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Modification, characterization and structure–anticoagulant activity relationships of persimmon polysaccharides

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ABSTRACT

Six chemical sulfated polysaccharide derivatives (PFP-S) with variable degree of substitution (DS) and molecular weights (Mw) were prepared from the fresh persimmon fruits by chlorosulfonic acid-pyridine and ultrasonic degradation methods, and the effectiveness of the reaction was monitored by the DS, FT-IR and ζ-potential analysis. The sulfation modification might cause advanced structure change to the samples, the SEM of PFP-S exhibited a polyphasic convoluted structure. Results of anticoagulant activities showed that PFP-S could prolong activated partial thromboplastin time (APTT) and thrombin time (TT), but not prothrombin time (PT), and the anticoagulant activity improved with the increasing DS and decreasing Mw.

1. Introduction

Heparin, a highly sulfated polysaccharide present in mammalian tissues, is one of the agents more largely used in the therapy and prophylaxis of thrombosis. However, alternatives to heparin have became an important field of research because of its side-effects and other disadvantages, such as bleeding, chemical inhomogeneity, variability of its physiological activities, contaminated with highly infectious bovine spongiform encephalopathy. Polysaccharides and their derivatives, e.g., sulfated polysaccharide, have attracted considerable attention as possible heparin substitutes (Athukorala, Jung, Vasanthan, & Jeon, 2006). The sulfation of polysaccharides could not only enhance the water solubility but also increase electrostatic binding to receptor sites on cells and/or ligands, resulting in the alteration of their anticoagulant activities (Lu, Wang, Hu, Huang, & Wang, 2008; Pushpamali et al., 2008).

The structure–activity relationships showed that the anticoagulant of sulfated polysaccharides was dependent upon the chemical structure of the carbohydrate biopolymer, such as the monosaccharide composition, molecular weight (Mw), degree and pattern of substituting groups, configuration and conformation of the uronic acid, and degree and size of the branch points, and the conformation of the polysaccharides itself (Alban & Franz, 1994; Alban, Schauerte, & Franz, 2002). SO_4^{2-} content of sulfated polysaccharide is one of the important effective factors for its anticoagulant action. It was previously reported that the anticoagulant effects of β-glucan was critically dependent on the presence of sulfate groups (Bae, Chang, Kim, & Lee, 2008; Chang, Lee, Yoo, & Lee, 2006). In some cases increasing the degree of sulfation was beneficial to the activity, e.g., the anticoagulant activity with respect to thrombin time (TT) of pullulan sulfate dramatically increased up to a level similar to heparin when the degrees of substitution (DS) of the sulfate groups ranged from 0.47 to 1.80 per glucose unit (Alban, 1992).

Polysaccharides from persimmon fruits (PFP) was a group of heteropolysaccharides with a mean molecular weight of 1.3×10^5 Da, mainly composed of arabinose, mannose, rhamnose, galactose, and glucose (Asgar, Yamauchi, & Kato, 2003, 2004). We have now prepared a kind of sulfated PFP (PFP-S) with variable DS (0.8, 1.7 and 2.5, respectively) by chlorosulfonic acid-pyridine method, however, little is known about the structure–activity relationships of anticoagulation of PFP-S.

The Mw of polysaccharide is also one of the most important structural factors. Because of their high molecular weights, high apparent viscosity and poor water solubility, polysaccharides are difficult to pass through organizational barriers and enter the interior of the cell, which limits their biological activity. Therefore, degradation of polysaccharides into low-molecular-weight oligosaccharides is expected to improve their biological activity. In the present study, three sulfated polysaccharide fragments with low molecular weight were prepared from the sulfated polysaccharides by ultrasonic degradation. The chemical characteristics and anticoagulant activities of the six sulfated polysaccharides were investigated.

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2. Experimental

2.1. Materials

Fresh persimmon fruits were collected from Lintong, a famous persimmon producing area in Shaanxi Province, China, during October to December. DEAE-cellulose and dialysis membranes (10 kDa) were purchased from Sigma Co. (St. Louis, MO, USA). Sephadex G-200, dextrans of different molecular weights were from Pharmacia Biotech Ltd. (Uppsala, Sweden); heparin (140 U/mg) was from Shuangxuan microorganism culture media factory (Beijing, China). APTT, PT and TT assay reagents and calcium chloride (0.025 mol/l) were from Shanghai Sunbiotech Co. (Shanghai, China). All other reagents used were of analytical grade and made in China.

