give sulfated *n*-dodecyl β-maltopentaoside. The yield was 0.163 mg which corresponded to 78% based on the theoretical yield of fully sulfated *n*-dodecyl β-maltopentaoside. NMR measurements and elementary analyses of the sulfated alkyl oligosaccharides were carried out. The observed sulfur content and the degree of sulfation calculated from the elementary analysis of C, H, and S are given in Table 1. Relative molecular masses of the sulfated compounds were determined in phosphate buffer solution by gel permeation chromatography (GPC) using Toyo Soda HLC-303D equipped with columns of G2000SW, G3000SW and G4000SW. Standard pullulan and maltopentaose were used as relative molecular mass reference.

Cell lines. Human T-lymphotropic virus type I (HTLV-I)-positive human T cell line, MT-4, was subcultured twice a week at a concentration of 3×10^5 cells/mL in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 IU/mL of penicillin and 100 μg/mL of streptomycin. Peripheral blood lymphocytes (PBLs) were also used as target cells according to the method of Schinazi *et al.* [19]. PBLs were separated from normal human peripheral blood by Ficoll Hypaque gradient centrifugation. PBLs were stimulated with phytohemagglutinin (PHA) for 3 days.

Viruses. HIV-$1_{HTLV\text{-}IIIB}$, HIV-1_{A012B} [4], HIV-1_{A012D} [4] and HIV-2_{ROD} [20] were used in the anti-HIV assay. HIV-$1_{HTLV\text{-}IIIB}$ was prepared from the culture supernatant of MOLT-4/HTLV-IIIB cells which were persistently infected with HTLV-IIIB. HIV-$1_{HTLV\text{-}IIIB}$ stocks were titrated into MT-4 cells in 50% tissue culture infectious doses ($TCID_{50}$) and plaque formation units and stored at −80° until used. HIV-1_{A012B} and HIV-1_{A012D} which were kindly supplied by Dr B. A. Larder were the clinical isolates with high and reduced sensitivity to AZT, respectively.

Anti-HIV assay. The anti-HIV activity of test compounds in fresh cell-free HIV infection was determined by the protection from HIV-induced cytopathic effects (CPE). MT-4 cells were infected with HTLV-IIIB at the multiplicity of infection (MOI) of 0.01. HIV- or mock-infected MT-4 cells (1.5×10^5 cells/mL, 200 μL) were placed into 96-well microtiter plates and incubated in the presence of various concentrations of the test compounds. The dilution step from one concentration to another was two or five times, and nine different concentrations of each compound were examined. All experiments were carried out in triplicate. After 5 days culture at 37° in a CO_2 incubator, cell viability was quantified by a colorimetric assay which monitors the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to a blue-colored formazan product according to Pauwels *et al.* [21]. The absorbances were determined in a microcomputer-controlled photometer (Titertek Multiskan, Labsystem Oy, Helsinki, Finland) at two wavelengths (540 and 690 nm). The absorbance measured at 690 nm was

Table 1. Synthesis and anti-HIV activity of sulfated *n*-alkyl oligosaccharides and sulfated oligosaccharides

Sample name	*n*-Alkyl oligosaccharide		Sulfated *n*-alkyl oligosaccharide		Biological activity[b]		
	Number of glucose units (N)	Carbon number of *n*-alkyl chain (M)	Sulfur content (%)	Degree of sulfation[a]	Anti-HIV activity[c] (EC_{50}) (μg/mL)	Cytotoxic effect[d] (CC_{50}) (μg/mL)	SI (CC_{50}/EC_{50})
Malto-oligosaccharide							
M5S0	5	None	17.6	4.5[e]	267	>1000	>4
M5S10	5	10	ND	ND	10.2	—	—
M6S0[f]	6	None	16.7	2.2	207	>1000	>5
M6S12[g]	6	12	14.4	2.7	2.4	>1000	>420
M6S18	6	18	14.5	1.9	0.6	748	1250
Laminari-oligosaccharide							
L5S0	5	None	19.7	4.3[e]	163	>1000	>6
L5S12	5	12	16.1	3.1	0.7	>1000	>1470
L5S16	5	16	17.4	4.9[e]	12	471	40
LOS12	11.3[h]	12	15.8	2.6	0.4	>2000	>5000
LOS12E	9.4[i]	12	14.7	5.8[e]	1.9	>1000	>530
CS[j]	—	—	14.1	1.3	0.43	>1000	>2330
AZT[k]	—	—	—	—	0.0019	6.43	3400

