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Synthesis and Structural Analysis of Curdlan Sulfate with a Potent Inhibitory Effect in Vitro of AIDS Virus Infection

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ABSTRACT: The inhibitory effects against a human immunodeficiency virus (HIV) in vitro and the structural studies on curdlan sulfate were investigated. Curdlan, a natural linear (1→3)-β-D-glucan, was sulfated by piperidine-*N*-sulfonic acid in dimethyl sulfoxide to give curdlan sulfates with several molecular sizes and different sulfur contents. Curdlan sulfate with a sulfur content of 14.4% completely inhibited the infection of HIV in the drug concentration of as low as 3.3 μg/mL. This is one of the most potent anti-AIDS viral polysaccharides. The high-resolution NMR analysis including COSY and RELAYED-COSY experiments revealed that for a polymer with the number of sulfate groups per glucose unit (DS) of 1.6 the sulfate group was introduced to C6, C4, and C2 positions of the glucose unit, respectively.

Introduction

The acquired immunodeficiency syndrome (AIDS) is a disease caused by a virus called human immunodeficiency virus (HIV), which destroys a part of the body's immune system.[1] It is urgent to discover a drug against the AIDS virus, because an extreme number of patients and virus carriers has been reported. Since the discovery of 3′-azido-2′,3′-dideoxythymidine (AZT) as a potent inhibitor of HIV, several other 2′,3′-dideoxynucleosides have also been shown to inhibit HIV.[2,3] However, it is known that these analogues as well as AZT cause toxic side effects such as a headache, anemia, leukopenia, etc.

We have synthesized stereoregular polysaccharides with blood anticoagulant activity by ring-opening polymerization of anhydro sugars.[4,5] Recently, it was found that these synthetic polysaccharides, i.e., sulfated ribofuranan and xylofuranan and sulfated natural dextrans, completely inhibited the infection of AIDS virus in the concentration of as low as 10 μg/mL and were not cytotoxic in vitro.[6]

Natural branched (1→3)-β-D-glucans, lentinan[7] and schizophyllan,[8] are especially noteworthy for their antitumor activity. It was also found that lentinan sulfate showed a potent anti-HIV activity in the drug concentration of more than 3.3 μg/mL.[9,10] Recently, we found that a sulfated curdlan with a linear 1,3-β-linked glucose backbone has a strong inhibitory effect against AIDS virus infection in vitro.[11] In the present study, curdlan is sulfated to afford curdlan sulfates with several molecular sizes and sulfur contents, in which anti-AIDS viral activity is assayed in vitro. Also reported are the structural studies of curdlan sulfates to determine the position of sulfate groups in the glucose unit by using a combination of ^{1}H and ^{13}C NMR and 2-dimensional correlation experiments (Chart I).

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GX-270 spectrometer working, respectively, at 270 and 67.8 MHz at 37 °C. A solution of the polysaccharides in D_2O was freeze-dried several times, and samples were made up as 10–20% solutions in D_2O with 4,4′-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal reference (=0.015 ppm for ^{1}H spectra and 0.00 ppm for ^{13}C spectra from the TMS reference). The RELAYED-COSY experiments were performed with the JEOL-supplied pulse sequence. The size of the matrix was 128

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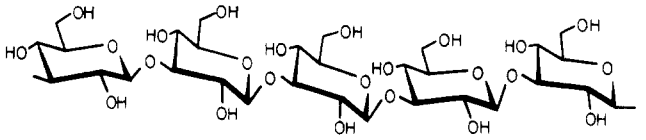
Chart I
Curdlan



× 128 and zero-filled into 512 × 512 data points. Total acquisition time was about 2 h for the single, 4 h for the double, and 24 h for the triple RELAYED-COSY spectra. Specific rotations were measured on a Perkin-Elmer 241 polarimeter in a 1-dm cell (10-cm length). Molecular weights were estimated by gel permeation chromatography (GPC) and calculated by using polystyrene and dextran standards as references for tetrahydrofuran-soluble and water-soluble compounds, respectively.

