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ScienceDirect
Food Chemistry 110 (2008) 807–813
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Structural characterisation and antimutagenic activity of a novel polysaccharide isolated from *Sepiella maindroni* ink

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Received 25 November 2007; accepted 11 February 2008

Abstract

A new heteropolysaccharide, named as SIP, was isolated from the ink of cuttlefish, *Sepiella maindroni*, by enzymolysis, anion-exchange and gel-permeation chromatography and tested for its antimutagenic activity. It was homogeneous with a molecular weight of 1.13×10^4 Da by HPSEC–MALLS analysis. SIP contained glucuronic acid, mannose, *N*-acetylgalactosamine, and fucose in a molar ratio of 1:1:2:2. Its structural characteristics were investigated and elucidated by methylation analysis, GLC–MS, and NMR (^{1}H, ^{13}C, H–H COSY, HMQC, HMBC, TOCSY and NOESY). The hexasaccharide repeating unit of SIP was found to be a backbone composed of fucose, *N*-acetylgalactosamine and mannose in a molar ratio of 2:2:1, and with a single branch of glucuronic acid at the C-3 position of mannose. According to the micronucleus test, SIP could significantly reduce the frequency of micronucleated cells in polychromatic erythrocytes and reticulocytes induced by cyclophosphamide in tumor-bearing mice, which revealed that SIP presented strong antimutagenic activity.

Keywords: Cuttlefish; *Sepiella maindroni*; Ink; Polysaccharide; Structure; Antimutagenic activity

1. Introduction

None of the currently available anticancer drugs act solely on carcinoma cells. Anticancer drugs are usually extremely toxic, and kill malignant and normal cells. Cyclophosphamide (CP), for example, a cytotoxic alkylating agent, is extensively used as an antineoplastic agent for the treatment of various cancers, as well as an immunosuppressive agent for organ transplantation, systemic lupus erythematosus and other benign diseases (Selvakumar, Prahalathan, Sudharsan, & Varalakshmi, 2006). However, despite its wide spectrum of clinical uses, CP is known to cause several adverse effects, including reproductive toxicity in humans and experimental animals (Anderson, Bishop, Garner, Ostrosky-Wegman, & Selby, 1995). These limits on the use of chemotherapeutic agents thus constrain their use in effective therapy. The present study focusses on developing a more potent therapeutic regimen for carcinoma by combining immunotherapy with chemotherapy.

Cuttlefish, *Sepiella maindroni* de Rochebruns, is a popular seafood with a yearly output of 40,000–70,000 tons in China (Shen, Xie, & Xu, 2007). Cuttlefish ink, the by-product of marine-product processing, is a natural substance released by cuttlefish. It is mainly composed of melanin and proteoglycan. Due to its antitumor, immunomodulation, and hemostasia effects, cuttlefish ink is widely used in traditional Chinese medicine.

We have devised a method to isolate the carbohydrate compounds from the ink of the cuttlefish, *S. maindroni*, and found a novel polysaccharide, which has not been reported previously. We named it *S. maindroni* ink

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doi:10.1016/j.foodchem.2008.02.026

polysaccharide (SIP). The present paper is concerned with the isolation, chemical characterization and evaluation of the antimutagenic activity of SIP.

2. Materials and methods

2.1. Materials

S. maindroni were collected from the Bohai Sea, China and transported to the laboratory packed in ice. The ink was kept frozen at −20 °C. The voucher specimen of this marine animal is deposited in the School of Pharmaceutical sciences, Shandong University, Jinan, China.

2.2. General methods

The specific rotation was determined at 20 °C ± 1 °C with an automatic polarimeter (Model WZZ-2B, China). UV–Vis absorption spectra were recorded with an Agilent 8453 spectrophotometer. The FTIR spectra (KBr pellets) were recorded on a Nicolet 470 FTIR spectrophotometer. Elemental analysis was conducted on an Elementar Vario EL III instrument. Total carbohydrate content was determined by the phenol–sulfuric acid method as D-glucose equivalents (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Uronic acid content was determined according to a *m*-hydroxydiphenyl colorimetric method in which neutral sugars do not interfere (Filisetti-Cozzi & Carpita, 1991). Protein was analyzed by the method of Bradford (1976).

