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Specific biological activities of Chinese lacquer polysaccharides

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Abstract

The specific biological activities such as blood coagulant, anti-tumor, anti-HIV, and anticoagulant activities of a Chinese lacquer polysaccharide, a branched acidic polysaccharide, before and after sulfonation were investigated. The lacquer polysaccharide at a concentration of 0.016 mg/ml was found to shorten the coagulation time of bovine plasma more than 1 min by comparison with that of a blank, 5 min and 25 s, suggesting that the lacquer polysaccharide had a blood coagulant-promoting activity. The linear acidic polysaccharides, sodium hyarulonate and alginate, delayed the coagulation time. The lacquer polysaccharide in the dose of 50 mg/kg by an oral administration to rat reduced the half weights of the Sarcoma 180 tumor. After sulfonation, sulfonated lacquer polysaccharides showed no anti-tumor activity. However, it was revealed that sulfonated lacquer polysaccharides had potent anti-HIV activity represented by the 50% protecting concentration (EC_{50}) around 0.5 μg/ml and lower blood anticoagulant activity than that of standard dextran sulfonate. These results suggest that the lacquer polysaccharides have specific biological activities originated from the acidic branched structure and are expected to be a candidate for anti-HIV drugs as naturally occurring and reproductive resources.

Keywords: Chinese lacquer polysaccharide; Sarcoma 180 tumor; Sulfonation

1. Introduction

Lacquer has been used in Japan and China for thousands of years as not only a coating material but also a traditional medicine based on experiences. Although constituents in the sap and the polymerization mechanism of lacquers have been revealed (Kumanotani, 1995), biological activities are still unclear. The structure of lacquer polysaccharides in the sap of lacquer tree was characterized by sugar (Oda, Ishida & Honnda, 1962), methylation (Oshima & Kumanotani, 1984), and NMR analyses (Lu et al., 1999), indicating a 1,3-β-galactopyranosidic main chain having complex branches with 4-*O*-methyl glucuronic acid in the terminal. The monosaccharide components were D-galactose, 4-*O*-methyl-D-glucuronic acid, L-arabinose, and L-rhamnose.

In general, neutral branched 1,3-β glucans provide a strong anti-tumor activity. Lentinan and Schizophyllan, isolated from mushrooms, *Lentinus edodess* and *Schizophyllum commune*, respectively, have 1,3-β-D-glucopyranosidic main chain having 1,6-β-D-glucopyranosidic branches, and have been used as anti-tumor drugs for stomach cancer in Japan since 1985 (Kaneko, Yamamoto & Uryu, 1990a; Kaneko et al., 1990b; Taguchi & Furue, 1985).

We have successively synthesized sulfonated polysaccharides having potent anti-HIV activity (Uryu, 1993), and the relationship between structure and activity of polysaccharides has been investigated (Yoshida et al., 1999). Curdlan sulfonate, which was prepared by sulfonation of a natural linear 1,3-β glucan, curdlan, had potent anti-HIV activity both in vitro and in vivo (Kaneko et al., 1990a,b; Yoshida et al., 1990). In 1994, the phase I/II test was revealed to increase the number of CD4 positive T4 lymphocytes in HIV carriers (Gordon, Guralnik, Kaneko, Mimura, Baker & Lang, 1994). The anti-HIV mechanism of sulfonated polysaccharides proposed that the electrostatic interactions of negatively charged sulfonate groups in the polysaccharides and positively charged amino acid residues in an envelope glycoprotein gp120 of HIV occurs to cause a

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Table 1
Sulfonation of Chinese lacquer polysaccharides

