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A comparative study of the anticoagulant activities of eleven fucoidans

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ABSTRACT

Seven fucoidans that differed only with respect to the average molecular weight and four fucoidans that differed with respect to both the molar ratio of fucose to galactose and the average molecular weight were obtained. The anticoagulant activities of these fucoidans were determined in order to understand the effects of the average molecular weight and the content of galactose. Fucoidans Y5–Y11, with the average molecular weight in the range of 50.1–8.4 kDa, showed decreasing activity at three concentrations and dose-dependent activity in the APTT and TT assays. Y1–Y4, which differed with respect to the average molecular weight and the molar ratio of fucose to galactose, exhibited complicated results in the APTT and TT assays. It was shown that not only the average molecular weight but also the molar ratio of fucose to galactose affected the anticoagulant activity of fucoidans.

1. Introduction

Fucoidans, a family of sulfated heteropolysaccharides, are found in brown algae and marine invertebrates. The amount of fucose present is dependent on the origin of the algae, the time of harvest and the species of algae. Owing to the complexity of fucoidan, it is still difficult to elucidate the relationship between the structure of this compound and its activities. However, there is abundant information on the structure and the activities. Patankar, Oehninger, Barnett, Williams, and Clark (1993) revised the structure of fucoidan extracted from *Fucus vesiculosus*. The structure of this fucoidan was a branched 1 → 3 linked α-L-fucan with sulfation at position O-4, and every two or three fucose residues had a branched fucose at the 2 or 3 position. Chevolot, Mulloy, Ratiskol, Foucault, and Colliec-Jouault (2001) observed that fucoidans from *Ascophyllum nodosum* and *Fucus vesiculosus* consisted of the repeating disaccharide unit [→3)-α-L-Fuc(2SO_3^-)-(1 → 4)-α-L-Fuc(2,3-diSO_3^-)-(1]. A structure of alternating 3-linked α-L-fucopyranose 2,4-disulfate and 4-linked α-L-fucopyranose 2-sulfate units was also identified from *Fucus distichus* (Bilan et al., 2004). Chandía and Matsuhiro (2008) obtained fucoidan from *Lessonia vadosa* (Phaeophyta) with a backbone of 1 → 3 linkages with sulfate groups primarily at position O-4 with infrequent sulfation at position O-2. The fucoidan from *Saccharina latissima* was made up of α-(1 → 3) linked fucopyranose residues, which were sulfated at C-4 and/or at C-2 and branched at C-2 with singly sulfated α-L-fucopyranose residues (Bilan et al., 2010).

Fucoidans have diverse biological activities, such as anticancer activity (Cho, Lee, & You, 2011; Ermakova et al., 2011), immunomodulating activity (Caipang, Lazado, Berg, Brinchmann, & Kiron, 2010), antiviral activity (Karmakar, Pujol, Damonte, Ghosh, & Ray, 2010), antiangiogenic activity (Koyanagi, Tanigawa, Nakagawa, Soeda, & Shimeno, 2003), antitumor activity (Sokolova, Ermakova, Awada, Zvyagintseva, & Kanaan, 2011; Synytsya et al., 2010) and antioxidant activity (Wang, Zhang, Zhang, Song, & Li, 2010). The anticoagulant activity of these compounds is by far the most widely studied (Croci, Cumashi, Ushakova, Preobrazhenskaya, & Piccoli, 2011; Pomin, 2004). This activity depends on the structural features, the sulfate position, the composition of the monosaccharides and the molecular weight (Barros et al., 2011). It has been reported that the structural features influence not only the anticoagulant activity of fucoidan but also the mechanism of this activity (Pereira, Mulloy, & Mourão, 1999). Chandía and Matsuhiro found that 4-O-sulfate 1 → 3 linked fucan exerted anticoagulant activity, whereas 2-O-sulfate 1 → 3 linked fucan had a deleterious effect. In addition, 2,4-disulfate 1 → 3 linked fucan had high anticoagulant activity (Chandía & Matsuhiro, 2008). It was confirmed that the difference in the anticoagulant activities of sulfated polysaccharides might depend on the different patterns of sulfation of the fucose branch of the chondroitin sulfate, especially 2,4-O-disulfation (Chen et al., 2011). Cumashi et al. (2007) examined the anticoagulant activity of nine polysaccharides from *Laminaria saccharina* (*Saccharina latissima*), *L. digitata*, *Fucus evanescens*, *F. serratus*, *F. distichus*, *F. spiralis*, *Ascophyllum nodosum*, and *Cladosiphon okamuranus*. They found that only the

