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Sulfation and biological activities of konjac glucomannan

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ABSTRACT

The sulfation of konjac glucomannan and its anti-HIV and blood anticoagulant activities were investigated. Konjac glucomannan is a polysaccharide occurring naturally in konjac plant tubers and has high molecular weights. Solubility in water is very low, and the aqueous solutions at low concentrations have high viscosity. Before sulfation, hydrolysis by diluted sulfuric acid was carried out to decrease the molecular weights of $\bar{M}_n = 19.2 \times 10^4 - 0.2 \times 10^4$. Sulfation with piperidine-*N*-sulfonic acid or SO_3-pyridine complex gave sulfated konjac glucomannans with molecular weights of $\bar{M}_n = 1.0 \times 10^4 - 0.4 \times 10^4$ and degrees of sulfation (DS) of 1.3–1.4. It was found that the sulfated konjac glucomannans had potent anti-HIV activity at a 50% effective concentration, (EC_{50}) of 1.2–1.3 μg/ml, which was almost as high as that of an AIDS drug, ddC, whose EC_{50} = 3.2 μg/ml, and moderate blood anticoagulant activity, AA = 0.8–22.7 units/mg, compared to those of standard sulfated polysaccharides, curdlan (10 units/mg) and dextran (22.7 units/mg) sulfates. Structural analysis of sulfated konjac glucomannans with negatively charged sulfated groups was performed by high resolution NMR, and the interaction between poly-L-lysine with positively charged amino groups as a model compound of proteins and peptides was measured by surface plasmon resonance measurement, suggesting that the sulfated konjac glucomannans had a high binding stability on immobilized poly-L-lysine. The binding of sulfated konjac glucomannan was concentration-dependent, and the biological activity of the sulfated konjac glucomannans may be due to electrostatic interaction between the sulfate and amino groups.

1. Introduction

The konjac glucomannan of konjac plant tubers is an abundant and easily available heteropolysaccharide with high molecular weights (Cescutti, Campa, Delben, & Rizzo, 2002; Chua, Baldwin, Hocking, & Chan, 2010; Dave & McCarthy, 1997). Konjac glucomannan has a linear structure composed of 1,4-β-linked D-glucopyranose and D-mannopyranose with a small number of branches and partially acetylated hydroxyl groups in the sugar units. A small amount of konjac glucomannan is soluble in water and gives a highly viscous solution; therefore, konjac glucomannan has been used in foods (Albrecht et al., 2011), food additives (Iglesias-Otero, Borderias, & Tovar, 2010), wrapping films when mixed with cellulose or curdlan (Lu, Zhang, & Xiao, 2004; Wu et al., 2012), cation-exchange resin supports (Zhou et al., 2012), and water-absorbent polymers (Li, Ji, & Li, 2012). Although studies on its biological activities are few, use as a carrier in a drug delivery system was reported among the application studies (Liu et al., 2012; Wang, Fan, Liu, & He, 2010).

Fundamental studies of konjac glucomannan have mainly focused on the structural analysis and gelation mechanisms. Katsuraya et al. (2003) reported in detail the structure of konjac glucomannan by methylation analysis and NMR spectroscopic measurements, indicating that a small proportion of branches existed at the C6 carbon of a glucosyl main chain with 1,6-β glucosyl branches. The ratio of glucose to mannose units in the main chain was about 2–1 and in the branches was about 8% glucose. Luo, Hu, and Lin (2011) described the gelation mechanism of konjac glucomannan in NaOH solution. Sodium hydroxide solution restrained expansion of the molecular chain and promoted gelation, probably due to the obvious effects of deacetylation, self-aggregation, and entanglement. On the other hand, Liu et al. (2012) developed a carrier for a pulsatile drug delivery system based on a highly impermeable capsule of konjac glucomannan. A 5-aminosalicylic acid drug was detected in plasma 5 h after oral administration in the capsule. The pulsatile capsule may have therapeutic potential for a colon-specific drug delivery system.

