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Macromolecular properties and hypolipidemic effects of four sulfated polysaccharides from sea cucumbers

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ABSTRACT

The present study investigates the relationship between the high-order structure and hypolipidemic activity of four well-defined sulfated fucans from sea cucumber. The chain conformation, determined by a combination of AFM and SEC-MALLS-RI, indicate that fucosylated chondroitin sulfate (fCS) from *Pearsonothuria graeffei* (fCS-*Pg*) and *Isostichopus badionotus* (fCS-*Ib*), and fucoidan from *P.graeffei* (fuc-*Pg*) were assigned as a random coil conformation with polysaccharide chain outstretched, while *I. badionotus* (fuc-*Ib*) was assigned as a spherical conformation and exhibited high viscosity. Fuc-*Pg* and fuc-*Ib* with higher molecular weights had a greater impact in inhibiting pancreatic lipase activity *in vitro*. However, fCS-*Pg*, fCS-*Ib and* fuc-*Pg* with random linear conformation exhibited excellent hypolipidemic activity in Sprague-Dawley rats (SD rats) fed on high-fat diet (HFD), whereas fuc-*Ib* showed only a modest effect. Our results indicate that structural characteristics, including side branch and sulfation pattern can affect the chain conformation of polysaccharides, which determine their physicochemical properties and hypolipidemic activity.

1. Introduction

Polysaccharides are common ingredients in many kinds of food, including citrus, mushrooms, and sea cucumbers, and these polysaccharides possess a wide variety of activities including anti-tumor (Nagamine et al., 2009), anticoagulant (Gracher, Cipriani, Carbonero, Gorin, & Iacomini, 2010), antiviral (Wang, Wang, & Guan, 2012), and immunostimulatory (Chlubnova et al., 2011; Xu, Yan, Tang, Chen, & Zhang, 2014) activities. Deeper insight of the structure of polysaccharides and their biological activities have led researchers to focus on structure–function relationships over the past several years. The primary structure of polysaccharides include their monosaccharide composition, glycosidic linkages, molecular weight, degree of branching, and the presence of functional groups. All these factors can impact the conformation of polysaccharides, thus, determining their biological activities (Ferreira, Passos, Madureira, Vilanova, & Coimbra, 2015).

Sulfated polysaccharides represent an important class of glycans. These polysaccharides are often endowed high bioactivity related to their sulfate functional groups, which can interact with many positively charged biological macromolecules, such as enzymes. In recent years, sulfated polysaccharides from sea cucumbers have aroused the interest researchers because of their significantly anticoagulant and antithrombotic activities (Buyue & Sheehan, 2009; Zhao et al., 2015). Sulfated polysaccharides from sea cucumbers have also been found to prevent metabolic diseases resulting from diet intervention (Shiwei et al., 2013; Wang, Hu et al., 2016; Wang, Wang et al., 2016; Wang, Ma et al., 2016). These sulfated polysaccharides alleviate metabolic syndromes caused by high fat-high sucrose diet (HFSD), such as lowering serum glucose, triglyceride (TG), total cholesterol (TC), TC, body weight gains, improving adiponectin, and inhibiting insulin resistance (Wang, Hu et al., 2016; Wang, Wang et al., 2016; Wang, Ma et al., 2016). However, little research has investigated the structure-function relationship of these sulfated polysaccharides. Past reports describing the preliminary function-structure relationship focus on the effects of sulfation pattern, molecular size, fucose branching and content of sulfated fucose on anticoagulant activity (Pomin, 2009). However, few studies have examined the relationships between the macromolecular properties, especially the higher order conformation structures, and the hypolipidemic effect of sulfated polysaccharides.

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It has been widely reported that the higher order conformational structure of β-glucan polysaccharides from mushroom, or medical fungus, are important for its immunology and anti-hyperlipidemic activity. The triple-helix structure of these β-glucans are important for maintaining their biological activity (Falch, Espevik, Ryan, & Stokke, 2000). However, there have been many fewer studies on sulfated polysaccharides from marine organisms.

In our previous work, four sulfated polysaccharides of regular structure were extracted from two sea cucumber species. Fucosylated chondroitin sulfate (fCS) derived from *Pearsonothuria graeffei* (fCS-*Pg*), fucosylated chondroitin sulfate from *Isostichopus badionotus* (fCS-*Ib*), fucoidan from *Pearsonothuria graeffei* (fuc-*Pg*), and fucoidan from *Isostichopus badionotus* (fuc-*Ib*) were studied (Chen et al., 2012; Hu et al., 2015; Wu et al., 2013). We found the sulfation pattern is important for their anticoagulant, antithrombotic and anti-hyperlipidemic activities (Chen et al., 2012, 2013; Wu et al., 2016). However, the *in vivo* and *in vitro* results were quite different, which suggested the mechanisms of action for these polysaccharides were quite complex and based not simply on their primary structure but also on their higher order conformational structure. In the present work, we focus our investigation on the spatial structures and the flow behavior of four sulfated polysaccharides. The hypolipidemic activity of these sulfated polysaccharides was investigated through both *in vitro* and *in vivo* experiments. This investigation on the effect of the higher order conformational structure on hypolipidemic activity provides a novel insight into the structure function relationship of these molecules.

2. Materials and methods

2.1. Materials

Two species of sea cucumbers, *Pearsonothuria graeffei* (from Indo-Pacific) and *Isostichopus badionotus* (from Western Atlantic Ocean) were purchased from a local market in Qingdao, Shandong, China.