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polysaccharide from *C. okamuranus*, which had substantial levels of 2-O-α-D-glucuronpyranosyl branches in the linear (1 → 3)-linked α-L-fucopyranoside chain, did not display anticoagulant activity as determined by the activated partial thromboplastin time (APTT). All of the other polysaccharides exhibited different anticoagulant activities. The effect of fucoidan on thrombin inactivation mediated by heparin cofactor II was reduced dramatically by a slight decrease in the molecular size of the sulfated fucan. A total of 178 monosaccharide units were necessary for the interaction with heparin cofactor II, and 408 monosaccharide units were necessary for complete thrombin inhibition (Pomin, 2004).

According to a previous study (Wang, Zhang, Zhang, Zhang, & Niu, 2010), the backbone of fucoidan LF2 extracted from the brown seaweed *Laminaria japonica* (*Saccharina japonica)*, consisting of 1 → 3 linkages (75%) and 1 → 4 linkages (25%). The branched points are at C-4, with β-D-galactopyranose residues (35%), or at C-2, with 4-sulfate fucopyranose residues (65%). Sulfation is sometimes present at position C-4 or C-2 with fucopyranose residues and C-3 and/or C-4 with galactopyranose residues.

In this study, we prepared seven polysaccharides (Y5–Y11) that differed only with respect to the average molecular weight and four polysaccharides (Y1–Y4) that differed with respect to both the molar ratio of fucose to galactose and the average molecular weight. We used these polysaccharides to further study the relationship between structure and anticoagulant activity. Based on the results of a previous study (Cumashi et al., 2007), it was hypothesized that the branching units of the 2-O-α-D-glucuronpyranosyl residues had a negative effect on anticoagulant activity. In this study, the relationship between the molar ratio of fucose to galactose and the anticoagulant activity was elucidated for the first time. According to our results, it is necessary to consider the content of galactose when evaluating the quality of commercially available crude fucoidans for use as anticoagulant agents. Although there have been many reports on the relationship between molecular weight and anticoagulant activity, this study was the first to determine the anticoagulant activities of samples that differed only with respect to the average molecular weight. We found that both the average molecular weight and the molar ratio of fucose to galactose influenced the activity of fucoidans in the APTT and TT assays. The effect was not as obvious in the PT assay.

2. Materials and methods

2.1. Materials

The brown algae *S. japonica* was collected in Shazikou, Qingdao, China, in April 2011. The standards (L-fucose, D-galactose, D-mannose, D-glucuronic acid, L-rhamnose monohydrate, D-xylose and D-glucose) were purchased from Sigma. 3-Methyl-1-phenyl-2-pyrazolin-5-one (99%) was obtained from Aldrich Chemistry.

2.2. Preparation and purification of fucoidans

Crude fucoidans were prepared according to the previously reported methods (Wang, Zhang, Zhang, & Li, 2008). The crude polysaccharides were separated by anion-exchange chromatography on a DEAE-Bio Gel agarose FF (12 cm × 70 cm) column with elution by water (30 L) (F0), 0.5 M NaCl (35 L) (F0.5), 1 M NaCl (35 L) (F1) and 2 M NaCl (35 L) (F2), respectively. Then, F2 was ultrafiltered, concentrated and precipitated with 72% ethanol before further precipitation with 95% ethanol. After analyzing the composition of F2, we obtained the polysaccharides (Y1–Y4), which were used to elucidate the relationship between the molar ratio of fucose to galactose and the anticoagulant activity.

