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SYNTHESIS OF SULFATED POLYSACCHARIDES AND OLIGOSACCHARIDE DERIVATIVES WITH POTENT ANTI-AIDS VIRUS ACTIVITY

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ABSTRACT

Sulfated synthetic polysaccharides (with both high anti-AIDS virus activity and high anticoagulant activity) were prepared by sulfating such synthetic polysaccharides as ribopyranan, ribofuranans, and dextrans. Sulfated natural polysaccharides with high anti-AIDS virus activity but low anticoagulant activity were synthesized from lentinan and curdlan. It is assumed that curdlan sulfate will be helpful as an AIDS drug. In addition, sulfated alkyl oligosaccharides with high anti-AIDS virus activity were prepared.

INTRODUCTION

Ever since the causative agent of the acquired immunodeficiency syndrome (AIDS) was revealed to be the human immunodeficiency virus (HIV) [1], the development of drugs and therapeutics for AIDS has been eagerly pursued. Possible curing drugs are classified into 4 groups. These are (a) vaccines, (b) reverse tran-

scriptase inhibitors, (c) fusion inhibitors of the virus to T cells, and (d) protease inhibitors.

Azidothymidine (AZT), dideoxyinosine (DDI), and dideoxycytidine (DDC) belonging to the nucleoside drug have been clinically used. They have function (b). However, it was revealed that long-term use of AZT produces AZT-resistant viruses [2]. Accordingly, it is necessary to develop new AIDS drugs which work by different action mechanisms.

During the search for AIDS drugs with existing drugs and chemicals, dextran sulfate was found to have inhibitory effects on reverse transcriptase activity and therefore to possibly possess anti-HIV activity [3]. Its inhibitory effects in vitro of the virus infection to T cells were demonstrated [4]. It was also reported that polyanionic compounds having sulfate or sulfonate groups have anti-HIV activities [5]. Since we had been investigating the synthesis of polysaccharides and their sulfation [6, 7], the anti-HIV activity of sulfated synthetic polysaccharides was examined. These sulfated polysaccharides exhibited considerable anti-HIV activities [8]. Before that time, the synthesis of high anticoagulant sulfated polysaccharides was unknown. As the AIDS virus exists in human blood, the anticoagulant activity intrinsicially possessed by sulfated polysaccharides was assumed to be a side effect against anti-HIV activity. This assumption was recently shown to be correct because intravenous injection of dextran sulfate with anticoagulant activity caused severe side effects to HIV-carrying patients [9].

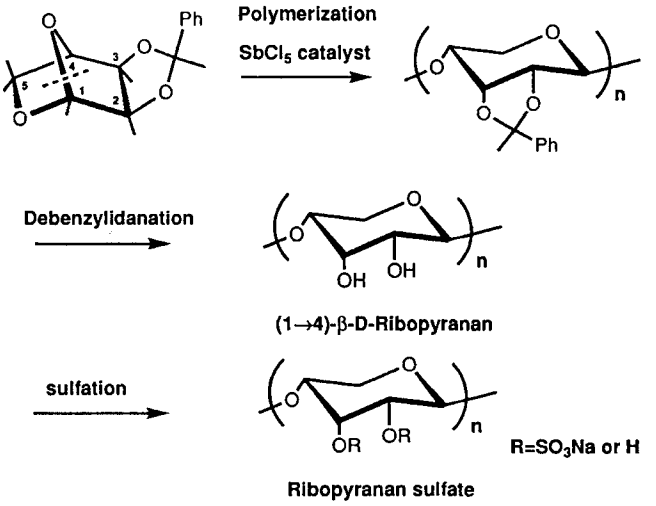
Since we anticipated such side effects of dextran sulfate, we aimed at synthesizing a highly anti-HIV active but low anticoagulant sulfated polysaccharide. After preparing various kinds of sulfated synthetic and natural polysaccharides, it was discovered that a sulfated lentinan has such biological properties [10]. Subsequently, a sulfated curdlan with high anti-HIV activity but considerably low anticoagulant activity was prepared [11]. The Phase I/II test (toxicity test) for curdlan sulfate has been carried out for AIDS virus carriers since December 1992 in the United States. It was recently reported that the intravenous injection of curdlan sulfate to HIV-infected patients induces short-time dose-related increases in CD4 lymphocytes [12].