2.2. Preparation of sea cucumber polysaccharides

Crude sea cucumber polysaccharide was prepared based on a previously described method (Chen et al., 2012). Briefly, the dry sea cucumber body wall (ca. 100 g) was minced and homogenized. The homogenate was digested with papain at 60 °C for 10 h in a solution containing 5 mM EDTA and 5 mM cysteine, and then subjected to centrifugation (2000 × *g* for 15 min at 4 °C). Polysaccharide in the clear supernatant fractions was precipitated with 160 mL of 10% cetylpyridinium chloride solution. After incubation at room temperature for 24 h, the mixture was centrifuged (2000 × *g* for 15 min). The precipitated sulfated polysaccharide was dissolved in 1000 mL of 3 M NaCl: ethanol (100:15, v/v) solution and then 600 mL of 95% ethanol were added to precipitate chondroitin sulfate. After centrifugation (2000 × *g* 15 min) and removal of the precipitate, another 900 mL of ethanol was added to the supernatant to a final concentration of 60%. The precipitate formed was collected by centrifugation (2000 × *g*, 15 min) and dissolved in water before dialysis against water for 24 h. The retained solution was lyophilized and crude fucoidan was obtained. The crude polysaccharides were purified according to our former methods (Hu et al., 2015).

2.3. AFM microscopy

Four sulfated polysaccharides water solutions at 10 μg/mL were diluted with distilled water, and filtered through a 0.45 μm filter (NYL, 13 mm syringe filter, Whatman, Inc., USA). A 10 μL drop was deposited onto freshly cleaved mica and allowed to dry in air for 24 h at room temperature and was observed by a Digital Instrument atomic force microscope (AFM, Bruker Daltonics Inc., USA). All images were acquired in tapping mode at a typical scan rate of 1 Hz with a scan range of 1–5 μm. All the measurements are made in ambient conditions.

2.4. Molecular structural characteristics measured by SEC-MALLS-RI system

Chain conformation of four sulfated polysaccharides were determined by a combined SEC-MALLS-RI system. The mobile phase consisted of 0.2 M NaCl solution, which was passed through a membrane filter (0.45 mm pore size; Millipore Corp., MA, USA) and degassed at reduced pressure for 1 h at room temperature. The polysaccharide solutions (1 mg/mL) were stretched in the shake slowly for 24 h. Before being injected into the SEC system, the polysaccharide solution was passed through a 0.45-mm membrane filter (Millipore Corp., MA, USA). The polysaccharide solution (1 mg/mL, 50 μL) was injected into a Waters Ultrahydrogel 500 column. Ultrahydrogel 500 column protected by a similarly packed guard column (Waters, USA), and detected by online MALLS (DAWN HELEOS II λ_0 = 658 nm, Wyatt Technology, USA) and refractive index (Waters 2414,USA) detectors at 40 °C. Bovine serum albumin was used to determine the delay volume between MALLS and RI (0.235 mL). RI (dn/dc) was set as 0.138 mL/g. The data obtained by SEC-MALLS-RI were analyzed by ASTRA 6.1 software (Wyatt Technology, CA, USA).

2.5. Rheological measurement of sulfated polysaccharides

The rheological properties of sulfated polysaccharides were performed on a Haake Rheostress 6000 rheometer (Thermo Scientific Instruments, Inc. Germany) fitted with a cone-plate geometry (diameter = 25 mm, cone angle = 2) with a gap of 0.104 mm. The apparent viscosity as a function of the shear rate was determined in the range from 0.1 to 100 s^{-1}. Samples were individually loaded on the measuring geometry and allowed to stand for 2 min prior to testing. All measurements were performed at 25 ± 0.1 °C and performed in triplicate.

2.6. Determination of pancreatic lipase activity in vitro

Pancreatic lipase activity of the sulfated polysaccharides were determined using the former reported method with some modifications (Zeng et al., 2015). Sodium phosphate buffer (0.1 mol/L, 5 mL, pH 7.2), polysaccharide solution (8 mg/mL, 1 mL in water), and 1 mL olive oil emulsion were mixed at 40 °C for 5 min then, one milliliter of pancreatic lipase (1 mg of pancreatic lipase mixed with 1 mL of sodium phosphate buffer; Sigma, USA) was added and mixed at 40 °C for 1 h. Following enzyme inactivation in a boiling water bath for 5 min, the mixture was cooled in cold running water for 3 min and centrifuged at 2850 x g for 15 min. The free fatty acids released by enzymatic action in the reaction mixture were titrated with 0.05 M NaOH, using phenolphthalein as an indicator. Water (1 mL) instead of polysaccharide solution was used as control. Pancreatic lipase activity was determined by the production rate of free fatty acids and expressed by the ability to inhibit pancreatic lipase activity compared with control. All analyses were performed in triplicate.

2.7. Animals and experimetal design

Fifty-six Sprague–Dawley rats, male, weighting from 180 to 220 g, were purchased from the Animal Lab Center of Zhejiang Chinese Medical University (Certificate No. SCXK131(Hu)2007-2005, China). The rats were housed in an air-conditioned room at 25 ± 2 °C

on a 12 h light–dark cycle. The animals were fed with a commercial mice chow for 3 days to acclimatize to animal facilities. Then, animals were weighed and randomly divided into 7 groups of 8 mice. Group (1) was normal control while group (2) served as hyperlipidemic control and group (3) had the standard drug (simvastatin, 5 mg/kg) treated animals that served as positive control. Groups (4), (5), (6), (7) received fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, fuc-*Ib* in doses of 40 mg/kg. After the period of acclimation ended, group (1) continued to be provided with the common commercial mice chow and others were fed with a high fat diet (HFD) for 30 days. At the same time, groups (3)–(7) were given different doses of simvastatin, fCS-Ib and fCS-Pg by oral administration for 28 days. The mice were allowed free access to food and water during the experimental period. The composition of HFD was 1% cholesterol, 10% lard, 10% yolk powder, 0.2% bile salt and 78.8% commercial chow. The weight gains of rats were measured once per week.