Materials. Commercial curdlan (Wako Pure Chemical Industries, Ltd. (Tokyo)) was used. Piperidine-*N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to the method of Nagasawa and Yoshidome.[12]

Sulfation.[13] A typical procedure for the sulfation of curdlan is as follows. A suspension of curdlan (0.50 g, 3.1 mmol, calculated as glucose) in anhydrous dimethyl sulfoxide (DMSO; 40 mL) was stirred for 2 h at room temperature. To a clear solution was added piperidine-*N*-sulfonic acid (1.16 g, 6.2 mmol). The temperature of the mixture was gradually elevated to 85 °C for 5 min, and then the mixture was stirred for another 45 min. After it was cooled in an ice bath, the reaction mixture was neutralized by saturated $NaHCO_3$ solution (40 mL) and then acetone (300 mL) was added until a precipitate appeared. The precipitate was collected by centrifugation, washed with acetone three times, redissolved into water (90 mL), and dialyzed against deionized water overnight. The dialyzate was concentrated to approximately 20 mL and freeze-dried to give curdlan sulfate: yield 0.72 g; $[\alpha]^{25}_D = -0.2°$ (H_2O, *c* 1); $\bar{M}_n = 2.8 \times 10^4$. Elem anal. found: C, 21.58; H, 3.23; S, 14.8.

Anti-HIV Assay.[6,9] The MT-4 cell is a human T4-positive cell line carrying HTLV-1 (human T cell lymphotropic virus type 1). Anti-HIV activity of curdlan sulfates was determined by the prevention of HIV-induced cytopathic effects (CPE) and the expression of virus-specific antigens. MT-4 cells infected with HIV at a multiplicity of infection of 0.002 were cultured for 3 and 6 days in the presence of various concentrations of curdlan sulfate. The initial cell number was adjusted to 3×10^5 cells/mL. The anti-HIV effect of curdlan sulfate on the expression of HIV-specific antigen was carried out by the indirect immunofluorescence (IF) method using a seropositive anti-HIV human serum and an antihuman IgG conjugated with a fluorescent substrate. The number of uninfected cells and percentage of IF-positive cells were determined by counting actually more than 500 cells under a fluorescence microscope.

Results and Discussion

Sulfation of Curdlan. Curdlan is a naturally occurring linear polysaccharide from *Alcaligenes faecalis var. myxogenes* 10C3 strain[14] and is made up of chains of 1,3-β-linked D-glucose units. Curdlan was sulfated with piperidine-*N*-sulfonic acid in DMSO to give curdlan sulfate. Table I summarizes the results of sulfation. When the sulfation was carried out at 60 °C (CS-1 in Table I), the obtained curdlan sulfate had a very low content of sulfate groups (DS = 0.35) based on elemental analysis and was hardly soluble in water. Increasing the reaction temperature and amounts of piperidine-*N*-sulfonic acid gave curdlan sulfates with relatively high contents of sulfate groups, but it caused a decrease in the molecular weight. The specific rotation of curdlan sulfates was close to 0°. Contamination with water should be prevented in the sulfation to avoid hydrolysis of the main chain.

Anti-HIV activity of curdlan sulfates was assayed on the prevention of HIV-induced cytopathic effects (CPE). MT-4 cells and HIV-infected MT-4 cells in the multiplicity of 0.002 were cultured in the presence or absence of various concentrations of curdlan sulfates in the CO_2 incubator. The number of viable cells and the percentage of viable antigen-positive cells were measured by the trypan blue dye exclusion method and the indirect immunofluorescence (IF) method, respectively.[6,9]

Figure 1 demonstrates the effects of curdlan sulfates on cell growth and HIV-induced CPE, and the percentage of IF-positive cells on the 3rd and 6th days after infection. The sulfur contents of curdlan sulfates in Figure 1 are as follows: (A) CS-2, S = 8.9% (DS = 0.8), (B) CS-3, S = 12.1% (DS = 1.1), (C) CS-8, S = 14.4% (DS = 1.6), and (D) CS-9, S = 14.7% (DS = 1.6). Curdlan sulfates showed no inhibitory effects on the cell growth in the concentration of 5000 μg/mL (except for CS-9), suggesting quite low cytotoxicity. CS-2 had no anti-HIV activity at a concentration of less than 1000 μg/mL. However, the anti-HIV activity increased with increasing sulfur content. CS-8 and CS-9 completely protected MT-4 cells from HIV-induced CPE in a drug concentration of as low as 3.3 μg/mL, and the cultured cells had no HIV-specific antigens (less than 1%). CS-3 had slightly lower anti-HIV activity than those of CS-8 and CS-9. After 6 days with culture in the presence of curdlan sulfate at the concentration below 1.0 μg/mL, the almost cells were dead by the infection of HIV and no multiplication of MT-4 cells in the culture was observed. The anti-HIV effect of curdlan sulfates was also confirmed by the different method such as counting the HIV-infected cells, which fluoresced under the microscope. The percentage of IF-positive cells was determined by the ratio of infected and normal cells in more than 500 cells under an observation of a fluorescence microscope. It was concluded that the anti-HIV activity of the sulfated polysaccharides depends on the molecular weight and sulfur content as well as the structure of the polysaccharide backbone.

