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Structural characterization of a homogalacturonan from *Capparis spinosa L.* fruits and anti-complement activity of its sulfated derivative

Huijun Wang · Hongwei Wang · Songshan Shi · Jinyou Duan · Shunchun Wang

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Abstract A water-soluble polysaccharide CSPS-2B-2 with a molecular mass of 8.8 kDa, was obtained from the fruits of *Capparis spinosa L.* Chemical and NMR spectral analysis verified CSPS-2B-2 was a linear poly-(1-4)-α-D-galactopyranosyluronic acid in which 12.9±0.4 % of carboxyl groups existed as methyl ester and 2.6±0.1 % of D-Gal*p*A residues were acetylated. A sulfated derivative Sul-2B-2 with a sulfation degree of 0.88±0.02 was prepared *via* the substitution of C-2 and/or C-3 of Gal*p*A residues in CSPS-2B-2. Bioassay on the complement and coagulation system demonstrated that Sul-2B-2 (CH_{50}: 3.5±0.2 μg/mL) had a stronger inhibitory effect on the activation of complement system through the classic pathway than that of heparin (CH_{50}: 8.9±0.3 μg/mL). Interestingly, Sul-2B-2 at low dose even middle dose (for example 52 μg/mL) had no effect on coagulation system, which was totally different from heparin. Thus, our observation indicated that Sul-2B-2 was more efficient than heparin in inhibiting the activation of the complement system through classical pathway and exhibiting a relatively less anti-coagulant activity. These results suggested that the sulfated derivative Sul-2B-2 prepared from the homogalacturonan in the fruits of *Capparis spinosa L*, might be a promising drug candidate in case of necessary therapeutic complement inhibition.

Keywords Anti-complement · Homogalacturonan · Sulfated derivative · Inhibition hemolysis · Anti-coagulant

Abbreviations

HG	homogalacturonan
HPGPC	high performance gel permeation chromatography
NMR	nuclear magnetic resonance spectroscopy
CP	classical pathway
AP	alternative pathway
MBL	mannan binding lectin pathway
ARDS	acute respiratory distress syndrome
TFA	trifluoroacetic acid
TLC	thin-layer chromatography
GPC	gel-permeation chromatography
GC-MS	gas chromatograph-mass spectrometer
GC-FID	gas chromatograph-flame ionization detector
CMC	1-cyclohexyl-3-(2-morpholinoethyl) carbodiimidemetho-p-toluenesulphonate
DMSO	dimethyl sulfoxide
IR	infrared absorption spectroscopy
NHS	normal human serum
PPP	platelet poor plasma
BBS	barbital buffered saline
RT	recalcification time
TT	thrombin time
DS	degree of substitution
CH_{50}	50 % complement hemolysis

J. Duan
College of Science, Northwest A&F University,
Yangling 712100 Shaanxi, China

H. Wang · H. Wang · S. Shi · S. Wang
Institute of Chinese Materia Medica,
Shanghai University of Traditional Chinese Medicine,
Shanghai 201203, China

S. Wang (✉)
Shanghai University of Traditional Chinese Medicine,
1200 Cailun Road,
Shanghai 201203, China
e-mail: shunchunwang@126.com

Introduction

The complement system plays an important role in host immune defense against infection through the clearance of antigen-antibody complexes from the bloodstream. It can be activated by a cascade signaling pathways including the classical pathway (CP), alternative pathway (AP), and the mannan binding lectin pathway (MBL). However, its excessive activation might result in various diseases such as system lupus erythematosus, rheumatoid arthritis, and acute respiratory distress syndrome (ARDS), *etc.* [1].

In 1929, Ecker and Gross found the anti-complement effect of heparin, a highly sulfated copolymer composed of uronic acid and glucosamine. Now heparin is recognized as one of the best characterized molecule that shows profound anti-complement activity. However, at the concentration sufficient to inhibit the complement system, heparin will demonstrate undesirable anti-coagulant side effect, which limits its application in the treatment of anti-complementary diseases [2]. In recent years, many studies focus on Chinese Traditional Medicine to search for the anti-complementary compounds with little anti-coagulant activity. These active molecules include polysaccharides, flavones [3], and triterpenes *etc.* For instance, fucan [4], a sulfated polysaccharide extracted from brown seaweed has certain resistance to excessive complement activity and exhibits moderate anti-coagulant activity. Again, Zhu *et al.* [1] isolated and characterized an anti-complementary protein-bound polysaccharide EWD-1 with limited anti-coagulant activity from the stem barks of *Eucommia ulmoides*.

Capparis spinosa L. belongs to the family Capparidaceae and is widespread over the dry regions in west or central Asia. In Chinese Traditional Medicine, it has long been used to treat rheumatism and poultice [5, 6]. In this study, a pectic substance designated CSPS-2B-2, was isolated from the water extract of this plant fruits. The structural analysis reveals it is a partially methyl-esterified homogalacturonan (HG). A sulfated HG derivative Sul-2B-2 was prepared, characterized and its anti-complementary and anti-coagulant were described.