2.8. Serum analysis

Levels of serum lipids including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were determined by assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) as the manufacturer's instructions. glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (AKP), and total bile acid (TBA) were also measured using commercial kits from the same company.

2.9. Statistical analysis

All of the numeric results are the mean ± SD. Repeated measures ANOVA was used to evaluate any changes in food utilization among groups. Other comparisons among the groups were performed with one-way ANOVA followed by an LSD (Least Significant Difference) or Duncan's test. SPSS 20 was used for all analysis. Differences were defined as statistically significant for values of $p < 0.05$.

3. Results and discussion

3.1. Macromolecular properties of four sulfated polysaccharides from sea cucumbers

3.1.1. Images of AFM

AFM has become a useful tool for directly observing the macromolecular conformation of polysaccharides. Sphere, random linear chain, random chain with branches, and rods are commonly reported conformations of polysaccharides. From AFM images (Fig. 1), these sulfated polysaccharides showed different conformations. Basically, fCS-*Pg* (Fig. 1**a** and **e**), fCS-*Ib* (Fig. 1**b** and **f**) and fCS-*Pg* (Fig. 1**c** and **g**) are primarily random linear chains with a few spherical aggregations. However, fuc-*Ib* showed totally spherical structure (Fig. 1**a** and **b**). For fCS-*Pg* and fCS-*Ib*, polysaccharides intertwined with each other and formed huge aggregates, which can be attributed to their fucose branches, providing hydroxyl groups (Liu et al., 2016) more accessible to interact with other molecules. Fuc-*Pg* showed random linear chain conformation, which was dispersely distributed on the surface of mica. The lengths of the chains of fCS-*Ib*, fCS-*Pg* and fuc-*Pg* were in the range of 100–1000 nm as observed by AFM. The widths of chains were much higher than that of a single polysaccharide chain (about 0.1–1.0 nm), suggesting that molecular aggregation was taking place. Fuc-*Ib* formed sphere in the surface of mica with a diameter at around 100 nm without linear chains observed.

3.1.2. Molecular structural characteristics of sulfated polysaccharides measured by SEC-MALLS-RI system

The absolute molecular weights of fCS-*Pg*, fCS-*Ib*, fuc-*Pg* and fuc-*Ib* were 4.91×10^4, 7.04×10^4, 6.55×10^5, and 6.38×10^5 g/mol, respectively, determined by SEC-MALLS-RI system (see **Supplementary** Table 1), which are different from the values measured by HPLC-SEC-RI. The single RI signal of the four sulfated polysaccharides indicated they were homogeneous components (**Supplementary** Fig. 1). Another important piece of information obtained from SEC-MALLS-RI system is structural conformation of a macromolecule. Chain conformation of the polysaccharides in aqueous solution can be obtained by calculating the slope between molar mass and molecular radius. When the slope of this plot is close to 0.33, the polymer in solution has a spherical conformation. When the slope of the line is 0.5–0.6, the polymer in solution has

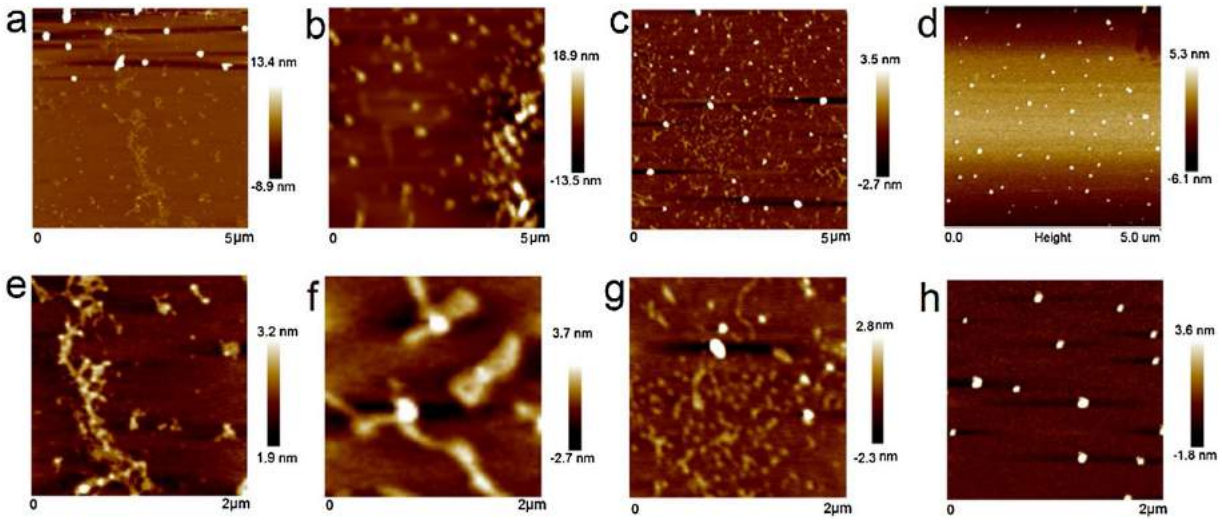

Fig. 1. AFM image of fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* dissolved into distilled water to be 10 μg/mL, deposited onto mica, and dried in air for two days. Image size: (a) to (d) 5 μm × 5 μm; (e) to (h) 2 μm × 2 μm.

a random coil conformation, and when the slope of the line is >1, the polymer exhibits a rod-like conformation (Wyatt, 1993; Zeng et al., 2016). The slope of fCS-*Pg* was not detected by SEC-MALLS-RI, which may be attributed to its low molecular weight. The slope of fCS-*Ib* was 0.50 ± 0.01 (Fig. 2**a**), which indicated fCS-*Ib* a random coil conformation. The slopes of fCS-*Pg* (Fig. 2**b**) and fuc-*Pg* (Fig. 2**c**) at 0.51 ± 0.01and 0.63 ± 0.01 also suggested random linear chains. A slope of 0.39 ± 0.00 suggests that fuc-*Ib* has a spherical conformation Fig. 2**d**. The data of was not obtained, which may be attributed to its low molecular weight. These results were consistent with the results of AFM.