Next, sulfated alkyl oligosaccharides with high anti-HIV activities were synthesized by use of oligosaccharides ranging individually from pentoses to nonaoses [13–15]. Although sulfated oligosaccharides themselves exhibited low anti-HIV activities, the binding of long alkyl groups at the reducing end of oligosaccharides causes a remarkable increase in the anti-HIV activity of the sulfated alkyl oligosaccharide. The relationship between the structure of the sulfated alkyl oligosasccharide and its biological activities was examined.

RESULTS AND DISCUSSION

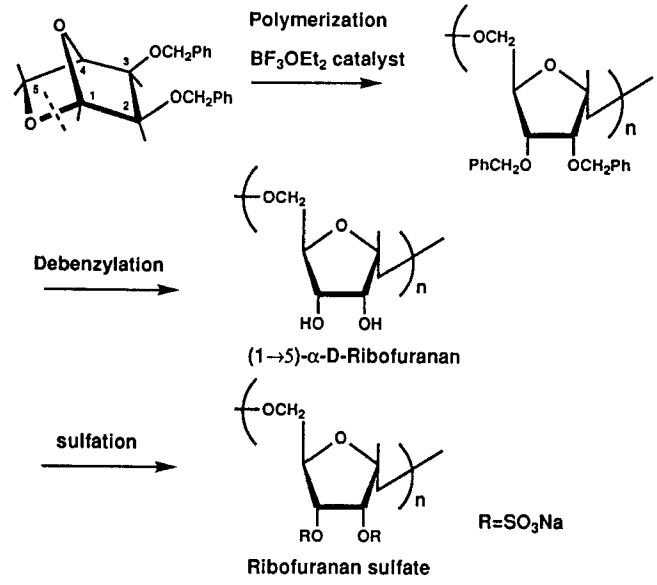
Synthesis of Sulfated Synthetic Polysaccharides

A linear cellulose-type polysaccharide (1→4)-β-D-ribopyranan was synthesized by selective ring-opening polymerization of 1,4-anhydro-2,3-*O*-benzylidene-α-D-ribopyranose with antimony pentachloride as catalyst (Scheme 1) [16]. The ribopyranan was sulfated with piperidine *N*-sulfonic acid to give ribopyranan sulfate [7]. Depending on the substituent on the 2 and 3 positions and the catalyst, the 1,4-



SCHEME 1. Synthesis of ribopyranan sulfate starting from polymerization of benzylidenated 1,4-anhydro-α-D-ribopyranose.

anhydro-ribose is polymerized into a (1→4)-β-D-ribopyranan or a (1→5)-α-D-ribofuranan. Another selective, i.e., 1,5-α selective, ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-benzyl-α-D-ribopyranose (= 1,5-anhydro-2,3-di-*O*-benzyl-β-D-ribofuranose) produced (1→5)-α-ribofuranan (Scheme 2) [17]. In the case of a combination of benzyl substituent and $BF_3 \cdot OEt_2$ catalyst, the 1,5-α-ring scission



SCHEME 2. Synthesis of ribofuranan sulfate starting from polymerization of benzylated 1,4- anhydro-α-D-ribopyranose (= 1,5-anhydro-β-D-ribofuranose).

was induced to produce the ribofuranan. This synthetic polysaccharide was also sulfated to afford ribofuranan sulfate.