The concentration of sulfated polysaccharide that completely inhibits the HIV infection was defined as the minimum effective concentration. Table II summarizes the physical properties and the minimum effective concentration of curdlan sulfates against HIV, where curdlan sulfates with high sulfur content exhibited very high anti-HIV activity, i.e., a minimum effective concentration of 3.3 μg/mL. However, low sulfur content curdlan sulfate showed a low anti-HIV activity even though they had high molecular weights.

The blood anticoagulant activity of curdlan sulfates was determined by using bovine plasma according to a modification of the procedure in the United States Pharmacopoeia[15] and was less than 10 units/mg compared with commercial dextran sulfate (Meito Sangyo, NC-1032, 20.6 units/mg).

Structural Analysis of Curdlan Sulfates. The structural studies of curdlan sulfates were attempted by a combination of high-resolution 1D and 2D NMR techniques, including RELAYED-COSY experiments.[16,17] The COSY experiment identifies direct *J*-couplings. On the other hand, RELAYED-COSY spectroscopy proposes a method for establishing connectivities between more than two spin systems through a linear network of couplings.

The first stage in an assignment of curdlan sulfates was to establish the identification of free curdlan in water. Since free curdlan, however, hardly dissolves in water, the assignment was carried out by using laminaripentaose, i.e., (1→3)-β-D-linked pentaglucoside, instead of curdlan, as shown in Figure 2. The peaks were assigned by H–H and C–H COSY spectra, and the arrow peaks were in complete agreement with the absorptions due to unsulfated glucose residues included in curdlan sulfates with low sulfur

Table I
Sulfation of Curdlan

curdlan[a]	PSA,[b] g (mol per glucose)	temp, °C	time, min	yield, g	$\bar{M}_n \times 10^{-4}$	$[\alpha]^{25}_D$,[d] deg	elem anal., % C	H	S	DS
CS-1	1.16 (2.0)	65	30	0.54			31.88	5.07	5.6	0.35
CS-2	4.0 (6.9)	75	45	0.92	6.8		24.77	4.24	8.9	0.8
CS-4	1.16 (2.0)	85	30	0.53	12.1	-3.8	24.05	3.58	12.1	1.1
CS-5	0.93 (1.6)	85	45	0.31	15.7	-2.3	22.39	3.33	12.5	1.3
CS-8	1.16 (2.0)	85	45	0.40	4.6	+0.1	21.02	3.17	14.4	1.6
CS-9	2.32 (4.0)	85	60	0.66	2.0	-1.5	21.19	3.38	14.7	1.6

[a] 0.5 g of curdlan was used. [b] Piperidine-*N*-sulfonic acid. [c] Determined by GPC using phosphate buffer as solvent. [d] Measured in H_2O (*c* 1).