2.2. Isolation and sulfation of polysaccharides

A total of 500 g of fresh persimmon was used for extraction with petroleum ether in a Soxhlet apparatus. The residue was dried and suspended with hot water followed by incubation for 6 h at 90 °C. Centrifugation was performed at 4000 rpm for 10 min, and the supernatant was concentrated and mixed with 85% ethanol for precipitation followed by incubation at 4 °C overnight. The sediments were filtered, washed and lyophilized, and then dissolved in water. The solution was filtered and the protein in the filtrate was removed with Sevag method. Then, the filtrate was dialyzed against distilled water (exclusion limit: 10 kDa) and the cold ethanol of threefold volume was added to the afforded dialysate, followed by precipitation and lyophilization after standing at 4 °C overnight. The polysaccharides (700 mg) were subjected to the DEAE-cellulose column (2.6 cm × 50 cm) which was eluted with distilled water and a gradient of 0.2 M NaCl at a flow rate of 1.0 ml/min. Then the solution was dialyzed, concentrated and further fractionated over a Sephadex G-200 column (2.0 cm × 50 cm) eluted with 0.05 M NaCl at a flow rate of 0.3 ml/min to yield a kind of purified polysaccharide (PFP).

Chemical sulfation of PFP was carried out using the chlorosulfonic acid (CSA) method. To obtain sulfated derivatives with variable DS, three kinds of sulfating reagents (the ratio of CSA to formamide was 1:8, 1:4 and 1:2) were prepared using dry formamide and CSA according to the reported method (Lu et al., 2008). In brief, the PFP samples (200 mg) were suspended in anhydrous formamide (20 ml) at room temperature followed by stirring for 30 min, and the sulfating reagents were added drop by drop. The mixture was maintained at 50 °C for 2 h with continuous stirring. After the reaction was finished, the mixture was cooled, neutralized with 2.5 M NaOH, and incubated with ethanol. The resulting precipitates were dissolved in water and dialyzed against distilled water for 72 h. The retained nondialysate was treated with ethanol. The precipitates were then dissolved in water, loaded to Sephadex G-200 column (2.0 cm × 50 cm), and eluted with water and 0.5 M NaCl solution. Based on the colorimetric test for total carbohydrate by phenol–sulfuric acid method, the main fraction was collected, dialyzed and lyophilized to give sulfated PFP (PFP-S). According to the method described above, three kinds of sulfated derivatives of PFP were obtained, and named as PFP-SI, PFP-SII and PFP-SIII.

2.3. Depolymerization of the sulfated polysaccharides by ultrasonication

An ultrasonic processor (Model JY96-ultrasonic generator, Xinzhi Bio-technology Institute, Shanghai, China,) with a 1/2-in. probe was used to sonicate PFP-S solutions. The sulfated derivative was treated by varying the ultrasonic time to obtain the different Mw samples. Polysaccharide solution readily prepared in deionised water in a screw-capped tube was placed in a thermostatic water bath (45 ± 0.5 °C) and then irradiated by ultrasonic (35 kHz, 300 W/cm^2). To eliminate the experimental errors caused by uneven power transfer and temperature fluctuation during ultrasonication, the sample was located just above the ultrasonic source and the temperature was equilibrated by coupling with an additional water circulator.

2.4. Characterizations

Total sugar content was estimated by the phenol–sulfuric acid assay using D-glucose as standard (Michel Dubois et al., 1956). The degree of substitution with sulfate was established on the basis of the sulfate content, determined by barium chloride–gelatin method (Kawai, Seno, & Anno, 1969). The molecular weight of these derivatives was determined by Gel-Filtration Chromatography (GFC) using the method of Yamamoto, Numome, Yamauchi, Kato, and Sone (1995) on Sephadex G-200 gel column (2.0 cm × 50 cm). The column was eluted with 0.1 M of NaCl at a flow rate of 0.3 ml/min, and the elution was monitored by the phenol–sulfuric acid method. The column was calibrated with standard dextran (molecular weight: 10, 40, 70, 133, 482, and 2000 kDa) and a standard curve was established before sample analysis. Fourier-transform infrared spectra (FT-IR) were recorded on a Nicolet Impact410 spectrometer using the KBr-disk method. Zeta (ζ)-potential of PFP and PFP-S was performed by a modified procedure using a Particle Size Analyzer (MALVEN Zetasizer Nano-ZS90). A 0.4% (w/w) PFP-S solution was adjusted to the pH 7.0 with HCl and NaOH. All experiments were carried out at 25 °C with the laser beam operation at 659.0 nm and 1.330 as the refractive index. The measurements were carried out in triplicate with three runs of 2 min each and 5 s between each run. The surface morphology of PFP and PFP-S was observed using a Quanta 200 scanning electron microscope (SEM) operated at 20 kV.