No.	Lacquer polysaccharide			SR[a] (g (mmol))	Temp. (°C)	Time (min)	Sulfonated lacquer polysaccharide						
	mg (mmol to galactose unit)	$\bar{M}_n$ (×10^3)	$[\alpha]_D^{25}$ (°)				Yield (mg)	$\bar{M}_n$ (×10^3)	$[\alpha]_D^{25}$ (°)	Elemental analysis			DS[b]
										C (%)	H (%)	S (%)	
SR: Piperidine N-sulfonic acid[c]													
1	94 (0.58)	86.0	−7.56	0.97 (0.58)	85	60	0.12	10.7	+14.58	22.66	3.61	14.68	1.5
2	212 (1.31)	86.0	−7.56	1.70 (10.3)	85	60	0.27	10.9	+14.93	21.44	3.24	14.25	1.5
3	212 (1.31)	86.0	−7.56	1.70 (10.3)	85	60	0.26	10.6	+13.88	24.31	4.02	13.42	1.3
4[d]	94 (0.58)	71.4	−2.64	0.97 (0.58)	85	60	0.17	42.7	+6.63	30.08	5.19	10.63	1.0
5[d]	84 (0.52)	71.4	−2.64	0.97 (0.58)	85	60	0.16	16.4	+5.95	28.31	4.58	13.38	1.1
6[d]	84 (0.52)	71.4	−2.64	1.43 (8.67)	85	60	0.17	14.9	+5.98	29.69	5.10	15.48	1.6
SR: DMF–SO_3 complex[e]													
7	212 (1.31)	86.0	−7.56	0.85 (5.55)	70	40	0.25	4.4	−6.4	33.85	5.48	3.89	0.3
8	212 (1.31)	86.0	−7.56	1.00 (6.53)	70	40	0.25	5.2	−7.3	28.21	5.16	8.56	0.7
9	212 (1.31)	86.0	−7.56	1.05 (6.86)	70	40	0.27	6.5	−5.2	23.33	4.75	11.58	1.1
10	212 (1.31)	86.0	−7.56	1.10 (7.18)	70	40	0.25	5.0	−5.7	23.08	4.50	13.33	1.3
11	212 (1.31)	86.0	−7.56	1.20 (7.83)	70	40	0.26	3.4	−5.4	24.01	4.47	14.12	1.3
12	212 (1.31)	86.0	−7.56	1.40 (9.14)	70	40	0.25	3.1	−5.1	19.05	3.91	16.96	2.0
13	212 (1.31)	86.0	−7.56	1.50 (9.79)	70	40	0.24	2.9	−3.3	16.07	3.33	16.32	2.3
14	212 (1.31)	86.0	−7.56	0.85 (5.55)	60	60	0.26	3.9	−6.8	23.01	4.19	12.82	1.3
15	212 (1.31)	86.0	−7.56	1.50 (9.79)	60	120	0.23	3.3	−3.4	16.54	2.85	14.77	2.0
16	212 (1.31)	86.0	−7.56	0.85 (5.55)	50	240	0.26	4.5	−7.2	26.34	4.41	10.25	0.9

[a] Sulfonating reagent.
[b] Degree of sulfonation.
[c] Condition: solvent; DMSO 25 ml.
[d] Reduced lacqure polysaccharide.
[e] Condition: solvent; DMF 30 ml.

conformational change in the gp120 (Uryu et al., 1992). By the interaction, sulfonated polysaccharides inhibit the binding of HIV to the CD4 positive T-lymphocytes. NMR studies revealed that curdlan sulfonate interacted strongly with polylysine, a model compound of gp120, to change the chemical shifts of absorptions due to polylysine, suggesting that the anti-HIV activity originated from the ionic interactions (Jeon, Katsuraya, Kaneko, Mimura & Uryu, 1997).

Recently, we synthesized 3-amino-3-*O*-deoxy-(1 → 6)-α-D-allopyranans by the ring-opening polymerization and copolymerization of benzylated 1,6-anhydro-3-azido-3-*O*-deoxy-α-D-allopyranose (Hattori, Yoshida & Uryu, 1997). Sulfonated amino polysaccharides were revealed to exhibit high anti-HIV activity and low cytotoxicity (Hattori et al., 1998). In addition, we found that both anti-HIV and blood anticoagulant activities decreased with decreasing aminoallose unit in the sulfonated polysaccharide backbone, concluding that the amino or sulfonamide substituent played an important role in the biological activities.

Although blood anticoagulant activity is another important biological activity for sulfonated polysaccharides (Hatanaka et al., 1987), it became a side-effect for AIDS drugs. Heparin is a natural mucopolysaccharide having strong anticoagulant activity (Kjellen & Lindahl, 1991). Curdlan sulfonate had low anticoagulant activity below 10 unit/mg by comparison with that of a standard dextran sulfonate (22.7 unit/mg) (Yoshida et al., 1990).

Although the lacquer has been used as a traditional medicine in Japan and China, there are few reports on the biological activities. In this paper, we wish to report for the first time in detail the specific biological activities of a Chinese lacquer polysaccharide such as blood coagulation, antitumor, anti-HIV, and anticoagulant activities.