2.3. Extraction and fractionation of polysaccharide

A total of 1 kg (wet weight) of cuttlefish ink was dissolved in water (500 ml), and then treated with 1‰ of trypsin and 2‰ of pronase in turn. The supernatants were collected by centrifugation and deproteinated by the Sevag method five times. The crude polysaccharide fraction (3.02 g) was obtained through precipitation with 4 vol of ethanol and desiccation in vacuo. The precipitate was redissolved in distilled water and applied to a DE52 (OH^-) anion-exchange chromatography column (3.5 × 30 cm), eluting at a flow rate of 1.5 ml/min successively with distilled water and a gradient of 0 → 2 M NaCl. The yielded fractions were combined according to the total carbohydrate content quantified by the phenol–sulfuric acid method. The main peak was further fractionated on a Sephadex G-25 column (1.8 × 80 cm) eluted with 0.05 M NaCl at a flow rate of 0.5 ml/min and two completely separated fractions were obtained. The main fraction was collected, dialyzed and lyophilized to get a white purified *S. maindroni* ink polysaccharide (SIP, 1.88 g, 62.2% of the crude polysaccharide).

2.4. HPSEC–MALLS analysis

The homogeneity and molecular weight of SIP were determined, as described previously(Rioux, Turgeon, & Beaulieu, 2007; Zhang, Zhang, Cheung, & Ooi, 2004), in a ThermoFinnigan high-performance size-exclusion chromatography (HPSEC) apparatus equipped with Two TSK columns, G3000 PWXL and G4000 PWXL, in series, coupled to a refractive index detector (RID) and a Wyatt Technology Dawn-EOS multi-angle laser light scattering detector (MALLS). The carrier solution was 0.2 M NaCl, and the samples were dissolved in 0.2 M NaCl with stirring. The carrier and sample solutions were made dust-free by passing through a 0.45 μm Millipore filter and degassed before use. The injection volume was 500 μl, and the flow rate was 0.6 ml/min. The normalisation of RID was done with bovine albumin monomer. The specific RI increment (dn/dc) at 690 nm and 25 °C was determined using an interferometric refractometer (Optilab/DSP, Wyatt Technology, USA). The dn/dc value was averaged to 0.1412 ml/g and was assumed to be constant during the sample elution. ASTRA software was utilized for data acquisition and analysis.

2.5. Monosaccharides analysis

For sugar analysis, the polysaccharide sample was hydrolyzed with 2 M CF_3COOH at 120 °C for 2 h in an oven, the products were reduced with an excess of $NaBH_4$ (20 °C, 2 h), acetylated with an Ac_2O–pyridine mixture (1:1, 100 °C, 1 h) (Blakeney, Harris, Henry, & Stone, 1983) and analyzed by GLC on a Hewlett–Packard HP 6890 chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm) and a flame-ionization detector, and a temperature gradient of 12 °C/min from 120 °C to 280 °C. Peaks were identified and estimated with myo-inositol as the internal standard. Quantitation was carried out from the peak area, using response factors.

For determination of the absolute configurations of the monosaccharides, a SIP sample was subjected to methanolysis (1 M HCl/MeOH, 100 °C, 16 h). The products were heated with (+)-2-octanol (100 μl) in the presence of CF_3COOH (15 μl) at 120 °C for 16 h, acetylated and analyzed by GLC–MS (Gerwig, Kamerling, & Vliegenthart, 1978; Leontein, Lindberg, & Lonngren, 1978).

2.6. Carboxyl-group reduction

The carboxyl-group of the polysaccharide was reduced by the method of Taylor and Conrad (1972). SIP (100 mg) dissolved in water (20 ml) was adjusted to pH 4.7 with 0.05 M HCl and, following the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (300 mg), the stirred reaction mixture was maintained at pH 4.7 over 4 h by the slow addition of 0.05 M hydrochloric acid. Following the addition of sodium borodeuteride (300 mg) the mixture was stirred for 12 h at 20 °C. The reaction mixture was brought to pH 7.0 with acetic acid and, after dialysis against running tap water, the reduced polysaccharide (SIP-r, 83 mg) was recovered by lyophilization. The carboxyl-reduced polysaccharide was hydrolyzed with 2 M

CF_3COOH for 2 h at 120 °C, and, after the usual treatment, the sugars were estimated by GLC.