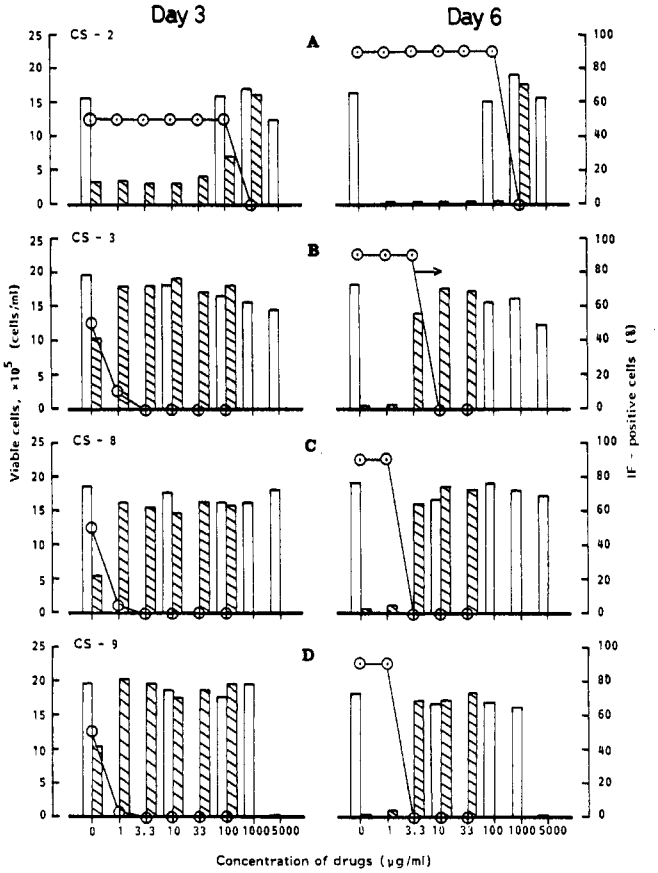


Figure 1. Effects of curdlan sulfates with different sulfur content on cell growth and HIV-induced cytopathic effects (CPE): (A) CS-2, S = 8.9%, (B) CS-3, S = 12.1%, (C) CS-8, S = 14.4%, (D) CS-9, S = 14.7%. Open bars: MT-4 cells. Slash bars: MT-4 cells and HIV-infected MT-4 cells (0.2%). Open circles: The percentage of IF-positive cells was determined by counting the IF-positive cells per approximately 500 cells after 3 and 6 days of infection.

Table II
Anti-AIDS Viral Activity of Curdlan Sulfates

curdlan sulfate	S content, %	DS	$\bar{M}_n \times 10^{-4}$	$[\alpha]^{25}_D$, deg	min effective concn,[a] μg/mL
CS-1	5.6	0.35			not effective
CS-2	8.9	0.8	6.8		1000
CS-3	12.1	1.1	8.1	-1.7	10
CS-4	12.1	1.1	11.8	-3.8	3.3
CS-5	12.5	1.3	15.7	-2.3	3.3
CS-6	13.6	1.4	3.4	-0.8	3.3
CS-7	14.1	1.6	2.1	-1.9	3.3
CS-8	14.4	1.6	4.6	+0.1	3.3
CS-9	14.7	1.6	2.0	-1.5	3.3

[a] Minimum effective concentration of curdlan sulfate on complete inhibition of HIV infection.

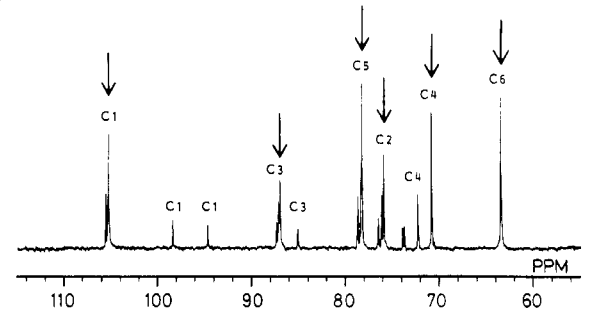


Figure 2. 67.8-MHz ^{13}C NMR spectrum of laminaripentaose in D_2O at 37 °C. The peaks were assigned by H–H and C–H COSY experiments.

content (CS-1 in Table I). The assignment was the same as previously reported.[18]

^1H NMR spectra (270 MHz) of curdlan sulfates with various sulfur content, i.e., (A) CS-7 (DS = 1.6), (B) CS-4 (DS = 1.1), and (C) CS-1 (DS = 0.35), are given in Figure 3, where an increase in sulfur content in curdlan sulfate tended to cause splitting of the H1 signal and the H6 and H6′ signals shifted downfield, suggesting that the sulfate groups occuppied the C6 position in glucose units. When the number of sulfate groups per glucose unit increased to 1.6 (CS-8), the H1 signal separated into five peaks, of which the c and d peaks overlapped. ^1H NMR spectra of curdlan sulfates were difficult to analyze in further detail, because of poorly resolved resonances.

Figure 4 shows ordinary H–H COSY (D) and the H1 regions of RELAYED-COSY (C), double RELAYED-COSY (B), and triple RELAYED-COSY (A) spectra of curdlan sulfate 8 (CS-8), which has 14.4% of sulfur content. In Figure 4D, the cross-peaks between H1 and H2 separated in two parts in the spectrum around 3.58 and 4.26 ppm, respectively, suggesting that the low-field H2 signals, H2a and H2b, exhibited the direct substitution of sulfate groups and the high-field signals, H2c, H2d, and H2e, showed no substitution of the sulfate group. Unfortunately, the connectivity was not further obtained from the H–H COSY spectrum, because of overlapping in the range of 3.7–4.0 ppm.

