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Note

Synthesis of curdlan sulfates having inhibitory effects in vitro against AIDS viruses HIV-1 and HIV-2

Takashi Yoshida [a,*], Yuichi Yasuda [a], Toru Mimura [b], Yutaro Kaneko [b], Hideki Nakashima [c], Naoki Yamamoto [c], Toshiyuki Uryu [d]

[a] *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-10 Nishi-8 Kita-ku, Sapporo 060, Japan*

[b] *Ajinomoto Company, Inc., Chuo-ku, Tokyo 108, Japan*

[c] *Tokyo Medical and Dental University School of Medicine, Yushima, Bunkyo-ku, Tokyo 113, Japan*

[d] *Institute of Industrial Science, University of Tokyo, Roppongi, Minato-ku, Tokyo 106, Japan*

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Nucleoside analogues such as azidothymidine (AZT), dideoxyinosine (ddI), and dideoxycytidine (ddC) [1] inhibit the viral reverse transcriptase (RT) of human immunodeficiency virus (HIV) and terminate DNA chain synthesis from viral RNA inside the infected cells. However, these nucleosides manifest serious side-effects such as bone-marrow toxicity and the appearance of AZT-resistant viruses upon long-term treatment [2]. It is difficult to make an effective vaccine owing to the multiplicity of AIDS viruses. Therefore, anti-AIDS compounds having a different blocking mechanism are of interest.

We have synthesized sulfated polysaccharides demonstrating high anti-AIDS virus activity by sulfation of synthetic and natural-occurring polysaccharides [3–6]. The mechanism of action of sulfated polysaccharides is assumed to be different from that of the nucleosides, that is, they bind to HIV virions and prevent them from penetrating into target cells [7–9]. However, sulfated polysaccharides such as dextran sulfate have high blood anticoagulant activity, leading to undesirable side-effects when used as anti-AIDS drugs [10].

* Corresponding author.

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An antitumour polysaccharide lentinan [11], with a branched β-(1 → 3)-linked glucan backbone and the linear polysaccharide curdlan [12], having the same β-(1 → 3) glucan backbone were sulfated with piperidine-*N*-sulfonic acid to give the respective lentinan and curdlan sulfates having various degrees of sulfation and molecular masses. Lentinan sulfates displayed high anti-AIDS virus activity, completely inhibiting AIDS virus infection of T lymphocytes at concentrations as low as 3.3 μg/mL in vitro. Curdlan sulfates also completely inhibited the infection at 3.3 μg/mL concentration. The half-life of curdlan sulfates [13] in plasma has been found to depend on their molecular masses, being 60 and 180 min for molecular masses of 7×10^4 and 17×10^4, respectively. It was also found that these curdlan sulfates were transported to tissues such as liver, kidney, lymph node, and bone marrow within 1 h, and remained in the tissues without degradation for 10 days [14]. To maintain the concentration of curdlan sulfates in plasma for a longer time, unnatural sugars such as L-glucose and L-mannose were reacted with curdlan to give L-glycosyl-branched curdlans [15]. However, the retention time of the L-branched sulfates was almost the same as that of the linear ones, indicating that the L-glucose had no effect. The anticoagulant activity of curdlan sulfate is lower (10 units/mg) than that of standard dextran sulfate (20.6 units/mg). Curdlan sulfate was thus expected to have potential as an AIDS drug through inhibition of virus entry in the early stage of infection. The Phase I/II test of curdlan sulfate has been carried out in the USA for the past 2 years. It was recently shown that curdlan sulfate produced dose-related increases in CD4 lymphocytes in HIV-infected patients after a 4 h infusion and no clinical side effects were observed at any dose tested [16].

Two kinds of AIDS viruses are known, HIV type 1 (HIV-1) and HIV type 2 (HIV-2) [17]. HIV-2, which is genetically distinct from HIV-1, has been less studied than HIV-1. The purpose of this study was to synthesize anti-AIDS-active curdlan sulfates by three sulfation methods, using piperidine-*N*-sulfonic acid (PSA method), SO_3–pyridine complex in pyridine (SPC method), and chlorosulfonic acid in pyridine (CSA method), and to evaluate the in vitro inhibitory effects of both HIV-1 and HIV-2 infections by these compounds. In addition, precise structural analysis of the curdlan sulfates was performed by means of high-resolution NMR measurements.