[a] The number of hydroxyl groups substituted by sulfate groups per glucose residue having three hydroxyls.
[b] Measured by cell viability experiment.
[c] Drug concentration effective for 50% inhibition of virus infection in 5-day HIV-infected MT-4 cell culture.
[d] Drug concentration for 50% cytotoxicity in 5 day MT-4 cell culture.
[e] Too high degree of sulfation might be due to remaining sodium sulfate or to formation of diester.
[f] Number-average molecular mass: calculated, 2425; observed by GPC in phosphate buffer solution, 4690.
[g] Calculated M_n, 2481; observed, 4085.
[h] Biologically synthesized laminari-oligomer with number-average degree of polymerization of 11.3.
[i] Laminari-oligomer obtained by degradation of curdlan with degree of polymerization of 9.4.
[j] Curdlan sulfate with M_r of 79×10^3 used in measurement of anti-HIV activity as reference.
[k] EC_{50} and CC_{50} of AZT are expressed in μM.

automatically substracted from that at 540 nm, to eliminate effects of non-specific absorptions. All data represent the mean values of triplicate wells. These values were then translated into percentage cytotoxicity and percentage antiviral protection, from which 50% cytotoxic concentration (CC_{50}), 50% effective concentration (EC_{50}) and selectivity index ($SI = CC_{50}/EC_{50}$) were calculated.

The PHA-stimulated PBLs at a concentration of 1×10^6 cells/mL were infected with HIV-$1_{HTLV\text{-}IIIB}$ at the MOI of 0.1 for 1 hr at 37°. Unadsorbed viruses were completely removed by centrifuging the cell-containing suspension five times with fresh medium. The HIV-infected PBLs were incubated with different concentrations of the compound in the RPMI-1640 medium containing 5 ng/mL of interleukin-2, as well as the supplements used in the case of MT-4 culture, in a CO_2 incubator at 37°. A half to a third of the medium was changed every 4 days. For PBLs, the anti-HIV-$1_{HTLV\text{-}IIIB}$ activity was determined by measuring the amount of HIV p24 antigen contained in the culture supernatant on the 12th day using a sandwich enzyme-linked immunosorbent assay kit (Abbott, Chicago, Ill, U.S.A. [22, 23].

Immunofluorescence staining. After MT-4 cells had been incubated with HTLV-IIIB at a MOI of 0.01 at 37° for 3 days, HIV-infected cells were washed with phosphate-buffered saline (pH 7.2), fixed with cold methanol and stained using an indirect two-step immunofluorescence method, as described elsewhere [7, 24]. The primary antibody was a human polyclonal anti-HIV-1-positive serum. The second antibody was a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (Cappel Organon Teknika Co., West Chester, PA, U.S.A.).

Flow cytometry. Flow cytometry was performed with an Epics Profile II (Coulter Electronic Inc., Hialeah, FL, U.S.A.) equipped with 488 nm Argon laser and with 15 mW light output. Before analysis of the samples the instrument was calibrated using fluorescent beads. FITC emission signals were collected by using the standard FITC/RD filter set and amplified logarithmically. Ten thousand events per sample were collected in list mode fashion, stored and analysed.