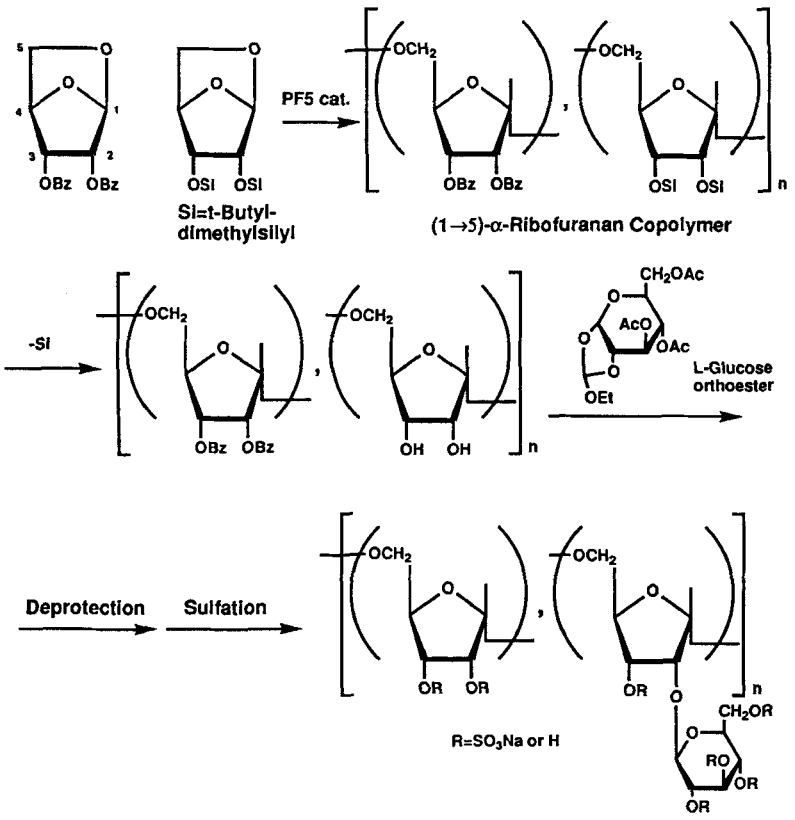
Ribofuranan and ribopyranan sulfates with the molecular weights of more than 10,000 were obtained.

By using a similar synthetic route, a benzylated 1,4-anhydro-α-D-xylopyranose prepared from D-xylose was polymerized into xylofuranan which was subsequently sulfated to give xylofuranan sulfate.

Sulfated branched polysaccharides were prepared starting from the copolymerization of 1,4-anhydro-riboses with two different substituents, followed by sulfation [18, 19]. As a representative case, the synthesis of sulfated L-glucose-branched ribofuranan is demonstrated in Scheme 3.

t-Butyldimethylsilyl group can be easily removed by refluxing the polymer with $(n\text{-Bu})_4NF$ in tetrahydrofuran. A L-glucose orthoester was reacted with the recovered hydroxyl group to produce glucose branchings. The branched poly-ribose was sulfated to give a sulfated L-glucose-branched ribofuranan.

Synthetic dextrans were obtained by the ring-opening polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose followed by debenzylation [20].



SCHEME 3. Synthesis of L-glucose-branched ribofuranan sulfate by ring-opening copolymerization.

They were sulfated with piperidine *N*-sulfonic acid to give synthetic dextran sulfates [21].

The chemical structures of ribopyranan and ribofuranan sulfates are shown in Fig. 1.

Preparation of Sulfated Natural Polysaccharides

Dextran sulfate is clinically used in Japan as a drug having lipemia clearing, anticoagulant, and fibrinolytic activities. The dextran sulfate is industrially produced by sulfating a natural bacterial dextran with chlorosulfonic acid. A commercial dextran sulfate has a relatively low molecular weight of about 7000. Both