1. Experimental

Materials.—Commercial curdlan ($\overline{M}_n = 8.9 \times 10^4$, Wako Pure Chemical Industries, Tokyo), pyridine–SO_3 complex (SO_3–pyridine) (Tokyo Chemical Industry, Tokyo), and dry Me_2SO (Aldrich Chemical, Milwaukee, WI) were used without further purification. Piperidine-*N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to the method of Nagasawa and Yoshidome [18].

Measurements.—^1H and ^{13}C NMR spectra were recorded on a JEOL JMN GX-270 spectrometer at 270 and 67.8 MHz, respectively, by using 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as the internal standard (0.015 ppm for ^1H and 0.00 ppm for ^{13}C spectra). ^1H NMR spectra (600 MHz) of curdlan sulfates in D_2O were taken on a Bruker AMX 600 spectrometer at 37°C. Specific rotations were measured on a Perkin–Elmer 241 polarimeter with a 1-mL cell (10 cm length) at 25°C. Molecular masses were

determined by aqueous phase GPC (column: Toso TSK-gel, G2000SW, G3000SW, G4000SW, 7.6 mm × 600 mm × 3) using pullulan standards (Shodex Standard P-82) as references (eluent, 66.7 mmol of phosphate buffer, pH 6.86).

Sulfation.—Curdlan was sulfated by the use of piperidine-*N*-sulfonic acid in Me_2SO (PSA method), SO_3–pyridine complex in pyridine (SPC method), and chlorosulfonic acid (CSA method) in pyridine. The degree of sulfation was calculated from the elemental analysis. Typical procedures are as follows.

PSA method.—Curdlan (0.5 g, 3.1 mmol, based on one glucose residue) was dissolved in dry Me_2SO (40 mL) and piperidine-*N*-sulfonic acid (2.32 g, 9.3 mmol) was added to the solution. The mixture was stirred for 60 min at 85°C, cooled with an ice bath, made neutral with saturated $NaHCO_3$ (40 mL) and then acetone was added until a precipitate appeared. The precipitate was collected by centrifugation, washed three times with acetone, redissolved in water (100 mL), and dialyzed overnight against deionized water. The dialyzate was concentrated to ca. 30 mL and freeze-dried to give curdlan sulfate; yield 0.66 g; $[\alpha]_D^{25}$ −1.5° (H_2O, *c* 1%), $\overline{M}_n = 2.0 \times 10^4$. Anal. Found: C, 21.19; H, 3.38; S, 14.8%.

SPC method.—To a suspension of curdlan (0.5 g, 3.1 mmol) in pyridine (40 mL) was added SO_3–pyridine complex (6.0 g, 47.1 mmol) and the mixture was stirred for 2 h at 85°C. After cooling with an ice bath, 10% NaOH (50 mL) was added to the mixture with vigorous stirring and then acetone was added until the acetone layer separated. The lower aqueous layer was washed three times with acetone, with subsequent dialysis against deionized water overnight. The dialyzate was concentrated and freeze-dried from water to give curdlan sulfate: yield 1.30 g; $[\alpha]_D^{25}$ −13.9° (H_2O, *c* 1%), $\overline{M}_n = 6.3 \times 10^4$. Anal. Found: C, 14.59; H, 2.38; S, 16.5%.

CSA method.—Curdlan (0.5 g, 3.1 mmol) was added to chlorosulfonic acid (4.4 g, 33.2 mmol) in dry pyridine (80 mL) and the mixture was stirred for 1 h at 100°C. Curdlan sulfate was obtained after the same treatment as just described: yield 1.22 g; $[\alpha]_D^{25}$ −22.0° (H_2O, *c* 1%), $\overline{M}_n = 1.3 \times 10^4$. Anal. Found: C, 14.80; H, 2.31; S, 19.4%.