RESULTS

Anti-HIV activity assay in the MT-4 cell–HIV-$1_{HTLV\text{-}IIIB}$ virus system

The synthesis of alkyl oligosaccharides, i.e. glycosidation in which a long chain alcohol reacts with the reducing end of an acetylated oligosaccharide, was carried out successfully with Lewis acid or phosphotungustic acid as catalyst, although the acetal linkage binding saccharide residues are known to be relatively labile under acidic conditions. The hydroxyl groups of the alkyl oligosaccharide obtained were then sulfated using piperidine *N*-sulfonic acid or sulfur trioxide–pyridine complex as sulfating agent to give a sulfated alkyl oligosaccharide, the general structure of which is shown in Fig. 1. Such synthetic chemistry will be published elsewhere in detail. The inhibitory effects of sulfated alkyl oligosaccharides on HIV infection were assessed by the protection from HIV-induced cytopathogenicity of MT-4 cells *in vitro*. In this study, an automatic cell quantification method [22] was adopted in which viable cells converted a MTT dye into a blue-colored product by chemically reducing it and the quantity of viable cells was measured photometrically. The HIV-$1_{HTLV\text{-}IIIB}$-infected MT-4 cell line can not survive under virus-induced cytopathic effects in a 5-day culture. For several compounds, the inhibitory effects on HIV infection are shown in Fig. 2. In the MTT experiments, the EC_{50} value designates the concentration of compound inhibiting 50% of cytopathogenicity in HIV-infected MT-4 cells. Results of the bioassays are summarized in Table 1.

The EC_{50} values of such sulfated oligosaccharides without a terminal alkyl group as sulfated maltopentaoside M5S0, sulfated maltohexaoside M6S0 and sulfated laminaripentaoside L5S0 were 267, 207 and 163 μg/mL, respectively. The curdlan sulfate which has been reported to inhibit completely HIV infection at a concentration as low as 3.3 μg/mL [11] showed an EC_{50} of 0.43 μg/mL which was obtained as an average value from several bioassays. Thus, as expected, the anti-HIV activity of the sulfated oligosaccharides is much lower than that of curdlan sulfate.

The anti-HIV activity was to a great extent enhanced by the binding of alkyl group to the sulfated oligosaccharide. In the case of malto-oligosaccharides, sulfated decyl maltopentaoside M5S10 which has a decyl group ($=C_{10}H_{21}$) at the reducing end showed an EC_{50} of 10.2 μg/mL, which was about 25 times as high as that of M5S0.

When the number of saccharide residues and the carbon number of alkyl groups were increased, sulfated dodecyl ($=C_{12}H_{25}$) maltohexaoside M6S12 and sulfated octadecyl ($=C_{18}H_{37}$) maltohexaoside M6S18 with much higher anti-HIV activities (EC_{50} of 2.4 and 0.6 μg/mL, respectively) were obtained.

The cytotoxic effects of the compounds were low as the 50% cytotoxic concentration CC_{50} was more than 1000 μg/mL, except for M6S18 which had a slightly higher cytotoxicity, i.e. CC_{50} of 748 μg/mL.

For 1,3-β-linked laminari-oligosaccharides, high anti-HIV activities were also obtained. Sulfated dodecyl laminaripentaoside L5S12 and sulfated dodecyl laminari-oligosaccharide LOS12 exhibited high activities, i.e. EC_{50} of 0.7 and 0.4 μg/mL, respectively. In the latter compound, laminari-oligosaccharide with the degree of polymerization of 11.3, which was prepared by enzymic degradation of a polysaccharide curdlan, was used as a starting oligosaccharide. Because it had a relative molecular mass distribution, a thorough glycosidation and the separation of alkylated oligosaccharides were not possible. The number-average molecular mass of the acetylated dodecyl laminari-oligosaccharide was calculated by the use of NMR spectroscopy to be 3360. Also, the proportion of alkyl group attached to the oligosaccharide was approximately 65%, suggesting that the activity of this compound might be increased if a complete alkyl binding is achieved.

