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Chain conformational and physicochemical properties of fucoidans from sea cucumber

Xiaoqi Xu, Changhu Xue, Yaoguang Chang*, Jun Wang, Kunhao Jiang

College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, China

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ABSTRACT

Although fucoidans from sea cucumber (SC-FUCs) have been proven as potential bioactive polysaccharides and functional food ingridents, their chain conformation and physicochemical properties were still poorly understood. This study investigated the chain conformation of fucoidans from sea cucumber *Acaudina molpadioides* (*Am*-FUC), *Isostichopus badionotus* (*Ib*-FUC) and *Apostichopus japonicus* (*Aj*-FUC), of which primary structure has been recently clarified. Chain conformation parameters demonstrated that studied SC-FUCs adopted random coil conformation in 150 mM NaCl solution (pH 7.4). Based on the worm-like cylinder model and atomic force microscopy, the chain stiffness of SC-FUCs was further evaluated as *Am*-FUC ≈ *Ib*-FUC > *Aj*-FUC. It was suggested that the existence of branch structure increased the chain flexibility, while sulfated pattern exerted limited influence. SC-FUCs demonstrated shear-thinning rheological behavior and negative charge. *Am*-FUC possessed a higher thermostability than *Ib*-FUC and *Aj*-FUC. These results have important implications for understanding the molecular characteristics of SC-FUCs, which could facilitate their further application.

1. Introduction

Fucoidan, a polysaccharide with substantial percentages of L-fucose and sulfate groups (Vo & Kim, 2013), is one of the major components of the sea cucumber body wall. Various biological activities of sea cucumber fucoidans (SC-FUCs) have been established, including anticoagulation (Ribeiro, Vieira, Mourão, & Mulloy, 1994), osteoclastogenesis inhibition (Kariya et al., 2004), neural stem/progenitor cell proliferation (Zhang, Song et al., 2010), protection against ethanol-induced gastric ulcer (Wang et al., 2012), anti-adipogenic effect (Xu et al., 2014), insulin resistance modification (Hu et al., 2014; Xu et al., 2015) and intestinal mucositis preventative effect (Zuo et al., 2015). SC-FUCs could also be utilized as functional ingredients in food industry. For instance, it has been recently verified that fucoidan from *Acaudina molpadioides* (*Am*-FUC) is potential in frabicating multilayer nano-emulsion as a coating agent (Chang & McClements, 2015).

Recently, many researchers have focused on polysaccharide chain conformation, which could substantively affect their bioactivities (Blaschek, Käsbauer, Kraus, & Franz, 1992; Falch, Espevik, Ryan, & Stokke, 2000; Kojima, Tabata, Itoh, & Yanaki, 1986). It has been reported that the anti-tumor activity of triple helical lentinan ((1 → 3)-β-D-glucan) significantly decreases with the transition from triple helical structure to a single chain conformation (Maeda, Watanabe, Chihara, & Rokutanda, 1988; Surenjav, Zhang, Xu, Zhang, & Zeng, 2006; Zhang, Li, Xu, & Zeng, 2005). Furthermore, polysaccharide chain conformation is also related to its physicochemical properties. The κ-carrageenan in random coil exhibits liquid-like viscoelastic behavior; by increasing the concentration of KCl in solution, the rigidity of κ-carrageenan molecule chains increases and they aggregates toward a dimensional network, which is consistent with the rheological behavior changing to a self-supporting gel (Núñez-Santiago, Tecante, Garnier, & Doublier, 2011). Knowledge of the chain conformation and physicochemical propeties is essential for successful interpretation of functions of a polysaccharide, which are ciritical for its application. Althogh functions and primary structure of SC-FUCs from various species of sea cucumber have been reported, few studies involved their chain conformations and physicochemical properties.

Fucoidans from sea cucumbers *Isostichopus badionotus* (*Ib*-FUC) and *Am*-FUC are clarified as linear polysaccharides consisting of α 1 → 3 linked tetrafucose repeating units with different sulfation patterns (Fig. 1) (Chen et al., 2012; Yu et al., 2014; Yu et al., 2013). Fucoidan from *Apostichopus japonicus* (*Aj*-FUC) (Yu et al., 2015) is a branched polysaccharide with pentasaccharide repeating units as its major structural component. Chain conformation of polysaccha-

* Corresponding author.
E-mail address: changyg@ouc.edu.cn (Y. Chang).

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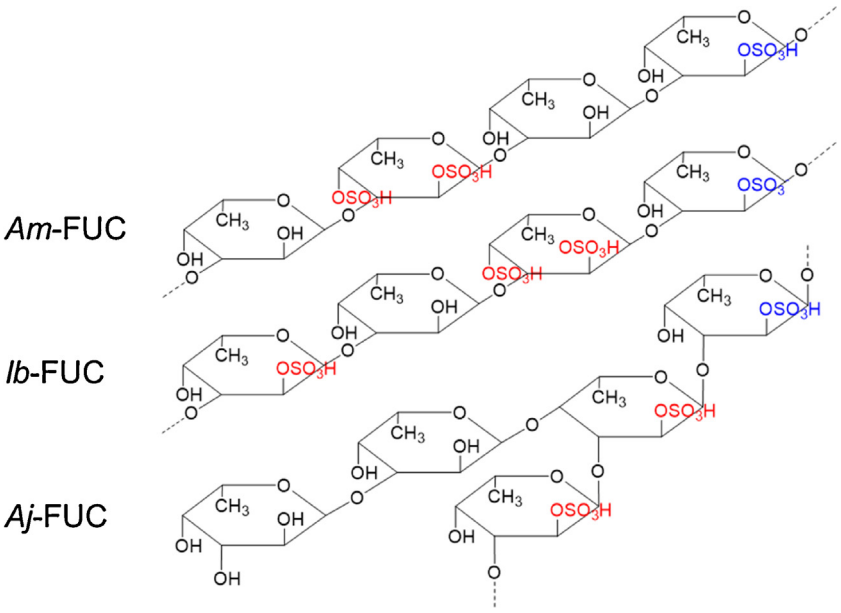