2.5. Clotting assays

Normal human plasma was collected from healthy donors without history of bleeding or thrombosis. Nine parts of human blood collected by venipuncture were drawn into one part of 3.8% sodium citrate aqueous solution. The plasma was centrifuged at 2400 × *g* for 20 min, and the citrated plasma was collected and stored at −80 °C until used. For APTT assay, 72 μl of the citrated human plasma was mixed with 8 μl of the sample solutions at various concentrations and incubated at 37 °C for 1 min. Then, 20 μl APTT reagent was added to the mixture and incubated at 37 °C for 5 min. Pre-warmed (37 °C) 10 μl of 0.025 mol/l $CaCl_2$ aqueous solution was added and the time of clot formation was measured on an automated coagulometer. For TT assay, undiluted plasma (45 μl) was first mixed with sample (5 μl) and incubated at 37 °C for 1 min before incubation with TT reagent (50 μl) for 2 min; meanwhile the time of clot formation was recorded. For PT assay, citrated normal human plasma (45 μl) was mixed with sample (5 μl) and incubated at 37 °C for 2 min before incubation with PT reagent (100 μl) for 10 min. All coagulation assays were performed with six individual replicates, and the mean values were taken. The anticoagulant activity was expressed as the clotting time. All samples including heparin were dissolved in 0.9% NaCl aqueous solution.

2.6. Statistical analysis

Data were presented as mean ± S.D. and statistical analysis was performed with the SPSS 11.0 software (SPSS Inc., Chicago, USA). One-way ANOVA followed by a post hoc Dunnett's test was applied for the comparisons and a value of $P < 0.05$ was considered as statistically significant.

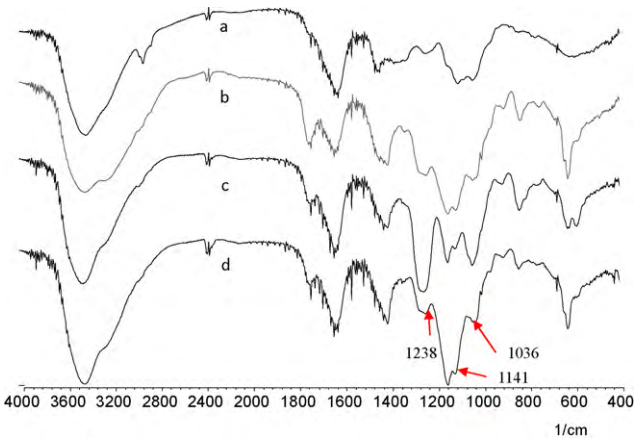

Fig. 1. IR spectra of PFP and its sulfated derivatives: (a) PFP; (b) PPF-SI; (c) PFP-SII and (d) PFP-SIII.

3. Results and discussion

3.1. Characterizations

The sulfated polysaccharides (PFP-S) were obtained by the chlorosulfonic acid/pyridine method and the effectiveness of the reaction was monitored by the DS FT-IR and ζ-potential analysis (Table 1 and Fig. 1). The sulfonation of PFP was conducted in formamide and relatively mild conditions by controlling the time and temperature of the reaction to minimize possible degradation of the polymeric chain. Three sulfated derivatives (PFP-SI, PFP-SII and PFP-SIII) with different DS were obtained by varying the ratio of CSA to formamide in the sulfating reagent. The DS showed an increase with the ratio of CSA to formamide under constant reaction conditions. They all showed a symmetrical peak on Sephadex G-200, and the average molecular weights of PFP-SI, PFP-SII and PFP-SIII were determined to be 53, 51 and 48 kDa, respectively, in reference to standard T-Dextran.