Y4 was degraded by hydrogen peroxide and ascorbic acid. Briefly, 1 g of Y4 was dissolved in 200 mL water, and then different concentrations of hydrogen peroxide and ascorbic acid were added at different temperatures. The details of this protocol will be described elsewhere. The degraded polysaccharides (Y5–Y11) were dialyzed against distilled water and then lyophilized.

The average molecular weight of the polysaccharides was determined by HPLC by elution with 0.05 M sodium sulfate on a TSKgel G3000PWXL column with refractive index detection. Ten different molecular weight dextrans purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China) were used as standard.

2.3. Composition analysis

The molar ratio of monosaccharides and the content of fucose were determined according to the previously published method (Zhang, Zhang, Wang, Shi, & Zhang, 2009). The level of sulfation was determined by ion chromatography. Uronic acid was analyzed by a modified carbazole method (Bitter & Muir, 1962).

2.4. NMR and IR spectroscopy

Polysaccharides (50 mg) were co-evaporated with deuterium oxide (99.9%) twice before dissolving in deuterium oxide (99.9%) containing 0.1 μL deuterated acetone. NMR spectra were recorded at a Bruker AVANCE III 600 MHz at 25 °C. The chemical shifts were adjusted to the internal standard (deuterated acetone, 2.05 and 29.92 ppm, respectively). The parameters used for DEPTQ were as follows: Pulprog (deptqsp); AQ_mod (DQD); TD (Size of fid: 65536); NS (Number of scans: 256); DS (Number of dummy scans: 8).

IR spectra were determined on a Nicolet-360 FTIR spectrometer (36 scans, at a resolution of 6 cm^{-1}) between 400 and 4000 cm^{-1} using powders pressed into KBr pellets.

2.5. Clotting time assay

2.5.1. Anticoagulant activity measured by the activated partial thromboplastin time (APTT)

The APTT (assay kit from SUNBIO, China) was measured according to the manufacturer's instructions. Briefly, platelet-poor plasma (0.1 mL) was mixed with a solution (50 μL) of fucoidans at different concentrations (3.6 μg m L^{-1}, 7.2 μg m L^{-1} or 14.4 μg mL^{-1}) in 0.9% NaCl, and then preheated APTT reagent (0.1 mL) was added. The mixture was incubated for 5 min at 37 °C. At the same time, a solution (0.1 mL) of 0.025 mol L^{-1} $CaCl_2$ was also preheated at 37 °C. Finally, the two solutions were mixed together, and the time required for clot formation was recorded. The controls were 0.9% NaCl.

2.5.2. Anticoagulant activity measured by the prothrombin time (PT)

The PT (assay kit from SUNBIO, China) was determined according to the manufacturer's instructions. First, the PT reagent (0.2 mL) was incubated for 5 min. Then, platelet-poor plasma (0.1 mL) was mixed with a solution (50 μL) of fucoidans with different concentrations (3.6 μg mL^{-1}, 7.2 μg mL^{-1} or 14.4 μg mL^{-1}) in 0.9% NaCl. The mixed solution was preheated for 3 min. Finally, the two preheated solutions were mixed together, and the time required for clot formation was recorded. The controls were 0.9% NaCl.

2.5.3. Anticoagulant activity measured by the thrombin time (TT)

The TT (assay kit from SUNBIO, China) was determined according to the manufacturer's instructions with some modifications. Briefly, platelet-poor plasma (0.2 mL) was mixed with a solution (50 μL) of fucoidans with different concentrations (3.6 μg mL^{-1},

Table 1
Chemical compositions and molecular weights of Y1–Y11.