Materials and methods

Materials

The dried fruits of *Capparis spinosa L.* were collected in Urumqi of Xinjiang Province in China by Prof. C.H. Wang, and identified by Prof. L.H. Wu at Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai. DEAE-cellulose column was purchased from Amersham Biosciences. Superdex-200 HR was obtained from GE Healthcare Bio-Sciences. Trifluoroacetic acid (TFA) and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimidemetho-p-toluenesulfonate (CMC) were purchased from Fluka. All other reagents were of analytical grade as available. Sheep erythrocytes were collected in Alsevers' solution. Rabbit erythrocytes were obtained from the heart of New Zealand white rabbits.

General methods

IR spectra (Nujol mulls) were recorded on a Perkin Elmer 2000 FTIR spectrometer. HPGPC was performed with an Agilent 1100 instrument fitted with the GPC software, using KS-805 and KS-804 connection in series (Shodex CO.) as the Chromatographic Colum. ^{13}C-NMR spectra were obtained in the FT mode with a Bruker AM 400 spectrometer at room temperature. Gas chromatography (GC) was performed with a Thermo TRACE GC apparatus equipped with a DB-624 column (length: 30 m×0.32 mm; thickness of liquid phase: 1.8 μm) for determination of methyl or acetyl groups in the esterification. GC-MS was carried out with a Thermo TRACE DSQ apparatus equipped with a TR-5 column (length: 60 m×0.25 mm; thickness of liquid phase: 0.25 μm). Uronic acid content was determined by the *m*-hydroxydiphenyl method [7, 8].

Isolation and purification of polysaccharides

CSPS-2B-2 was isolated according to the scheme shown in Fig. 1. The dried fruits of *Capparis spinosa L.* were defatted with 95 % EtOH for 24 h and were extracted with boiling water three times (3 h for each time). The aqueous extract was concentrated and precipitated with 4 Vol. of 95 % EtOH to obtain the crude polysaccharide CSPS. CSPS was fractionated on a column of DEAE-cellulose column (50 cm×5 cm, Cl^- form), and eluted stepwise with distilled water, 0.1, 0.2, and 0.5 M NaCl solutions. The eluted curve as shown in Fig. 2a. The fraction eluted with 0.2 M NaCl (CSPS-2) was further separated on a column of Superdex-200 HR (100 cm× 2.6 cm) and eluted with water to give two major fractions CSPS-2A (70–110 min) and CSPS-2B (160–215 min), respectively, as shown in Fig. 2b. The second fraction CSPS-2B was further purified on a column of Superdex-200 HR to yield CSPS-2B-2.

Homogeneity and molecular weight [9]

Measurements were performed by HPGPC on two columns of KS-805 and KS-804 in series. The column was calibrated by standard Dextrans with known molecular weight (P-5, P-10, P-20, P-50, P-100, P-200, P-400, P-800, Shodex CO.). The column temperature was kept at 40.0±0.1 °C. 0.2 M NaCl was used as eluant and the flow rate was kept at 0.8 mL/min.

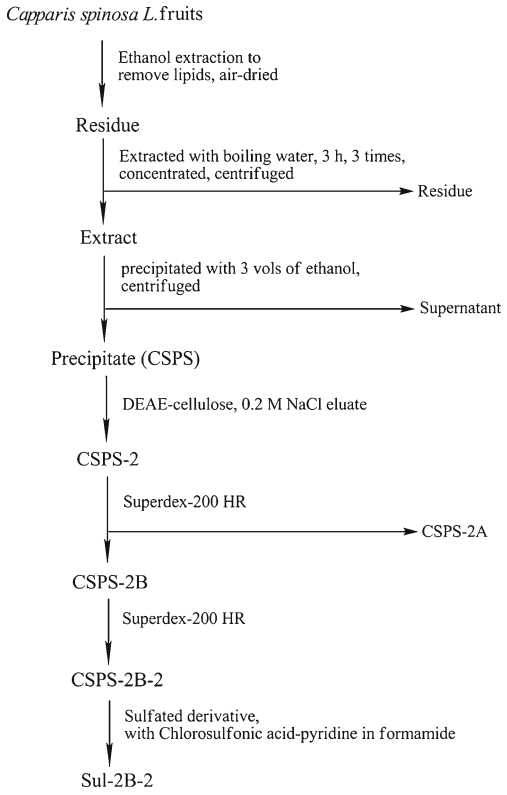

Fig. 1 Scheme of isolation of polysaccharide CSPS-2B-2 from *Capparis spinosa L.* and its Sulfated Derivative Sul-2B-2. The dried fruits of *Capparis spinosa L.*were defatted with EtOH and extracted with boiling water, the aqueous extract was precipitated to give CSPS. After successive separation by DEAE-cellulose anion-exchange and Superdex-200 HR gel permeation chromatographic steps, it afforded the carbohydrate fraction CSPS-2B-2. A sulfated derivative Sul-2B-2 was prepared by CSPS-2B-2 reacting with chlorosulfonic acid-pyridine in formamide

All samples were prepared as 2 mg/mL solutions, and 20 μL aliquot was injected for each run.