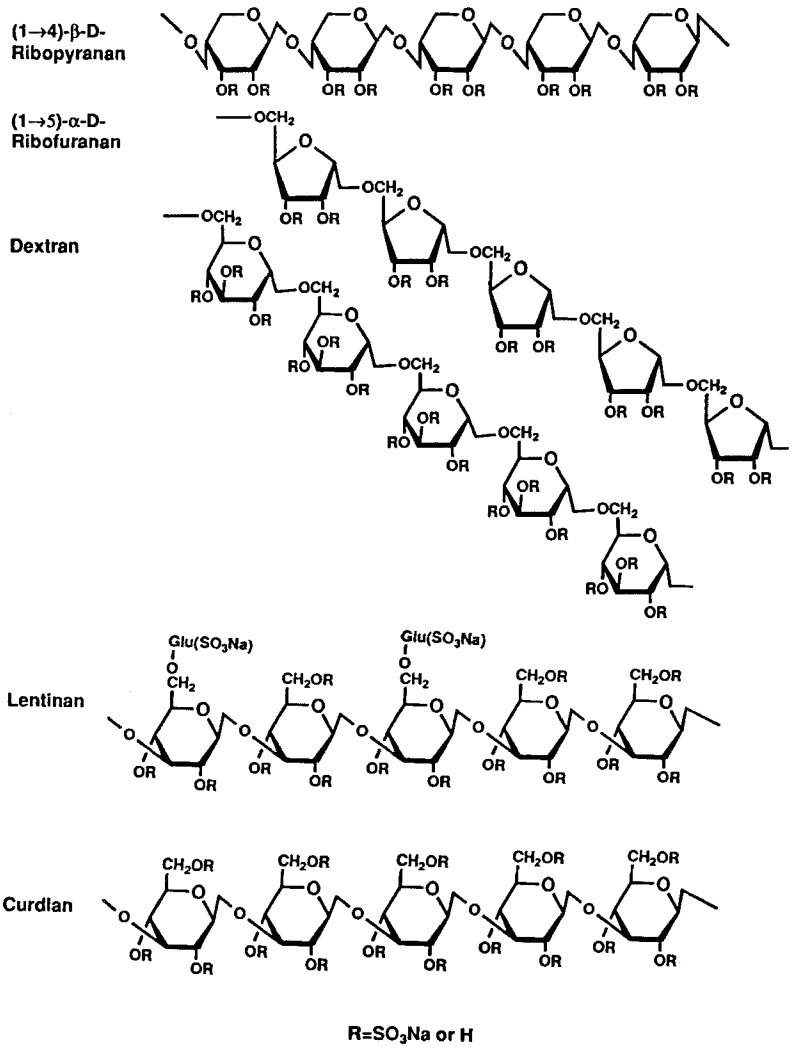


FIG. 1. Chemical structure of anti-AIDS virus active sulfated polysaccharides.

natural and synthetic dextrans were sulfated with piperidine *N*-sulfonic acid. According to the chemical structure shown in Fig. 1, the dextran sulfate is a 1,6-α linked polyglucose, and the degree of sulfation, i.e., the number of sulfate groups per glucose residue, is less than 2.

A branched 1,3-β linked polyglucose lentinan, produced by extraction from a Japanese edible mushroom and clinically used as an antitumor drug in Japan, was sulfated with piperidine *N*-sulfonic acid in dimethyl sulfoxide (DMSO).

A linear 1,3-β linked polyglucose curdlan industrially produced by bacterial fermentation in Japan was also sulfated with piperidine *N*-sulfonic acid in DMSO. Both sulfated polysaccharides have not been reported before (Fig. 1). The unsubstituted 1,3-β glucans are known to have a helical conformation [22].

Synthesis of Sulfated Alkyl Oligosaccharides

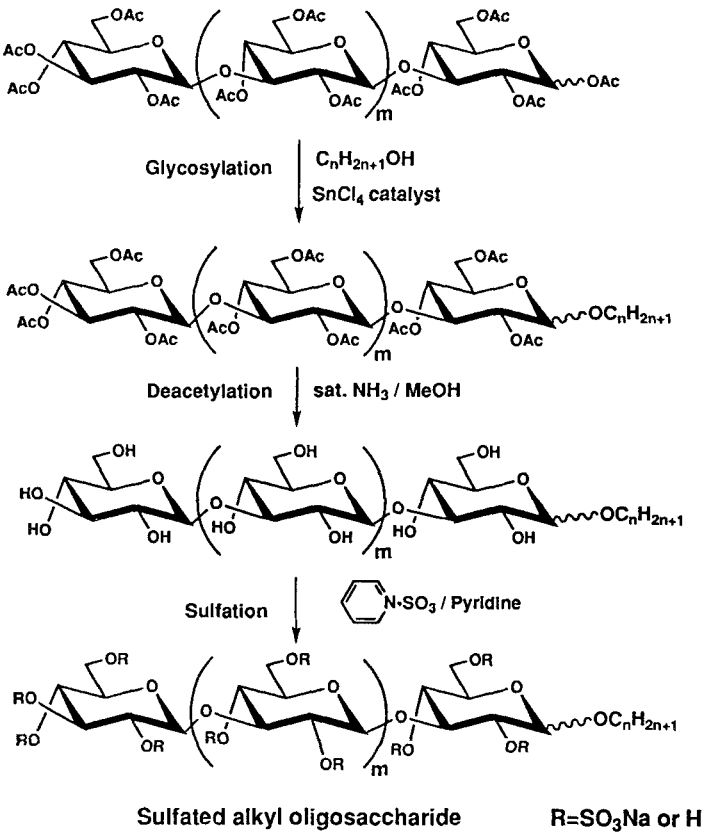
Maltooligosaccharides and laminarioligosaccharides prepared by acidic hydrolysis of starch and curdlan, respectively, were used as starting oligosaccharides [14]. Binding of a long alkyl group to the reducing end of the oligosaccharide was carried out by reacting the peracetylated oligosaccharide with an alcohol, using a Lewis acid or heteropolyacid as catalyst. The alkyl oligosaccharide was sulfated with a sulfur trioxide–pyridine complex or piperidine *N*-sulfonic acid to afford sulfated alkyl oligosaccharides with a high degree of sulfation (Scheme 4).