Samples	Fucose (%)	Uronic acid (%)	Mw (kDa)	Sulfate (%)	Neutral sugar (molar ratio)						
					Fuc	Gal	Man	Glc A	Rha	Xyl	Glc
Y1	37.2	0	49.3	40.82	1	0.32	–	–	–	–	–
Y2	37.05	0	73.1	41.19	1	0.24	–	–	–	–	–
Y3	37.31	0	49.2	40.93	1	0.16	–	–	–	–	–
Y4	36.67	0	90.1	39.88	1	0.08					
RSD (%)	0.75	0	–	1.40	–	51.64					
Y5	40.96	0	50.1	46.89	1	0.086	–	–	–	–	–
Y6	40.65	0	36.0	46.96	1	0.083	–	–	–	–	–
Y7	41.43	0	29.5	48.82	1	0.085	–	–	–	–	–
Y8	41.06	0	22.4	48.33	1	0.086	–	–	–	–	–
Y9	42.07	0	15.4	45.15	1	0.085	–	–	–	–	–
Y10	40.59	0	11.3	47.63	1	0.089	–	–	–	–	–
Y11	40.95	0	8.4	43.74	1	0.083	–	–	–	–	–
RSD (%)	1.24	0	–	3.83	–	2.41					

7.2 μg mL^{-1} or 14.4 μg mL^{-1}) in 0.9% NaCl, and then the mixed solution was preheated for 3 min. Then, the TT reagent (0.2 mL) was added to the solution, and the time required for clot formation was recorded. The controls were 0.9% NaCl.

3. Results and discussion

3.1. Preparation and purification

The molar ratios of monosaccharides, contents of uronic acid and fucose, sulfate content and average molecular weight of Y1–Y4 are presented in Table 1. The relative standard deviations for the contents of fucose and sulfate of Y1–Y4 were 0.75% and 1.40%, respectively. These low standard deviations suggested that the primary compositions of Y1–Y4 might be the same. The monosaccharide composition analysis showed a clear difference in the molar ratios of fucose to galactose. However, as shown in Table 1, the average molecular weight of Y1–Y4 were in the order Y4 > Y2 > Y1 ≈ Y3, which resulted from the processing of the crude fucoidan. Therefore, it was hypothesized that the differences among Y1–Y4 included both the molar ratio of fucose to galactose and the average molecular weight.

As shown in Table 1, the relative standard deviations in the contents of fucose and sulfate and in the molar ratios of fucose to galactose for Y5–Y11 were 1.24%, 3.83% and 2.41%, respectively. These samples were prepared by the degradation of Y4, suggesting that their structures were similar. The only difference was the molecular weight, which ranged from 8.4 kDa to 50.1 kDa. It was difficult to obtain polysaccharides with lower molecular weight due to the loss of the sulfate group under the harsh conditions. The relationship between the content of sulfate groups and the anticoagulant activity has not been elucidated previously. Therefore, in this report, the lower molecular weight polysaccharides with lower contents of sulfate are not discussed.

The IR spectra (Fig. 1) showed that all samples had the same infrared absorption properties, suggesting that all samples contained the same functional groups. As reported previously (Barros et al., 2011; Chandía & Matsuhiro, 2008; Chen et al., 2011; Rioux, Turgeon, & Beaulieu, 2010; Wijesinghe, Athukorala, & Jeon, 2011), the band at 1240–1260 cm^{-1} corresponded to the S=O stretching vibration, and the band at approximately 850 cm^{-1} was assigned to the C—O—S vibration. These vibrations confirmed the presence of the sulfate group linked at the C-4 axial position on fucose or galactose. It was suggested the fucoidans were mainly sulfated at C-4 on fucose or galactose. The absorption at approximately 579 cm^{-1} suggested that there were a significant number of sulfate groups.

3.2. Anticoagulant activities

The anticoagulant activities of all samples were determined by the APTT, PT and TT assays, and the results were summarized in Table 2.