Composition analysis and determination of esterification (O-methyl and O-acetyl groups)

(a) Sugar composition: The acidic polysaccharide was hydrolyzed with 2 M TFA at 121 °C for 2 h. After repeated evaporation with MeOH to remove TFA, the residue was dissolved in 0.1 mL of distilled water and analyzed on a PEI-cellulose plate (E. Merck), developed with 5:5:1:3 (v/v) EtOAc-pyridine-HOAc-water. The plate was visualized by spraying with *o*-phthalic acid reagent and heating at 100 °C for 5 min [10]. The remaining hydrolysate was reduced by $NaBH_4$ for 3 h at room temperature and acetylated with Ac_2O for 1 h at 100 °C. The resulting alditol acetates were subjected to GC-MS analysis. Reduction of carboxyl groups was carried out with CMC-$NaBH_4$ for three times as described [11, 12]. The reduced polysaccharide (2B-2re, 2 mg) was hydrolyzed and converted into alditol acetates as described above.

The configuration of reduced polysaccharide (2B-2re) was determined by comparing with standard D-galactose and L-galactose using the double hydrolysis/reductive amination method of Cases *et al.* [13].

(b) Determination of esterification: Internal calibration curves for quantification of methanol and acetic acid were prepared by adding the standards in the concentration range between 20 and 100 μg/mL for methanol and acetic acid, and isopropanol as the internal standard. The saponification of the polysaccharide (CSPS-2B-2) occurred by the addition of 0.8 mL of 2 M NaOH at 25 °C, After 1 h, the reaction was terminated by the addition of 0.8 mL of 2 M HCl, and pH was adjusted to 2.0, as described [14]. The GC oven temperature program used for methanol was: stay at 65 °C (held for 8 min) and program used for acetic acid was: stay at 100 °C (held for 8 min). The flow rate of the carrier gas (N_2) was set at 2 mL/min.

Methylation analysis

The reduced polysaccharide (2B-2re, 2 mg) was methylated three times using the method developed by Needs [15] with minor modifications. After drying with P_2O_5 overnight, 2B-2re was stirred in 2.0 mL of DMSO, and 200 mg of sodium hydroxide powder was gradually added. After 30 min, methyl iodide (1.5 mL) was added slowly for methylation. The sample was kept in darkness for 1 h before 2.0 mL of distilled water was used to decompose the remained methyl iodide. The methylated polysaccharide was extracted by chloroform, washed by distilled water for three times, hydrolysed and acetylated for sugar composition described, and analysed by GC-MS.

Preparation of sulfated polysaccharide

CSPS-2B-2 was derived by the chlorosulfonic acid-pyridine method [16] at 0 °C for 2 h and then room temperature for 3 h. The molar ratio between chlorosulfonic acid and pyridine was 1:1. After the reaction, the mixture was adjusted to pH 7-8, dialyzed first against saturated $NaHCO_3$ overnight, then against deionized water extensively. The retentate was lyophilized to give Sul-2B-2.

NMR analysis

CSPS-2B-2 and Sul-2B-2 (50 mg) were deuterium-exchanged and dissolved in 0.4 mL of D_2O (99.8 % D), respectively. Samples were deuterium exchanged by

