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Anticoagulant and FGF/FGFR signal activating activities of the heparinoid propylene glycol alginate sodium sulfate and its oligosaccharides

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ABSTRACT

Propylene glycol alginate sodium sulfate (PSS), prepared by chemical sulfation of alginate, has been used for treating cardiovascular diseases in China for nearly 30 years. In the current study, the PSS was hydrolyzed partially by an environment-friendly solid phase acid degradation method, and then separated by using a Bio-Gel P6 chromatographic column. Thirteen PSS oligosaccharide fractions were obtained and characterized by ESI-MS. The results of different coagulation assays showed that a high molecular weight and a higher degree of sulfation were essential for the anticoagulant activity of the PSS because the PSS oligosaccharides exhibited no detectable anticoagulant activity. In contrast, not only PSS but also certain oligosaccharides showed significant activities in stimulation of FGF1, 2, 7, 8, 9 or 10 induced cell proliferation in FGFR1c-expressing BaF3 cells. Such properties made the PSS and its oligosaccharides promising compounds in the regulation of FGF-dependent development, treatment of cancer, and wound healing processes.

1. Introduction

With the discovery that the anticoagulant activity of heparin resides in a unique pentasaccharide sequence and its anti-thrombin III (AT-III) binding properties (Cross, Sobel, McAdory, & Harris, 1996; Lindahl, Bäckström, Thunberg, & Leder, 1980), there has been an increasing interest in the preparation of heparin oligosaccharides and investigation of the relationship between oligosaccharide sequences and their biological activities. Studies have shown that heparin oligosaccharides decreases anticoagulant activity (Ma et al., 2002). However, the decreased anticoagulant activity is desirable since the oligosaccharides can thus be used as anti-tumor and anti-allergic compounds (Ahmed, Smith, & Abraham, 2014).

Propylene glycol alginate sodium sulfate (PSS), prepared by chemical sulfation of low-molecular-weight alginate extracted from brown algae, has been commonly used as a drug for the treatment of cardiovascular diseases in China for nearly 30 years (Zeng et al., 2014). As a structural analog of the natural blood anticoagulant heparin, PSS shows various heparinoid activities such as anticoagulation and reduction of blood cholesterol/glucose levels (Li, Su, & Guan, 2012). However, there are limited reports about the preparation and structure–activity studies of the PSS oligosaccharides.

It is a well-established fact that sulfated polysaccharides not only play important roles in the regulation of blood coagulation, but also interact with hundreds of signaling molecules (Zhang, 2010), such as growth factors, chemokines, and cytokines. Sulfated polysaccharides were reported to facilitate many important signaling transduction pathways, including fibroblast growth factor (FGF) and FGF receptor (FGFR) pathway (Itoh & Ornitz, 2011). In the presence of glycosaminoglycans (GAGs), FGFs stably bind FGFRs and leads to the formation of FGF-FGFR-GAG dimers which enable the cytoplasmic kinase domains to transphosphorylate one another and become activated (Pellegrini, 2001; Presta et al., 2005). FGFR activation results in the stimulation of various signal transduction cascades that have been implicated in multiple aspects of vertebrate and invertebrate embryonic development (Martin et al., 2006), tumor growth (Fearon, Gould, & Grose, 2013; Pan, Deng, & Zhou, 2012), wound healing (Lee et al., 2012), and physiology.

In this study, the PSS oligosaccharides were prepared and their anticoagulant and FGF/FGFR signal activating activities were

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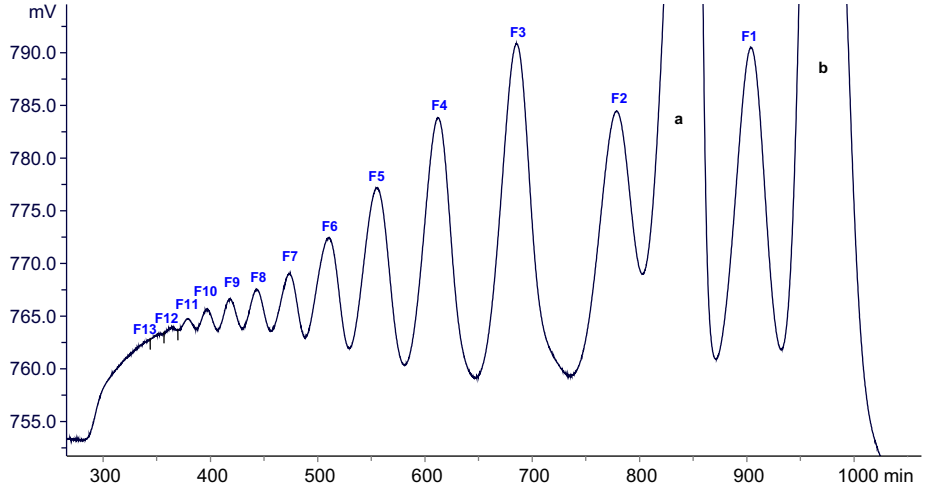

Fig. 1. Separation chromatogram of PSS oligosaccharides on a Bio-Gel P6 column (F1 to F13 – thirteen fractions of PSS oligosaccharide; a – sodium sulfate; b – sodium chloride).

evaluated. The PSS and its oligosaccharides showed promising applications in the regulation of FGF-dependent development, treatment of cancer, and wound healing processes.