Next, the proportion of infected cells was measured using the indirect immunofluorescence method, after the MT-4 cells had been infected

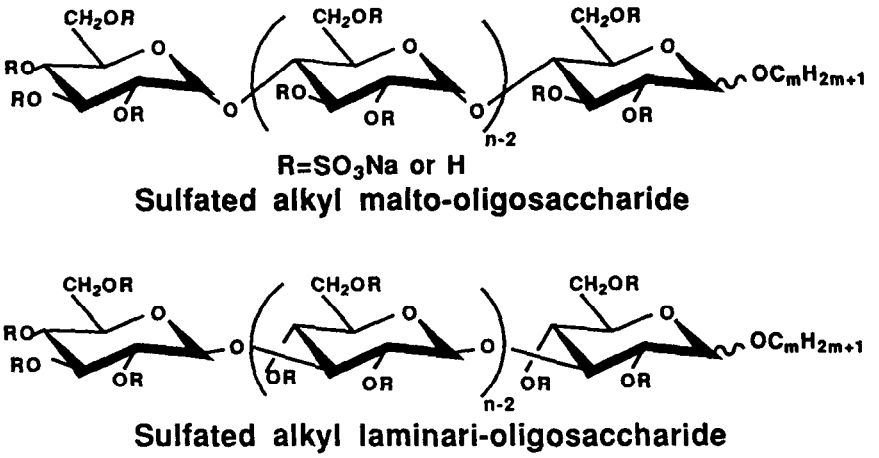

Fig. 1. Structure of sulfated alkyl oligosaccharide.