The APTT assay was used to determine the effects of fucosidase on intrinsic factors such as II, V, VIII, IX, XI and XII and/or common pathways. The prolongation of the APTT was indicative of the inhibition of the intrinsic factors and/or the common pathways. The results (Table 2) of the APTT assay showed that all samples had *P* values of less than 0.01 and prolonged coagulation times compared with the control sample treated with saline, suggesting that all samples inhibited the intrinsic factors and/or common pathways. However, only two samples, Y5 (3.6 μg mL^{-1}) and Y1 (7.2 μg mL^{-1}), did not have significant P values compared with the Y4 sample, suggesting that these two samples had anticoagulant activities similar to that of Y4. The results for Y4–Y11, shown in Fig. 2, demonstrated that the anticoagulant activity decreased as the average molecular weight decreased. Y4 exhibited the best anticoagulant activity. This tendency was not obvious at a low concentration (3.6 μg mL^{-1}). Thus, it was hypothesized that the ability to inhibit intrinsic factors and/or common pathways depends on both the concentration and the average molecular weight (8.4–50.1 kDa).

The results of the APTT assay, shown in Fig. 2, were complicated because there were two variables for Y1–Y4: the molar ratio of fucose to galactose and the average molecular weight. Comparing Y1 and Y3, which differed only with respect to the molar ratio of fucose to galactose, revealed that the anticoagulant activity of Y1

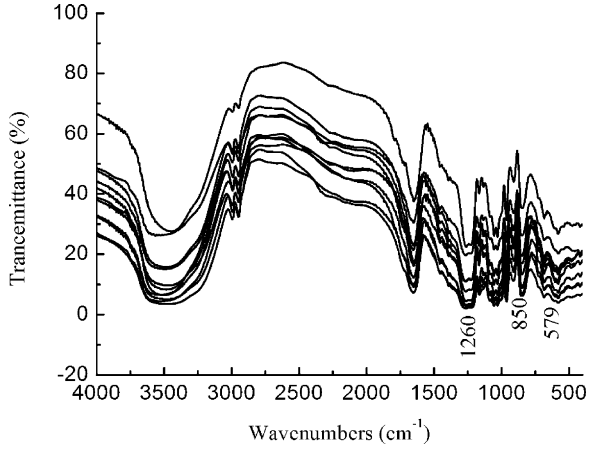

Fig. 1. IR spectra of Y1–Y11 from top to bottom.

Table 2
Anticoagulant activities of Y1–Y11.