FT-IR spectra of the sulfated polysaccharides were shown in Fig. 1. The strong absorb band at 1036 cm^{-1} was probably due to the symmetrical S=O stretching vibration, and the strong absorb band at 1141 cm^{-1} could originate from the asymmetrical C–O–S stretching vibration. The two bands overlapped with the absorb bands of C–C and C–O stretching vibrations. The spectra showed two characteristic absorption bands, one at 1238 cm^{-1} describing an asymmetrical S=O stretching vibration and the other at 831 cm^{-1} with a discernible shoulder at 857 cm^{-1} indicating a symmetrical C–O–S vibration associated to a C–O–SO_3 group. Compared with the FT-IR spectrum of PFP, the absorbance of these bands increased, which indicated that the DS increased. These results indicated that the sulfation reaction had actually occurred.

The surface-charged density of colloids is often estimated by the ζ-potential, which can be derived from the electrophoretic mobility of the particles. The ζ-potential values of PFP-S in saline were listed in Table 1. As anticipated, all surface-sulfated PFP exhibited negative ζ-potential. The magnitude of the ζ-potential, which should correspond to the charge density, conformed to the extent of surface modification as expressed in terms of DS. The negative ζ-potential value decreased with an increase in DS for all fractions. It was confirmed further that the sulfatation of the samples have occurred. This outcome was in accord with the results reported on crosslinked N-sulfofurfuryl chitosan membranes (Liu, Zhang, Cheng, Cao, & Yao, 2004).

Ultrasonic irradiation has been used to depolymerize various biopolymers, including dextran, DNA, and starch, without modification of their chemical structure. The current opinion is that low-frequency cavitation generates hydrodynamic shear forces that are able to split macromolecules mid-chain in a non-random way (Price & Mason, 1990). Compared with chemical degradation, there is no excess of any substances introduced into the system, which significantly simplifies the separation and purification process. Three reduced molecular weight PFP-S were produced by ultrasonic degradation from PFP-SII in 10 min, 20 min and 40 min, respectively. As shown in Table 1, ultrasonic treatments increased the negative ζ-potential because the galacturonic acids were digested and lost from the main backbone. Ultrasonic depolymerization did not lead to significantly desulfation in our experimental conditions, as previously noted by Tatsuhara et al. for chondroitin sulfate (Tohoru & Sadao, 1973). These degradation products had decreased viscosity and increased water solubility. This showed how the ultrasonic technique could successfully degrade macromolecules in solution.

The SEM of the native and sulfated PFP was shown in Fig. 2(a)–(d). The native PFP exhibited a sheet appearance on which a large number of protruding microfibrils were evident. PFP-SI has particles similar to rod-shape, whereas the PFP-SII and PFP-SIII looked like Judas-ear. When the same was treated as below, the preparation might cause damage to the samples as some rigid fragments appear in the micrographs. The SEM of sulfated PFP exhibited a polyphasic convoluted structure. The solvent, water or diluted sodium hydroxide solution, did not affect the shape or surface smoothness of the polysaccharides.

3.2. Anticoagulant activities of PFP-S

Anticoagulant activity of PFP-S was evaluated by the classical coagulation assays of APTT, TT and PT, using heparin as a reference standard. The results were listed in Tables 2–4. All the fractions significantly prolonged APTT regards to control (saline solution) in a dose-dependent manner. The clotting times of PFP-SI, PFP-SII and PFP-SII at 20 μg/ml reached 113 s, 149 s and 286 s, respectively, while the APTT of PFP-SMH, PFP-SMM and PFP-ML was 256 s, >300 s and >300 s, respectively. Moreover, the TT of PFP-SI, PFP-SII, PFP-SII, PFP-SMH, PFP-SMM and PFP-ML (at 20 μg/ml) reached 39 s, 46 s, 43 s, 45 s, 40 s and 43 s, respectively. The anticoagulant activity of the sulfated polysaccharide was weaker than that of heparin, and higher concentrations were required to achieve the same effect as with heparin in the APTT and TT assays. However, the effect of the sulfated polysaccharide on PT was markedly different from that of heparin (Table 4). No clotting inhibition was observed in PT assay even at the concentration at which APTT and TT were prolonged. The APTT and TT of PFP-S and its fractions were statistically significantly different from the control value ($P < 0.01$), but the PT of PFP-S was not. The results suggested that PFP-S was a rather poor inhibitor of the extrinsic coagulation pathway.