2. Experimental

2.1. General

100 MHz ^{13}C NMR spectra were recorded on a JEOL JNM α-400 spectrometer in D_2O with sodium 3-(trimethylsilyl)propanesulfonate (DSS) as an internal standard. Specific rotations were measured on a JASCO DIP-140 digital polarimeter in water. Molecular weights were determined at 40°C by means of aqueous phase GPC (column; TOSOH TSK-gel G2500PW$_{XL}$, G3000PW$_{XL}$, and G4000PW$_{XL}$, ϕ7.6 mm × 300 mm × 3 mm; eluent, 66.7 mmol/l of phosphate buffer, pH = 6.86) running on HPLC system with the RI detector using TOSOH pullulan standards having molecular weights of 0.58×10^4, 1.22×10^4, 2.37×10^4, 4.80×10^4, 10.00×10^4, 18.60×10^4, 38.00×10^4, and 85.30×10^4. The elution rate and pressure of the phosphate buffer eluent were 0.8 ml/min and 42 kgf/cm^2, respectively.

2.2. Materials

The Chinese lacquer polysaccharide was isolated from the sap of the Chinese lacquer tree at Maoba in Hubei province of China according to a method reported previously (Oshima & Kumanotani, 1984). The GPC profile of the polysaccharide shows two peaks with number-average molecular weights of 29.0×10^3 and 93.0×10^3, respectively, which were impossible to separate. Piperidine *N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to a method in the literature (Nagasawa & Yoshidome, 1969).

2.3. Reduction of Chinese lacquer polysaccharide

The lacquer polysaccharide was reduced according to a method of Taylor and Conrad (1972). The clear solution of the polysaccharide (84 mg) in deionized water (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) (212 mg, 1.1 mmol). The solution was kept at pH = 4.75 by 0.1 N HCl. After stirring for 2 h at room temperature, 2 M $NaBH_4$ aqueous solution (25 ml) was added dropwise and then the pH of the solution was kept at 7.0 by 4 N HCl solution. The mixture was stirred at room temperature for further 1 h, acidified with 4 N HCl, and then dialyzed against deionized water for 24 h to give 64 mg of a reduced lacquer polysaccharide.

2.4. Sulfonation

2.4.1. By piperidine N-sulfonic acid

The EDC-esterified polysaccharide (94 mg) was dissolved in dry DMSO (20 ml) and then the mixture was stirred at 85°C for 1 h with piperidine *N*-sulfonic acid (99 mg, 0.6 mmol). The reaction mixture was cooled to room temperature by an ice bath, neutralized with 10% NaOH solution, and then dialyzed for 30 h with deionized water. The dialyzate was concentrated to 30 ml and then freeze-dried to give a sulfonated polysaccharide in 120 mg yield.

The reduced lacquer polysaccharide (94 mg) after drying was also sulfonated with piperidine *N*-sulfonic acid (99 mg, 0.6 mmol). The reaction mixture was neutralized with saturated $NaHCO_3$ solution, and then dialyzed to give sulfonated reduced polysaccharides in 168 mg yield after freeze-drying.

2.4.2. By DMF–SO_3 complex in DMF

The lacquer polysaccharide (200 mg) was suspended in dry DMF (30 ml) under nitrogen atmosphere. After stirring at 70°C for 10 min, DMF–SO_3 complex (1.53 g, 10 mmol) was added to the DMF solution. The mixture was stirred at 70°C for 40 min, and then neutralized with 2 N NaOH solution below 5°C. The neutralized solution was poured into ethanol (500 ml) to give a precipitate, which was collected by centrifugation. The precipitate was dissolved in 50 ml of deionized water and then dialyzed for 24 h to give a sulfonated polysaccharide (261 mg) after freeze-drying from water. The results are shown in Table 1.