Sample	Concentration ($\mu g\,mL^{-1}$)	APTT/s	PT/s	TT/s
0.9%NaCl		24.16 ± 0.55	8.03 ± 0.02	18.05 ± 0.44
Heparin	0.24	91.03 ± 0.15$^{**,\Delta}$	50.27 ± 0.07$^{**,\Delta\Delta}$	65.84 ± 0.32$^{**,\Delta\Delta}$
Y1	3.6	35.16 ± 0.16$^{**,\Delta\Delta}$	8.98 ± 0.68**	26.43 ± 0.28$^{**,\Delta\Delta}$
	7.2	53.09 ± 1.26**	9.66 ± 0.05**	32.89 ± 0.26$^{**,\Delta\Delta}$
	14.4	94.35 ± 0.24$^{**,\Delta\Delta}$	10.60 ± 0.15**	50.84 ± 0.32$^{**,\Delta\Delta}$
Y2	3.6	31.32 ± 0.80$^{**,\Delta\Delta}$	8.91 ± 0.1**	24.54 ± 0.21$^{**,\Delta\Delta}$
	7.2	51.63 ± 0.74$^{**,\Delta\Delta}$	9.73 ± 0.15**	27.41 ± 0.42$^{**,\Delta\Delta}$
	14.4	86.46 ± 0.62$^{**,\Delta\Delta}$	10.15 ± 0.14$^{**,\Delta\Delta}$	39.48 ± 0.59$^{**,\Delta\Delta}$
Y3	3.6	36.87 ± 0.55$^{**,\Delta\Delta}$	8.92 ± 0.12**	26.74 ± 0.35$^{**,\Delta\Delta}$
	7.2	48.28 ± 1.28$^{**,\Delta\Delta}$	9.62 ± 0.19**	30.13 ± 0.24$^{**,\Delta\Delta}$
	14.4	85.67 ± 0.41$^{**,\Delta\Delta}$	10.33 ± 0.21$^{**,\Delta}$	41.03 ± 0.54$^{**,\Delta\Delta}$
Y4	3.6	34.60 ± 0.27**	8.68 ± 0.14**	28.50 ± 0.07**
	7.2	53.49 ± 0.70**	9.60 ± 0.2**	34.00 ± 0.35**
	14.4	91.95 ± 0.22**	10.86 ± 0.17**	53.87 ± 0.34**
Y5	3.6	33.89 ± 0.13**	8.86 ± 0.05**	27.50 ± 0.09$^{**,\Delta\Delta}$
	7.2	51.03 ± 0.37$^{**,\Delta\Delta}$	9.56 ± 0.06**	32.63 ± 0.16$^{**,\Delta\Delta}$
	14.4	84.03 ± 0.20$^{**,\Delta\Delta}$	10.33 ± 0.07$^{**,\Delta}$	50.30 ± 0.11$^{**,\Delta\Delta}$
Y6	3.6	33.18 ± 0.20$^{**,\Delta\Delta}$	9.07 ± 0.05**	26.95 ± 0.06$^{**,\Delta\Delta}$
	7.2	45.75 ± 0.31$^{**,\Delta\Delta}$	9.85 ± 0.5**	31.61 ± 0.13$^{**,\Delta\Delta}$
	14.4	69.74 ± 0.27$^{**,\Delta\Delta}$	10.73 ± 0.3**	44.29 ± 0.39$^{**,\Delta\Delta}$
Y7	3.6	32.74 ± 0.27$^{**,\Delta\Delta}$	9.44 ± 0.29$^{**,\Delta\Delta}$	26.72 ± 0.18$^{**,\Delta\Delta}$
	7.2	43.04 ± 1.11$^{**,\Delta\Delta}$	10.18 ± 0.35$^{**,\Delta}$	30.90 ± 0.03$^{**,\Delta\Delta}$
	14.4	59.58 ± 0.71$^{**,\Delta\Delta}$	10.84 ± 0.31**	43.77 ± 0.31$^{**,\Delta\Delta}$
Y8	3.6	30.92 ± 1.28$^{**,\Delta\Delta}$	8.79 ± 0.15**	26.38 ± 0.04$^{**,\Delta\Delta}$
	7.2	38.29 ± 1.04$^{**,\Delta\Delta}$	9.47 ± 0.3**	28.23 ± 0.32$^{**,\Delta\Delta}$
	14.4	57.87 ± 0.14$^{**,\Delta\Delta}$	10.26 ± 0.15$^{**,\Delta}$	41.79 ± 0.04$^{**,\Delta\Delta}$
Y9	3.6	30.39 ± 0.44$^{**,\Delta\Delta}$	9.11 ± 0.26**	25.64 ± 0.35$^{**,\Delta\Delta}$
	7.2	34.02 ± 1.21$^{**,\Delta\Delta}$	9.79 ± 0.19**	27.40 ± 0.38$^{**,\Delta\Delta}$
	14.4	46.59 ± 0.45$^{**,\Delta\Delta}$	10.54 ± 0.36**	35.49 ± 0.20$^{**,\Delta\Delta}$
Y10	3.6	29.72 ± 1.32$^{**,\Delta\Delta}$	8.63 ± 0.26**	22.30 ± 0.36$^{**,\Delta\Delta}$
	7.2	33.56 ± 0.93$^{**,\Delta\Delta}$	8.73 ± 0.17$^{**,\Delta\Delta}$	24.38 ± 0.42$^{**,\Delta\Delta}$
	14.4	42.79 ± 1.30$^{**,\Delta\Delta}$	9.37 ± 0.44$^{**,\Delta\Delta}$	31.73 ± 0.25$^{**,\Delta\Delta}$
Y11	3.6	28.50 ± 0.17$^{**,\Delta\Delta}$	8.83 ± 0.2**	20.84 ± 0.38$^{**,\Delta\Delta}$
	7.2	30.79 ± 1.10$^{**,\Delta\Delta}$	8.69 ± 0.5$^{**,\Delta\Delta}$	24.07 ± 0.24$^{**,\Delta\Delta}$
	14.4	36.48 ± 0.63$^{**,\Delta\Delta}$	8.85 ± 0.12$^{**,\Delta\Delta}$	29.73 ± 0.54$^{**,\Delta\Delta}$