The molecular weight, chain length, charge density and the three-dimensional structure of the sulfated polysaccharide influence its interactions with the coagulation proteins (Olson, Bjork, & Bock, 2002). Literature reports indicated that the anticoagulant activity always increased with the DS and DS of above 0.8 was necessary (Yang, Du, Huang, Wan, & Wen, 2005). In this study, PFP-SIII had a higher APTT and TT activity than PFP-SI and PFP-SII. PFP-SI, PFP-SII and PFP-SIII had almost identical Mw, their DS were 0.8, 1.7 and 2.5, respectively. The anticoagulant activity improved with increasing DS, indicating that sulfate esters played a major role in the anticoagulation activity.

It is generally accepted that the anticoagulant activity of the sulfated polysaccharides partly results from the strong interaction between the negatively charged sulfate groups and some positively charged peptidic sequences (Huynh, Chaubet, & Jozefonvicz, 2001). The negative ζ-potential value, which should correspond to the charge density, decreased with an increase in DS for all fractions.

Table 1
Sulfation of PFP and molecular characterization of sulfated polysaccharides.

Fractions	V_{CSA}/V_{Pyr}[a]	Time (h)	Mw (kDa)	DS[b]	ζ-Potential
PFP-SI	1:4	–	58	0.86	−19.8
PFP-SII	1:8	–	55	1.73	−23.2
PFP-SIII	1:12	–	48	2.52	−27.1
PFP-SMH	1:8	10 min	35	1.73	−29.4
PFP-SMM	1:8	20 min	17	1.74	−27.6
PFP-SML	1:8	40 min	9.2	1.76	−31.6

[a] *n*(SR)/*n*(SU): molar rate of sulfating reagent/sugar unit.
[b] DS, degree of sulfation.

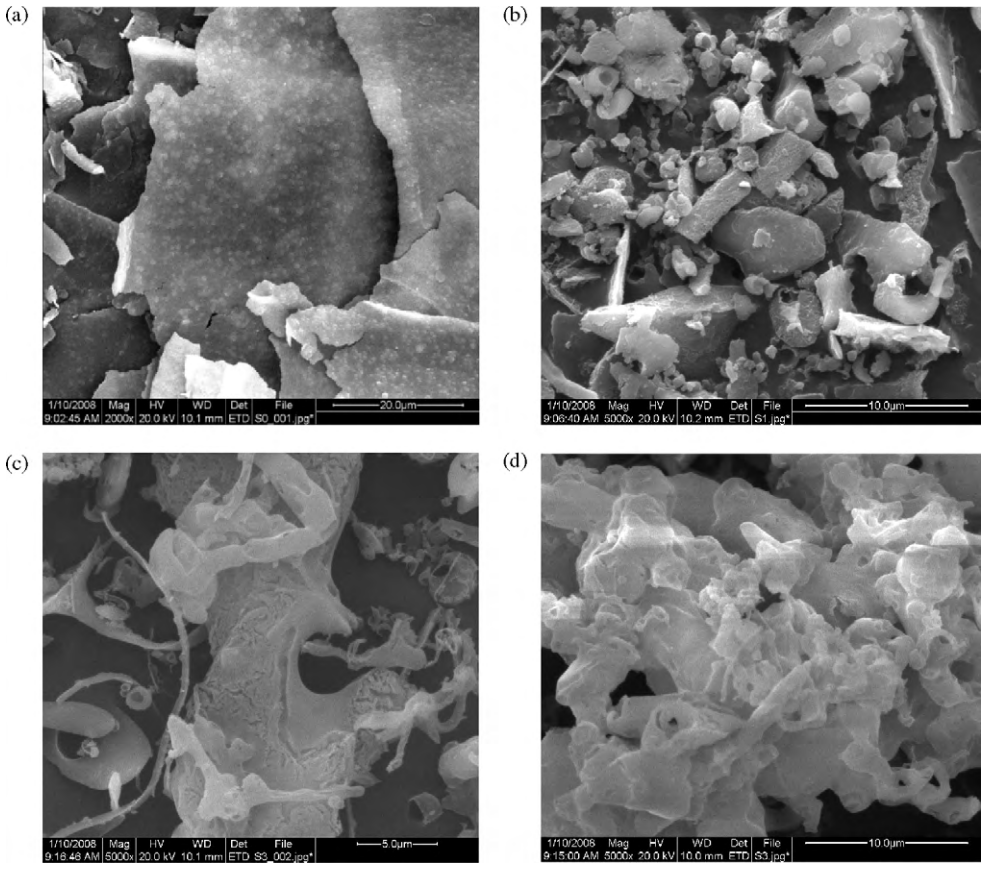

Fig. 2. Scanning electron microscopic photographs of PFP (a), PFP-SI (b), PFP-SI (c) and PFP-SIII (d).