All of the assignment could be made by RELAYED-COSY experiments. A comparison with Figure 4 revealed that the additional cross-peaks between H1 signals and H3, H4, and H5, appeared (circled), respectively, in the RELAYED-COSY spectra. In Figure 4B, the cross-peaks between H1e and H4e appeared in a low-field region at 4.18 ppm due to doubly relayed magnetization transfer from H1 to H4 through H2 and H3, suggesting that the sulfate group was introduced to the H4 position in the glucose unit. A large low-field shift of the proton absorption indicates the direct substitution of the sulfate group with the negative charge.

The other cross-peak between H1e and H4e should also be around 3.6 ppm, owing to no sulfate substitution, which overlapped with the cross-peak between H1e and H2e. The degree of sulfate substitution at the C4 position was

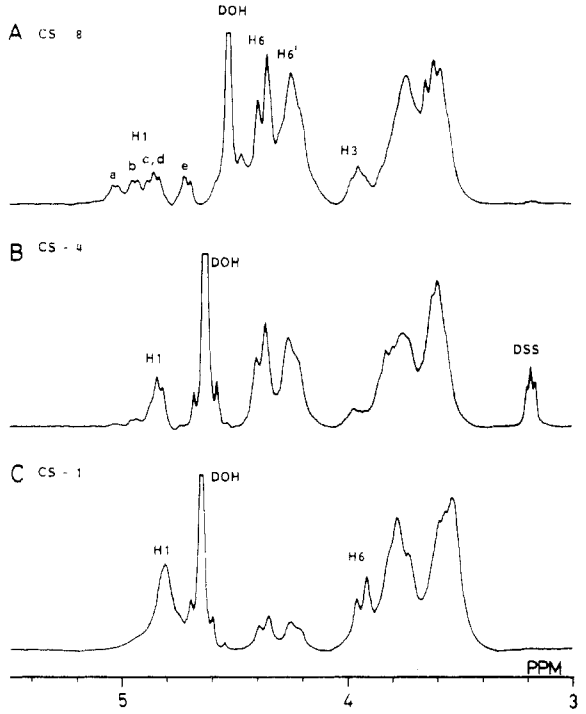


Figure 3. 270-MHz ^{1}H NMR spectra of curdlan sulfates in D_2O at 37 °C: (A) CS-8, S = 14.4% (DS = 1.6), (B) CS-4, S = 12.1% (DS = 1.1), (C) CS-1, S = 5.6% (DS = 0.35). (a–e) H1 signals that were separated by the sulfate substitution in glucose unit.

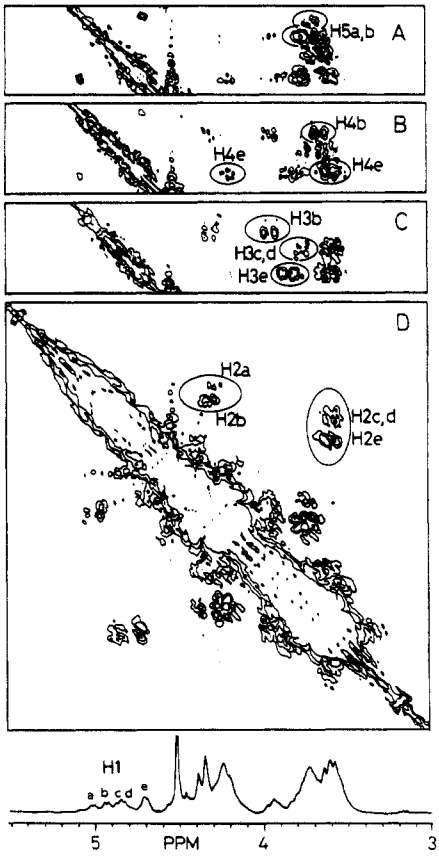


Figure 4. Counter plots of H–H COSY and H1 regions of RELAYED-COSY spectra: (A) triple RELAYED-COSY, (B) double RELAYED-COSY, (C) single RELAYED-COSY spectra, (D) ordinary COSY spectrum of curdlan sulfate 8 (CS-8) in D_2O at 37 °C.

assumed less than 5%. As a result, it was revealed that the sulfate groups of CS-8 were substituted at the C2, C4, and C6 positions in the glucose unit, and the C6 position was almost completely sulfated. A small number of sulfate