Values are depicted as means ± SD ($n = 3$).
** $P < 0.01$ compared to the negative control.
$^{\Delta}$ $P < 0.05$ compared to the Y4.
$^{\Delta\Delta}$ $P < 0.01$ compared to the Y4.

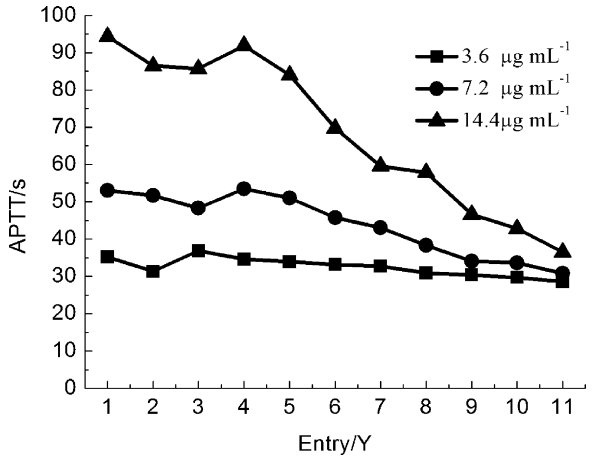

Fig. 2. Comparison of Y1–Y11 at the three concentrations on APTT. Values are expressed as mean ± SD of three determinations.

was greater than that of Y3 at the concentration of 14.4 $\mu g\,mL^{-1}$. This result suggested that the higher content of galactose enhances the anticoagulant activity. However, at the lower concentrations, this tendency was not obvious. The average molecular weight of Y2 and Y4 was 73.1 kDa and 90.1 kDa, respectively. It was not appropriate to use the results from above analysis because these samples were outside of the average molecular weight range. Comparing Y2 with Y4 revealed that the anticoagulant activity of Y2 was lower than that of Y4 at the three concentrations (3.6, 7.2 and 14.4 $\mu g\,mL^{-1}$), suggesting that the effect of the average molecular weight on the anticoagulant activity was more pronounced than the effect of the molar ratio of fucose to galactose for lower contents of galactose. Therefore, it was concluded that the anticoagulant activity in the APTT depended not only on the average molecular weight but also on the molar ratio of fucose to galactose and that the former had a more significant effect in the APTT assay than the latter. Y1–Y4 also showed dose-dependent effects.