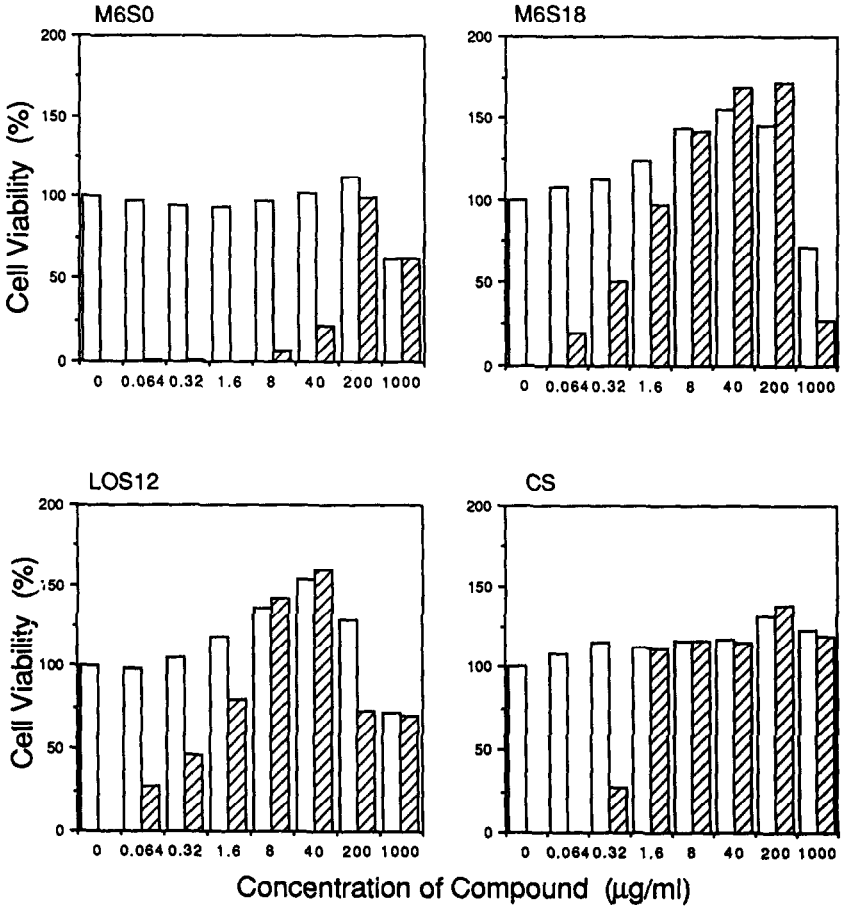

Fig. 2. Effects of M6S0, M6S18, LOS12 and curdlan sulfate (CS) on cell viability and cytopathic effect. MT-4 cells (open bars) and HIV-infected MT-4 cells (slash bars) were adjusted at 1.5×10^5 cells/mL and cultured in the presence of various concentrations of the drugs for 5 days.

Table 2. The proportion of inhibition of HIV infection determined by indirect immunofluorescence method and calculated IC_{50} values.

Concentration of compound (μg/mL)	Inhibition of HIV antigen expression (%)					IC_{50}* (μg/mL)
	40.0	8.0	1.6	0.32	0.064	
Curdlan sulfate	99.9	99.7	97.4	72.4	0	0.2
M6S12	96.4	92.0	35.1	6.4	4.6	2.4
M6S18	100.0	97.1	51.4	5.3	7.1	1.5
L5S12	97.2	93.2	83.9	38.8	1.1	0.5
LOS12	100.0	99.0	88.6	53.1	14.9	0.3

* Concentration of compound causing 50% inhibition of HIV antigen expression.

Table 3. Inhibitory effects of sulfated alkyl oligosaccharides against HIV-$1_{HTLV-IIIB}$ and HIV-2_{ROD} infection of MT-4 cells

Compound	HIV-$1_{HTLV-IIIB}$			HIV-2_{ROD}		
	EC_{50} (μg/mL)	CC_{50} (μg/mL)	SI (CC_{50}/EC_{50})	EC_{50} (μg/mL)	CC_{50} (μg/mL)	SI (CC_{50}/EC_{50})
M6S12	1.0	990	990	0.9	990	1100
M6S18	0.4	550	1380	1.4	550	390
LOS12	0.5	830	1650	0.4	830	2080

with HIV-$1_{HTLV-IIIB}$ in the presence of various concentrations of the compounds for 3 days. The result is shown in Table 2. The concentration of compound causing 50% inhibition of HIV infection IC_{50}, was calculated from the proportions. The IC_{50} corresponds to the EC_{50} calculated from the protection from HIV-induced CPE. The IC_{50} of curdlan sulfate was 0.2 μg/mL, which is close to its EC_{50} of 0.43 μg/mL.

Of sulfated alkyl maltohexaosides, the dodecyl and octadecyl compounds exhibited fairly high anti-HIV activities, i.e. IC_{50} of 2.4 and 1.5 μg/mL, respectively. In the case of laminari-oligosaccharides, sulfated dodecyl laminaripentaoside L5S12 and sulfated dodecyl laminari-oligosaccharide LOS12 had high anti-HIV activities showing an IC_{50} of 0.5 and 0.3 μg/mL, respectively. The latter activity, especially, is very high compared to that of the highly active curdlan sulfate.

Anti-HIV activity assay in the MT-4 cell–HIV-2_{ROD} virus system

Since high anti-HIV activities of sulfated alkyl oligosaccharides were exhibited against HIV-1 virus, the inhibitory effect of three representative sulfated alkyl oligosaccharides on HIV-2_{ROD} [4] infection of MT-4 cells was measured in terms of HIV-induced CPE using the MTT method. The result is summarized in Table 3. All three compounds showed high activities against HIV-2 and their inhibitory values are almost equivalent to those against HIV-1. The EC_{50} values of sulfated dodecyl laminari-oligosaccharide LOS12 were 0.5 μg/mL and 0.4 μg/mL against HIV-$1_{HTLV-IIIB}$ and HIV-2_{ROD}, respectively.