2.7. Methylation analysis

SIP and SIP-r were methylated three times by the Needs and Selvendran (1993) method. The pre-methylated products were depolymerized with 90% HCOOH at 100 °C for 6 h and further hydrolyzed with 2 M CF_3COOH at 110 °C for 3 h. The partially methylated residues were reduced and acetylated. The resulting products were analyzed by GLC–MS. The GLC temperature programme was isothermal at 150 °C, followed by a 3 °C/min gradient up to 220 °C and 30 °C/min up to 280 °C. Methylated alditol acetates were identified by their fragment ions in GLC–MS and by relative retention time on GLC and the molar ratios were estimated from the peak areas and the response factors.

2.8. NMR spectroscopy

The freeze-dried polysaccharide was kept over P_2O_5 in a vacuum for several days and dissolved in 99.96% D_2O. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX Avance 600 MHz spectrometer (operating frequencies 600.13 MHz for ^1H NMR and 150.92 MHz for ^{13}C NMR) at 35 °C. Chemical shifts were reported relative to internal DSS at δH 0.00 ppm for ^1H spectra and δC 21.777 ppm for ^{13}C spectrum. Standard homo- and hetero-nuclear correlated 2D techniques were used for general assignments of SIP: COSY, NOESY, HMQC, and HMBC (Brisson et al., 2003). The TOCSY was recorded at a mixing time of 150 ms. Complete assignment required several TOCSY experiments having mixing times ranging from 60 ms to 300 ms. The NOESY mixing delay was 300 ms. The delay time in the HMBC experiment was 80 ms.

2.9. Micronucleus test

Thirty-two male Kunming mice (6–8 weeks old) were purchased from the Center for New Drugs Evaluation of Shandong University (Jinan, China. Quality certificate Number: SCXK2003-0004). The mice were housed under normal laboratory conditions, namely, room temperature, 12/12 h light–dark cycle with free access to standard rodent chow and water. Seven-day-old Sarcoma 180 (S180) ascites cells (0.2 ml, 2×10^6 cells) were transplanted subcutaneously into the right axilla of each mouse. All the mice were randomly divided into four groups, each group consisting of 8 animals. The mice were treated as follows: control group (normal saline); cyclophosphamide(CP) group(15 mg/kg body weight); two CP + SIP groups(CP: 15 mg/kg body weight; SIP: 20, 10 mg/kg body weight) All the groups were administered by intraperitoneal injection in 0.2 ml every day for seven days, starting 24 h after tumor implantation.

The micronucleus assay from bone marrow or peripheral blood cells was performed according to the protocol described by MacGregor et al. (1987) and by Hayashi, Morita, Kodama, Sofuni, and Ishidate (1990), respectively. The number of micronucleated cells (MN) was counted in 1000 polychromatic erythrocytes (PCEs) and reticulocytes (RETs) per animal. The slides were analyzed in a blind test, using a light microscope with a 100× immersion objective for PCEs and RETs. The percentage of reduction in the frequency of MN was calculated according to Delmanto et al. (2001).

Data analysis and evaluation of statistical significance among different values determined were done using one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Isolation and structural analysis

The yield of the crude water-soluble polysaccharide from the ink of cuttlefish was 3.02% of the fresh material. The crude polysaccharide was separated and sequentially purified through DE52 and Sephadex G-25 and detected by the phenol–sulfuric acid assay. The main fraction (SIP) was collected for subsequent analyses.

SIP appeared as a white powder. The specific rotation of the polysaccharide was −24.4° (c 0.1, H_2O) at 20 °C. It had a negative response to the Bradford test and no absorption at 280 nm or 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid. Elemental analysis (wt%) Found: C, 38.86; H, 7.02; N, 2.73. The HPSEC-RID elution profile showed a single and symmetrically sharp peak, indicating that SIP was a homogeneous polysaccharide. The weight-average molecular weight of SIP was 1.13×10^4 Da by HPSEC–MALLS analysis.

There are two types of end carbon-glucoside bonds: α- and β-styles, which can be judged by FTIR. In the FTIR spectrum of SEP, the C–H bond in α-style has an absorption peak near 838.49 cm^{-1}, while that of the C–H bond is in β-style near 887.82 cm^{-1} (Barker, Bourne, Stacey, & Whiffen, 1954). The absorption at 1644 and 1552 cm^{-1} is attributed to the stretching vibration of the carbonyl bond and the bending vibration of the N–H bond, respectively. The FTIR spectrum also showed a strong band at 3303.83 cm^{-1} attributed to the hydroxyl stretching vibration of the polysaccharide. The band at 2918.20 cm^{-1} was due to C–H stretching vibration.