We have reported the synthesis, structural analysis, and biological activities of naturally occurring and synthetic polysaccharides obtained by a ring-opening polymerization of anhydro sugar monomers (Yoshida, 2001, 2005). Previously, we found that

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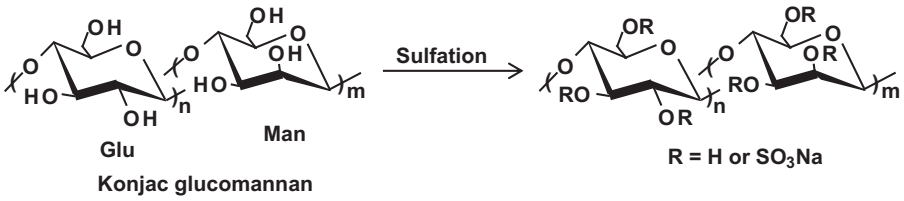

Scheme 1. Sulfation of konjac glucomannan.

sulfated polysaccharides had high anti-HIV and blood anticoagulant activities. In particular, curdlan sulfate, which was prepared by sulfation of curdlan, a naturally occurring polysaccharide, with a linear 1,3-β pyranoside structure, and produced by a bacterial strain, completely inhibited infection of MT-4 cells by HIV at concentrations as low as 3.3 μg/ml and with low cytotoxicity at concentrations as high as 1000 μg/ml (Yoshida et al., 1990). Therefore, an alkyl curdlan sulfate was prepared recently by ionic interaction between a positive didodecyldimethyl ammonium bromide and a negative sulfate group of curdlan sulfate, and then fixed on a membrane filter by a hydrophobic interaction with the long alkyl chain. The alkyl curdlan sulfate-coated membrane filters decreased the concentration of influenza A virus to below 1/4–1/32, suggesting that the membrane filter effectively removed influenza A virus by electrostatic interaction between negatively charged sulfate groups and the positively charged envelope protein of the viruses (Tegshi, Han, Kanamoto, Nakashima, & Yoshida, 2011).

Although there are many reports on the structure and applications of konjac glucomannan, few reports on the biological activities have been published. Sulfated polysaccharides are expected to have specific biological activities that are antiviral and heparin-like (Lane & Lindahl, 1989). In this paper, we report the sulfation of konjac glucomannan and its biological activities such as anti-HIV and blood anticoagulant activities. In addition, we describe the preliminary results on the interaction between the sulfated konjac glucomannan and poly-L-lysine as a model compound of proteins and peptides by using surface plasmon resonance (SPR) to elucidate the biological mechanisms.

2. Experimental

2.1. Measurement and materials

The ^{1}H NMR and ^{13}C NMR spectra were recorded with a JEOL ECM-400 spectrometer at 400 MHz and 100 MHz, respectively, in D_2O or 2.5% NaOH D_2O solution at 50 °C with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as an internal standard or in DMSO-d_6 at 60 °C. Infrared spectra were measured by a Perkin-Elmer Spectrum One FT-IR spectrometer using a KBr pellet. The molecular weight of hydrolyzed konjac glucomannan was determined by an aqueous phase GPC (column; Tosoh TSK-gel G2500PW$_{XL}$, G3000PW$_{XL}$, and G4000PW$_{XL}$, 7.6 mm × 300 mm × 3 mm eluted with 66.7 mM of phosphate buffer, pH = 6.86) with a Tosoh RI detector using pullulan as a standard. Optical rotation was measured by using a JASCO DIP-140 digital polarimeter in aqueous 2.5% NaOH solution at 25 °C in a water-jacketed 10 ml quartz cell. Elemental analysis was carried out with a CE-440 elemental analyzer (System Engineering Inc.). A surface plasmon resonance (SPR) spectrum was taken on a Biacore X100 instrument at 25 °C using a CM5 sensor chip.

Konjac glucomannan was obtained from Chengdu Root Industry (China). Poly-L-lysine with $\bar{M}_w = 1000 - 5000$ and anhydrous dimethyl sulfoxide were purchased from Sigma, Inc. Piperidine-*N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to the method of Nagazawa and Yoshidome (Nagasawa & Yoshidome, 1969).

2.2. Hydrolysis of konjac glucomannan

A typical procedure for hydrolysis of konjac glucomannan is as follows. Konjac glucomannan (0.5 g) was added into 90 ml of deionized water and then stirred vigorously for 3 h at 70 °C. Sulfuric acid (25%, 10 ml) was added dropwise to the viscous konjac glucomannan solution (the final concentration of sulfuric acid in solution was 2.5%), and the mixture was further stirred for 2 h at 70 °C. After cooling to room temperature, the reaction mixture was neutralized by saturated $NaHCO_3$ solution, dialyzed against deionized water for 24 h, and then freeze-dried to give 0.41 g of a low molecular weight konjac glucomannan ($\bar{M}_n = 3.7 \times 10^4$).