Anti-HIV Activity and Anticoagulant Activity of Sulfated Polysaccharides

Anti-HIV activities of sulfated polysaccharides were determined by measuring the cytopathic effects of HIV against MT-4 cells [8, 23]. The drug concentration which protects 50% of the cytopathic effects induced by HIV infection is denoted EC_{50} [23]. In early investigations the drug concentration for protecting 100% of the HIV infection was determined and denoted as IC_{100} [8, 10, 11]. Since the sulfated synthetic polysaccharides previously obtained had low anti-HIV activities [8], we tried to synthesize sulfated polysaccharides having high HIV activities. The anti-HIV activities of various sulfated polysaccharides are summarized in Table 1.

Ribopyranan sulfates (RPS) which possess S contents of more than 15% and molecular weights of more than 10,000 had high anti-HIV activities: EC_{50} = 0.1–1.5 μg/mL. A sulfated polysaccharide composed of a mixed structure (RPFS) which had a high S content (14.7%) and a high molecular weight (1.4×10^4) showed high anti-HIV activity. Both D-glucose- and L-glucose-branched ribopyranan sulfates exhibit high activities in the EC_{50} = 0.4–0.9 μg/mL range. Linear ribofuranan sulfate (RFS) and D-mannose-branched ribofuranan sulfate have high IC_{100} activities (3.3 μg/mL). Accordingly, both ribopyranan and ribofuranan give highly active sulfated polysaccharides. Dextran sulfates obtained from synthetic dextrans show high anti-HIV activities represented by IC_{100} = 3.3 μg/mL.

However, these sulfated polysaccharides possess high anticoagulant activities which are assumed to cause side effects if they are injected into human blood. For ribopyranan sulfates and ribofuranan sulfates, it is in the range of 26–47 and 55–56 unit/mg, respectively. Dextran sulfates have higher activities of 49–85 unit/mg. Therefore, it was revealed that sulfated polysaccharides belonging to this category



SCHEME 4. Synthesis of sulfated alkyl laminarioligosaccharides.

have both high anti-HIV activity and high anticoagulant activity. It was reported that the intravenous injection of a dextran sulfate to HIV-carrying patients caused severe side effects [9].

When various sulfated polysaccharides were prepared and their anti-HIV activities were examined in vitro, it was found that lentinan sulfates with molecular weights of 1.8×10^4 to 4.8×10^4 have a very potent anti-HIV activity, i.e., IC_{100} = 3.3 μg/mL, and a relatively low anticoagulant activity of 21 unit/mg (Table 2) [10]. On the other hand, dextran sulfate obtained from natural dextran possesses a high anticoagulant activity of 56 unit/mg, although it showed high anti-HIV activity.

Curdlan, which is industrially produced in Japan and has the same backbone structure as lentinan, i.e., 1,3-β-glucan, was sulfated into curdlan sulfate. As shown in Table 3, curdlan sulfates with an S content of 13 to 15% (CS-3 to 5) has a high anti-HIV activity of IC_{100} = 3.3 μg/mL.

Moreover, in spite of their high molecular weights (2.1×10^4 to 7.9×10^4), they exhibit remarkably low anticoagulant activities of <10 or 14–16 unit/mg [11, 27]. In addition, the toxicities of curdlan sulfate are low [11]. It was recently reported that branched curdlan sulfates also show high activities of EC_{50} (0.3–

TABLE 1. Anti-HIV Activity and Anticoagulant Activity of Sulfated Synthetic Polysaccharides[a]