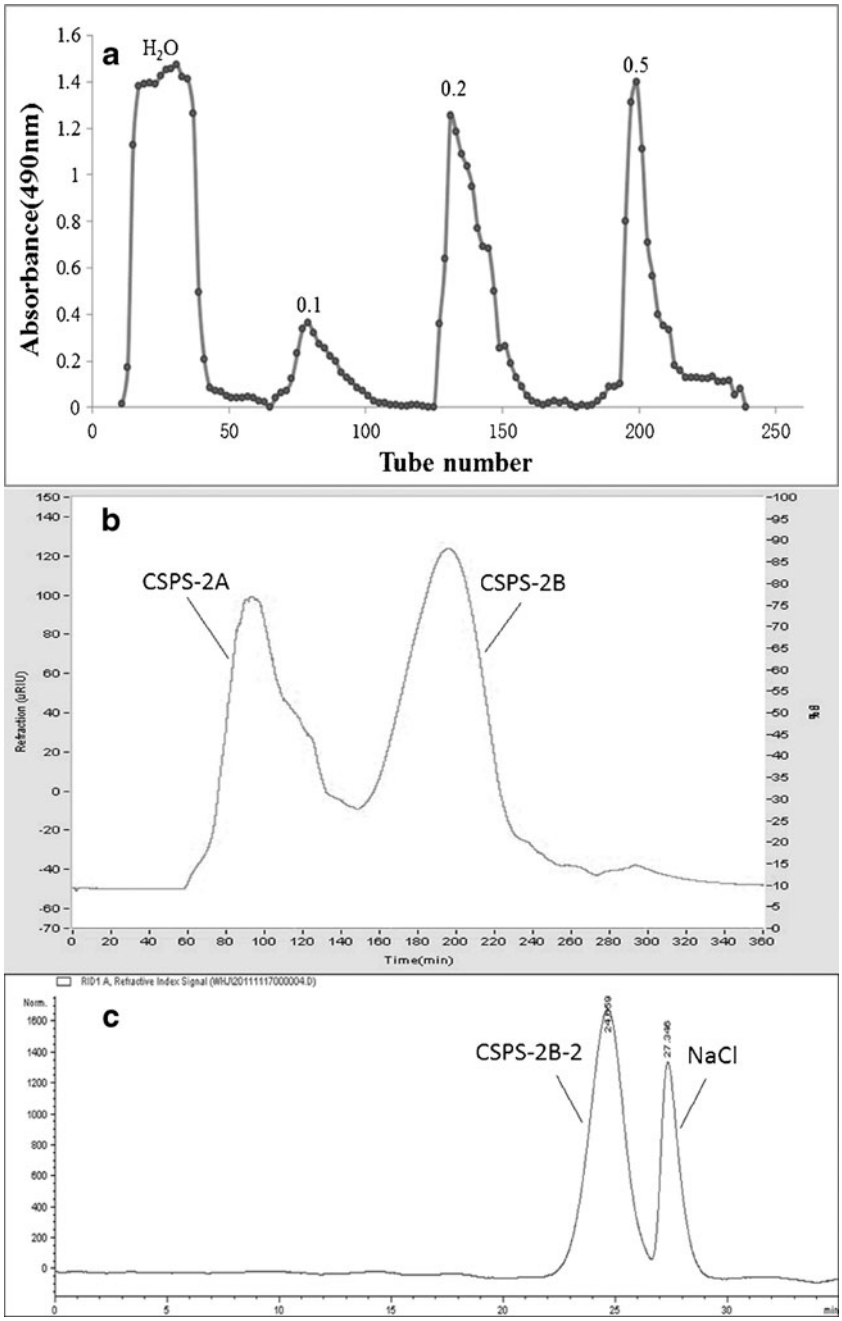

Fig. 2 Profile of CSPS in DEAE-Cellulose, CSPS-2 in superdex-200 HR, and CSPS-2B-2 in HPGPC. **a** Profile of crude CSPS in DEAE. The sample was analyzed by a DEAE-cellulose column (50 cm×5 cm) and eluted with water and different gradients NaCl at 1.0 mL/min. **b** Profile of CSPS-2 in superdex-200 HR. The sample was analyzed by a Superdex-200 HR column (100 cm×2.6 cm) and eluted with water at a flow rate of 1.0 mL/min. **c** Profile of CSPS-2B-2 in HPGPC. The sample was analyzed by a shodex series-connected KS-805 and KS-804 gel filtration column (30 cm×7.8 mm) and eluted with 0.2 M NaCl at 0.8 mL/min

lyophilization twice with D_2O, and chemical shifts are expressed in ppm using acetone as the internal standard at 31.50 ppm.

Anti-complementary activity through the classical pathway

As described [17], sensitized erythrocytes (EAs) were prepared by incubation of sheep erythrocytes (4.0×10^8 cells/mL) with equal volumes of rabbit anti-sheep erythrocyte antibody in barbital buffered saline (BBS). Samples and heparin (used as positive control) were dissolved in BBS. Normal human serum (NHS)was used as the complement source. The 1:10 diluted NHS was chosen to give submaximal lysis in the absence of complement inhibitors. Various dilutions of tested samples (100 μL) were pre-incubated with 100 μL NHS and 200 μL BBS at 37 °C for 10 min. Then 100 μL EA was added and the mixture was incubated at 37 °C for 30 min. The different assay controls were incubated in the same conditions: (1) vehicle control, 100 μL EAs in 500 μL BBS; (2) 100 % lysis, 100 μL EAs in water (500 μL); (3) sample control, 100 μL dilution of each sample in 500 μL BBS. The reaction mixture was centrifuged immediately. Optical density of the supernatant was measured at 405 nm with a spectrophotometer (Wellscan MK3, Lab systems Dragon). Subtract sample control