3.1.3. Rheology properties of sulfated polysaccharides

The flow properties of the four sulfated polysaccharides are shown in Fig. 3 under steady-shear conditions. A concentration of 3% was chosen to compare the behavior of these four sulfated polysaccharides. In addition, the flow curves in Fig. 3 were fitted to the Power-law equation as follows, $\sigma = K \cdot \gamma^n$, where σ is the shear stress (Pa), γ is shear rate (s^{-1}), K is consistency index ($Pa \cdot s^n$), and n is the flow behavior index. The n values of fCS-*Pg*, fCS-*Ib*, fuc-*Pg* and fuc-*Ib* were 0.982 ± 0.002, 0.977 ± 0.003, 0.987 ± 0.001, and 0.997 ± 0.005. Correspondingly, all flow behavior indices of four sulfated polysaccharides were less than 1.0, approaching Newtonian pseudoplastic behavior (Wang, Hu et al., 2016; Wang, Wang et al., 2016; Wang, Ma et al., 2016). All samples showed a same shear thinning fluid behavior. However, the *K* values of fCS-*Pg*, fCS-*Ib*, fuc-*Pg* and fuc-*Ib* were 0.2743 ± 0.027, 0.2749 ± 0.001, 0.02897 ± 0.003, and 0.4231 ± 0.019, respectively. The higher *K* value of fuc-*Ib* indicated higher apparent viscosity than other three polysaccharides (Tzoumaki, Moschakis, & Biliaderis, 2013).

3.2. Effects of four sulfated polysaccharides on pancreatic lipase activity in vitro

The effects of four sulfated polysaccharides are shown in Fig. 4. The pancreatic lipase activities were calculated based on polysaccharide capacity of lipase inhibition. At a concentration of 8 mg/mL, the pancreatic lipase inhibitory activity of fCS-*Pg*, fCS-*Ib*, fuc-*Pg* and fuc-*Ib* were 24.13 ± 4.59%, 6.76 ± 1.11%, 62.07 ± 3.45%, and 60.92 ± 5.86%, respectively. The lipase inhibitory activities of four sulfated polysaccharides were different. The results showed that these four sulfated polysaccharides had different lipase inhibitory activities. Fuc-*Pg* and fuc-*Ib* had stronger inhibitory ability compared with fCS-*Pg* and fCS-*Ib* ($P < 0.05$). In addition, fCS-*Ib* showed almost no effects on pancreatic lipase activity, and there was no significant differences between fCS-*Pg* and fCS-*Ib*.

3.3. Effects of sulfated polysaccharides on SD rats fed on HFD

3.3.1. Effects of sulfated polysaccharides on body and liver weight of SD rats

All groups of rats gained body weight, 2-fold of their initial body weight, and the hyperlipidemia group had 20% weight gains over the normal group (Table 2). This indicated that HFD induced an increasingly abnormally body weight. Positive control group and fCS-*Pg*, fCS-*Ib*, fuc-*Pg* and fuc-*Ib* fed groups showed different effects on body weight gains in HFD fed rats (Fig. 5**a**). Among these, fuc-*Pg* exhibited best effect on weight control, which had even lower body weight gains than normal chow-fed group ($P > 0.05$, compared with normal group). FCS-*Pg* and fCS-*Ib* have similar effect on reducing body weight gains ($P < 0.05$, compared with normal group; $P < 0.05$, compared with only HFD-fed group). However, fuc-*Ib* exhibited only a slight effect on preventing weight gains caused by HFD ($P < 0.05$, compared with normal group; $P > 0.05$, compared with only HFD-fed group). From these results it is possible to conclude that the structure of sulfated polysaccharides at dose of 40 mg/kg determine their effects of protecting SD rats from the dietary disturbance observed in HFD-fed rats.

Liver is important organ for lipid metabolism. In the liver, fat accumulation can be toxic. Nonalcoholic fatty liver disease (NAFLD) is caused by accumulation of fat in hepatocytes (Kakimoto & Kowaltowski, 2016). The liver weight was 26.7 ± 1.39 g in the HFD group, which was much higher than normal chow-fed group (19.8 ± 1.01 g) (Fig. 5**b**). This results indicated that HFD caused lipid accumulation in the liver. The liver weights all the five groups (simvastatin, fCS-*Pg*, fcs-*Ib*, fuc-*Pg*, and fuc-*Ib* groups) were significantly lower than hyperlipidemia group ($P < 0.05$). More importantly, there was no significant difference between fCS-*Ib*, fuc-*Pg* fed groups and normal group ($P > 0.05$). As for fuc-*Ib* and fCS-*Pg* groups, liver weights were also lower than HFD group ($P < 0.05$).

3.3.2. Effects of sulfated polysaccharides on serum lipids in HFD induced hyperlipidaemia SD rats

Next, serum TC, TG, HDL-C and LDL-C levels were investigated to value the lipid changes in the serum. Serum TC, TG and LDL-C levels increased and serum HDL-C level decreased significantly in HFD group as compared with normal chow-fed group (Table 1). High TG and TC are risk factors that can cause hyperlipidemia. LDL-C is principal plasma carrier of cholesterol for delivering to peripheral tissues, having atherogenic potential. HDL-C is carrier of cholesterol from peripheral tissues to livers and then cholesterol is synthesized into bile acids, HDL-C therefore giving protection against hyperlipidemia (Jain, Kathiravan, Somani, & Shishoo, 2007).