2.3. Sulfation of konjac glucomannan and its biological activities

Konjac glucomannan was sulfated by piperidine-*N*-sulfonic acid or SO_3-pyridine complex (Scheme 1). Typical methods are as follows.

For the sulfation by piperidine-*N*-sulfonic acid (no. 2 in Table 2), konjac glucomannan (0.25 g, 1.5 mmol, $\bar{M}_n = 0.8 \times 10^4$) was dissolved in anhydrous DMSO (25 ml) solution at 85 °C and then piperidine-*N*-sulfonic acid (1.0 g, 6.1 mmol) was added. The mixture was stirred for 2 h at 85 °C, then cooled and neutralized by a 5% NaOH solution, and then the alkaline solution was dialyzed against deionized water for 2 d. The dialysate was freeze-dried to give 0.26 g of sulfated konjac glucomannan with the number-average molecular weight of $\bar{M}_n = 0.7 \times 10^4$. Found for C: 19.7%, H: 3.0%, S: 14.7%.

For the sulfation by SO_3-pyridine complex (no. 3 in Table 2), konjac glucomannan (0.25 g, 1.5 mmol, $\bar{M}_n = 0.8 \times 10^4$) was dissolved in anhydrous DMSO (25 ml) with stirring at 60 °C and then SO_3-pyridine complex (1.5 g, 9.4 mmol) was added. The mixture was stirred for 45 min further at 60 °C. After cooling to room temperature, the mixture was neutralized with saturated $NaHCO_3$ solution, dialyzed against deionized water for 2 d, and the dialysate was freeze-dried to give 0.57 g of sulfated glucomannan with the number-average molecular weight of $\bar{M}_n = 0.8 \times 10^4$. Found for C: 17.2%, H: 2.9%, S: 17.3%.

2.4. Biological activities

The anti-HIV activity was assayed in vitro by the MTT method (Pauwels et al., 1988). The activity was evaluated at an EC_{50} value, which is the half maximal inhibitory concentration of sulfated konjac glucomannan to prevent the infection of MT-4 cells by HIV. The cytotoxicity was determined as the 50% cytotoxic concentration (CC50) value, of sulfated konjac glucomannan on MT-4 cells.

The blood anticoagulant activity was measured using bovine plasma according to the modified method of the U.S. Pharmacopeia (U.S. Pharmacopoeia National Formulary, 1985), and the activity was calculated in comparison with that of a standard dextran sulfate (H-39) at 22.7 unit/mg.

Table 1
Hydrolysis of konjac glucomannan by diluted sulfuric acid.[a]

No	Konjac glucomannan				Hydrolyzed konjac glucomannan		
	g	H_2SO_4 (%)	Temp (°C)	Time (h)	Yield (g)	$\bar{M}_n$[b]	$\bar{M}_w/\bar{M}_n$
1	0.25	0.5	50	4	0.17	19.2	1.8
2	1.5	2.5	50	0.5	1.01	5.6	2.1
3	0.5	2.5	70	0.5	0.41	3.7	2.1
4	3.0	5	60	2	2.02	0.8	2.4
5	0.5	5	70	2	0.05	0.2	1.5

[a] Before hydrolysis, glucomannan was stirred for 3 h in water at 70 °C.
[b] The molecular weights of soluble parts were measured by aqueous GPC using phosphate buffer as solvent.

2.5. Interaction of sulfated konjac glucomannan with polylysine by SPR

Interaction between the sulfated konjac glucomannan and poly-L-lysine was measured quantitatively by a Biacore X100 surface plasmon resonance (SPR) instrument at 25 °C. Poly-L-lysine was dissolved in the acetate buffer (10 mM sodium acetate, pH 5.5) to prepare a ligand solution with the concentration of 5000 μg/ml. The poly-L-lysine solution was immobilized on the CM5 sensor chip by an amine coupling reaction method as described by the manufacturer. The final immobilization rate was 1839 RU. The sulfated konjac glucomannans was dissolved in a HBS-EP running buffer (10 mmol HEPES, 0.15 M NaCl, 3.0 mmol EDTA, 0.05% (v/v) Surfactant P20, pH7.4) and the solution was poured over the surface of the sensor chip at a flow rate of 30 μl/min for 120 s, followed by allowing the buffer to flow at the same rate for 600 s. The ligands were regenerated with 50 mM NaOH. The association rate (k_a) and dissociation rate (k_d) constants were calculated using Biacore-supplied software provided by GE Healthcare UK Ltd.