2.5. Blood coagulation time

The complete coagulation time of blood was measured by using bovine plasma. The polysaccharide sample solutions in saline such as natural lacquer polysaccharide, sodium alginate, hyaluronic acid, and sulfonated lacquer polysaccharide were prepared by diluting from 0.16 mg/ml to 0.016 μg/ml, respectively. In the test tube (15 ml), each sample solution (0.8 ml) and bovine plasma (1 ml) were stirred homogeneously and then 2% $CaCl_2$ solution (0.2 ml) in deionized water was added. The coagulant time was compared at 37°C with a blank solution without polysaccharides (4 min and 25 s). The standard dextran sulfonate (Meito Sangyo Co. Ltd, Japan, H-039) ($\bar{M}_n = 8.5 \times 10^3$, S content; 18.4%) with anticoagulant activity (AA) of 22.7 unit/ml was used as a reference. To the mixture of H-039 (0.16 mg/ml, 0.05 ml) in 0.75 ml of saline and bovine plasma (0.8 ml) was added to 2% $CaCl_2$ solution (0.2 ml). The complete coagulant time was 7 min and 35 s.

2.6. Anti-tumor assay

Anti-tumor activity of the natural lacquer, reduced, and sulfonated polysaccharides was assayed in vivo by using rats. Lentinan, a drug for stomach cancer, was used as a standard. Five normal and healthy rats, 8-week old, were used in each group. Sarcoma 180 ascites cells (5×10^6) were inoculated subcutaneously into the groin of the rat. The test polysaccharides were provided once a day by intraperitoneal injection (i.p.) or by oral administration (p.o.) for 7 days from the day seven after transplantation. After the days 7, 9, 11, 14, 16, 18, 21, 28, and 35 of transplantation, the rats were sacrificed, and then tumors were extirpated and weighted. The results are exhibited in Fig. 3.

2.7. Anti-HIV assay (Pauwels et al., 1988)

Anti-HIV activity of sulfonated polysaccharides was assayed in vitro by MTT method. In the 96 well-microplate, MT-4 cells were added to each well at a proportion of 2.5×10^4 cells/well, and then HIV-infected MT-4 cells at the multiplicity of 0.01 was added. HIV-1-infected and -uninfected MT-4 cells with sulfonated polysaccharides having various concentrations were incubated for 5 days at 37°C in a CO_2 incubator. The viability of both HIV-1-and mock-infected cells were measured spectrophotometrically by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT). The anti-HIV activity was defined by EC_{50}, which means 50% protecting concentration of the test compounds from HIV infection to MT-4 cell. The 50% cytotoxic concentration of the test compounds to MT-4 cell was determined by CC_{50}. Curdlan sulfonate ($\bar{M}_n = 79.0 \times 10^3$, S content; 14.10%, $EC_{50} = 0.13$ μg/ml) was used as a standard.

2.8. Blood anticoagulant activity

Blood anticoagulant activity was measured by a slightly modified method according to US Pharmacopoeia using bovine plasma (USP XXI, 1985). The standard dextran sulfonate (H-039) with an anticoagulant activity of 22.7 unit/mg was used as a reference.

3. Results and discussions

3.1. Reduction of Chinese lacquer polysaccharide

As described later, in order to examine the effects of carboxylic acid in the lacquer polysaccharide on the biological activities such as blood anticoagulant, anti-tumor, and anti-HIV activities, reduction was performed with $NaBH_4$ in aqueous solution after esterification of the glucuronic acid with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) to give a reduced polysaccharide. In IR and ^{13}C NMR spectra, the absorption due to the carboxylic acid disappeared.

3.2. Sulfonation of Chinese lacquer polysaccharide

Sulfonated polysaccharides are known to have blood anticoagulant and anti-HIV activities. In particular, since sulfonated 1,3-β-glucan, curdlan, had a high anti-HIV activity and low blood anticoagulant activity as well as low cytotoxicity (Hatanaka et al., 1989; Yoshida et al., 1988; Yoshida et al., 1990), the biological activities of the lacquer polysaccharide having a branched 1,3-β galactopyranosidic structure were assayed before and after sulfonation.

As shown in Table 1, sulfonation of the lacquer polysaccharide was attempted initially with piperidine *N*-sulfonic acid in DMSO, however, sulfonated lacquer polysaccharides could not be obtained, because of the insolubility of the lacquer polysaccharide in DMSO. Therefore, after esterification and/or subsequent reduction, sulfonation was performed with piperidine *N*-sulfonic acid to give sulfonated polysaccharides with a degree of sulfonation of 1.5 and 1.3 (nos. 1–3) for the esterificated polysaccharides and of 1.0–1.6 (nos. 4–6) for the reduced polysaccharides, respectively. The ester groups were hydrolyzed to recover the carboxylic acids during sulfonation. Number-average molecular weights were relatively high in the range 10×10^3–42×10^3. Specific rotations were around $[\alpha]_D^{25} = +15°$ and +6° for the esterificated and reduced polysaccharides, respectively.