Polysaccharide	S content, %	DS[b]	$10^{-4}\,\overline{M}_n$[c]	Anti-HIV activity				AA,[h] unit/mg
				IC_{100},[d] μg/mL	EC_{50},[e] μg/mL	CC_{50},[f] μg/mL	SI[g]	
RPS[i]	16.5	1.5	0.9	nd	1.5	510	340	26
RPS[i]	17.9	1.6	1.2	nd	0.1	420	4200	nd
RPFS[j]	14.7	1.7	1.4	nd	0.5	>1000	>2000	29
RPDGS[k]	16.7	1.5	1.5	nd	0.4	706	1660	34
RPLGS[l]	16.8	1.7	1.2	nd	0.9	524	530	47
RFS[m]	17.6	1.9	1.7	3.3	nd	nd	nd	56
RFDMS[n]	16.1	1.7	1.2	3.3	nd	nd	nd	55
DS[o]	12.4	1.2	4.2	3.3	nd	nd	nd	49
DSM[p]	15.2	1.9	2.4	10	nd	nd	nd	85

[a]Sulfating agent: piperidine *N*-sulfonic acid.
[b]Degree of sulfation denotes the number of sulfate groups per glycose unit.
[c]Number-average molecular weight.
[d]Minimum drug concentration for 100% inhibition of the virus infection.
[e]Effective concentration for 50% inhibition of the virus infection (reference: standard curdlan sulfate, 0.43 μg/mL).
[f]Drug concentration inducing 50% cytotoxic effect.
[g]Selectivity index denotes CC_{50}/EC_{50}.
[h]Anticoagulant activity (reference: dextran sulfate, 20.6 unit/mg).
[i]Ribopyranan sulfate (β 100%).
[j]Sulfated polyribose composed of 26% 1,4-β units and 74% 1,5-α units.
[k]D-Glucose-branched ribopyranan sulfate (D-glucose branching 38%).
[l]L-Glucose-branched ribopyranan sulfate (L-glucose branching 23%).
[m]Ribofuranan sulfate.
[n]D-Mannose-branched ribofuranan sulfate.
[o]Synthetic dextran sulfate.
[p]D-Mannose-branched synthetic dextran sulfate (D-mannose branching 50%).

1.2 μg/mL). However, although these L-glucose- or L-mannose-branched curdlan sulfates were synthesized in order to increase the half-life in the blood, the binding of nonnatural L-glucose branches has almost no effect on the time the activity is retained [24].

The Phase I/II test for the curdlan sulfate has been carried out in the United States for two years. It was reported that an increase in the number of CD-4 cells was found for a short time after intravenous injection to HIV-carrying patients [12]. The mechanism of action of curdlan sulfate was examined [25, 26], and it was suggested that curdlan sulfate interacts with the AIDS virus protein.

Anti-HIV Activity of Sulfated Alkyl Oligosaccharides

By making use of sulfated oligosaccharides with very low anti-HIV activities, sulfated alkyl oligosaccharides with high anti-HIV activities were synthesized [13]. As shown in Table 4, the EC_{50} of the sulfated dodecyl maltohexaoside (M6S12) was

TABLE 2. Anti-HIV Activity and Anticoagulant Activity of Lentinan Sulfate and Natural Dextan Sulfate[a]

Polysaccharide	S content, %	DS[b]	$10^{-4}\overline{M}_n$[c]	Anti-HIV activity				AA,[h] unit/mg
				IC_{100},[d] μg/mL	EC_{50},[e] μg/mL	CC_{50},[f] μg/mL	SI[g]	
LS-1[i]	6.8	0.5	>10	>100	nd	nd	nd	0
LS-2[i]	13.9	1.7	4.8	3.3	nd	nd	nd	54
LS-3[i]	16.2	2.0	1.8	3.3	nd	nd	nd	21
LS-4[i]	16.4	2.1	1.9	3.3	nd	nd	nd	21
DS-1[j]	13.5	1.4	6.6	10	nd	nd	nd	56
DS-2[j]	14.0	1.4	10.7	3.3	nd	nd	nd	nd
DS-3[k]	18	nd	0.7	nd	0.9	>1000	>1100	20

[a-h]As in Table 1.
[i]Lentinan sulfate.
[j]Natural dextran sulfate.
[k]Commercial dextran sulfate.