3. Results and discussion

3.1. Decrease of molecular weight of konjac glucomannan

A konjac glucomannan has a naturally occurring polysaccharide with an almost linear structure and a molecular weight of more than 1 million. The constituent sugars are glucose and mannose with a 1, 4-β pyranosidic linkage similar to cellulose, so it does not easily dissolve in water and organic solvents. An aqueous solution of a low concentration produces a viscous liquid. Therefore, konjac glucomannan was hydrolyzed by an aqueous sulfuric acid to decrease the molecular weights and then to increase the solubility in water and diluted NaOH solution. Table 1 shows the results of the hydrolysis of konjac glucomannan by diluted sulfuric acid. When 0.5% aqueous H2SO4 was used at 50 °C for 4 h, the molecular weight decreased to $\bar{M}_n = 19.2 \times 10^4$, and the yield was 0.17 g from 0.5 g of the original material. It was found that the molecular weights decreased to $\bar{M}_n = 5.6 \times 10^4$ to 0.2×10^4 with increasing concentrations of sulfuric acid. With 5% H_2SO_4 at 70 °C, the molecular weight decreased to $\bar{M}_n = 0.2 \times 10^4$; however, the yield was low, 0.05 g from 0.5 g of konjac glucomannan. In the preparation of cellulosic bioethanol, acid hydrolysis of cellulose to glucose is a general method, and acid concentration, temperatures and time must be controlled to avoid sugar decomposition to by-products (Uryu et al., 2006). Under the mild hydrolysis conditions of this work, we found that diluted H_2SO_4 (2.5%) hydrolysis of konjac glucomannan for 0.5 h at 50 °C (no. 2 in Table 2) gave low molecular weight konjac glucomannans without by-products like furfurals.

Fig. 1 shows the aqueous-phase GPC profiles of the low molecular weight konjac glucomannan. After hydrolysis, the konjac glucomannan was dissolved partially in water to give a viscous solution, and the molecular weights of the soluble part were measured by gel permeation chromatography (GPC), indicating that the elution time was delayed with decreasing molecular weight.

Fig. 2B shows the ^{13}C NMR spectrum of the hydrolyzed konjac glucomannan with the molecular weight of $\bar{M}_n = 0.8 \times 10^4$ in 2.5% NaOH D_2O solution at 50 °C. The C1 signals due to glucose and mannose units appeared at 103 and 106 ppm, respectively, and their intensities revealed that the glucose and mannose units of the konjac glucomannan were present in almost the same proportions. The carbon signal due to the acetyl group appeared at 183 ppm.

3.2. Sulfation and biological activities of konjac glucomannan

The original and low molecular weight konjac glucomannans were sulfated with piperidine-*N*-sulfonic acid in DMSO or

Table 2
Sulfation and biological activities of konjac glucomannan.[a]

No	Konjac glucomannan			Sulfated konjac glucomannan									
	$\bar{M}_n \times 10^4$	Temp (°C)	Time (min)	Yield (g)	$\bar{M}_n \times 10^4$[d]	$[\alpha]_D^{25}$ (deg)[e]	Elemental analysis (%)			DS[f]	EC_{50} (μg/ml)[g]	CC_{50} (μg/ml)[h]	AA (unit/mg)[i]
							C	H	S				
1[b]	0.8	100	120	0.21	0.4	−12.2	21.6	3.2	14.2	1.3	1.4	62.6	8.0
2[b]	0.8	85	120	0.26	0.7	−14.1	19.7	3.0	14.7	1.4	1.3	331.8	13.8
3[c]	0.8	60	45	0.57	0.8	−16.3	17.2	2.9	17.3	1.9	1.6	680	n.d.
4[c]	5.6	rt	60	0.51	2.1	−18.4	18.4	3.0	15.7	1.6	0.7	649	n.d.
Dextran sulfate					0.9	+92.1			18.4	2.1	3.2	105.2	22.7
Curdlan sulfate					7.9	−0.3			14.1	1.4	0.1	518.2	10
AZT (μmol)											0.05	210.4	
ddC (μmol)											1.2	2216.5	

[a] Konjac glucomannan (0.25 g) was used. Sulfation was carried out with piperidine-*N*-sulfonic acid (b) or sulfur trioxide pyridine complex (c).
[d] Determined by GPC using phosphate buffer as solvent.
[e] Measured in H_2O (c 1%).
[f] Degree of sulfation (maximum, 3.0).
[g] 50% effective concentration of sulfated glucomannan on HIV.
[h] 50% cytotoxic concentration on MT-4 cell.
[i] Anticoagulant activity compared to standard dextran sulfate H-039 with 22.7 unit/mg.