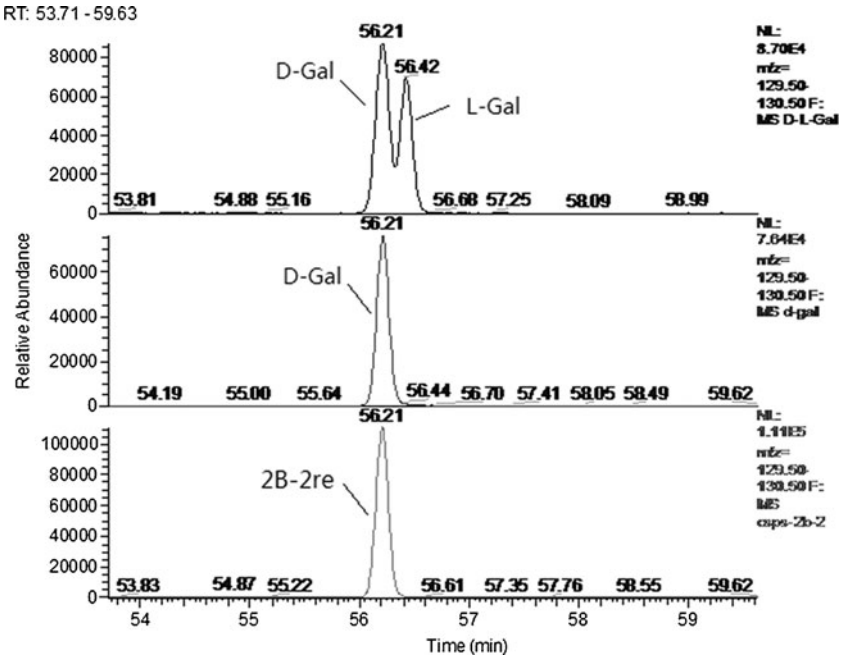

Fig. 3 Configuration of 2B-2re, CSPS-2B-2 was reduced three times to give 2B-2re. The peak time of D-Gal and L-Gal is 56.21 min and 56.42 min, respectively; and the peak time of 2B-2re is 56.21 min. We concluded that the determination of the configuration of 2B-2re had D configuration. Furthermore, it inferred that CSPS-2B-2 was D configuration

absorbance from each value to obtain corrected absorbance and the percent inhibition was calculated.

Influence on recalcification time (RT) and thrombin time (TT) [1]

150 μL of platelet poor plasma (PPP) (obtained from blood of heart of New Zealand white rabbits) was added into 15 μL of BBS alone, Sul-2B-2 at different concentrations of 1040 μg/mL, 52 μg/mL and 5.2 μg/mL, and CSPS-2B-2 at 1040 μg/mL. Heparin at 5.2 μg/mL was used as positive control. The mixtures above were incubated at 37 °C for 5 min, and then 150 μL of 0.025 mol/L $CaCl_2$ solution was added. The time from addition of $CaCl_2$ to clot formation was recorded as plasma recalcification clotting time, briefly called recalcification time (RT). The determination of TT was identical with RT except the substitution of $CaCl_2$ with thrombin.

Results and discussion

Isolation of CSPS-2B-2

CSPS-2B-2 was isolated according to the scheme shown in Fig. 1. The boiling water extract of the dried fruit of *Capparis*

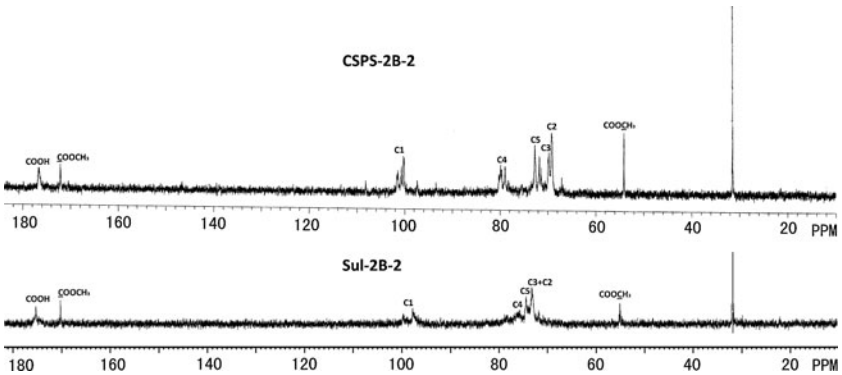

Fig. 4 ^{13}C-NMR spectrum of CSPS-2B-2 and its sulfated derivative Sul-2B-2 obtained at 25 °C in D_2O. For CSPS-2B-2, the low-field signal at 176 ppm corresponded to C-6 of (1-4)-linked Gal*p*A. The signals at 172 ppm and 54 ppm suggested partial Gal*p*A residues might be existed as methyl-esterified. The anomeric signal at 100–101 ppm was assigned to C-1 of (1-4)-linked Gal*p*A, indicating a α-configuration for the Gal*p*A residues. The signal at 73 ppm attributed to C-5 of (1-4)-linked Gal*p*A. For Sul-2B-2, the chemical shift of C-2 and/or C-3 of Gal*p*A was downshifted about 4 ppm from 69.2 ppm and 69.8 ppm to 73.5 ppm, indicating the sulfonyl substitution was exclusively at C-2 and/or C-3 on 1, 4-linked Gal*p*A. Meanwhile, the presence of sulfate group at the C-2 shifts the signal from the anomeric carbon to higher field (from 100.7 ppm in the initial galacturonan to 97.7 ppm in the sulfated galacturonan), and the presence of sulfate group at the C-3 is demonstrated by the shift of the signal of atom C-4 to a higher field (from 79.9 ppm in the initial galacturonan to 76.2 ppm in the sulfated galacturonan). The signal at 74.3 ppm was assigned to C-5 of sulfated galacturonan