Anti-AIDS virus assay.—The anti-AIDS virus activity was determined by the protection from HIV-induced cytopathic effects in vitro [19]. Human T-lymphotropic virus type-I positive human T cell line (MT-4 cell) was infected with $HIV\text{-}1_{HTLV\text{-}IIIB}$ or $HIV\text{-}2_{ROD}$ strain at the multiplicity of infection of 0.01. The HIV-1 and HIV-2 strains were prepared from the supernatant of MOLT-4/$HIV\text{-}1_{HTLV\text{-}IIIB}$ and MOLT-4/$HIV\text{-}2_{ROD}$ cells, respectively. HIV-infected and uninfected MT-4 cells (3×10^5 cells/mL) were co-cultured in the presence of various concentrations of curdlan sulfates for 5 days at 37°C in a CO_2 incubator. The viability of both HIV- and mock-infected cells was assayed by a spectrophotometric method using the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue formazan product for the concentration of viable cells. The dose achieving 50% protection of MT-4 cells from HIV infection was defined as EC_{50} and the 50% cytotoxic concentration was designated as CC_{50}.

Blood anticoagulant activity.—The anticoagulant activity testing of curdlan sulfates was measured by use of bovine plasma according to a modification of the US Pharmacopeia [20] and was calculated in comparison with the activity of standard dextran sulfate (NC-1032), 20.6 units/mg.

2. Results and discussion

Sulfation of curdlan.—The highly ordered structure of curdlan was demonstrated by solid-state NMR studies indicating that curdlan powder was composed of ~ 10% triple-standed helical portions and 90% flexible single-standed helical chains [21]. Because the helical structure causes low solubility, curdlan is insoluble in water and common organic solvents, but it is soluble in Me_2SO. The results of sulfation are summarized in Table 1. Sulfation of curdlan with PSA in Me_2SO proceeded homogeneously to give curdlan sulfates having specific rotations around 0° (Nos. 1–4). Taking into account the homogeneous sulfating conditions and the low specific rotations, the conformation of the curdlan sulfates obtained by this method might be a random coil rather than the initial helical structure. With increasing temperature, time, and amounts of PSA, the degree of sulfation increased and the molecular mass decreased. The degrees of sulfation calculated from elemental analysis for curdlan sulfates prepared by the PSA method were in agreement with the results of NMR measurements.

The other two sulfating reagents [SO_3–pyridine complex (SPC) and chlorosulfonic acid (CSA)] were used in suspension. The SPC was added to a suspension of curdlan in pyridine to afford curdlan sulfates having a degree of sulfation of 2.2–2.6 and number-average molecular masses of 4.7×10^4–12.4×10^4 (Nos. 5–8). Sulfation with CSA gave curdlan sulfates having molecular masses of 1.2×10^4–3.5×10^4 and degrees of sulfation of 2.6–3.1 (Nos. 9–12). The specific rotations were relatively large and negative (−7.1 to −23.4°); curdlan sulfates prepared with PSA had values +0.1° to −3.8°. Sulfation by SPC and CSA in pyridine proceeded heterogeneously and the curdlan sulfates might have retained the original helical structure. Sulfations with SPC

Table 1
Sulfation of curdlan

Curdlan [a]	Sulfating reagent [b] [g (mol per glucose)]	Temp. (°C)	Time (min)	Yield (g)	$\bar{M}_n \times 10^4$	$[\alpha]_D^{25}$ [c] (°)	Elemental analysis (%)			DS
							C	H	S	
1	1.16 (2.0)	65	30	0.54	–	–	31.88	5.07	5.6	0.4
2	1.16 (2.0)	85	30	0.53	12.1	−3.8	24.05	3.58	12.1	1.1
3	1.16 (2.0)	85	45	0.40	4.6	+0.1	21.02	3.17	14.4	1.6
4	2.32 (4.0)	85	60	0.66	2.0	−1.5	21.19	3.38	14.8	1.6
5	3.0 (7.6)	85	120	0.43	4.7	−7.1	16.19	2.68	15.6	2.2
6	3.0 (7.6)	85	240	0.79	12.4	−19.9	16.35	2.72	17.7	2.5
7	6.0 (15.2)	85	60	1.20	5.4	−14.9	16.22	2.59	16.5	2.4
8	6.0 (15.2)	85	120	1.30	6.3	−13.9	14.59	2.38	16.5	2.6
9	2.2 (6.3)	100	60	1.08	1.2	−16.0	15.84	2.40	18.0	2.6
10	2.2 (6.3)	100	120	0.96	1.2	−15.4	15.03	2.48	19.0	2.8
11	4.4 (12.6)	100	60	1.22	1.3	−22.0	14.80	2.31	19.4	2.9
12	17.5 (50.0)	100	60	0.90	3.5	−23.4	14.62	2.49	20.0	3.1

[a] Curdlan: 0.5 g.
[b] 1–4, Piperidine-*N*-sulfonic acid; 5–8, Pyridine–SO_3 complex; 9–12, Chlorosulfonic acid.
[c] Measured in H_2O (*c* 1%).