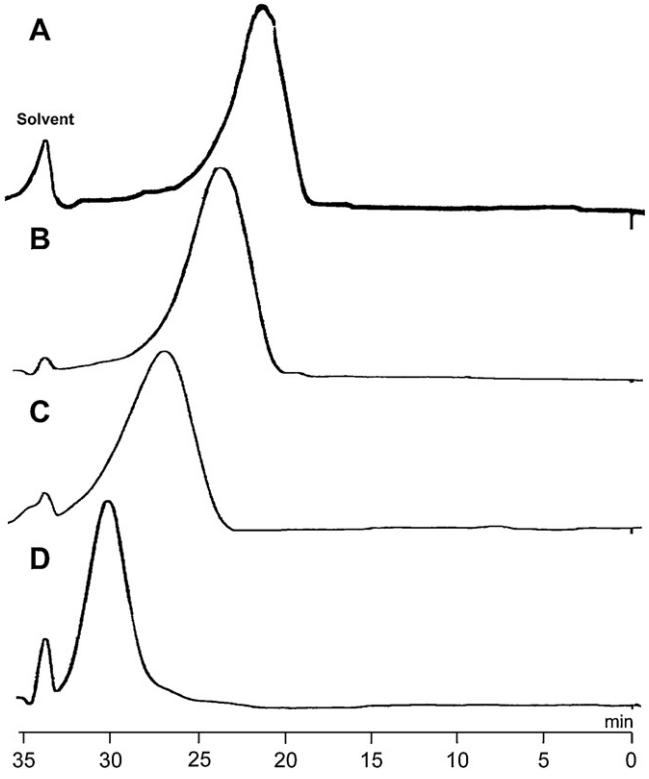

Fig. 1. Aqueous GPC profiles of hydrolyzed konjac glucomannans by diluted sulfuric acid. (A) $\bar{M}_n = 19.2 \times 10^4$ ($\bar{M}_w/\bar{M}_n = 1.82$), (B) $\bar{M}_n = 5.6 \times 10^4$ ($\bar{M}_w/\bar{M}_n = 2.07$), (C) $\bar{M}_n = 0.8 \times 10^4$ ($\bar{M}_w/\bar{M}_n = 2.39$), (D) $\bar{M}_n = 0.2 \times 10^4$ ($\bar{M}_w/\bar{M}_n = 1.53$).

