Catalytic synthesis of sulfated polysaccharides. II: Comparative studies of solution conformation and antioxidant activities

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ABSTRACT

Sulfated derivatives of *Artemisia sphaerocephala* polysaccharide (ASP) with high degree of substitution (DS) were synthesized using 4-dimethylaminopyridine (DMAP)/dimethylcyclohexylcarbodiimide (DCC) as catalyst. Size exclusion chromatography combined with multi-angle laser light scattering (SEC-LLS) results showed a decrease in fractal dimension (d_f) values of sulfated ASP (SASP). Compared to ASP and SASP with low DS (0.51–1.01), SASP$_{cata2}$ exhibited an internal structure between rigid rod and random coil with a DS of 1.24. DS had greater influence on its conformation in aqueous solution. Circular dichroism (CD), methylene blue (MB) and Congo red (CR) spectrophotometric method and atomic force microscopy (AFM) results confirmed that the degradation of ASP and $-SO_3H$ groups improved significantly the stiffness of the chains due to the electrostatic effect. Furthermore, antioxidant experiments revealed that high DS could enhance the scavenging activities of radicals and reducing power of SASP in vitro. The extended chain conformation was beneficial to enhance the biological activity of sulfated polysaccharides.

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1. Introduction

It is well known that the activities of polysaccharides are depended upon the chemical structure, such as monosaccharide composition, average molecular weight, degree and pattern of substituting groups and solution conformation (Chen, Xu, Zhang, & Kennedy, 2009; Tao, Zhang, Yan, & Wu, 2007; Wu, Li, Cui, Eskin, & Goff, 2012). Water soluble polysaccharides exhibit different chain conformation in solutions, such as random coil, single helix, double helix, triple helix, aggregate and sphere-like conformation (Zhang, Li, Wang, Zhang, & Peter, 2011). It has been shown by Zhang et al. that the relationship between biological activity and solution conformation are complicated due to the structure varieties (Cui et al., 2008; Wang, Zhang, Zhang, & Ding, 2009; Xu, Zhang, et al., 2009; Xu, Chen, Wang, & Zhang, 2009). The conformation of polysaccharides in solutions can be investigated according to the theory of dilute polymer solutions. Considerable attention has been paid to the solution properties of natural polysaccharides (lentinan and schizophyllan) and the driving force for the conformation transition, such as hydrogen bonding, solvent, temperature and pH value

(Tao et al., 2007; Yang and Zhang, 2009; Yi et al., 2012; Zhang et al., 2011). It is reported that triple helix lentinan exhibited a relatively high inhibition ratio against the proliferation of tumor cells, whereas the bioactivity of its single flexible chains almost disappeared (Tao et al., 2007). The results of Chen et al. also indicate that the extended chain conformation were beneficial to enhance the anti-tumor activity, as a result of the increasing of the interaction between polysaccharide and immune system (Chen, Xu, Zhang, & Zeng, 2009). Therefore, a basic understanding of the molecular conformation is essential for successful interpretation of the bioactivities mechanism of water soluble polysaccharides.

Among the natural and synthetic biopolymers, sulfated polysaccharides represent a class of macromolecules of particular interest. The activity of sulfated polysaccharides also depends on structural parameters such as the degree of substitution (DS), the average molecular mass, the position of sulfation and solution conformation (Liu et al., 2009; Wang, Zhang, Zhang, Zhang, & Li, 2009; Wang et al., 2013). It is also shown that the structure of chemical modified polysaccharides is depended on the reaction conditions (Qi et al., 2005). This suggests that upon interaction with reaction reagent, sulfated polysaccharide under-goes complex conformational changes that subsequently modify the biological activities. However, as one of the important factors influencing the activities of sulfated polysaccharide, the studies on the relation between chemical structure and solution conformation have not received much attention in recent articles. Little information on the

conformation of modified polysaccharides with different DS and average molecular mass in aqueous solution has been reported (Wang, Zhang, Zhang, et al., 2009). The main reason is that the building blocks of sulfated polysaccharides are rather complex toward the harsh conditions of sulfation and that some glycosidic linkages can be hydrolyzed relatively easily. Therefore, it is very important to clarify the structures and solution properties of sulfated polysaccharide.

It is the purpose of the present investigation to explore systematically the solution conformation of sulfated polysaccharide with different structure features. In our previous studies, sulfated derivatives of *Artemisia sphaerocephala* polysaccharide (SASP) with high DS were prepared using 4-dimethylaminopyridine (DMAP)/dimethylcyclohexylcarbodiimide (DCC) as catalyst (data is being considered for publication). The chemical structure of SASP was analyzed by FT-IR, X-ray photoelectron spectroscopy (XPS) and ^{13}C NMR spectroscopy. The DS (0.51–1.28) and weight average molecular mass (0.626–4.771 × 10^4 Da) of SASP were listed in Table 1. *A. sphaerocephala* polysaccharide (ASP) and SASP had the same monosaccharide composition (Ara:Xyl:Man:Glc:Gal) but different molar ratio (1:4.21:45.9:9.74:11.43 and 1:5.43:12.41:14.62:6.85, respectively).