2. Materials and methods

2.1. Materials

PSS was provided by Chiatai Haier Pharmaceutical Co., Ltd (Qingdao, China). Bio-Gel P6 (fine, 45–90 μm) was purchased from Bio-Rad company (California, USA). Anticoagulation determination kits were purchased from MD Pacific Technology Co., Ltd (Shanghai, China). Also, 96-well tissue culture plates (TP92696) were purchased from MIDSCI, USA. Resazurin (R7017) was purchased from Sigma–Aldrich, USA. FGFR1c-expressing BaF3 cell line was provided by Professor David D. Ornitz of Washington University in St. Louis (USA). All the FGFs used in the current study were purchased from Gold Biotechnology Inc., St. Louis (USA). All other chemicals and solvents used were of analytical grade unless otherwise specified.

2.2. General analysis

The content and uronic acid composition (M/G ratio) was determined by pre-column derivatization HPLC method reported in a previous literature (Wu et al., 2014). The molecular weight (Mw) of PSS was determined by high performance gel permeation chromatography (HPGPC) method (Zhao et al., 2007). The sulfate content was determined using ion chromatography method (Wang, Raptis, & Yeh, 2004) and calculated by the weight of SO_3Na/total weight. High performance thin layer chromatography (HPTLC) analysis was carried out on a silica gel HPTLC plate. The developing solvent is n-butanol/formic acid/water (4:6:1, v/v/v) and the chromogenic agent is aniline-diphenylamine (Dreisewerd, Kölbl, Peter-Katalinić, Berkenkamp, & Pohlentz, 2006; Hu et al., 2013).

2.3. Preparation of PSS oligosaccharides

PSS oligosaccharides were prepared by a solid phase acid method as previously described (Zhao et al., 2008). Briefly, 2 g of PSS were dissolved in 100 mL distilled water and 2 g of solid phase acid (732# resin, H^+ form) was added. Depolymerization was carried out at 100 °C in an oil bath while refluxing and stirring gently for 6 h. The hydrolysate was neutralized to pH 7.0 and filtered by a 0.8 μm cellulose membrane to remove resin, then freeze-dried to obtain PSS oligosaccharides mixture.

2.4. Separation of PSS oligosaccharides

The AKTA UPC100 purification system (GE, USA) was used for the separation of PSS oligosaccharides. Lyophilized mixture of PSS oligosaccharides (0.25 g) was dissolved in 2 mL of 0.1 mol/L NH_4HCO_3 and applied to a Bio-Gel P6 column (2.6 × 100 cm). The column was eluted with 0.1 mol/L NH_4HCO_3 at a flow-rate of 0.5 mL/min (Hu et al., 2013). The eluents were detected by an online refractive index detector (RID) and collected by a fraction collector (6 mL/tube). Fractions containing the same oligosaccharide were pooled and the NH_4HCO_3 was removed by speed vacuum at 40 °C.

2.5. ESI-MS analysis

Electrospray ionization mass spectrometry (ESI-MS) analysis of PSS oligosaccharides was carried out on an electrospray mass spectroscopy instruments (LTQ Orbitrap XL, Thermo Fisher Scientific, USA) in negative-ion mode. Each fraction of PSS oligosaccharides was dissolved in acetonitrile/1% NH_4CO_3 (1:1, v/v) and was delivered by a syringe pump at a flow rate of 5 μL/min (Zhang et al., 2006). Solvent volatilization temperature and capillary temperature were 275 °C, the sheath flow gas flow rate was 8 arb.

2.6. Anticoagulant activity of PSS and the oligosaccharides

The anticoagulant activities of PSS and the oligosaccharides were determined *in vitro* by the sheep plasma-based clotting assays. The experiments were carried out strictly according to the instruction of the manufacturer. For activated partial thromboplastin time (APTT) assay, sheep plasma (90 μL) was mixed with PSS or oligosaccharide aqueous solution (10 μL) at different concentrations, then 100 μL APTT reagent was added to the mixture and incubated at 37 °C for 5 min. After that, pre-warmed (37 °C) $CaCl_2$ solution was added to the mixture quickly and the clot formation time was measured by an automated blood coagulation instrument. For thrombin time (TT) assay, the plasma (180 μL) and the PSS or oligosaccharide sample solution (20 μL) were mixed and incubated at 37 °C for 3 min, the clotting time was recorded after adding 100 μL TT reagent. For prothrombin time (PT) assay, the plasma (90 μL) and sample solution (10 μL) were mixed and incubated at 37 °C for 3 min. Following the addition of 200 μL PT reagent