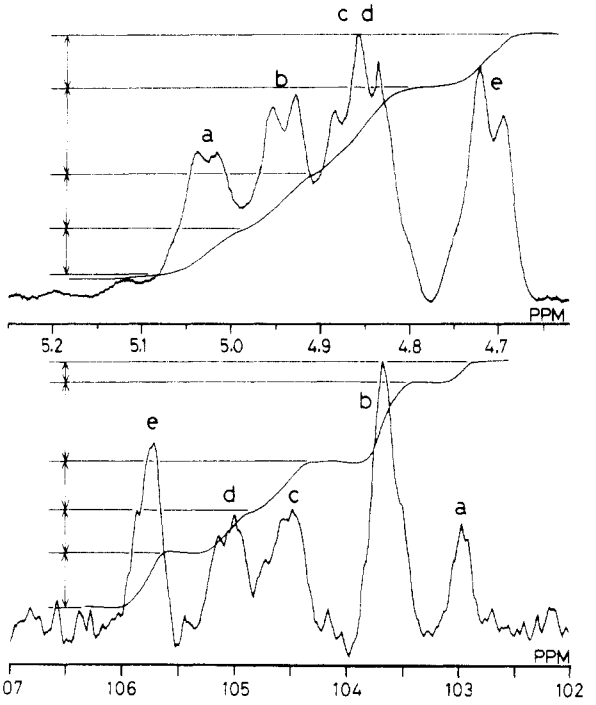


Figure 5. Anomeric proton (270-MHz) and carbon (67.8-MHz) regions of curdlan sulfate 8 (CS-8).

groups was introduced at the C4 position.

The expanded spectra of anomeric proton and carbon regions of CS-8 are shown in Figure 5. There were five H1 and C1 signals in the region of 4.7–5.1 and 103–106 ppm, respectively, where the same numbered signals corresponded to each other (determined from C–H COSY spectra). Signals a and b showed H1 or C1 absorption of the two sulfate substitutions at the C6 and C2 positions in the glucose unit; the c and d signals were the one sulfate substitution at the C6 position. The cross-peak between H1e and C1 appeared as only one absorption in the C–H COSY spectrum, but the H1e signal in Figure 5 (top) could be overlapped. Consequently, the H1e signal exhibited H1 peaks of the glucose residue with the two sulfate groups at the C6 and C4 positions and also with the one sulfate substitution at the C6 position. The peak intensity in the anomeric region suggested that the ratios of sulfate groups of CS-8 (DS = 1.6) in the C6 and C2 positions were 1 and 0.4, respectively. Although the substitution of a sulfate group at the C4 position could not be determined exactly because of overlapping, it was estimated to be less than 5% sulfate substitution. The ^{1}H NMR chemical shifts for CS-8 are given in Table III, where the assignment was directly carried out by the COSY or RELAYED-COSY spectrum as mentioned above.

^{13}C NMR spectra of curdlan sulfates with various sulfur contents are given in Figure 6, where the peak assignment was performed by the C–H COSY measurement. As the number of sulfate groups increased, the C6 signal completely disappeared, and the C6′ signal, which is the sulfated C6 carbon downfield, and the C5 signal observed upfield shift due to a γ-effect of the C6 sulfate group. In Figure 6A, the C1 absorption splits into five peaks corresponding to the following substitution patterns; i.e., peaks substituted as follows: (a) C6 and C2, (b) C6 and C2, (c) C6, (d) C6, (e) C6 and C4, respectively, which were assigned by the C–H COSY spectrum. The ratio of substitution at the individual protons, calculated by the integration of C1 carbons in Figure 5, was in good agreement with the result from the H1 integration value. The substituted C4 signal (C4′) was presumed to be

Table III
^{1}H NMR Chemical Shifts for Curdlan Sulfate 8[a] (CS-8, DS = 1.6)

H1 peak	substitution position[b]	H1	H2	H3	H4	H5	H6	H6′
a	6 and 2	5.02	4.26	nd	3.72	3.71	4.21	4.37
b	6 and 2	4.94	4.28	4.94	3.63	3.80	4.24	4.37
c	6	4.86	3.60	3.77	3.65	3.64	4.24	4.37
d	6	4.84	3.60	3.77	3.65	3.64	4.24	4.37
e	6	4.71	3.60		3.60		4.24	4.37
e	6 and 4	4.71	3.60	4.84	4.19	3.92	4.24	4.37

[a] Chemical shifts were measured in ppm and given with reference to DSS (0.015 ppm): solvent, D_2O; temp, 37 °C. [b] The substitution pattern of the sulfate groups in a glucose unit.