The components of SIP hydrolyzate were tentatively identified as fucose (Fuc), mannose (Man), 2-amino-2-deoxygalactose (GalN) and glucuronic acid by thin-layer chromatography (TLC). Monosaccharide analysis by GLC–MS of the alditol acetates derivatives of SIP hydrolyzate showed the presence of Fuc, Man, and GalN in the ratios 2:1:2. The carboxyl-reduced polysaccharide on hydrolysis, followed by GLC–MS examination of the corresponding alditol acetates, showed the presence of fucose, mannose, 2-amino-2-deoxygalactose (GalN), and glucose

in a molar ratio of 2:1:2:1. The absolute configurations were determined by GLC analysis of their acetylated (+)-2-octyl derivatives, and the result showed that Man, GalN and GlcA had the D-configuration, whereas Fuc had the L-configuration.

The fully methylated product of SIP was hydrolyzed with acid, converted into alditol acetates, and analyzed by GLC and GLC–MS. The polysaccharide showed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylfucitol, 1,3,5,6-tetra-*O*-acetyl-2, 4-di-*O*-methylmannitol and 1,4,5-tri-*O*-acetyl-2-(*N*-methylacetamido)-2-deoxy-3, 6-di-*O*-methylgalactitol. These results indicate that →4)-L-Fuc*p*-(1→, →3,6)-D-Man*p*-(1→, and →4)-D-Gal*p*NAc-(1→ are present in the polysaccharide. The carboxyl-reduced polysaccharide was methylated, and alditol acetates of the methylated sugars were identified by GLC–MS analysis which showed the presence of the above peaks and a new peak of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-gluctitol in a molar ratio of 2:1:2:1. This result indicates that (1→)-linked D-GlcpA is also present in the polysaccharide.

The evidence indicated that the SIP was constituted of repeating hexasaccharide units, composed of L-fucose, D-mannose, D-2-amino-2-deoxygalactose and D-glucuronic acid, a conclusion substantiated by NMR analysis.

The ^{1}H NMR spectrum (600 MHz) (Fig. 1) of this polysaccharide at 35 °C, supported by the ^{1}H,^{13}C HMQC spectrum of the SIP (data not shown), showed four anomeric proton signals at δ 5.437, 5.273, 5.162, and 4.561 ppm in a 1:1:2:2 molar ratio. Besides the ring proton region at δ 3.3–4.5, the ^{1}H NMR spectrum showed two methyl groups (C-6 of L-Fuc*p* and CH_3 of *N*-acetyl group) at δ 1.28 and 2.02, respectively.

The ^{13}C spectrum (Fig. 2) showed four anomeric signals δ 106.491, 102.148, 101.666, and 99.379 ppm in a molar ratio of nearly 2:1:2:1. Other characteristic signals in the ^{13}C NMR spectrum of SIP were as follows: The ^{13}C NMR spectrum of SIP revealed one nitrogen-bearing carbon (C-2 of D-Glc*p*NAc) at δ 52.427, two methyl groups (C-6 of L-Fuc*p* and CH_3 of an *N*-acetyl group) at δ 17.801 and 24.8, respectively, and two carboxyl groups at δ 176.7 and 177.3. These data indicate that the hexasaccharide repeating unit of SIP consisted of L-Fuc, D-Man, D-GalNAc, and D-GlcA. As judged by the $J_{1,2}$ coupling constants <3 Hz, Man, GalNAc and GlcA were α-linked, whereas Fuc was β-linked ($J_{1,2} \sim 7$ Hz).

The complete structural characterisation of the SIP was retrieved from the results of 2D NMR analysis involving ^{1}H, ^{1}H COSY, TOCSY, NOESY and ^{1}H, ^{13}C HSQC and HMBC experiments, which were used to assign the chemical shifts and anomeric configurations of the four sugar residues present in the repeating unit (glycose residues labelled A–D in order of decreasing anomeric proton chemical shifts) (Table 1) according to the published methodology (Duus, Gotfredsen & Bock, 2000; Perry, Bundle, MacLean, Perry, & Griffith, 1986).