As shown in Table 2, all samples had the ability to prolong the time required for clot formation, suggesting that the extrinsic pathway of coagulation was inhibited. However, the anticoagulant activity was weak. A previous study (Mourão et al., 2001) showed

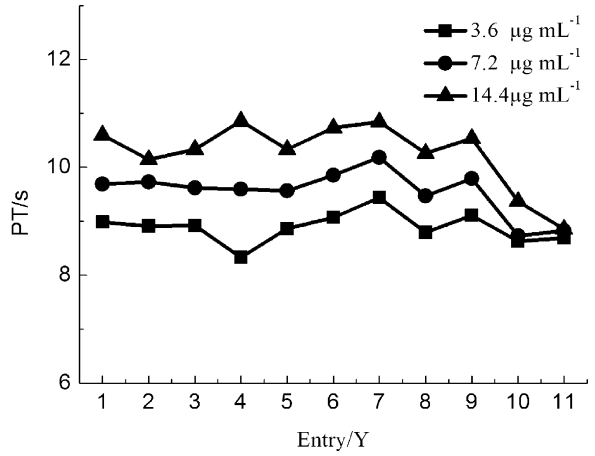

Fig. 3. Comparison of Y1–Y11 at the three concentrations on PT. Values are expressed as mean ± SD of three determinations.

that fucosylated chondroitin sulfate had no effect on the PT, suggesting that the mechanism of coagulation was different from that of fucosylated chondroitin sulfate. The results presented in Fig. 3 showed that the ability to inhibit the extrinsic pathway of coagulation was not influenced by the molecular weight but was affected by the dose. When the molecular weight exceeded 15.4 kDa, the anticoagulant activity was dose dependent. However, when the molecular weight was approximately 11.3 kDa, this activity was not dose dependent. Furthermore, when the molecular weight was 8.4 kDa, the activity did not change with dose, suggesting that the content of sulfate or the structure determined the ability of the polysaccharide to inhibit the extrinsic pathway of coagulation. Thus, it was concluded that the molecular weight is not essential to the ability to inhibit the extrinsic pathway of coagulation. When the effect of the molar ratio of fucose to galactose was considered, the results were similar to those for the APTT assay. Therefore, it was concluded that the molar ratio of fucose to galactose partly contributed to the polysaccharide's ability to inhibit the extrinsic pathway of coagulation.

The results of the TT assay, shown in Fig. 4, were similar to the results of the APTT assay. However, the mechanisms were different. The prolongation of the TT reflected the inhibition of thrombin activity or fibrin polymerization, whereas the prolongation of the APTT reflected the inhibition of the intrinsic factors and/or the common pathways.

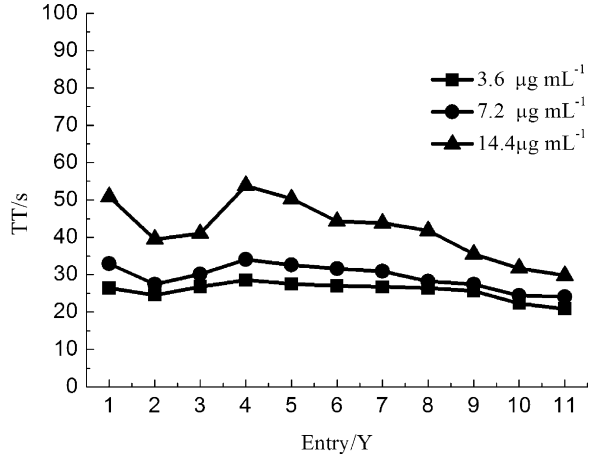

Fig. 4. Comparison of Y1–Y11 at the three concentrations on TT. Values are expressed as mean ± SD of three determinations.

4. Conclusions

Fucoidans that differed with respect to the average molecular weight and the molar ratio of fucose to galactose were prepared by anion-exchange chromatography. Y4 was degraded in the presence of hydrogen peroxide and ascorbic acid. Finally, seven fucoidans that differed only with respect to the average molecular weight were obtained. Fucoidan, a sulfated polysaccharide, possessed numerous biological activities, such as anticancer activity, immunomodulating activity and antiviral activity. In this study, we determined the effects of the average molecular weight and the content of galactose on the anticoagulant activity of fucoidans. Our results showed that the average molecular weight and the content of galactose affected the anticoagulant activities of fucoidans. This activity also exhibited dose dependence in the APTT and TT assays.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.07.067.

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