TABLE 3. Anti-HIV Activity and Anticoagulant Activity of Curdlan Sulfate

Polysaccharide	S content, %	DS[b]	$10^{-4}\overline{M}_n$[c]	Anti-HIV activity				AA,[h] unit/mg
				IC_{100},[d] μg/mL	EC_{50},[e] μg/mL	CC_{50},[f] μg/mL	SI[g]	
CS-1[i]	8.9	0.8	6.8	>1000	nd	nd	nd	nd
CS-2[i]	12.1	1.1	8.1	10	nd	nd	nd	<10
CS-3[i]	13.6	1.4	3.4	3.3	nd	nd	nd	<10
CS-4[i]	14.1	1.6	2.1	3.3	nd	nd	nd	<10
CS-5[i]	15.2	nd	7.9[j]	3.3	nd	nd	nd	14–16
CSDG-1[k]	13.2	nd	4.2	nd	0.9	>1000	>1030	19
CSDG-2[k]	14.4	nd	3.6	nd	0.3	>1000	>3430	nd
CSLG[l]	13.2	nd	4.7	nd	1.2	>1000	> 780	17
CSDM[m]	15.1	nd	1.7	nd	0.6	>1000	>1660	nd
CSLM[n]	15.2	nd	1.2	nd	0.5	>1000	>2000	nd

[a-h]As in Table 1.
[i]Curdlan sulfate.
[j]Weight-average molecular weight.
[k]D-glucose-branched curdlan sulfate (D-glucose branching, 39%).
[l]L-glucose-branched curdlan sulfate (L-glucose branching, 35%).
[m]D-Mannose-branched curdlan sulfate (D-mannose branching, 19%).
[n]L-Mannose-branched curdlan sulfate (L-mannose branching, 17%).

TABLE 4. Anti-HIV Activity of Sulfated Alkyl Oligosaccharides[a]

Sample name	Sulfated alkyl oligosaccharide: Number of glucose units	Carbon number of *n*-alkyl chain	Sulfur content, %	Biological activity: Anti-HIV activity, EC_{50},[b] μg/mL	Cytotoxic effect, CC_{50},[c] μg/mL	SI (CC_{50}/EC_{50})
			Maltooligosaccharide			
M5S0	5	None	17.6	267	>1000	>4
M5S10	5	10	nd	10.2	nd	nd
M6S0	6	None	16.7	207	>1000	>5
M6S12	6	12	14.4	2.4	>1000	>420
M6S18	6	18	14.5	0.6	748	1250
M7S0	7	None	15.9	80	>1000	>13
M7S12	7	12	14.9	10	820	79
M7S18	7	18	15.2	0.5	810	1600
			Laminarioligosaccharide			
L5S0	5	None	19.7	163	>1000	>6
L5S10	5	10	nd	46	>1000	>15
L5S12[d]	5	12	17.0	0.10[e]	>1000	>10000
L6S12[d]	6	12	14.2	0.18[e]	>1000	>5600
L7S12[d]	7	12	nd	0.14[e]	>1000	>7100
L7S18[d]	7	18	17.2	0.20[e]	180	900
L9S12[d]	9	12	nd	0.18[e]	> 1000	>5600
L9S18[d]	9	18	15.9	0.59[e]	240	410

[a]Sulfating agent: piperidine *N*-sulfonic acid.

[b]Effective concentration for 50% inhibition of the virus infection (reference: standard curdlan sulfate, 0.43 μg/mL).

[c]Drug concentration inducing 50% cytotoxic effect.

[d]Sulfating agent: sulfur trioxide–pyridine complex.

[e]Reference of EC_{50}: standard curdlan sulfate, 0.18 μg/mL.

2.4 μg/mL, which is about 80 times as high as that of sulfated maltohexaoside without alkyl group (M6SO). The anti-HIV activity of sulfated octadecyl maltoheptaoside was further increased to become EC_{50} = 0.5 μg/mL. A similar tendency was observed for the laminarioligosaccharide series. Sulfated dodecyl laminarioligosaccharides composed of the pentaoside to the nonaoside exhibited high anti-HIV activities of EC_{50} = 0.10–0.18 μg/mL. Furthermore, their cytotoxicity CC_{50} was as low as >1000 μg/mL. On the other hand, although both sulfated octadecyl laminariheptaoside (L7S18) and laminarinonaoside (L9S18) had high activities of EC_{50} = 0.20–0.59 μg/mL, they showed high cytotoxicities of CC_{50} = 180–240 μg/mL. It is assumed that the long alkyl group causes an increase in cytotoxicity. These compounds had no or very low anticoagulant activities [15].

The remarkable increase in anti-HIV activity by binding of the alkyl group might be ascribed to the formation of a hydrophilic–hydrophobic structure by which the sulfated alkyl ologosaccharide molecules are easily oriented and aggregated.

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