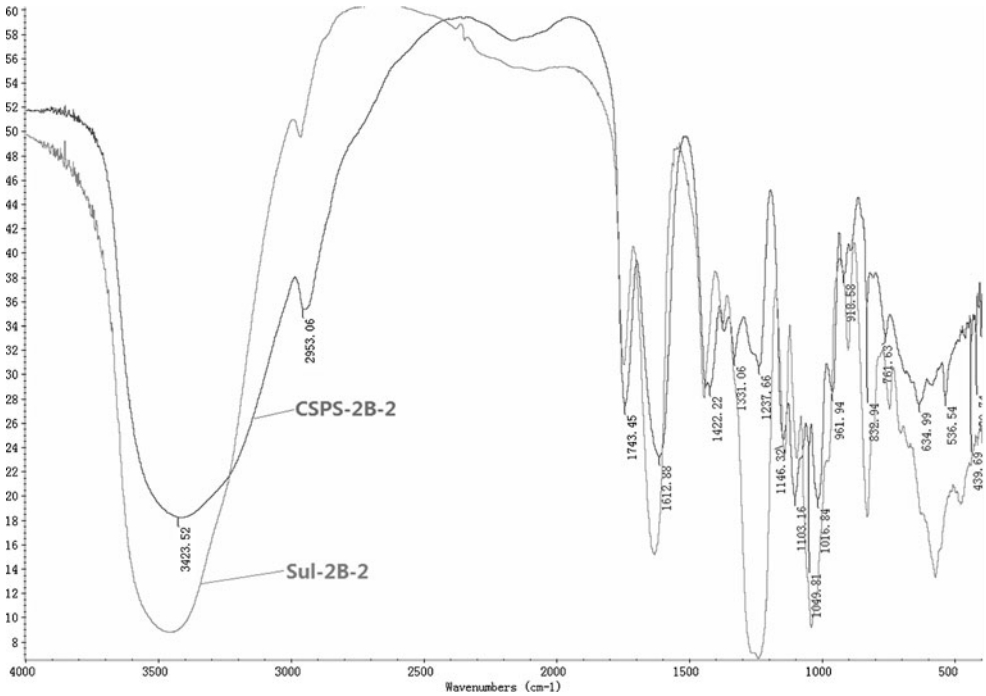

Fig. 5 IR spectrum of CSPS-2B-2 and Sul-2B-2, a homogalacturonan isolated from *Capparis spinosa L.* and its Sulfated Derivative. The IR spectrum of CSPS-2B-2 showed an absorption at 1730 cm^{-1}, which originates from the C=O of uronic acid. The absorptions at 3413, 2942, and 1418 cm^{-1} corresponded to the stretching from O-H, C-H and carboxyl C-O groups, and those at 1145, 1102 and 1015 cm^{-1} to various in-plane C-O vibrations, respectively. The IR spectrum of Sul-2B-2 showed a C-O-S absorption at 830 cm^{-1} and a S=O vibration at 1260 cm^{-1}, indicating that Sul-2B-2 was successfully sulfated

spinosa L. (5.0 kg) was precipitated with 4 vols. EtOH to give the crude polysaccharide CSPS (370 g, yield 7.4 %). After successive separation by DEAE-cellulose anion-exchange chromatography and Superdex-200 HR gel permeation chromatography, it afforded the carbohydrate fraction CSPS-2B-2 (0.759 % of the crude polysaccharide). CSPS-2B-2 showed a single symmetrical peak on high-performance gel permeation chromatography (HPGPC) (Fig. 2c). Its molecular weight was estimated to be 8.8 kDa, in reference to P-series Dextrans. The IR spectrum (Fig. 5) showed the absorption at 1730 cm^{-1} caused by stretching vibration of methyl-esterified carboxyl groups, the absorption at 1610–1619 cm^{-1} caused by the C=O stretching vibration of carboxyl groups, which suggested that CSPS-2B-2 might be a methyl-esterified acidic polysaccharide. The absorptions at 3413, 2942, and 1418 cm^{-1} are corresponding to the stretching from O-H, C-H, and carboxyl C-O groups, and those at 1145, 1102, and 1015 cm^{-1} are assigned to various in-plane C-O vibrations, respectively [18].

Monosaccharide analysis and determination of esterification CSPS-2B-2

Complete hydrolysis followed by TLC analysis showed that CSPS-2B-2 contains mainly uronic acid. The presence of 86.4 % uronic acid was confirmed by the 3-phenylphenol (*m*-hydroxybiphenyl) method [7, 8], with D-galacturonic acid as the standard. CSPS-2B-2 was reduced three times with CMC-sodium borohydride by the Taylor and Conrad method [12] to give the carboxyl-reduced polysaccharide 2B-2re. The GC-MS analysis of 2B-2re verified it contained mainly galactose resulted from galacturonic acid originally existed in CSPS-2B-2. The configuration of galactose in 2B-2re (Fig. 3) was assigned as the D configuration by using the GC-MS method of Cases *et al.* [13], indicating D-galacturonic acid as the major uronic acid in CSPS-2B-2.