Table IV
^{13}C NMR Chemical Shifts for Curdlan Sulfate 8[a] (CS-8, DS = 1.6)

C1 peak	substitution position	C1	C2	C3	C4	C5	C6
	unsubstitution[b]	105.35	75.90	87.00	70.83	78.35	63.40
a	6 and 2	105.72	81.46		71.10	76.30	69.90
b	6 and 2	105.05	81.46		71.10		
c	6	104.49	76.00		70.50		
d	6	103.69	76.00	86.68	70.50		
e	6	103.00		85.81			
e	6 and 4	103.00	75.35	85.81	81.46		

[a] Recorded in D_2O at 37 °C, using DSS = 0.00 ppm as internal reference. [b] Estimated from chemical shifts of laminaripentaose in D_2O at 37 °C.

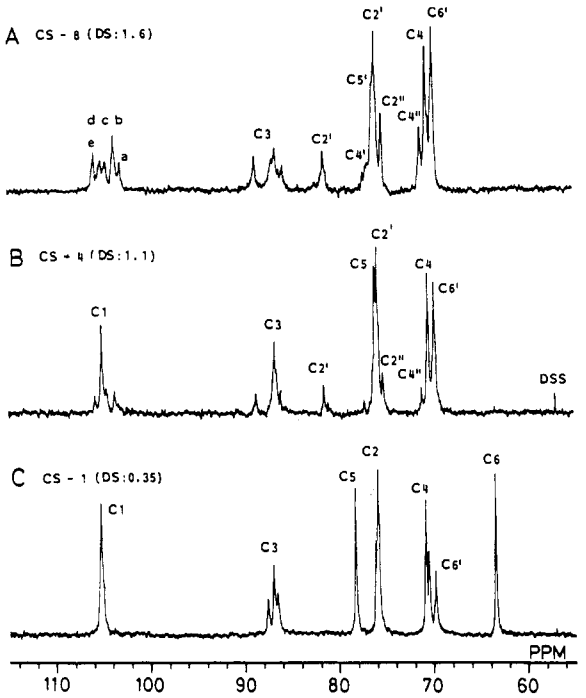


Figure 6. 67.8-MHz ^{13}C NMR spectra of curdlan sulfates in D_2O at 37 °C: (A) CS-8, S = 14.4% (DS = 1.6), (B) CS-4, S = 12.1% (DS = 1.1), (C) CS-1, S = 5.6% (DS = 0.35). (a–e) C1 signals that were separated by the sulfate substitution. C2′, C4′, and C6′ mean the sulfate-substituted carbons and C2″ and C4″ neighboring carbons to the substituted carbons.

assigned to a shoulder peak around 76.5 ppm in Figure 6A. Further details of the C4′ assignment will be reported elsewhere. The C2″ and C4″ absorptions (nonsubstituted peaks) exhibited a little upfield or downfield shift owing to the neighboring group participation of the substituted C4 and C2 carbons, respectively. It was assumed that a dispersion of C3 chemical shifts depended on a difference of tacticity of the polymer backbone. The ^{13}C chemical shifts of CS-8 are summarized in Table IV.

In conclusion, a curdlan sulfate (CS-8) completely inhibited the infection of AIDS virus in the concentration of 3.3 μg/mL. The structural analysis of the curdlan sulfate with a strong inhibitory effect was carried out by a combination of ^{1}H, ^{13}C, and 2D NMR including the RELAYED-COSY experiment. It was found that the sulfate groups were introduced to C6, C4, and C2 positions of the glucose units of curdlan in the ratio of 100%, ~5%, and 40%, respectively. It was revealed that the first sulfation to the glucose unit of curdlan occurred at the C6 position. The hydroxyl group at C6 is a primary alcohol and has a relatively lower steric hindrance than those of C2 and C4. As the degree of substitution increased, the sulfate group was introduced to the C2 and C4 positions. The sulfated polysaccharides with high molecular weights and high degrees of substitution showed higher anti-HIV activity but, however, also have cytotoxicity from our results (data did not show here). Curdlan sulfates as well as lentinan sulfate,[10] which have 1→3-linkages, have a high anti-HIV activity and a low cytotoxicity.