The present study is focused on the mechanisms governing the relations between chain conformation and structure features. For that purpose, size-exclusion chromatograph combined with multi-angle laser photometer (SEC-LLS), circular dichroism (CD), methylene blue (MB) and Congo red (CR) spectrophotometric method and atomic force microscopy (AFM) are employed to determine the chain conformation in solution and molecular morphology. Moreover, we also evaluate the effect of DS on the antioxidant activities of SASP in vitro and offer theoretical evidence for the synthesis of sulfate polysaccharides with different structure features. Antioxidant properties are assayed in terms of antioxidant activities in vitro, by testing the scavenging abilities on superoxide radicals, hydroxyl radicals, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and reducing power.

2. Materials and methods

2.1. Preparation of SASP

ASP was extracted and purified according to our previous studies (Wang, Zhang, & Wang, et al., 2009; Wang et al., 2010). ASP (500 mg) was suspended in anhydrous formamide (30 mL) at room temperature with stirring for 30 min, and the sulfating reagents (chlorosulfonic acid/pyridine) were added dropwise. Then, DMAP and DCC were added. For comparison, sulfation reactions without DMAP and DCC were also studied. The mixture was stirred for 3 h at different temperatures (Table 1). After the reaction, the mixture was cooled to room temperature and the pH value was adjusted to 7–8 with 2 mol/L NaOH solution. The mixtures were precipitated with ethanol (95%), washed, redissolved in water, and then dialyzed (molecular weight cutoff 8–12 kDa) against tap water for 48 h and distilled water for 24 h to remove pyridine, salt and potential degradation products. Sulfated ASP (SASP$_1$–SASP$_5$ for samples without catalyst and SASP$_{cata1}$–SASP$_{cata7}$ for samples using catalyst) with different DS (Table 1) were collected. The samples were stored in a dry box under room temperature till use.

2.2. Molecular mass and size characterization

The most commonly employed method for conformation studies are dynamic and static light scattering technique (Sumihito, Tomoko, & Takashi, 2001; Tomoko & Takashi, 2003; Yi et al., 2012).

Table 1
Molecular properties of ASP and its sulfated derivatives.

Samples	CSA:Pyr	DMAP/DCC (mg)	Temperature (°C)	DS	$M_W \times 10^4$	Relation equation	d_f	Conformation
ASP	–	–	–	–[a]	7.348	$\langle S^2 \rangle_z^{1/2} = 0.203 M_w^{0.35\pm0.027}$	2.86	Between hard sphere and random coil (fully swollen)
SASP$_1$	1:4	–	40	0.51	3.836	$\langle S^2 \rangle_z^{1/2} = 0.309 M_w^{0.36\pm0.034}$	2.77	Between hard sphere and random coil (fully swollen)
SASP$_2$	1:2	–	40	0.69	3.203	$\langle S^2 \rangle_z^{1/2} = 0.309 M_w^{0.36\pm0.034}$	2.63	Between hard sphere and random coil (fully swollen)
SASP$_3$	1:1	–	40	0.82	2.110	$\langle S^2 \rangle_z^{1/2} = 0.309 M_w^{0.36\pm0.034}$	2.08	Between hard sphere and random coil (not swollen)
SASP$_4$	2:1	–	40	0.87	0.645	$\langle S^2 \rangle_z^{1/2} = 0.309 M_w^{0.36\pm0.034}$	2.38	Between hard sphere and random coil (not swollen)
SASP$_5$	2:1	–/200	40	0.91	1.353	$\langle S^2 \rangle_z^{1/2} = 0.309 M_w^{0.36\pm0.034}$	1.96	Random coil
SASP$_{cata1}$	2:1	5/200	40	0.83	2.382	$\langle S^2 \rangle_z^{1/2} = 0.29 M_w^{0.37\pm0.048}$	2.70	Between hard sphere and random coil (fully swollen)
SASP$_{cata2}$	2:1	10/200	40	1.24	3.103	$\langle S^2 \rangle_z^{1/2} = -1.99 M_w^{0.88\pm0.55}$	1.13	Between rigid rod and random coil
SASP$_{cata3}$	2:1	20/200	40	1.01	1.292	$\langle S^2 \rangle_z^{1/2} = -0.056 M_w^{0.43\pm0.017}$	2.32	Between hard sphere and random coil (not swollen)
SASP$_{cata4}$	2:1	50/200	40	0.98	2.002	$\langle S^2 \rangle_z^{1/2} = -0.023 M_w^{0.31\pm0.005}$	3.22	Hard sphere
SASP$_{cata5}$	2:1	100/200	40	0.82	4.771	$\langle S^2 \rangle_z^{1/2} = 0.218 M_w^{0.37\pm0.029}$	2.70	Between hard sphere and random coil (fully swollen)
SASP$_{cata6}$	2:1	10/200	60	1.28	3.097	$\langle S^2 \rangle_z^{1/2} = 0.26 M_w^{0.28\pm0.036}$	3.57	Hard sphere
SASP$_{cata7}$	2:1	10/200	90	1.13	0.626	$\langle S^2 \rangle_z^{1/2} = 0.723 M_w^{0.15\pm0.018}$	6.67	Hard sphere

[a] Not detected.