When DMF–SO_3 complex was used as a sulfonating reagent (nos. 7–16) for the lacquer polysaccharide, sulfonated polysaccharides having a degree of sulfonation of 0.3–2.3 were obtained. At 70°C, the degree of sulfonation increased with increasing amount of sulfonating reagent but the molecular weight decreased. For instance, when 1.5 g (9.8 mmol) of DMF–SO_3 complex was used (no. 9), the molecular weight and the degree of sulfonation of the

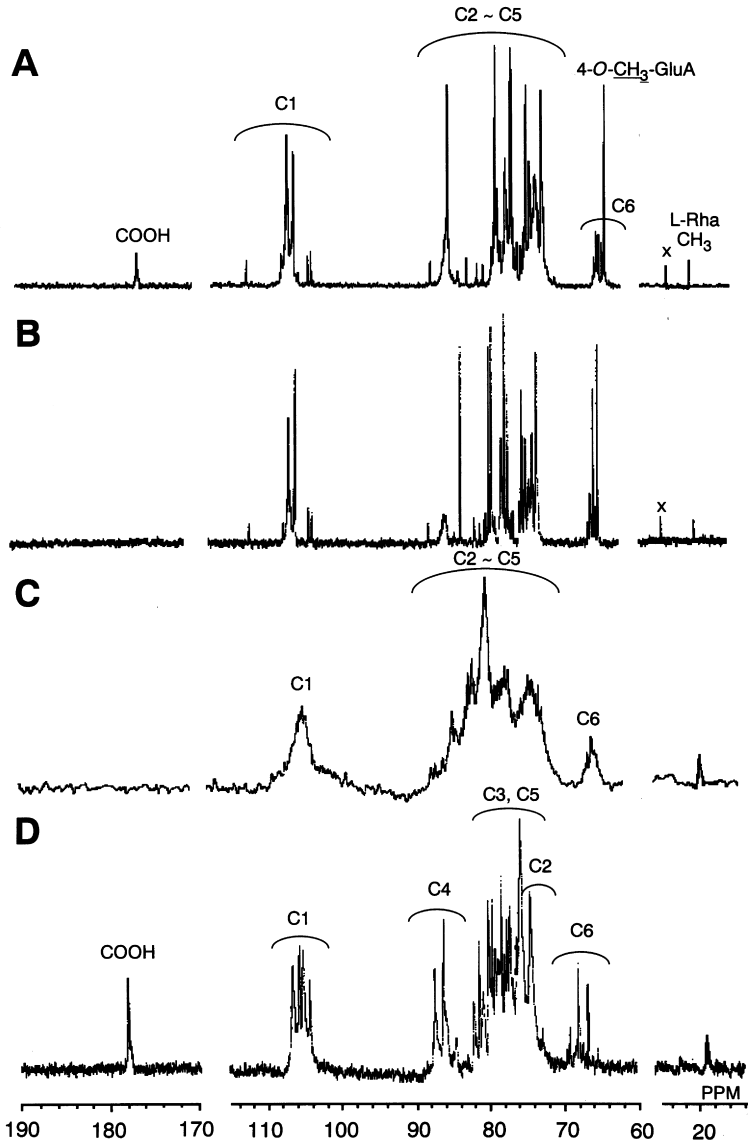

Fig. 1. 100 MHz ^{13}C NMR spectra of: (A) natural lacquer polysaccharide ($\bar{M}_n = 86.0 \times 10^3$, $[\alpha]_D^{25} = -7.6°$ (c1, H_2O)); (B) reduced polysaccharide ($\bar{M}_n = 71.4 \times 10^3$, $[\alpha]_D^{25} = -2.6°$ (c1, H_2O)); (C) sulfonated polysaccharide ($\bar{M}_n = 16.4 \times 10^3$, $[\alpha]_D^{25} = +5.9°$ (c1, H_2O)) (no. 5 in Table 1), with piperidine *N*-sulfonic acid; and (D) sulfonated polysaccharide ($\bar{M}_n = 3.9 \times 10^3$, $[\alpha]_D^{25} = -6.8°$ (c1, H_2O)) (no. 14 in Table 1), with DMF–SO_3 complex (D_2O, 37°C).

resulting polysaccharide were 6.5×10^3 and 1.1, respectively. In no. 13, the molecular weight decreased to 2.9×10^3 and the degree of sulfonation increased to 2.3. In order to prevent the degradation, the reaction temperature decreased. However, long reaction time was necessary to obtain sulfonated polysaccharides having a high degree of sulfonation and the molecular weights decreased (nos. 14 and 15). Furthermore, when the reaction temperature decreased to 50°C, the molecular weight increased to 4.5×10^3 but the degree of sulfonation decreased to 0.9 (no. 16).