It was confirmed further that the enhancement in anticoagulant activity might be due to the fact that the introduction of sulfate groups increased the negative charge density of the groups.

The molecular weight of polysaccharide is another important parameter influencing biology activity (Zhao et al., 2006; Zhou et al., 2004; Zhou, Sheng, Yao, & Wang, 2006). Among the four fractions with almost identical DS, the activities of PFP-SML and PFP-SM were even higher than that of the parent PFP-SII and PFP-SMH. Similar effects were also observed in the case of heparin-derived oligosaccharides and other sulfated polysaccharides, such as β-

Table 2
Anticoagulant activity measured by APTT assay.

Sample	Clotting time (s)					
	0 μg/ml	4 μg/ml	8 μg/ml	12 μg/ml	16 μg/ml	20 μg/ml
PFP-SI	67.3 ± 0.8	78.5 ± 2.3$^{\triangle}$	87 ± 0.6$^{\triangle}$	100.5 ± 1.4$^{*\triangle}$	108.5 ± 0.8$^{*\triangle}$	113 ± 2.3$^{*\triangle}$
PFP-SII	67.3 ± 0.8	73.6 ± 3.1$^{\triangle}$	98 ± 1.7$^{*\triangle}$	108 ± 2.1$^{*\triangle}$	120.5 ± 0.4$^{*\triangle}$	148.7 ± 1.1$^{*\triangle}$
PFP-SIII	67.3 ± 0.8	85.2 ± 0.6$^{\triangle}$	127.5 ± 0.2$^{*\triangle}$	188 ± 2.2$^{*\triangle}$	238 ± 2.6$^{*\triangle}$	286 ± 1.7$^{*\triangle}$
PFP-SMH	67.3 ± 0.8	92.7 ± 1.1$^{*\triangle}$	138 ± 3.1$^{*\triangle}$	166 ± 2.9$^{*\triangle}$	203 ± 2.6$^{*\triangle}$	256 ± 1.6$^{*\triangle}$
PFP-SMM	67.3 ± 0.8	99.3 ± 3.2$^{*\triangle}$	162 ± 1.3$^{*\triangle}$	217 ± 0.8$^{*\triangle}$	265 ± 1.5$^{*\triangle}$	>300$^{*\triangle}$
PFP-SML	67.3 ± 0.8	111 ± 2.7$^{*\triangle}$	187 ± 2.2$^{*\triangle}$	261 ± 1.5$^{*\triangle}$	>300$^{*\triangle}$	>300$^{*\triangle}$

Heparin	2 μg/ml	4 μg/ml	6 μg/ml
	89.5 ± 1.2	176.4 ± 0.6	>300

Note: Significant differences from the heparin (2 μg/ml) and the control were evaluated using Student's *t*-test: $^{*}P < 0.01$ versus heparin groups, $^{\triangle}P < 0.01$ versus controls.

Table 3
Anticoagulant activity measured by TT assay.

Sample	Clotting time (s)					
	0 μg/ml	4 μg/ml	8 μg/ml	12 μg/ml	16 μg/ml	20 μg/ml
PFP-SI	15.2 ± 0.8	16.6 ± 1.2	22.8 ± 0.8$^{\triangle}$	29.8 ± 0.7$^{\triangle}$	33.9 ± 0.2$^{\triangle}$	38.7 ± 0.5$^{\triangle}$
PFP-SII	15.2 ± 0.8	18.1 ± 0.3	29.9 ± 0.9$^{\triangle}$	34.8 ± 1.1$^{\triangle}$	43.2 ± 3.1$^{\triangle}$	46.0 ± 2.7$^{*\triangle}$
PFP-SIII	15.2 ± 0.8	20.1 ± 1.1$^{\triangle}$	27.9 ± 1.3$^{\triangle}$	31.1 ± 2.3$^{\triangle}$	40.2 ± 3.2$^{\triangle}$	43.2 ± 3.3$^{\triangle}$
PFP-SMH	15.2 ± 0.8	19.3 ± 1.2$^{\triangle}$	33.4 ± 0.6$^{\triangle}$	40.2 ± 2.1$^{\triangle}$	44.0 ± 0.4$^{\triangle}$	45.4 ± 2.6$^{\triangle}$
PFP-SMM	15.2 ± 0.8	18.1 ± 0.5	25.5 ± 0.7$^{\triangle}$	31.9 ± 1.2$^{\triangle}$	38.2 ± 1.3$^{\triangle}$	40.1 ± 0.8$^{\triangle}$
PFP-SML	15.2 ± 0.8	16.6 ± 0.3	24.8 ± 0.4$^{\triangle}$	30.0 ± 1.7$^{\triangle}$	38.7 ± 2.2$^{\triangle}$	43.2 ± 1.6$^{\triangle}$