The monosaccharide sequence within the repeating unit was established by NOESY and HMBC experiments. In the NOESY spectrum (figure not shown), the following inter-residual NOE correlations were observed: A1–B3, B1–C4, C1–B6/D4, and D1–D4/C4. As to the HMBC spectrum (figure not shown), the intra- and inter-residual connectivities of both anomeric protons and carbons of each of the glycosyl residues are summarized. Cross peaks were found between C-1 of residue A (δ 99.379) with H-3 of

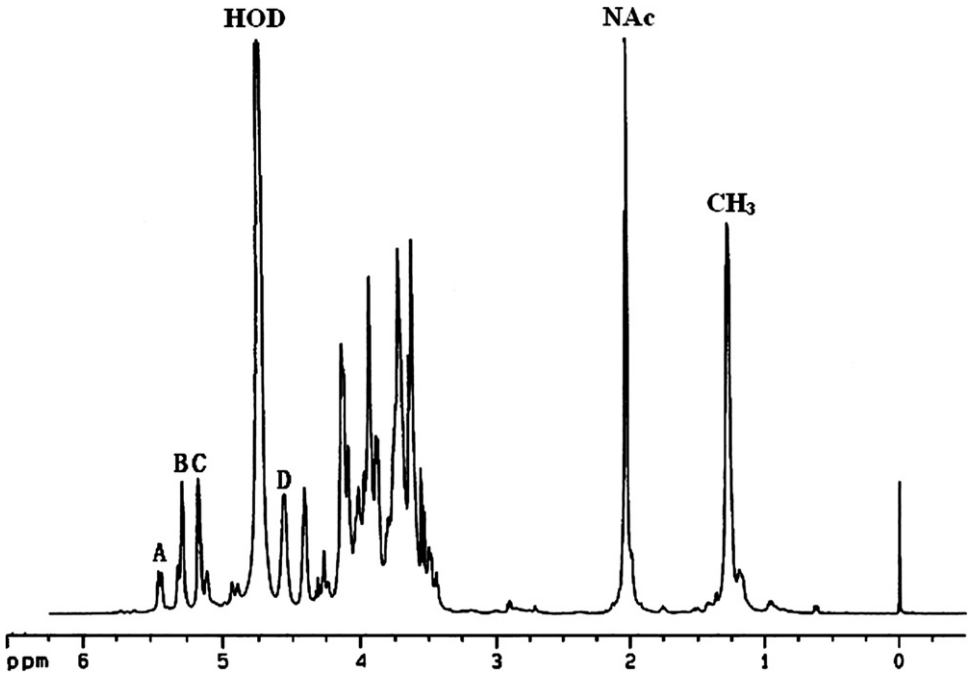

Fig. 1. ^{1}H NMR (600M, D_2O, 35 °C) spectrum of polysaccharide SIP isolated from *Sepiella maindroni* ink.

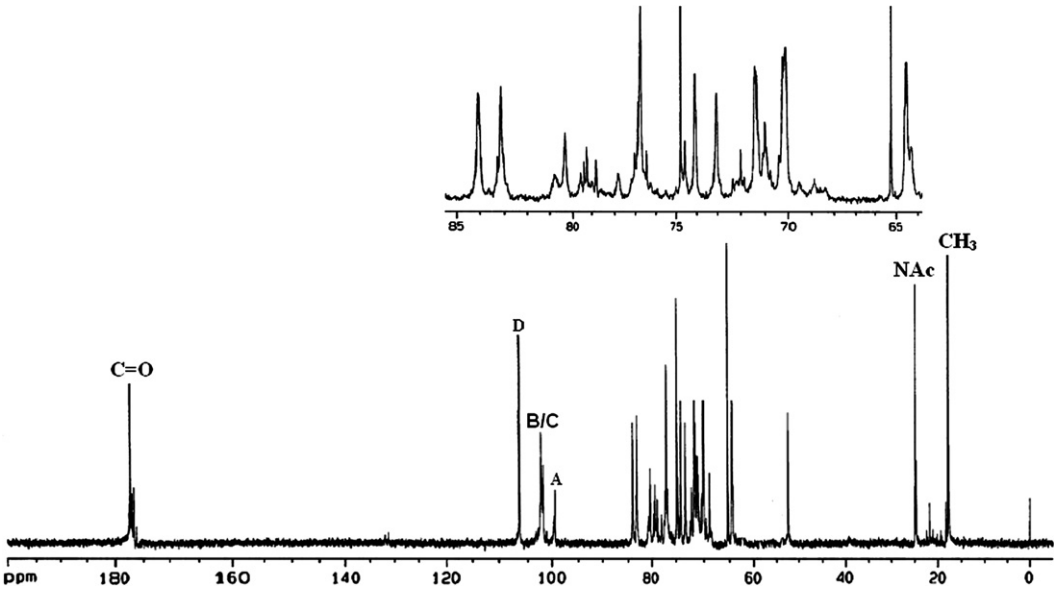

Fig. 2. ^{13}C NMR (150.9 M, D_2O, 35 °C) spectrum of polysaccharide SIP isolated from *Sepiella maindroni* ink.