As determined, about 12.9±0.4 % of carboxylic groups in galacturonic acid residues of CSPS-2B-2 existed as methyl ester. The degree of *O*-acetylation of the CSPS-2B-2 was determined to be about 2.6±0.1 %. It might be possible that galacturonic acid residues in CSPS-2B-2 are 2-and/or 3-*O*-acetylated, which is commonly observed in the homogalacturonan regions of pectic polysaccharides [9, 19].

Linkage analysis of CSPS-2B-2

2B-2re was methylated three times to give a permethylated product. Its hydrolysate was converted into the partially methylated alditol acetates and analyzed by GC-MS. The result revealed that 2B-2re is a linear (1-4)-linked galactan, indicating that the native CSPS-2B-2 is a linear (1-4)-linked homogalacturonan.

Table 1 Molecular weight and degree of substitution (DS) of CSPS-2B-2 and Its sulfated derivative Sul-2B-2

Sample	Molecular weight (Da)	DS[a]
CSPS-2B-2	8.8×10^3	0
Sul-2B-2	7.0×10^3	0.88±0.02

[a] DS is calculated as $176\times\%W/(96-80\times\%W)$; $\%W$ is content of SO_4^{2-}

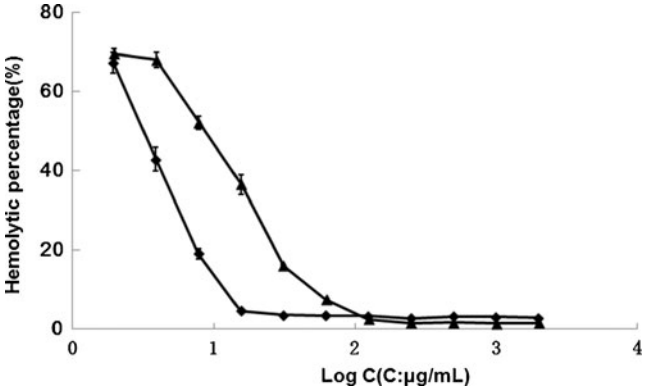

Fig. 6 Inhibition of classical pathway-mediated hemolysis of Sul-2B-2 and Heparin. Inhibition of classical pathway-mediated hemolysis of EAs in 1:10 diluted NHS in the presence of increasing amounts of Sul-2B-2 (♦). Heparin (▲) was used as reference. Results are expressed as hemolytic percentage. The concentrations that resulted in 50 % inhibition (CH_{50}) were 3.5±0.2 μg/mL and 8.9±0.3 μg/mL for Sul-2B-2 and heparin, respectively. The 100 % hemolysis corresponds to the hemolytic activity of HPS in the absence of Sul-2B-2. At a concentration of 15.5 μg/mL, Sul-2B-2 almost abolished all of the hemolytic activity. On the contrary, at the concentration of 2000 μg/mL, CSPS-2B-2 exhibited the complete hemolytic activity. Data are mean from 4 determinations ± S.E.M

NMR analysis of CSPS-2B-2

Acetone was used as the internal standard; the signals in the ^{13}C-NMR spectrum (Fig. 4) of CSPS-2B-2 were assigned, according to references [10, 20]. The signal at 176 ppm was corresponding to C-6 of (1-4)-linked Gal*p*A. The signals at 172 ppm and 54 ppm suggested partial Gal*p*A residues might be existed as methyl-esterified. The anomeric signal at 100–101 ppm attributed to C-1 of (1-4)-linked Gal*p*A, indicating the Gal*p*A residues possess α-configuration. The signal at 78–80 ppm was assigned to C-4, and signals at 69–72 ppm to C-2 and C-3. The signal at 73 ppm was attributed to C-5 of (1-4)-linked Gal*p*A.

Preparation of sulfated derivative from CSPS-2B-2

A sulfated derivative Sul-2B-2 was prepared by CSPS-2B-2 reacting with chlorosulfonic acid-pyridine in formamide. The IR spectrum of the derivative (Fig. 5) showed a C-O-S absorption at 830 cm^{-1} and a S=O vibration at 1260 cm^{-1}, indicating that Sul-2B-2 was successfully sulfated [9]. The degree of substitution (DS) of Sul-2B-2 was 0.88±0.02 (Table 1), as determined by the $BaCl_2$ gelatin method [21]. The ^{13}C-NMR spectrum of Sul-2B-2 (Fig. 4) demonstrates the presence of sulfate groups at the C2 and C3 of Gal*p*A residues, according to reference [22]. The chemical shifts of C-2 and C-3 of Gal*p*A were downshifted about 4 ppm from 69.2 and 69.8 ppm to 73.5 ppm, indicating the sulfation had exclusively happened at C-2 and C-3 on Gal*p*A. Meanwhile, the presence of sulfate group at the C-2 shifts the signal from the anomeric carbon to higher field (from 100.7 ppm in the initial galacturonan to 97.7 ppm in the sulfated galacturonan), and the presence of sulfate group at the C-3 is demonstrated by the shift of the signal of atom C-4 to a higher field (from 79.9 ppm in the initial galacturonan to 76.2 ppm in the sulfated galacturonan). The signal at 74.3 ppm was assigned to C-5 of sulfated galacturonan. Compared with those in CSPS-2B-2, the ^{13}C-NMR signals of methyl ester in Sul-2B-2 became weaker and molecular weight (Table 1) of Sul-2B-2 was slightly reduced, which may be accounted for by the partial destruction of CSPS-2B-2 due to the sulfation with chlorosulfonic acid [22].