Heparin	2 μg/ml	4 μg/ml	6 μg/ml
	43.9 ± 0.6	86.9 ± 0.5	147.8 ± 1.5

Note: Significant differences from the heparin (2 μg/ml) and the control were evaluated using Student's *t*-test: $^{*}P < 0.01$ versus heparin groups, $^{\triangle}P < 0.01$ versus controls.

Table 4
Anticoagulant activity measured by PT assay.

Sample	Clotting time (s)					
	0 μg/ml	4 μg/ml	8 μg/ml	12 μg/ml	16 μg/ml	20 μg/ml
PFP-SI	13.7 ± 1.5	14.4 ± 0.2	14.7 ± 1.3	16.3 ± 0.9	19.9 ± 0.6$^{*\triangle}$	20.6 ± 0.6$^{*\triangle}$
PFP-SII	13.7 ± 1.5	14.7 ± 1.1	15.1 ± 0.9	17.7 ± 1.4$^{\triangle}$	18.1 ± 1.5$^{\triangle}$	19.2 ± 2.1$^{\triangle}$
PFP-SIII	13.7 ± 1.5	15.6 ± 0.2	16.3 ± 0.7	17.9 ± 0.7$^{\triangle}$	18.9 ± 1.3$^{\triangle}$	18.0 ± 0.2$^{\triangle}$
PFP-SMH	13.7 ± 1.5	14.4 ± 0.3	14.6 ± 0.7	15.4 ± 0.6	16.4 ± 1.1	16.7 ± 1.9
PFP-SMM	13.7 ± 1.5	14.6 ± 0.6	15.5 ± 1.5	16.9 ± 1.1$^{\triangle}$	18.8 ± 0.7$^{\triangle}$	17.5 ± 1.7$^{\triangle}$
PFP-SML	13.7 ± 1.5	15.8 ± 1.4	16.1 ± 1.3	17.0 ± 2.3$^{\triangle}$	18.1 ± 0.9$^{\triangle}$	20.6 ± 0.6*

Heparin	2 μg/ml	4 μg/ml	6 μg/ml
	16.8 ± 0.5	47.3 ± 0.2	67.7 ± 0.4

Note: Significant differences from the heparin (2 μg/ml) and the control were evaluated using Student's *t*-test: $^{*}P < 0.01$ versus heparin groups, $^{\triangle}P < 0.01$ versus controls.

glucan sulfate (Alban & Franz, 1994; Bae et al., 2008; Chang et al., 2006), sulfated chitosan (Huang, Du, Yang, & Fan, 2003), sulfated galactan (Matsubara et al., 2001), and sulfated galactomannan (Mestechkina et al., 2008). These suggested that the anticoagulant activity improved with decreasing the molecular weights. In our studies, the sulfated polysaccharide with the DS of 1.7 and the lowest Mw of 1.0×10^4 was best in the prolongation of APTT and TT. It was speculated that higher Mw of PFP sulfates could prevent them from interacting with coagulation cofactors and their target proteases.

4. Conclusion

Various sulfated persimmon polysaccharides were prepared and their anticoagulant activity was investigated by the classical coagulant assays. The results indicated that the anticoagulant activity of sulfated persimmon polysaccharide could inhibit the intrinsic coagulant process and the thrombin-mediated fibrin formation, but they did not inhibit the extrinsic coagulant process. Sulfated derivatives inhibited the blood coagulation non-specifically due to their polyelectrolyte property and their anticoagulant activities were strongly dependent on the degree and position of sulfate groups and Mw. The sulfated polysaccharide from persimmon was a promising anticoagulant polysaccharide and a possible alternative for antithrombotic compound. To elucidate the relationship between anticoagulant activity and structure on the sulfated polysaccharide, an in-depth study of structural characteristics of the sulfated polysaccharide will indubitably play an indispensable role in the understanding of the anticoagulant activity.

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