As shown in Fig. 1, the structure of the sulfonated polysaccharides obtained was characterized by comparison with the NMR spectrum of the lacquer polysaccharide (spectrum A), the signals of which were assigned recently on the basis of two-dimensional NMR measurements (Lu et al., 1999). After reduction (spectrum B), a signal at 174 ppm due to carboxylic acid on glucuronic acid unit disappeared. In spectrum C, the sulfonated reduced polysaccharide had the degree of sulfonation of 1.3 and the peak broadening occurred, which is a common phenomenon for sulfonated polysaccharides (Yoshida, Nakashima, Yamamoto & Uryu, 1993). Sulfonation with DMF–SO_3 complex provided spectrum D, in which the signals appeared sharply, probably because of low molecular weight ($\bar{M}_n = 3.1 \times 10^3$). The degree of sulfonation was 0.9. The signal due to carboxylic acid appeared at 177 ppm.

3.3. Blood coagulant activity

In general, sulfonated polysaccharides have blood

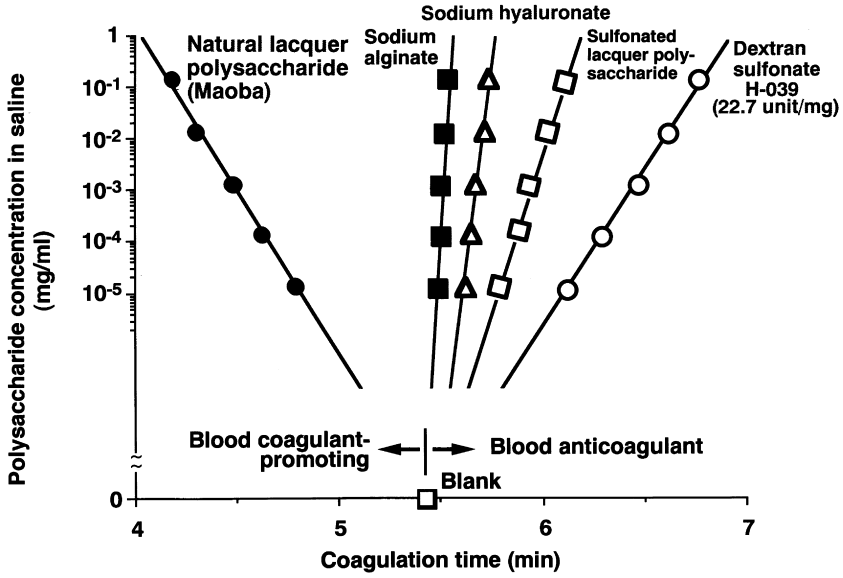

Fig. 2. Coagulation time of bovine plasma at 37°C in the presence of acidic polysaccharides. The concentration of sample solutions were made by 10 times dilutions with saline from 0.016 mg/ml to 0.016 μg/ml. Sample: 0.8 ml, bovine plasma; 1 ml, 2% $CaCl_2$; 0.2 ml. Dextran sulfonate: 0.05 ml, saline; 0.75 ml, 2% $CaCl_2$; 0.2 ml.

anticoagulant activity. Since the lacquer polysaccharide is an acidic polysaccharide, the coagulation time of bovine plasma should be delayed. Thus, we measured the coagulant time according to a slightly modified method described in the US Pharmacopoeia (USP XXI, 1985). However, the lacquer polysaccharide at a concentration more than 0.016 mg/ml was revealed to promote the blood coagulation more than 1 min on the blank (4 min and 25 s) as shown in Fig. 2.