Table 1
The ^{1}H and ^{13}C NMR chemical shifts for the polysaccharide SIP isolated from *Sepiella maindroni* in D_2O at 35 °C[a]

Glycosyl residue	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6
A α-D-Glc*p*A-(1→	5.437	3.974	4.313	4.120	3.750	
	99.379	71.881	79.264	71.999	73.051	176.755
B →3,6)-α-D-Man*p*-(1→	5.273	3.942	4.014	4.123	3.768	3.875[b] 3.892[c]
	102.148	71.418	76.765	74.079	74.767	70.110
C[d] →4)-α-D-Gal*p*NAc-(1→	5.1624	4.140	3.875	3.624	3.643	3.749[b] 3.676[c]
	101.666	52.427	70.203	84.036	74.079	64.497
D →4)-β-L-Fuc*p*-(1→	4.561	3.631	3.730	4.092	4.413	1.282
	106.491	65.242	73.051	83.125	70.11	17.801

[a] The ^{1}H NMR spectrum was recorded at 600 MHz and at 35 °C. The ^{13}C NMR spectrum was recorded at 150 MHz on a 600 MHz instrument at the same temperature. Chemical shifts were reported relative to internal DSS at δH 0.00 ppm for ^{1}H spectra and δC 21.777 ppm for ^{13}C spectrum.
[b] Chemical shift for H-6a.
[c] Chemical shift for H-6b.
[d] There are additional chemical shifts for NAc in the ^{1}H NMR spectrum at δ 2.02 ppm for CH_3, in the ^{13}C NMR spectrum at δ24.8 ppm for CH_3, and at 177.3 ppm for CO.

residue B (A C-1, B H-3), along with intraresidual coupling between H-1 of residue A with its own C-2 (A H-1, A C-2). Similarly cross peaks were found between H-1 of residue B (δ 5.273) and C-4 of residue C (B H-1, C C-4); C-1 of residue B (102.148) and H-4 of residue C (B H-1, C C-4; B C-1, C H-4), along with intraresidual cross couplings between H-1 of residue B with C-2 and C-3 (B H-1, B C-2; B H-1, B C-3). Cross peaks were also observed between H-1 of residue C (δ 5.162) and C-6 of residue B (C-H1, B C-6); C-1 of residue C (δ 101.666) and H-4 of residue D (C C-1, D H-4), along with intraresidual coupling between H-1 of residue C with C-5 (C H-1, C C-5). Cross peaks was observed between H-1 of residue D (δ 4.561) and C-4 of residue D (D H-1, D C-4); C-1 of residue D (δ 106.491) and H-4 of residue D (D C-1, D H-4) were observed, along with intraresidual cross coupling between C-1 of residue D and H-2 (D C-1, D H-2).

Based on all these chemical and spectroscopic findings, the structure of the repeating unit of the polysaccharide SIP was established as in Fig. 3.

3.2. Antimutagenic activity

The animals were killed by cervical dislocation for the evaluation of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow and micronucleated reticulocytes (MNRETs) in peripheral blood.

Table 2 shows the frequencies of MN in PCEs of tumor-bearing mice treated with a mixture of CP (15 mg/kg) and polysaccharide SIP (10 or 20 mg/kg). CP alone induced a

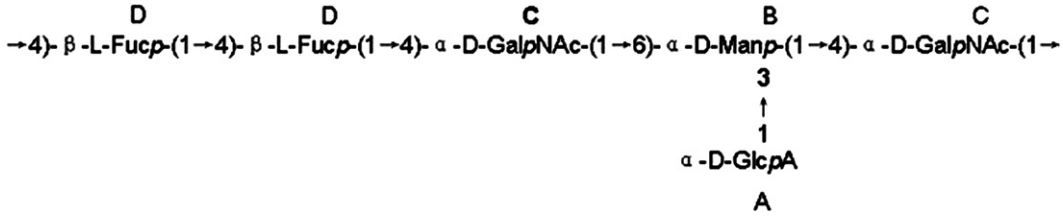

Fig. 3. Schematic structure of the repeating unit of polysaccharide SIP isolated from *Sepiella maindroni* ink.

