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Regioselective sulfation of *Artemisia sphaerocephala* polysaccharide: Characterization of chemical structure

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ABSTRACT

The biological activities of sulfated polysaccharides are related to the substitution positions of functional groups. In this study, regioselective sulfation of *Artemisia sphaerocephala* polysaccharides (S_{RS}ASP) was prepared by using triphenylchloromethane (TrCl) as protecting precursor. FT-IR spectra and X-ray photoelectron spectroscopy (XPS) showed that $-SO^{3-}$ group (S^{6+}, high binding energy of 168.7 eV) was widely present in sulfated polysaccharides. ^{13}C NMR spectroscopy showed that C-2 and C-3 substitution was occurred but not fully sulfation. Meanwhile, C-6 substituted signals near 65 ppm were not observed. The degree of substitution varied from 0.44 to 0.63 in S_{RS}ASP which could be attributed to the low reactivity at secondary hydroxyl. Monosaccharide composition result showed a decrease in the ratio of mannose/glucose, indicating the change of chemical composition in sulfated polysaccharides. In size-exclusion chromatograph analysis, a decrease in molecular weight and broadening of molecular weight distribution of sulfated polysaccharides was also observed. It could be attributed to the hydrolysis of polysaccharide in the sulfated reaction.

1. Introduction

Sulfated polysaccharides are one group of the most attractive components in carbohydrate. There has been growing interest in polysaccharide sulfate over the last decades due to their wide spectrum of biological activities and potential in biomedical applications (Liu et al., 2009). The biological activities of sulfated polysaccharides are dependent on the structure features, such as the compositions, average molecular weight, degree of substituting groups, substitution positions and chain conformation (Wu, Li, Cui, Eskin, & Goff, 2012; Yi et al., 2012; Tao, Zhang, Yan, & Wu, 2007).

It is interesting and imperative to synthesize polysaccharides with different functional group distributions. Therefore, new synthetic methods have to be developed for regionselective modification. Several publications have shown the interest in modified polysaccharides with different substitution positions by using regioselective synthesis, including glycosaminoglycan (Redouan et al., 2012), curdlan (Zhang & Edgar, 2014), amylase (Shen, Ikai, & Okamoto, 2010) and carrageenans (Araújo et al., 2013). Nishimura et al. report that the chitin and chitosan derivative using selective sulfation at C-2 and/or C-3 affords greater anti-AIDS activities than that by the known C-6 sulfated derivative in vitro. Whereas, C-6 sulfated derivative strongly inhibits blood coagulation (Nishimura et al., 1998). Regioselective silylation of starch is reported by Petzold et al. Thexyldimethylchlorosilane is employed as bulky silylating agent in the reaction system of *N*-methylpyrrolidone (NMP)/ammonia (Petzold, Einfeldt, Gunther, Stein, & Klemm, 2001). These results suggest that the biological activities of sulfated polysaccharides depend significantly on the positions of functional groups rather than on the degree of substitution (DS).

Sulfation of polysaccharide is known to occur predominantly at primary hydroxyl group. Regioselective synthesis of polysaccharide derivatives is a challenge due to the low reactivity of secondary hydroxyl groups at C-2 and C-3. The precursors for the regioselective protection of hydroxyl group at C-6 are typically bulky reagents such as triphenylmethyl chloride (TrCl) and thexyldimethylsilyl chloride. Bulky reagents react preferentially

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Table 1
Regioselective synthesis of sulfated ASP.

Samples	Triphenylmethylation			Sulfation reaction		Deprotection	
	TrCl (mg)	Yield (mg)	DS$_{Tr}$ [a]	Yield[b] (mg)	DS$_{S}$ [c]	Yield (mg)[d]	DS$_{S}$ [e]
ASP	–	–	–	–	–	–	nd [f]
SASP	–	–	–	596.0	0.93	–	–
ASP$_{Tr1}$	200	536.6	0.22	494.6	0.88	410.4	0.61
ASP$_{Tr2}$	500	578.9	0.34	451.8	0.79	380.9	0.57
ASP$_{Tr3}$	1000	640.4	0.52	473.7	0.83	446.1	0.63
ASP$_{Tr4}$	2000	761.8	0.89	460.3	0.71	400.9	0.58
ASP$_{Tr5}$	2500	728.6	0.83	450.4	0.67	420.6	0.44

[a] DS$_{Tr}$ was calculated from the weight of triphenylmethanol.
[b] Yield was calculated from ASP$_{Tr}$.
[c] DS was calculated by elemental analysis after sulfation reaction.
[d] Yield was calculated from SASP$_{Tr}$.
[e] DS was calculated by elemental analysis after deprotection.
[f] Not detected.

with the primary hydroxyl group at C-6 on the polysaccharide backbone rather than with the secondary hydroxyl groups at other positions. Furthermore, the bulky groups can be removed after functionalization of the hydroxyl groups at C-2/3 to afford modified polysaccharides with different substitution patterns (Fox, Li, Xu, & Edgar, 2011; Binette & Gagnon, 2007; Xu, Voiges, Elder, Mischnick, & Edgar, 2012; Kurita, Matsumura, Takahara, Hatta, & Shimojoh, 2011). Our earlier research also suggested that sulfation at C-2 and C-3 of guar gum increased the scavenging abilities of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical (Wang et al., 2013).