Fig. 1. Primary structure of *Am*-FUC, *Ib*-FUC and *Aj*-FUC.

ride is greatly influenced by its primary structural charactreristics, such as individual linkage patterns, branched structure, branching degrees (Wei et al., 2016). Since *Am*-FUC, *Ib*-FUC and *Aj*-FUC exihit subtle distinctions in primary structure, they might be suitable natural materials for elucidating the relationship between primary structure and chain conformation, *i.e*, a higher level structure of SC-FUCs.

The aim of this study was set to investigate the chain conformation and physicochemical properties of SC-FUCs, employing *Am*-FUC and *Ib*-FUC as the representitives of linear SC-FUCs and *Aj*-FUC as the representative of branched SC-FUCs. The chain conformation was studied by using high performance size exclusion chromatography combined with multi-angle laser scattering, differential refractive index detector and viscometer (HPSEC-MALLS-Visc-RI), and further interpreted based on the worm-like cylinder model. The physicochemical properties including rheological character, electrical property, and thermal stability were also clarified.

2. Experimental methods

2.1. Materials

The wild sea cucumber *A. molpadioide* was harvested from the South China Sea (Fujian, China) in May 2009 and dried using a hot air dryer. Dry body wall of *I. badionotus* and *A. japonicus* were purchased from a local market (Qingdao, China) in April 2015. In addition to the morphological identification, the species of sea cucumber sample was also confirmed by phylogenetic analysis utilizing the cytochrome oxidase subunit I gene (COI) as a housekeeping genetic marker (Arndt, Marquez, Lambert, & Smith, 1996; Byrne, Rowe, & Uthicke, 2010). All chemicals and reagents used were of analytical grade.

2.2. Extraction and purification of fucoidans from sea cucumbers

The SC-FUC samples were prepared according to Chang's method (Chang et al., 2010) with small modification. Briefly, the milled dried body wall of the sea cucumber was hydrolyzed by papain and precipitated by cetylpyridinium chloride to obtain crude sulfated polysaccharides. The crude polysaccharides were applied onto an Express-Ion D column (Whatman, USA) using the AKTA UPC 100 (GE, USA) system and eluted with 0–2.0 M linear gradient NaCl. The carbohydrate content in the eluate was measured by the H_2SO_4–phenol method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1951), and the monosaccharide compostion of fractions was analyzed by an HPLC method (Strydom, 1994). The fraction containing fucoidan (eluating NaCl concentration 1.2–1.5 M) was collected and further purified on a gel filtration column (HiPrep 26/60 Sephacryl S-500 HR column, GE Healthcare, USA), which was eluted with 0.2 M NH_4HCO_3. The finally purified fucoidan was collected, dialyzed (cut-off 8000 Da), lyophilized, and used in the following analysis.

2.3. HPSEC-MALLS-Visc-RI detection

The HPSEC-MALLS-Visc-RI system consisted of a pump (Agilent 1200, Agilent Technologies, Santa Clara, USA), a SEC column (OHpak SB-806 HQ, 8.0 mm × 300 mm, Shodex, Japan), an Optilab T-rEX differential refractive index detector (Wyatt Technology Co., Santa Barbara, USA), a Viscostar-II viscometer (Wyatt Technology Co., Santa Barbara, USA), and a multi-angle laser light scattering (MALLS) instrument equipped with He-Ne laser (λ = 633 nm) (DAWN DSP, Wyatt Technology Co., Santa Barbara, USA). A 10 mM phosphate buffer solution (pH 7.4) containing 150 mM NaCl (PBS), which is widely used in various bioactivity assays, was employed as the eluent. The flow rate and the column temperature were set at 0.4 mL min^{-1} and 25 °C respectively. *Am*-FUC, *Ib*-FUC and *Aj*-FUC were fully dispersed in PBS at a concentration of 2 mg mL^{-1} and filtered through a 0.2 mm syringe membrane (PTFE, Puradisc 13-mm Syringe Filters, Whatman, Little Chalfont, UK). Then, 50 μL sample solution was injected into the instrument. The instrumental software (ASTRA 6.1.2) was utilized for data acquisition and analysis. The weight-average molecular weight (M_w) and *z*-average radius of gyration (R_g) were calculated using the Zimm method. The specific refractive index increments (*dn/dc*) of SC-FUCs in PBS were determined using the refractive index detector (Optilab T-rEX) at 633 nm and 25 °C. Samples were dissolved in increasing concentration from 0.5 to 2.0 mg mL^{-1} to determine the increment slope. The *dn/dc* of *Am*-FUC, *Ib*-FUC and *Aj*-FUC was determined as 0.1106 ± 0.0007 mL g^{-1}, 0.1084 ± 0.0005 mL g^{-1} and 0.1147 ± 0.0012 mL g^{-1}, respectivly.