Table 2
The effect of treatment with SIP on the micronuclei induced by CP in bone marrow cells of tumor-bearing mice

Group	Dose (mg/kg)	MNPCEs		Reduction (%)[d]
		Total number	%[a]	
Control	–	69	8.63 ± 3.16	
CP	15	310	38.75 ± 6.54[b]	
CP + SIP	15 + 10	156	19.5 ± 5.53[c]	63.9
	15 + 20	110	13.75 ± 4.68[c]	83.0

[a] The results are expressed as means ± SD, $n = 8$, 1000 micronucleated polychromatic erythrocytes (MNPCEs) scored per animal.
[b] $P < 0.01$, compared with control group.
[c] $P < 0.01$, compared with CP group.
[d] $\text{Reduction}(\%) = \dfrac{\text{frequency of MN in A} - \text{frequency of MN in B}}{\text{frequency of MN in A} - \text{frequency of MN in C}} \times 100,$

where A is the group treated with CP (positive control); B the group treated with CP plus SIP solutions and C the group treated with 0.9% NaCl (control).

Table 3
The effect of treatment with SIP on the micronuclei induced by CP in peripheral blood cells of tumor-bearing mice

Group	Dose (mg/kg)	MNRETs		Reduction (%)[d]
		Total number	%[a]	
Control	–	23	2.88 ± 1.13	
CP	15	113	14.13 ± 4.22[b]	
CP + SIP	15 + 10	85	10.63 ± 1.85[c]	31.1
	15 + 20	55	7.88 ± 2.30[c]	64.4

[a] The results are expressed as means ± SD, $n = 8$, 1000 micronucleated reticulocytes (MNRETs) scored per animal.
[b] $P < 0.01$, compared with control group.
[c] $P < 0.01$, compared with CP group.
[d] $\text{Reduction}(\%) = \dfrac{\text{frequency of MN in A} - \text{frequency of MN in B}}{\text{frequency of MN in A} - \text{frequency of MN in C}} \times 100,$

where A is the group treated with CP (positive control); B the group treated with CP plus SIP solutions; C the group treated with 0.9% NaCl (control).

clear increase in MN frequencies ($P < 0.01$). Treatment with SIP led to a statistically significant reduction in the frequency of MN in PCEs induced by CP in both doses. Reduction was between 63.9% and 83.0% and significantly increased with the increasing SIP doses ($P < 0.01$), which indicated that the preventive effect of micronuclei in PCEs of mice was related to SIP.

Table 3 shows the frequencies of MN in RETs of tumor-bearing mice treated with a mixture of CP (15 mg/kg) and polysaccharide SIP (10 or 20 mg/kg). CP induced a clear increase in MN frequencies. Treatment with SIP led to a statistically significant reduction in the frequency of MN induced by CP in RETs. The reduction was between 31.1% and 64.4%, and significantly increased with the increasing SIP doses ($P < 0.01$), which also indicated that the preventive effect of micronuclei in RETs of mice was related to SIP.

4. Conclusions

SIP, first isolated from the ink of cuttlefish *S. maindroni*, was a heteropolysaccharide with a molecular weight of 1.13×10^4 Da. The repeating unit of SIP was found to be a backbone composed of fucose and *N*-acetylgalactosamine in a molar ratio of 2:2:1, and with a single branch of glucuronic acid at the C-3 position of mannose according to chemical analysis and NMR analysis. According to the micronucleus test, SIP could significantly reduce the

frequency of micronucleated cells in polychromatic erythrocytes and reticulocytes in a dose-dependent manner, induced by cyclophosphamide in tumor-bearing mice, suggesting its potential as an effective natural antimutagenic agent. However, further studies must be conducted to establish the mode mechanisms of antimutagenicity of the polysaccharide.

Acknowledgements

The authors are grateful for financial support from China Postdoctoral Science Foundation (No. 20060400984). They express their thanks to Dr. Bin Ma and Mrs. Jian Ren for NMR analysis.

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