All four polysaccharides reduced the concentration of TG in the serum. Among these, fCS-*Ib* and fuc-*Pg* had optimal effects on TG lowering ($P > 0.05$ compared with normal chow-fed group, $P < 0.05$ compared with HFD group). FCS-*Pg* and fuc-*Ib* showed weaker effects on TG levels ($P < 0.05$ compared with normal chow-fed group, $P < 0.05$ compared with HFD group). FCS-*Pg*, fCS-*Ib* and fuc-*Pg* showed the similar effects on lowering serum TC, but fuc-*Ib* showed no effects on that. Fuc-*Ib* also had no effect on reversing HDL-C and LDL-C levels, which were disrupted by HFD. For fCS-*Pg*, fCS-*Ib* and fuc-*Pg*, HDL-C levels increased 67.7%, 36.7%, and 25.8% and LDL-C levels decreased 13.2%, 32.9%, and 19.8%, respectively. The results indicated that sulfated polysaccharides from sea cucumbers exerted different effects on alleviating serum lipid dis-

Table 1
Effects of fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* on the serum lipids of HFD-fed rats.

Groups	TG(mmol/L)	TC(mmol/L)	HDL-C(mmol/L)	LDL-C(mmol/L)
Normal	2.56 ± 0.16*	3.24 ± 0.07*	1.45 ± 0.21*	0.74 ± 0.04*
HFD	4.04 ± 0.62#	4.41 ± 0.15#	0.93 ± 0.06#	0.91 ± 0.09#
simvastatin	3.56 ± 0.41#	4.22 ± 0.63#	1.13 ± 0.11#	0.60 ± 0.04*
fCS-*Pg*	3.32 ± 0.33*#	3.52 ± 0.32*	1.56 ± 0.20*	0.79 ± 0.11#
fCS-*Ib*	2.2 ± 0.66*	3.57 ± 0.42*	1.27 ± 0.26	0.61 ± 0.11*
fuc-*Pg*	2.86 ± 0.35*	3.28 ± 0.34*	1.17 ± 0.25#	0.73 ± 0.10*
fuc-*Ib*	3.25 ± 0.21*#	4.48 ± 0.5#	0.89 ± 0.11#	0.91 ± 0.13#

*P < 0.05: compared to hyperlipidemia control group. # P < 0.05: compared to normal control group. Data are presented as mean ± SD, n = 8. The normal group was supplied with common commercial chow and all the other groups were supplied with HFD.

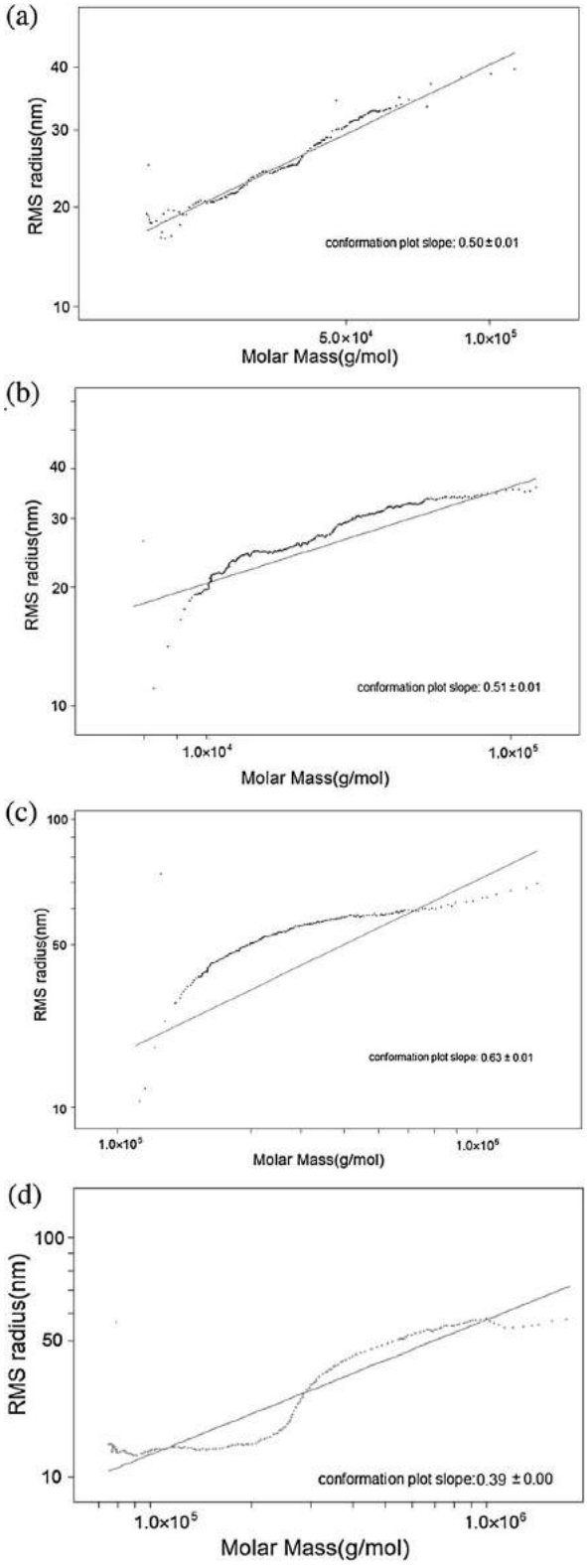

Fig. 2. Chromatograms of chain conformation in aqueous of three sulfated polysaccharide: (a) fCS-*Ib*; (b) fCS-*Pg*;(c)fuc-*Pg*; (d) fuc-*Ib*.