When 0.05 ml of dextran sulfonate (H-039) solution (0.16 mg/ml of saline) was added to the bovine plasma, the coagulation time was delayed to 6 min and 45 s. Linear acidic polysaccharides such as sodium hyaluronate and sodium alginate had weak anticoagulant activity, that is, the coagulation time was delayed slightly. The natural lacquer polysaccharide at a concentration as low as 0.016 μg/ml had still blood coagulant-promoting activity. If a complexion of the carboxylic groups with salt ions occurs, the salt concentration in the plasma must decrease to delay the coagulation time (Sakairi et al., 1998). Furthermore, by the results of viscosity measurements in deionized water and in NaCl solutions, we revealed that the lacquer polysaccharide had a swelled structure originated from the branched structure (Lu, Yoshida & Uryu, 1999). Thus, this branched structure having acidic groups in the terminal might be activated by some coagulant factors in the plasma. Further, exact investigations are in progress.

3.4. Anti-tumor activity

Since branched 1,3-β glucans have potent anti-tumor activity, the lacquer polysaccharides were assayed for the anti-tumor activity by using Sarcoma 180 tumor in rat as shown in Fig. 3. After 35 days of transplantation, the weights of tumor in rat without any polysaccharides increased to 20 g. When lentinan (1 mg/kg) provided to the rat by an intraperitoneal injection (i.p.), the tumor disappeared after 35 days of transplantation. However, lentinan had no anti-tumor effects by an oral administration (p.o.). For the natural lacquer polysaccharide, it was found that the weights of tumor decreased to 10, 11, and 13 g by 50 and 5 mg/kg by p.o., and 1 mg/kg by i.p., respectively. In particular, it was interesting that the oral administration of the lacquer polysaccharide was effective on decreasing weights of tumor, even though lentinan was not inhibiting the growth of the tumor. The anti-tumor activity of reduced and sulfonated lacquer polysaccharides decreased as shown in Fig. 3B and C. These results indicate that the glucuronic acid in the terminal of the branches might play an important role in the anti-tumor activity of the lacquer polysaccharide.

3.5. Anti-HIV activity

Anti-HIV activity was examined by the MTT method using MT-4 cell (Pauwels et al., 1988), which is a sensitive cell to HIV as given in Table 2. The natural lacquer polysaccharide exhibited no anti-HIV activity. It was revealed that the sulfonated lacquer polysaccharides having molecular weights more than 10×10^3 had high anti-HIV activity (nos. 21–26). The sulfonated reduced polysaccharides (nos. 24–26) also had a high activity of 0.5 and 0.6 μg/ml, suggesting that no carboxylic acids in the lacquer polysaccharide were concerned with the anti-HIV activity. In nos. 27–35, sulfonated polysaccharides having both low sulfur