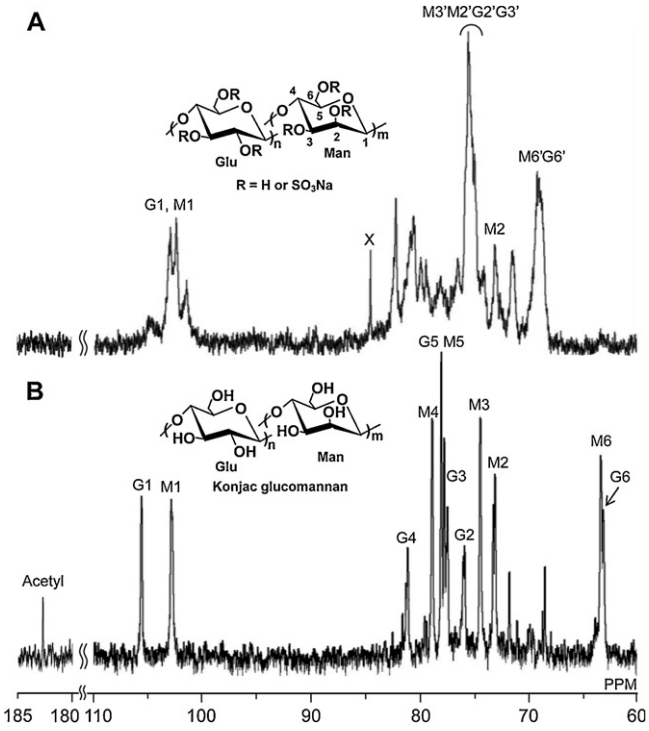

Fig. 2. 100 MHz ^{13}C NMR spectra of (A) sulfated konjac glucomannan with $\bar{M}_n = 0.8 \times 10^4$ and DS = 1.4 in D_2O at 50 °C and (B) konjac glucomannan with $\bar{M}_n = 0.8 \times 10^4$ in 2.5% NaOH D_2O solution at 50 °C. The signals were assigned by 2D NMR measurements.

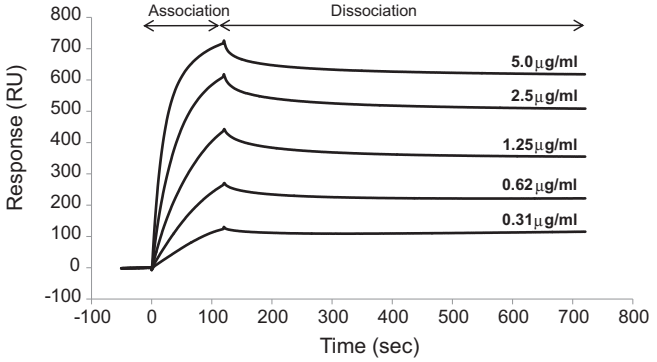

Fig. 3. SPR binding affinity of sulfated konjac glucomannan ($\bar{M}_n = 1.0 \times 10^4$, DS = 1.3) to poly-L-lysine. Sulfated konjac glucomannan (60 μl) was injected for 120 s at a flow rate of 30 μl/min of a HBS-EP running buffer at 25 °C and then the running buffer was further flowed for 600 s. Concentrations of sulfated konjac glucomannan were 5.0, 2.5, 1.25, 0.62, and 0.31 μg/ml, respectively.

SO_3-pyridine complex in pyridine at high temperatures to give sulfated konjac glucomannans with a degree of sulfation (DS) of 1.3–1.4 (maximum, 3) as shown in Table 2. The sulfated konjac glucomannans were easily soluble in water, and the molecular weights were $\bar{M}_n = 0.2 \times 10^4 - 1.0 \times 10^4$. Fig. 2A shows the ^{13}C NMR spectrum of a sulfated konjac glucomannan with the molecular weight of $\bar{M}_n = 0.8 \times 10^4$ and DS of 1.4 in D_2O solution at 50 °C. After sulfation, the C6 signals shifted the lower magnetic field from 64 ppm to 70 ppm and broadened it, suggesting that the sulfate group was introduced into the C6 hydroxyl groups. The C2 and C3 signals were also shifted and broadened.

Table 2 also shows the results of tests of anti-HIV and blood anticoagulant activity (AA) of the sulfated konjac glucomannan compared to those of standard dextran and curdlan sulfates and AIDS drugs used clinically. The anti-HIV activity was measured by the MTT method and evaluated at a 50% effective concentration (EC_{50}) and a 50% cytotoxic concentration (CC50) using MT-4 cells (Pauwels et al., 1988). It was found that sulfated konjac glucomannan had anti-HIV activity at the EC_{50} of 1.3–1.4 μg/ml, which was as high as that of standard dextran sulfate and the clinically used HIV drug ddC. The 50% cytotoxic concentration (CC50 = 62–331 μg/ml) was almost the same as that of standards. The blood anticoagulant activity (AA) was measured by using bovine plasma according to the modified method of the U.S. Pharmacopeia (U.S. Pharmacopoeia National Formulary, 1985) and comparison of the activity to that of the standard polysaccharides in Table 2 indicated that the anticoagulant activity was low to medium, 8.0–22.7 unit/mg. These biological results suggest that sulfated konjac glucomannan is a candidate for an antiviral polysaccharide because of its high anti-HIV and low to moderate blood anticoagulant activities.