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Synthesis and Properties of Polyamides Having Anti Head-to-Head Umbelliferone Dimer as a Component

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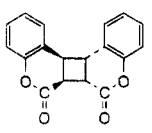
ABSTRACT: Anti head-to-head umbelliferone dimer (**6**) was synthesized from anti head-to-head 7-acetoxycoumarin dimer (**5**). The ring-opening polyaddition reaction of **6** with diamines in an aprotic polar solvent was carried out, and the properties of the resulting polyamides were investigated. Dimer **6** reacted successfully with aliphatic and aromatic diamines to give the corresponding high-molecular-weight polyamides (**8**). Upon photoirradiation, the cyclobutanes in the main chain of **8** were preferentially cleaved in an asymmetric manner to give fumaramide (or maleamide) units. In contrast, in an alkaline solution, the cyclobutanes in **8** were cleaved only in a symmetric manner to give low-molecular-weight products, accompanying the isomerization of the configuration of the substituents on the cyclobutanes. These reactions in alkaline medium are considered to occur through an intermediate of a quinoid enolate structure.

Introduction

In previous papers, we have reported on the reactions and properties of anti head-to-head coumarin dimer (**1**), its lactone-opened derivatives,[1-5] and polyamides **2** derived from **1**.[6-14] Dimer **1** is susceptible to lactone ring-



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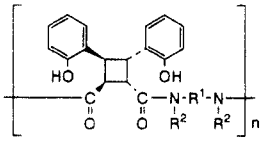

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opening reactions with various nucleophiles, because **1** has highly reactive lactone rings fused to a strained cyclobutane ring.[2] Moreover, **1** reacts easily with diamines to give the corresponding high-molecular-weight polyamides **2**.[8] Polyamides **2** show characteristic photobehavior and thermal behavior. The cyclobutanes in **2** are cleaved in an asymmetric manner by photoirradiation with UV light to give poly(fumaramide) and 2,2′-dihydroxystilbene.[6-8] In contrast, upon heating, the amide linkages of **2** are severed to regenerate lactone rings, followed by a successive cyclobutane cleavage.[5] Furthermore, optically active polyamides, prepared from optically active **1**, form an ordered conformation and show a high chiral recognition ability.[9-14] The phenolic hydroxyl groups in the side chain play an important role in revealing these characteristic properties.

In the present paper, we report the synthesis and behavior of a new type of polyamide consisting of anti head-to-head umbelliferone (7-hydroxycoumarin) dimer, which has more phenolic hydroxyl groups than those of the coumarin dimer **1**.

Results and Discussion

Synthesis of Anti Head-to-Head Umbelliferone Dimer (Scheme I). Anti head-to-head 7-acetoxycoumarin dimer (**5**) can be easily prepared from umbelliferone (7-hydroxycoumarin) (**3**) via 7-acetoxycoumarin (**4**) according to a procedure reported by Leenders et al.[15] The deacetylation of **5** was tried in order to synthesize anti head-to-head umbelliferone dimer (**6**), but the hydrolysis of **5** in an aqueous NaOH solution resulted in a cyclobutane cleavage in a symmetric manner to give 2,4-dihydroxycinnamic acid (umbellic acid) (see below). In contrast, the hydrolysis of **5** in an aqueous HCl solution, followed by relactonization in acetic acid, gave **6** in high yield. Dimer **6**, deposited from an acetic acid solution, contained a small amount of acetic acid. Dimer **6** is barely soluble in most organic solvents but is soluble in aprotic polar solvents such as *N*,*N*-dimethylacetamide (DMAc), *N*,*N*-dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), and dimethyl sulfoxide (DMSO). Recrystallization from DMF/$CHCl_3$ gave **6** free from acetic acid, though it contained DMF.

Ring-Opening Polyaddition Reaction of Anti Head-to-Head Umbelliferone Dimer with Diamines. The ring-opening polyaddition reaction of anti head-to-head umbelliferone dimer (**6**), recrystallized from DMF/$CHCl_3$, with 1,6-hexanediamine (**7a**) proceeded smoothly at room temperature in DMAc (Scheme II). However, the resulting polyamide (**8a**) did not possess a very high molecular weight (η_{inh} = 0.32 dL·g^{-1}, 0.3 g of polymer/dL in DMAc at 30 °C). Next, the ring-opening polyaddition reaction of **6**, crystallized from acetic acid, with diamines was carried out in the presence of triethylamine equimolar with acetic acid contaminated.

At first, the conditions for polymerization were optimized by using 1,4-phenylenediamine (**7b**) as a diamine

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