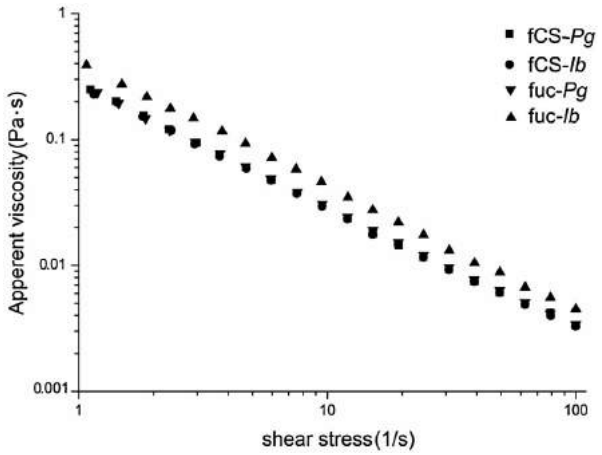

Fig. 3. (a) Flow behavior of fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* at concentration of 3% (w/v).

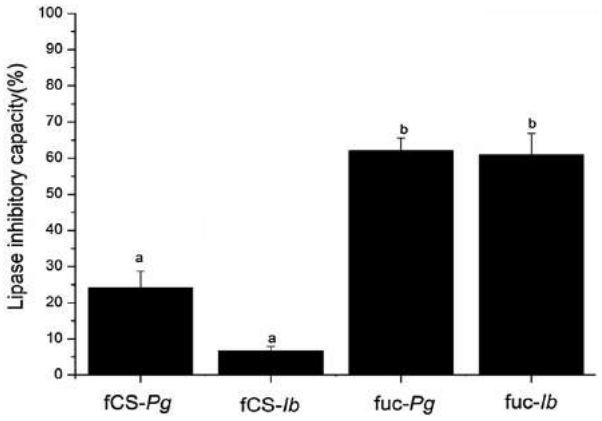

Fig. 4. Lipase inhibitor capacities of fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib*.

order caused by HFD. FCS-*Pg*, fCS-*Ib* and fuc-*Pg* could reduce TG, TC, and LDL-C levels and increase HDL-C level. Although these three sulfated polysaccharides showed different influence on the lipid profile, all of these polysaccharides could alleviate dyslipidemia caused by HFD and inhibit hyperlipidemia. However, fuc-*Ib* showed almost no effect on lipid disorder except lowering TG level.

3.3.3. Effects of sulfated polysaccharides on the indices of liver function

In this section, liver weight and the levels of GOT, GPT, TBA and AKP in serum of HFD-fed rats were studied to assess the effects of fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* on liver function.

GOT, GPT, TBA and AKP are synthesized mainly in the liver. When there is serious necrosis or damage in liver, these substances are released into the blood, thus, lifting their levels in the serum. Abnormally high levels of these substances can serve as serum biomarkers of hepatotoxicity (Ozer, Ratner, Shaw, Bailey, & Schomaker, 2008; Thomson et al., 2009). GOP, GPT, TBA, and AKP in the serum of HFD rats were much higher than normal group, increasing 71.3%, 72.3%, 168.5% and 59.4%, respectively (Table 2). All these indices indicate that HFD causes damage to liver function. Among the four sulfated polysaccharides treated groups, fCS-*Ib* shows a remarkably strong effect in decreasing GOP, GPT, TBA and AKP levels in the serum (no significant difference compare with normal group, $P > 0.05$). FCS-*Pg* and fuc-*Pg* had selective effects on reducing these substances.

Table 2
Effects of fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* on the indices of liver function.

Groups	GOP (IU/L)	GPT (IU/L)	TBA (μmol/L)	AKP (king units/100 mL)
Normal	53.3± 2.3*	35.7 ± 8.9*	33.0 ± 4.8*	424.1 ± 58.6*
HFD	91.3 ± 8.5#	61.5 ± 3.4#	88.6 ± 9.4#	676.1 ± 26.4#
simvastatin	58.8 ± 12.7*#	41.6 ± 10.4*	57.0 ± 11.8*#	537.1 ± 74.4
fCS-*Pg*	64.6 ± 14.3*#	38.9 ± 13.4*	61.5 ± 6.9*#	564.9 ± 97.7#
fCS-*Ib*	48.4 ± 11.3*	31.1 ± 3.91*	36.5 ± 7.0*	531.8 ± 90.3*
fuc-*Pg*	59.1 ± 10.7*#	38.4 ± 11.7*	56.5 ± 7.9*#	574.3 ± 114.9#
fuc-*Ib*	72.6 ± 18.7#	61.7 ± 8.4#	74.8 ± 8.8*#	686.4 ± 75.0#

*P < 0.05: compared to hyperlipidemia control group. # P < 0.05: compared to normal control group. Data are presented as mean ± SD, n = 8.