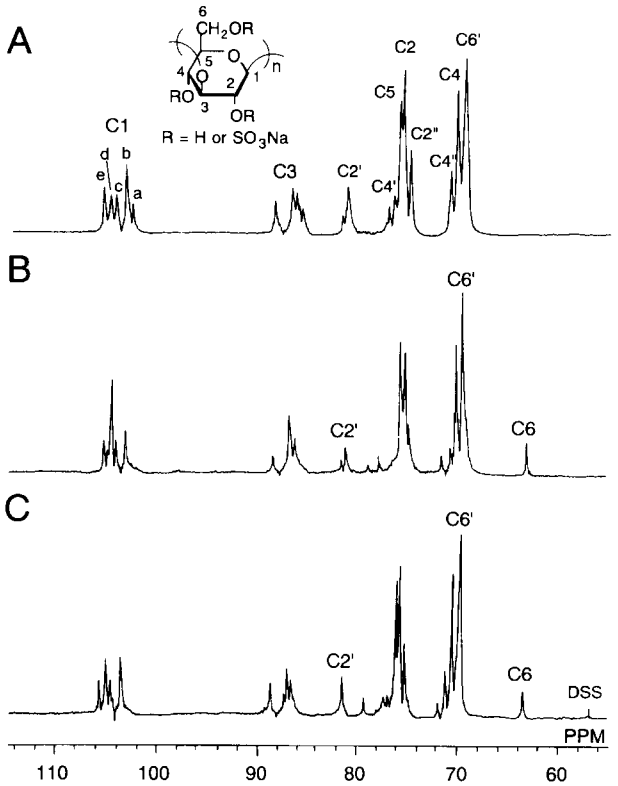

Fig. 1. 67.8 MHz ^{13}C NMR spectra of curdlan sulfates (in D_2O at 37°C): (A) prepared by piperidine-*N*-sulfonic acid (S 14.4%), (B) by the pyridine–SO_3 complex (S 16.5%), and (C) by chlorosulfonic acid (S 21.7%). C-2′, C-4′, and C-6′ denote the sulfate-substituted carbon signals and C-2″ and C-4″ are carbons adjacent to the sulfate-substituted carbons.

and CSA in pyridine required a large excess of sulfating reagents to give curdlan sulfates having high degrees of sulfation. The degrees of sulfation calculated from the elemental analyses appear high compared with those determined by ^{13}C NMR measurements (see later).

Structure of curdlan sulfates.—Fig. 1 shows the ^{13}C NMR spectra of curdlan sulfates with sulfur contents of 14.4, 16.5, and 21.7% prepared by the PSA (A), SPC (B), and CSA (C) methods, respectively. Fig. 1A shows peak assignments made from two-dimensional NMR measurements [12,22]. The original C-6 signal disappeared and was shifted downfield to 69.5 ppm, indicating complete sulfation at O-6. In contrast (Fig. 1B and 1C), a C-6 peak still remained at 63 ppm for the heterogeneously sulfated products suggesting that the primary OH groups on the internal side of the helix were not sulfated.

Splitting of the C-1 signal in the ^{13}C NMR spectra (Fig. 1B and 1C) was examined with reference to the previous structural analysis of curdlan sulfates obtained by the PSA method. Fig. 2 shows ^{13}C NMR spectra in the anomeric region of curdlan sulfates with