In our previous studies, chemical structure, solution conformation and antioxidant activities of sulfated derivatives of *Artemisia sphaerocephala* polysaccharide (SASP) with high DS were reported (Wang et al., 2015, 2014). The goal of our investigations is to explore systematically the relations between substitution positions and biological activities of sulfated ASP using regioselective synthesis. The present paper describes our attempts to achieve regioselective sulfation of ASP using triphenylchloromethane (TrCl) as protective group. The chemical structure of non-C6 substituted SASP (S$_{RS}$ASP) was characterized by FT-IR, X-ray photoelectron spectroscopy (XPS), monosaccharide composition analysis and ^{13}C NMR spectroscopy. Size-exclusion chromatograph combined with multi-angle laser photometer (SEC-LLS) was employed to determine the average molecular weight and polydispersity. The purpose of the current research is to probe into the effectiveness of regioselective sulfation using bulky reagent.

2. Materials and methods

2.1. Materials

Chlorosulfuric acid (CSA), pyridine (Py) and formamide were purchased from Jingchun Industry Co., Ltd. (Shanghai, China). Triphenylchloromethane (TrCl), L-arabinose, D-xylose, D-lyxose, D-mannose, D-glucose and D-galactose were purchased from Sigma Chemical Co. (USA). All chemicals used in this study were of analytical reagent or better grade without further purified.

ASP was extracted and purified according to our previous studies. The monosaccharide composition of ASP was L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose (the molar ratio of 1:4.2:45.9:9.7:11.4) with the weight average molecular weight (M_W) of 7.348×10^4 Da (Wang et al., 2009).

2.2. Regioselective synthesis of sulfated ASP

2.2.1. Triphenylmethylation of ASP

ASP (500 mg) was dissolved in 30 mL anhydrous formamide and 5 mL Py at room temperature. The polysaccharide solution was stirred for 30 min. Then, TrCl was added (Table 1) and stirred at 80 °C for 12 h under a nitrogen atmosphere. After that, ethanol (100 mL) was added in the solution to precipitate the product. 6-*O*-trityl-ASP (ASP$_{Tr1}$–ASP$_{Tr5}$) with different DS of triphenylmethyl (DS$_{Tr}$) was collected after lyophilizing and kept in dryness box. The DS$_{Tr}$ was calculated from the weight of triphenylmethanol obtained by hydrolysis (Kurita et al., 2011).

2.2.2. Synthesis of sulfating reagent

CSA was added dropwise in anhydrous Py filled in a three-necked flask, under agitating and cooling in ice water bath. The ratio of CSA/Py was 2:1. All determinations were completed in 40 min (Wang et al., 2010a).

2.2.3. Sulfation reaction of ASP$_{Tr}$

ASP$_{Tr}$ (500 mg) was added to anhydrous formamide (20 mL) at room temperature with stirred for 30 min. Then sulfating reagent was added at 60 °C. After 3 h, the mixture was neutralized (pH value was adjusted to 7–8 with 2 mol/L NaOH), precipitated, dialyzed (molecular weight cutoff 8–12 kDa) and lyophilized to give sulfated ASP$_{Tr}$ (SASP$_{Tr1}$–SASP$_{Tr5}$). For comparison, sulfation reaction without TrCl was also studied (SASP).

2.2.4. Deprotection

SASP$_{Tr}$ (500 mg) was added to dichloroacetic acid (15 mL) portionwise over a period of 10 min for dissolution to remove the Tr group. The solution was stirred at room temperature for 1 h. Then, ice water (100 mL) was poured slowly. The product was collected by filtration, washed with anhydrous ethanol and lyophilized to give deprotected SASP$_{Tr}$ (S$_{RS}$ASP$_1$–S$_{RS}$ASP$_5$).

Element Analysis (Euro Vector EA3000, Leeman) was employed to determine the sulfur contents of SASP$_{Tr}$ and S$_{RS}$ASP. The degree of substitution (DS$_S$) was calculated according to the equation:

$$DS = \frac{1.62 \times S\%}{32 - 1.02 \times S\%} \tag{1}$$

2.3. Characterization of sulfated polysaccharides

2.3.1. FT-IR analysis

FT-IR spectra were recorded on a Thermo Nicolet iS10 fourier transform infrared spectrometer with KBr pellets. Sixteen scans at a resolution of 4 cm^{-1} were recorded in the wavenumber range of 400–4000 cm^{-1} and referenced against air.

2.3.2. X-ray photoelectron spectroscopy (XPS)

XPS results were recorded with the PHI-5702 system for chemical composition analysis of sulfated samples using a monochromatic Al-Ka source (1486.7 eV) for excitation. The pow-