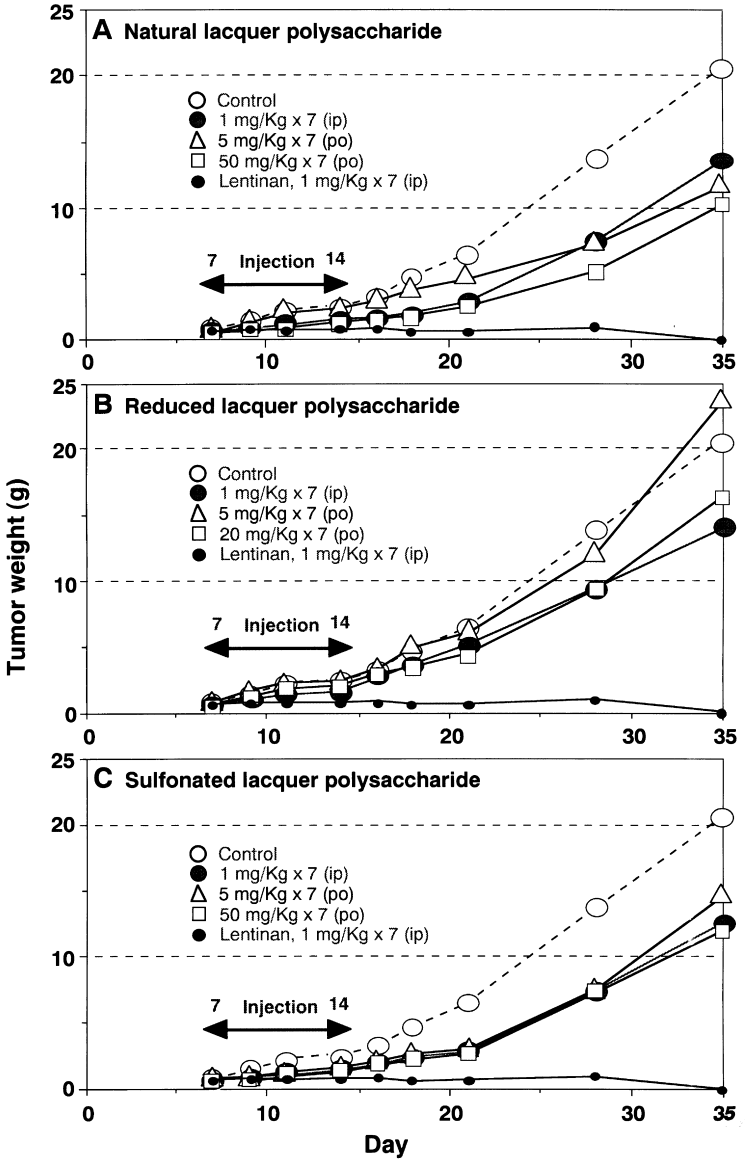

Fig. 3. Anti-tumor activity of lacquer polysaccharides in vivo. The polysaccharides were administrated by intraperitoneal injection (i.p.) or oral administration (p.o.) once in a day for 7 days from day 7 to 14 after transplantation of Sarcoma 180 tumor. Lentinan was used as a standard.

contents and low molecular weights showed low anti-HIV activity.

3.6. Blood anticoagulant activity

Blood anticoagulant activity was determined by comparison with that of a standard dextran sulfonate (22.7 unit/mg) according to a modified method of the US Pharmacopoeia (USP XXI, 1985) as also exhibited in Table 2. Before sulfonation, the lacquer polysaccharide had blood coagulant-promoting activity as described above. After sulfonation, the lacquer polysaccharides showed weak anticoagulant activity, which increased slightly with increasing degree of sulfonation (nos. 21–26). We revealed previously that the branched structure increased the blood anticoagulant activity (Yoshida et al., 1993). However, the sulfonated lacquer polysaccharides provided low anticoagulant activity, probably because of competition with anticoagulant activity originated from sulfonate groups and activation of coagulant factors by the branched structure. In nos. 27–36, for the polysaccharides having low molecular weights, the anticoagulant activity was low.

In this study, we described the specific biological activities of lacquer polysaccharides for the first time. It was revealed that the lacquer polysaccharide had the blood coagulant-promoting activity in vitro and anti-tumor activity in vivo. After sulfonation, the sulfonated lacquer polysaccharides provided weak anticoagulant and potent anti-HIV activities in vitro. Further detailed works on the biological activities are currently underway.

Table 2
Anti-HIV and anticoagulant activities of sulfonated Chinese lacquer polysaccharides

No.	Sulfonation of lacquer polysaccharides			EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	Anticoagulant activity (unit/mg)
	$\bar{M}_n$ ($\times 10^3$)	S content (%)	DS			
Natural	86.0	0		>1000	>1000	–
23	10.6	13.42	1.3	0.8	879	12
22	10.9	14.25	1.5	0.4	>1000	14
21	10.7	14.68	1.5	0.6	767	15
24[c]	42.7	10.63	1.0	0.5	554	9
25[c]	16.4	13.38	1.1	0.6	457	10
26[c]	14.9	15.48	1.6	0.6	566	15
27	4.4	3.89	0.3	>1000	>1000	5
28	5.2	8.56	0.7	37	>1000	6
36	4.5	10.25	0.9	655	>1000	7
29	6.5	11.58	1.1	109	>1000	7
34	3.9	12.82	1.3	143	>1000	7
30	5.0	13.33	1.3	32	492	8
31	3.4	14.12	1.3	155	>1000	11
35	3.3	14.77	2.0	29	>1000	12
33	2.9	16.32	2.0	35	>1000	17
32	3.1	16.69	2.0	48	>1000	20
DS[d]	8.5	18.40	1.8	0.84	>1000	22.7
CS[e]	79.0	14.10	1.6	0.13	>1000	10

[a] 50% Effective concentration.
[b] 50% Cytotoxic concentration.
[c] Reduced lacquer polysaccharide.
[d] Standard dextran sulfonate, H-039.
[e] Standard curdlan sulfonate.

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