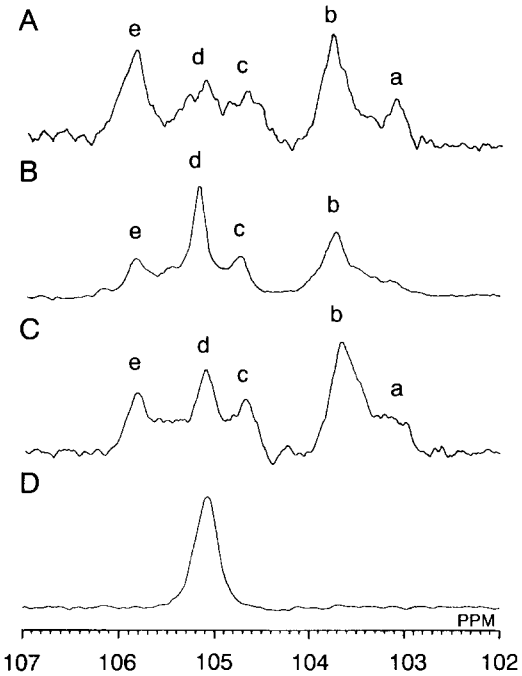

Fig. 2. 67.8 MHz ^{13}C NMR spectra of anomeric carbon region of curdlan sulfates (in D_2O at 37°C): (A) prepared by piperidine-*N*-sulfonic acid (S 14.4%), (B) by the pyridine–SO_3 complex (S 16.5%), (C) by chlorosulfonic acid (S 21.7%), and (D) by piperidine-*N*-sulfonic acid (S 5.6%).

(A) 14.4% sulfur content (DS 1.6), (B) 16.5% (DS 2.4), (C) 18.2% (DS 2.7), and 5.6% (DS 0.25), which were prepared by the PSA, SPC, CSA, and PSA methods, respectively. Spectra 2B and 2C were similar to each other, but were different from spectrum 2A; this difference might be mainly attributable to different substituent distributions of sulfate groups in the polymer backbone. Absorptions *a* and *b* in Fig. 2 were C-1 peaks with two sulfate substitutions at C-6 and C-2 in the glucose residue and signals *c*, *d*, and *e* those for one sulfate substitution at C-6. With reference to the spectrum of a low-sulfated curdlan (2D), the unsubstituted C-1 carbon absorption in spectra 2B and 2C was presumed to overlap peak *d* at 105.1 ppm. The anomeric proton signal in the 600 MHz ^1H NMR spectrum showed a symmetrical pattern (Fig. 3B), supporting the notion that the position of sulfate groups obtained by SPC is different from that by PSA (Fig. 3A).

Fig. 4 shows the C-H COSY spectrum of a curdlan sulfate having 16.5% sulfur content prepared by the SPC method. A large downfield shift of the proton absorption suggests substitution by an electron-withdrawing sulfate group. The cross-peaks for C-2–H-2 and C-6–H-6 were separated into two parts around 4.3 and 3.6 ppm for H-2, and 4.3 and 3.8 ppm for H-6, respectively, indicating that the low-field signals (indicated as C-6′) were due to the sulfate-substituted absorptions and the high field signals (C-6) were the unsubstituted group absorptions. Therefore, the peaks at 81.5 and

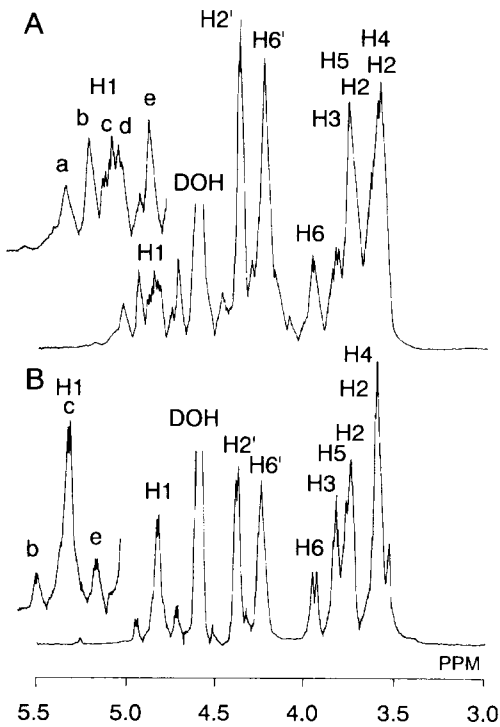

Fig. 3. 600 MHz ^{1}H NMR spectra of curdlan sulfates (in D_2O at 37°C): (A) prepared by piperidine-*N*-sulfonic acid (S 14.4%) and (B) pyridine–SO_3 complex (S 17.4%).