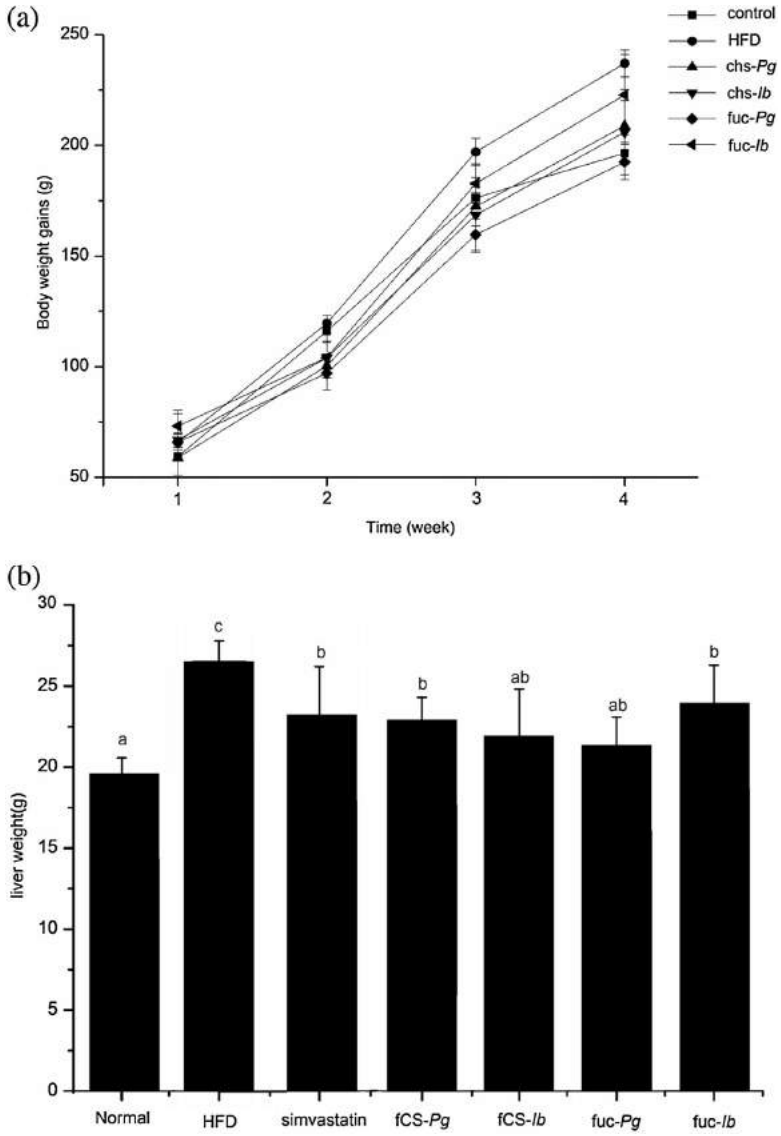

Fig. 5. (a) The body weight gains of normal group, HFD group, fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* group per week compared with initial weight. Body weight was measured every week; (b) Effects of simvastatin, fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib* on liver weight. All data are expressed as mean ± SD (n = 8).

While for fuc-*Ib*, the levels of GOP, GPT and AKP were no significant difference from HFD group (P > 0.05), and only TBA was decreased. These results indicated that fCS-*Ib* could efficiently protecting liver from HFD. Meanwhile, fCS-*Pg* and fuc-*Pg* showed weaker effects than fCS-*Ib*, while fuc-*Ib* almost no effects on protecting liver.

3.4. Further discussion for structure-function relationship of sulfated polysaccharides

Based on our former study (Chen et al., 2012; Hu et al., 2015; Wu et al., 2013), we demonstrated that all of these polysaccharides have specific repeating oligosaccharide units (Fig. 6), which is

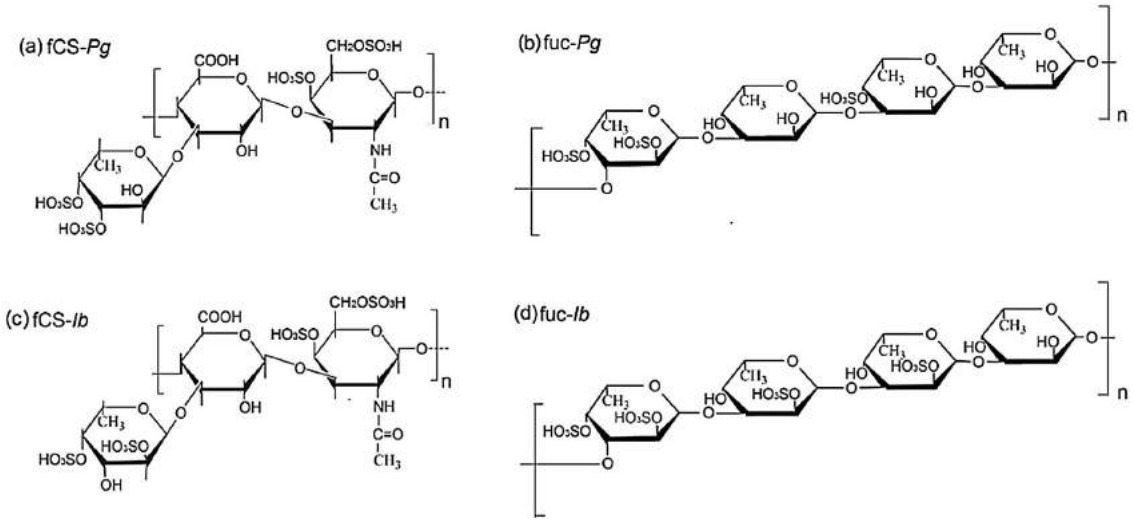

Fig. 6. The main structures of sulfated polysaccharides from sea cucumbers: fCS-*Pg*(a), fuc-*Pg*(b), fCS-*Ib*(c), and fuc-*Ib*(d).

useful for exploring the relationship between structure and function. Both fCS-*Pg* and fCS-*Ib* have a standard chondroitin sulfate E (CSE) backbone and fucose branches. The difference between fCS-*Pg* and fCS-*Ib* is the sulfation pattern of their fucose branches: fCS-*Ib* has two, 4-*O*-sulfo fucose branches while fCS-*Pg* has three, 4-*O*-sulfo fucose branches. Fuc-*Pg* and fuc-*Ib* are fucoidans with straight-chain structures. Fuc-*Pg* is sulfated with either two, 4-*O*-sulfo groups or 4-*O*-sulfo groups, while fuc-*Ib* is dominated by 2-*O*-sulfo esters. All the four species sulfated polysaccharides have well defined repeating structures. The different structures cause these polysaccharides to have different anticoagulant activities (Chen et al., 2013).