3.3. Interaction of sulfated konjac glucomannan with polylysine

The interaction of sulfated konjac glucomannan with poly-L-lysine was preliminarily elucidated by surface plasmon resonance (SPR) in water at 25 °C. Poly-L-lysine was used as a model compound of basic proteins and peptides on the surface of HIV and cells. Fig. 3 shows the typical binding curves of the sulfated konjac glucomannan ($\bar{M}_n = 1.0 \times 10^4$ and DS = 1.3) at the concentration range of 0.31–5.0 μg/ml to immobilized poly-L-lysine. The sulfated konjac glucomannan was found to be bound strongly and concentration-dependently to poly-L-lysine in the association phase. The dissociation rate was slow, suggesting that the binding between the sulfated konjac glucomannan and poly-L-lysine was very strong. Table 3 shows the apparent kinetic properties of the sulfated konjac glucomannans with poly-L-lysine calculated

Table 3
Apparent association and dissociation rates of sulfated konjac glucomannan.[a]

No	$\bar{M}_n \times 10^4$[b]	$[\alpha]_D^{25}$ (deg)[c]	S (%)	DS[d]	$k_{a1} \times 10^4$ (1/Ms)	$k_{d1} \times 10^{-2}$ (1/s)	$k_{a2} \times 10^{-2}$ (1/s)	$k_{d2} \times 10^{-4}$ (1/s)	$K_D \times 10^{-9}$ (M)
1	2.0	−20.5	16.0	1.6	104.0	3.6	2.4	2.9	0.4
2	2.1	−18.1	15.7	1.6	61.1	2.0	2.1	3.2	0.5
3	0.8	−16.3	17.3	1.9	16.2	1.5	1.9	3.6	1.7
4	1.0	−13.8	14.0	1.3	8.6	0.7	1.3	4.0	2.6
5	2.8	−12.3	12.1	1.0	6.6	0.7	1.1	8.1	7.8
6	0.9	−15.4	9.5	0.7	4.9	1.2	1.3	6.7	11.9
7	0.7	−14.1	14.7	1.4	3.8	1.0	1.2	6.6	14.5
8	0.5	−9.0	13.9	1.3	3.7	1.0	1.3	5.3	10.3
9	0.4	−12.2	14.2	1.3	1.7	1.0	1.1	7.3	34.7

[a] The apparent kinetic rates were calculated from the two-state model supplied by a Biacore software; k_a: association rate constant, k_d: dissociation rate constant, K_D: dissociation constant calculated by k_d/k_a.
[b] Determined by GPC using phosphate buffer as solvent.
[c] Measured in H_2O (c 1%).
[d] Degree of sulfation (maximum, 3).

from the two-state fitting model by Biacore-supplied software. The apparent kinetic results calculated from the two-state model that contains a conformational change of the complex provided good fittings compared to those of the 1:1 binding model. We assumed previously that the active antiviral mechanism of sulfated polysaccharides was that sulfated polysaccharides with negatively charged sulfate groups interacted strongly with the positively charged glycoprotein gp120 on the surface of HIV (Jeon et al., 2000; Kaneko et al., 1990) and then the conformation of the complex was changed to prevent the infection of T cells by HIV (Jeon et al., 2000). The binding of sulfated polysaccharides to HIV was expected to be a conformational change of the glycoprotein. Therefore, the two-state fitting model was preferred to the 1:1 binding model. The sulfated konjac glucomannan with a high molecular weight ($\bar{M}_n = 2.0 \times 10^4$) and high degree of sulfation (DS = 1.6) had the high association rate constant (k_{a1}) of 104.0×10^4 1/Ms and low dissociation constant (K_D) of 0.4×10^{-9} M (no. 1). The association rate constant decreased with both decreasing molecular weights and degrees of sulfation. The results shown in Table 3 indicate that the sulfated konjac glucomannans had fast association and slow dissociation rates on poly-L-lysine, suggesting the high stability of the interaction.

In conclusion, a naturally occurring konjac glucomannan was sulfated to give sulfated konjac glucomannan, and its anti-HIV and blood anticoagulant activities were investigated for the first time. We found that the sulfated konjac glucomannans had high anti-HIV activity below EC_{50} = 1.4 μg/ml, as high as that of the clinically used AIDS drug, ddC (1.2 μg/ml) and standard dextran sulfate (3.2 μg/ml). The interaction between the sulfated konjac glucomannan with poly-L-lysine as a model compound of proteins and peptides was investigated preliminarily by SPR measurement, indicating that the sulfated konjac glucomannan had a fast association rate and slow dissociation rate on the immobilized poly-L-lysine, suggesting a high stability for the interaction. Details on the interactions continue to be investigated.

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