69.5 ppm in the ^{13}C NMR spectrum could be assigned to the sulfate-substituted signals of C-2 and C-6, respectively. An intense cross-peak between C-4 and H-4 in the high-field region indicated no substitution at C-4. It was assumed that a splitting of the C-3 signal depends on the presence of a sulfate group in the polymer backbone. Elemental analysis indicated a degree of sulfation of 2.4. The curdlan sulfates prepared by the SPC and CSA methods were generally of higher degrees of sulfation than the curdlan sulfate obtained by PSA. The discrepancy between NMR results and elemental analysis suggest that inorganic sulfur derivatives from SO_3 might remain as impurities in the sulfated curdlan molecules. The unidentified sulfur derivatives could not be removed by dialysis against deionized water. Precise evaluation of the degree of sulfation by NMR spectroscopy is difficult because of peak overlap and poorly resolved resonances.

As already mentioned, the different conditions used for the preparation of curdlan sulfates resulted in structurally different products. We considered the PSA method to be the best because sulfation proceeded homogeneously under mild conditions to give pure curdlan sulfate of degree of sulfation of 1.6. However, it was assumed that the heterogeneous sulfation methods using SPC or CSA gave curdlan sulfate with the retention of highly ordered structures. Consequently, the structure–anti-AIDS and –anticoagulant activities relationships for both types were investigated.

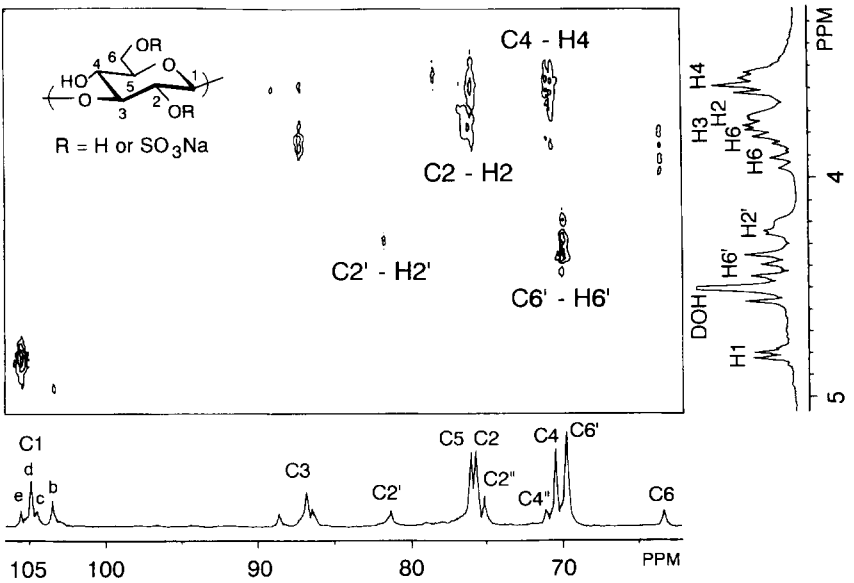

Fig. 4. Counter plots of the C-H COSY spectrum of curdlan sulfate prepared by the pyridine–SO_3 complex (S 16.5%) (in D_2O at 37°C). The abbreviations b, c, d, and e for the C-1 signals are the same as those in Fig. 2.

Anti-AIDS virus activities of curdlan sulfates against HIV-1 and HIV-2.—Several sulfated polysaccharides exhibit antivirus activities; thus agar and carrageenan inhibit influenza B and mumps viruses [23]. Anti-AIDS virus activity has been reported for sulfated polysaccharides extracted from marine algae [24,25]. As we have shown that curdlan sulfate prepared by PSA demonstrates potent anti-AIDS virus activities against HIV-1, the anti-AIDS virus activity of the curdlan sulfates obtained here were examined using both HIV-1 and HIV-2.