Combining the results of AFM and SEC-MALLS-RI, the chain conformation of fCS-*Pg*, fCS-*Ib* and fuc-*Pg* can be assigned to random coil conformation with polysaccharide chain outstretched, while fuc-*Ib* forms into spherical conformation in solution. Both fuc-*Pg* and fuc-*Ib* are linear polysaccharides without side branch, and fucose is the only monosaccharide they possess. The only difference of their structure is their sulfation patterns, which results in their conformational difference. Sulfates can provide negative charge and then affect the polysaccharides charge, solubility, and conformation (Ferreira et al., 2015; Wang & Zhang, 2009). There are sulfate groups present in every fucose unit of fuc-*Ib*, while there are fucose units without sulfate groups present in fuc-*Pg*. It has been reported that introduction of sulfate groups into polysaccharide backbone led to the intramolecular repulsions between the negatively charged sulfate groups and enhanced the steric hindrance between the polymer chains, leading to their relatively expanded chain conformation (Wang & Zhang, 2009). However in our studies, fuc-*Ib* with more sulfate groups forms a sphere in water and NaCl solution. This finding indicates it is sulfation pattern, not only sulfation contents, that determines the spatial structure of linear polysaccharides. Fucoidans have been reported to be random coil or spherical conformation in the former studies (Xu, Xue, Chang, Wang, & Jiang, 2016; You, Chen, Hyeonyong, & Booyong, 2010). Unlike fucoidan, fCS is a type of heteropolysaccharide with more complicated structure. Remarkably, fCS-*Pg* and fCS-*Ib* have fucose branches. This structural character may make these polysaccharides more outstretched and promote a random linear conformation in solution, even though the sulfation pattern of fCS-*Pg* and fCS-*Ib* is different. FCS from sea cucumber *Apostichopus japonicas* has been reported to be a random coil chain conformation in solution, which is consistent with our results (Xu, Xue, Chang, Feng, & Wang, 2016). Our findings indicate that the chain conformation of sulfated polysaccharides are affected by a combination of primary structural characteristics, including side branch and sulfation pattern. In addition, chain conformation endows polysaccharides different biological and physicochemical properties. The special chain conformation also makes fuc-*Ib* exhibit a high apparent viscosity.

Effects of sulfated polysaccharides on pancreatic lipase activity were evaluated *in vitro*. It has been reported that the lipase inhibitory capacity of polysaccharides are related to arrangement of anionic groups, molar concentration of monosaccharide groups along the polysaccharide backbone, and the molecular weight of polysaccharides (Edashige, Murakami, & Tsujita, 2008; Hu, Li, Decker, & Mcclements, 2010). According to our results, fucoidans with higher molecular weight have more powerful effects on inhibiting pancreatic lipase activity than fCSs. In the present work, the discrepancy of lipase inhibitory capacity may be mainly attributed to different molecular weight of sulfated polysaccharides. FCS-*Pg* and fCS-*Ib* were smaller than 10 kDa. However, fuc-*Pg* and fuc-*Ib* were far more than 30 kDa. Polysaccharides with larger molecular weight have been reported to have stronger lipase inhibitory capacity (Edashige et al., 2008).

Hypolipidemic activity was investigated *in vivo* using SD rats fed on high-fat diet (HFD). Under the same dose, of 40 mg/kg, these four sulfated polysaccharides exert different effects on alleviating lipid disorders caused by HFD. fCS-*Pg*, fCS-*Ib* and fuc-*Pg* have powerful effect on reducing weight gains, reversing lipid profile, and promoting liver function. Fuc-*Ib* showed a limited effect in reducing weight gains and TG level. It is interesting when considering that only fuc-*Ib* exhibit spherical conformation, whereas other three polysaccharides showed a linear conformation. Our findings indicate that macromolecular sulfated polysaccharides may exert different effects on regulating lipid *in vivo*. A spherical conformation for sulfated polysaccharides with more active groups embeded into the chain center may weaken the chance of contacting with lipids regulation related protein, thus resulted in reducing hypolipidemic activity. The other three polysaccharides with linear chain conformation might make more active site contact. Polysaccharides with higher chain stiffness are reported to have higher antitumor activities (Wang & Zhang, 2009), though fuc-*Ib* has more powerful capacity of lipase inhibition *in vitro* than fCS. When in animal

experiments, fuc-*Ib* has limited effects on SD rats feeding on HFD. This may be attributed to the low dose of fuc-*Ib*. In another perspective, the differences observed in the *in vivo* experiments are more convincing than those observed in the *in vitro* experiments.

4. Conclusion

The chain conformation of four sulfated polysaccharides from sea cucumbers, fCS-*Pg*, fCS-*Ib*, fuc-*Pg*, and fuc-*Ib*, were studied using AFM and SEC-MALLS. Their hypolipidemic effects were evaluated by both animal experiment and pancreatic lipase activity inhibition. Our results indicate that the chain conformation of these four sulfated polysaccharides are determined by their fucose branch and sulfation pattern. In addition, chain conformation endows polysaccharides different physicochemical properties. In consequence, these four sulfated polysaccharides exhibit different hypolipidemic effects on HFD rats. Sulfated polysaccharides with an outstretched linear conformation show greater activity. In this study, a relationship between primary structure and chain conformation was demonstrated and the hypolipidemic activity of sulfated polysaccharides with regular structure is determined by their chain conformation. Our study indicated that chain conformation may be a new perspective to explore structure-function relationship of sulfated polysaccharides, not only restricted to primary structure.

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.2017.05.063.

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