Inhibition of Sul-2B-2 on the classical pathway of complement system

The effects of CSPS-2B-2 and Sul-2B-2 on the activation of human complement system through the classical pathway were examined in 1:10 diluted NHS and heparin was used as reference. As shown in Fig. 6, the activation rate for the control was 98.75±1.32 %. Clearly, Sul-2B-2 with a CH_{50} of 3.5±0.2 μg/mL was more potent than heparin with a CH_{50} of 8.9±0.3 μg/mL in inhibiting the activation of the classical pathway. At a concentration of 15.5 μg/mL, Sul-2B-2 almost abolished all of the hemolytic activity of NHS (1:10) (inhibition of 93.60±2.34 %), indicating a strong inhibition effect on the complement activation through the classic pathway. On the contrary, at the concentration of 2000 μg/mL,

Table 2 The Influence of CSPS-2B-2 and Sul-2B-2 on coagulation system (RT: recalcification time, TT: thrombin time, x±SD, n=6)

Sample	Vehicle	Heparin	Sul-2B-2			CSPS-2B-2
		low dose 5.2 μg/mL	high dose 1040 μg/mL	middle dose 52 μg/mL	low dose 5.2 μg/mL	high dose 1040 μg/mL
(RT)S	329.3±14.3	621.3±18.8**	459.6±21.0**	353.5±9.2	322.5±10.6	327.5±3.5
(TT)S	150.3±3.4	225.0±14.6**	174.0±9.9*	147.5±3.5	154.5±3.5	152.5±3.5

*P<0.05, statistically different from the vehicle; **P<0.01, statistically significantly different from the vehicle

CSPS-2B-2 exhibited the complete hemolytic activity, and no inhibitory effect of CSPS-2B-2 was observed.

Influence of Sul-2B-2 on coagulation system

The data (Table 2) of coagulation assays demonstrated that Sul-2B-2 at the concentration of 1040 μg/mL had moderate effect on recalcification time (RT) and thrombin time (TT) compared to that of the vehicle control, whereas CSPS-2B-2 at the same concentration showed little effect. Additionally, anticoagulation activities of Sul-2B-2 at either a low dose (5.2 μg/mL) or a middle dose (52 μg/mL) were much lower than that of heparin at the corresponding doses. For instance, at the concentration of 5.2 μg/mL, heparin demonstrated a remarkable prolonged RT (621.3±18.8 s) and TT (225.0±14.6 s) in contrast to that of vehicle control (RT: 329.3±14.3 s, TT: 150.3±3.4 s). However, Sul-2B-2 at the low dose (5.2 μg/mL) had no significant effect on RT (322.5±10.6 s) and TT (154.5±3.5 s), as well as at a middle dose (52 mg/mL, RT: 353.5±9.2 s, TT: 147.5±3.5 s).

Discussion

Taken together, it was concluded that CSPS-2B-2 was a linear partially esterified poly-(1-4)-α-D-galactopyranosyluronic acid. The degree of methyl-esterification at carboxyl groups and O-acetylation were about 12.9±0.4 % and 2.6±0.1 %, respectively. Sul-2B-2 (DS: 0.88±0.02), the sulfated derivative of CSPS-2B-2, which was sulfated mainly at C-2 and/or C-3 of Gal*p*A, demonstrated a stronger inhibitory effect than that of heparin on the activation of complement system through the classic pathway. In contrast, native CSPS-2B-2 had no inhibition. This observation that the sulfated uronic acid of Sul-2B-2 played a crucial role in the anti-complement activity, was consistent with the concept that sulfate groups appeared to be necessary, although not sufficient to be required for anti-complementary effect of polysaccharides [4].

Heparin (5.2 μg/mL) markedly prolonged recalcification time (RT: 621.3±18.8 s) and thrombin time (TT: 225.0±14.6 s) when compared with vehicle control (RT: 329.3±14.3 s, TT: 150.3±3.4 s). However, high dose of Sul-2B-2 (1024 μg/mL) had mild effect on RT (459.6±21.0 s) and TT (174.0±9.9 s). At the concentration of 5.2 μg/mL and 52 μg/mL, Sul-2B-2 exhibited no effect on RT (s) and TT (s). These data indicated that Sul-2B-2 at low dose even middle dose (for example 52 μg/mL) exhibited no effect on coagulation system. These results indicated that Sul-2B-2 could be considered as a promising candidate of an anti-complement medicine, since this derivative in a certain concentration range is efficient to inhibit the complement system and couldn't induce undesirable anti-coagulant activity.

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