Fig. 5 demonstrates the effects of curdlan sulfate obtained by the PSA method on cell viability and cytopathic effect against (A) HIV-1 and (B) HIV-2 infection at various concentrations of the drug. The activity was taken by the MTT method [19] and was evaluated by the EC_{50} value, which is a concentration of MT-4 cells that protected 50% against HIV infection, after MT-4 cells had been infected with $HIV\text{-}1_{HTLV\text{-}IIIB}$ or $HIV\text{-}2_{ROD}$ in the presence of various concentrations of curdlan sulfates for 5 days. It was observed that curdlan sulfate (S 12.8%, DS 1.4, $\overline{M}_n$ 7.0×10^4) completely inhibited the infection of both HIV-1 and HIV-2 on MT-4 cells at the EC_{50} of 0.4 μg/mL (A) and 0.6 μg/mL (B), respectively. This result shows that MT-4 cells which escaped infection by both HIV-1 and HIV-2 viruses by the inhibitory effects of the curdlan sulfate proliferated at the same rate as that of the uninfected MT-4 cells (slash bars). These values are almost comparable to the complete inhibitory concentration of 3.3 μg/mL [26]. Cytotoxicity against HIV-uninfected MT-4 cells was not observed at concentrations of up to 1000 μg/mL, because MT-4 cells grew normally (open bars). The curdlan sulfate obtained by the PSA method had high anti-AIDS virus activities and lower cytotoxicities against both HIV-1 and HIV-2 strains.

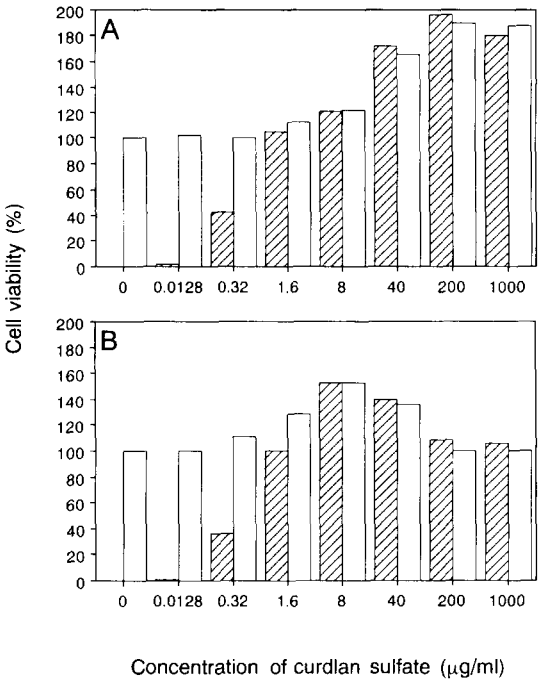

Fig. 5. Inhibitory effect of curdlan sulfate (S 12.8%, $\overline{M}_n$ 7.0×10^4) obtained by the PSA method against (A) HIV-1 and (B) HIV-2 at various concentrations and the direct cytotoxicity to MT-4 cells. MT-4 cells (open bars) and HIV-infected MT-4 cells (slash bars) were adjusted at 2.5×10^4 cells/mL and cultured in the presence of various concentrations of the curdlan sulfate for 5 days.

To investigate the relation between anti-AIDS virus activities and such structural factors as degree of sulfation, molecular mass, and method of preparation, a series of curdlan sulfates were synthesized by the SPC or CSA methods already described. The anti-AIDS virus activities of these curdlan sulfates (Fig. 6) are represented as their concentrations of $< EC_{50} = 1.0$ μg/mL (○, ◦, ●), 1.0–10 μg/mL (△, ▲), and > 10 μg/mL (×). The inhibitory effects to both HIV-1 and HIV-2 of curdlan sulfates prepared by the SPC and CSA methods were high. Products of molecular masses of $> 1.0 \times 10^4$ and degrees of sulfation of > 1.0 showed potent anti-AIDS virus activities against both HIV-1 and HIV-2 strains. Curdlan sulfates having relatively low degrees of sulfation (around 1.0) had high inhibitory effect against HIV-2 (Fig. 6B) compared with that against HIV-1. The difference was not substantial, and the infectious strength of the HIV-2 strain used in this experiment might have been weaker than that of the HIV-1 strain. These results indicate that structural differences seem not to be essential for anti-AIDS virus activities on HIV-1 and HIV-2, but the degree of sulfation and molecular mass of the curdlan sulfates are important. We have obtained similar results in recent investigations on the anti-AIDS virus activity of artificial sulfated polysaccharides [4–6]. However, it was found that the cytotoxicity of curdlan sulfates obtained by the SPC or CSA method was slightly higher than that of curdlan sulfate prepared by the