Table 4. Anti-HIV activities of sulfated alkyl oligosaccharides against AZT-sensitive HIV-1_{A012B} and HIV-1_{A012D} with reduced AZT sensitivity infection of MT-4 cells

	HIV-1_{A012B} (IC_{50},* mg/mL)	HIV_{A012D}
M6S12	0.2	0.1
M6S18	0.1	0.1
LOS12	0.01	0.1
AZT	0.0012†	0.143†

HIVs were kindly supplied by Dr Richman [20].

* Concentration of compound causing 50% inhibition of HIV antigen expression.

† IC_{50} values of AZT are expressed in μM.

Anti-HIV activity against clinically isolated HIV-1 with reduced sensitivity to AZT

Larder *et al.* [4] isolated HIV_{A012D} which had reduced sensitivity to AZT in AIDS patients treated by prolonged therapy with AZT. The anti-HIV activity of sulfated alkyl oligosaccharides against HIV_{A012D} and its unconverted virus HIV_{A012B} was examined by means of the indirect immunofluorescence method. The MT-4 cell was used as the target cell. The result is shown in Table 4. The IC_{50} values of AZT-sensitive virus, HIV-1_{A012B}, and a clinically isolated virus with reduced AZT sensitivity, HIV_{A012D}, were 0.0012 and 0.143 μM, respectively.

On the other hand, the three sulfated alkyl oligosaccharides exhibited high anti-HIV activity against AZT-resistant HIV_{A012D}. The IC_{50} of the

compounds was 0.1 μg/mL, which was almost equivalent to that against AZT-sensitive HIV_{A012B}, i.e. 0.01–0.2 μg/mL. It is assumed that since the sulfated alkyl oligosaccharide acted as an anti-HIV agent by a mechanism completely different to that of AZT, it worked effectively against various HIVs.

Anti-HIV activity assay using PBLs and HIV-$1_{HTLV-IIIB}$

The anti-HIV activity of three sulfated alkyl oligosaccharides against HIV-$1_{HTLV-IIIB}$ infection of PBLs was examined by means of the inhibition of p24 antigen expression. The EC_{50} values of sulfated dodecyl maltohexaoside (M6S12), sulfated octadecyl matohexaoside (M6S18) and sulfated dodecyl laminari-oligosaccharide (LOS12) were 11.7, 13.1 and 4.2 μg/mL, respectively. Although these activities were lower than those obtained by the bioassay using the MT-4 cell line because of the difference in the experimental conditions, the sulfated alkyl oligosaccharides exhibited considerably high activities against HIV infection of PBLs.

DISCUSSION

These findings indicate that highly anti-HIV active compounds might be the sulfated alkyl oligosaccharides which are composed of a sulfated oligosaccharide portion with a degree of polymerization of approximately 10 and a long chain alkyl group of carbon number 12–18 at the reducing end of the oligosaccharide.

The sulfated alkyl oligosaccharide has a structure characteristic of surface-active agents which are composed both of a hydrophilic portion, a sulfated oligosaccharide, and a hydrophobic portion, a terminal akyl group, and it showed a high foam-forming property. The sulfated oligosaccharide portion is assumed to have a high affinity to the envelope glycoprotein of HIV in analogy with sulfated polysaccharides [25]. Accordingly, two possible explanations may be considered for the activity of the medium relative molecular mass compounds in blocking HIV infection in T cells. First, the alkyl portion interacts with HIV lipid bilayers composed of similar alkyl chains, because such surface-active agents as polyethylene glycol and sodium dodecyl sulfate are routinely used for destroying lipid bilayers of bacteria and cells. Second, these surface-active agents have a tendency to associate leading to the formation of higher relative molecular mass aggregates. Although we attempted to measure the relative molecular masses of both sulfated alkyl oligosaccharides and sulfated oligosaccharides in aqueous solutions by GPC, these were always much higher than either those calculated or those measured in phosphate buffer solutions, suggesting that association of the sulfated compounds occurred in water.

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