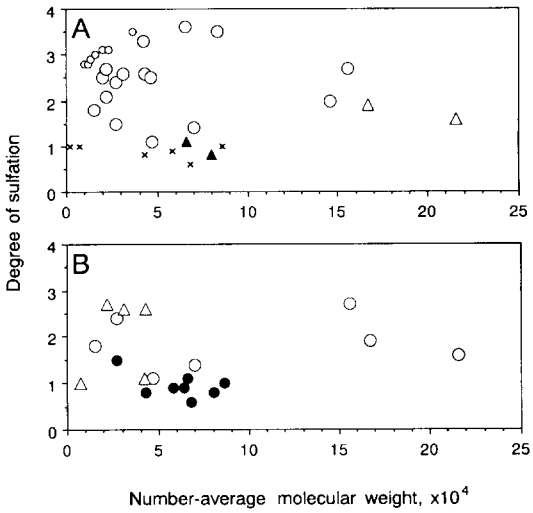

Fig. 6. Relation between anti-AIDS virus activity of curdlan sulfates against (A) HIV-1 and (B) HIV-2 and both the degree of sulfation and molecular mass of the compounds. The inhibitory effect was evaluated by the MTT method and represented by the EC_{50} concentration of < 1.0 μg/mL (○, ∘, ●), 1.0–10 μg/mL (△, ▲), and > 10 μg/mL (×). Symbols: ∘, curdlan sulfates prepared by the CSA; ○, △, by the SPC; ●, ▲, ×, by the PSA methods, respectively.

PSA method, suggesting that the unidentified inorganic sulfate impurities in the curdlan sulfates might be responsible for this cytotoxicity.

Anticoagulant activities of curdlan sulfates.—Table 2 shows the blood anticoagulant activities of curdlan sulfates obtained by sulfation with PSA, SPC, and CSA. The

Table 2
Anticoagulant activity of curdlan sulfates

No.	Sulfating reagent [a]	DS [b]	$\overline{M}_n$ [c] $\times 10^4$	$[\alpha]_D^{25}$ [d] (°)	AA [e] (units/mg)
1	PSA	1.6	2.1	−1.9	10 [f]
2	PSA	1.6	2.0	−1.5	10 [f]
3	SPC	2.4	2.7	−17.0	36
4	SPC	2.6	3.1	−24.0	36
5	SPC	3.3	4.2	−22.9	36
6	CSA	1.8	1.6	−5.0	22
7	CSA	1.9	5.0	−20.6	39
8	CSA	2.1	1.9	−22.9	34
9	CSA	2.4	2.8	−20.0	38

[a] PSA = piperidine-*N*-sulfonic acid; SPC = sulfur trioxide–pyridine complex; CSA = chlorosulfonic acid.
[b] Degree of substitution was calculated by elemental analysis.
[c] Determined by GPC.
[d] Measured in H_2O (*c* 1%).
[e] Anticoagulant activity; commercial dextran sulfate (NC-1036); 20.6 units/mg.
[f] Ref. [12].

anticoagulant activity measurement was performed at 37°C by using bovine plasma according to the US Pharmacopeia and compared with that of the commercial dextran sulfate (NC-1032) as a reference (20.6 units/mg). Curdlan sulfates obtained by the PSA method had lower anticoagulant activity (10 units/mg) than those of curdlan sulfates prepared by the SPC and CSA methods. The higher anticoagulation activities of curdlan sulfates obtained by the SPC or CSA methods might be attributed to their helical structure. Curdlan sulfates prepared by the PSA method show low anticoagulant activity and have rigid and rod-like structures. Consequently the curdlan sulfates with lower anticoagulant activities obtained by the PSA method are preferable to those by the SPC and CSA methods for use as anti-AIDS drugs.

In conclusion, different AIDS virus strains were used in this study to evaluate their inhibition by curdlan sulfates. Curdlan sulfates synthesized by the PSA, SPC, and CSA methods were equally effective inhibitors against HIV-1 and HIV-2, indicating that curdlan sulfates can be used to inhibit the infection of various types of AIDS virus strains. However, curdlan sulfates obtained by the SPC and CSA methods had higher anticoagulant activities and slightly higher cytotoxicities. Curdlan sulfates prepared by the PSA method, having low anticoagulant activity are preferred candidates for an anti-AIDS virus drug.

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