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Structural characterization of polysaccharides from *Hyriopsis cumingii*

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ABSTRACT

The structural characterization of purified *Hyriopsis cumingii* polysaccharides (HCPS) were studied by using periodic acid oxidation, Smith degradation, methylation combined with HPLC, GC, GC–MS, FTIR and NMR spectra. Results showed that sugar rings of purified HCPS were pyranose rings. In HCPS-1 and HCPS-2, glycosyl residues were linked mainly by α-configuration glycosidic bond. As to HCPS-3, sugar residues were connected by α- and β-configuration glycosidic bonds. Number of glycosyl residues in one repeat unit was 7, 9 and 10 for HCPS-1, HCPS-2 and HCPS-3 respectively. Backbone chains of purified HCPS were linked by 1 → 4 glycosidic bonds. In HCPS-1 and HCPS-2, there were one backbone chain and two branch chains (one linked by 1 → 3 glycosidic bond, another connected by 1 → 6 glycosidic bond) in one repeat unit. As to HCPS-3, three branch chains (two connected by 1 → 3 glycosidic bond, one linked by 1 → 6 glycosidic bond) were attached to backbone chain in one repeat unit.

1. Introduction

Hyriopsis cumingii, a member of freshwater pearl mussels, is widely cultivated in China because it can produce high quality pearls. According to traditional Chinese medicine, *Hyriopsis cumingii* is one type of the animals used as medicine and food simultaneously. However, the *Hyriopsis cumingii* flesh (waste of producing freshwater pearls) is used mainly as animal feeds and fertilizer. The pharmacological activity of *Hyriopsis cumingii* is not well developed and utilized. In fact, *Hyriopsis cumingii* can cure some diseases in traditional Chinese medicine. In addition, recent studies demonstrated that the *Hyriopsis cumingii* flesh was rich in polysaccharides (Dai, Zhang, Zhang, & Wang, 2009; Hu & Cao, 2003; Qiao, Hu, et al., 2009), which resulted in nutrient and pharmacological functions such as anti-tumor, anti-inflammation, anti-oxidation and anti-aging (Cheng, Wu, Lu, & Chi, 2007; Dai et al., 2009; Hu & Cao, 2003; Qiao, Ke, et al., 2009; Zhang, Wu, Di, & Chen, 2007).

Bioactivity of polysaccharides is correlated with its chemical structure. Relationship between structure and activity of polysaccharides has drawn much attention of some scientists. The chemical structures of different polysaccharides have been studied by using high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography–mass spectroscopy (GC–MS), Fourier transform-infrared (FTIR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy (Li et al., 2008; Maciel et al., 2008; Rout, Mondal, Chakraborty, & Islam, 2008; Zha, Luo, Luo, & Jiang, 2007). However, little attention has been thrown to the chemical structure of polysaccharides from *Hyriopsis cumingii* (HCPS). The relationship between structure and activity of HCPS remains unclear due to lacking of structural data.

Recently, we have reported the extraction, purification and preliminary characterization of HCPS. The antioxidant activities of HCPS were also reported (Qiao, Hu, et al., 2009; Qiao, Ke, et al., 2009). In this paper, we present the structural characterization of purified fractions of HCPS (HCPS-1, HCPS-2 and HCPS-3) by using periodic acid oxidation, Smith degradation, methylation combined with HPLC, GC, GC–MS, FTIR and NMR spectra.

2. Materials and methods

2.1. Materials and reagents

Purified fractions of HCPS (HCPS-1, HCPS-2 and HCPS-3) were prepared from *Hyriopsis cumingii* according to our previous method (Qiao, Hu, et al., 2009). Monosaccharide reference (arabinose, rhamnose, fucose, xylose, galactose, glucose and mannose) and glucan reference were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Determination of relative molecular weight and monosaccharide compositions

Determination of relative molecular weight was done according to the reported method of size-exclusive HPLC (Alsop & Vlachogiannis, 1982; Roy, Maiti, Mondal, Das, & Islam, 2008) with

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slight modification. HPLC (1100 Series HPLC, Agilent) equipped with a refractive index detector and a TSK-Gel G3000SW$_{XL}$ column (7.8 mm × 300 mm) (Tosoh Corp., Tokyo) was used in this work. Sample (20 μl of 1.0 mg/ml) was injected in and eluted with 0.1 M Na_2SO_4 (dissolved in 0.01 M phosphate buffer of pH 6.8) at a flow rate of 0.8 ml/min. Temperature of column and detector was set at 25 °C. Standard glucans (5.9 kDa, 11.8 kDa, 22.8 kDa, 112 kDa, 212 kDa, 404 kDa and 788 kDa) were used as reference.

Analysis of monosaccharide compositions was acted by using the reported method (Li et al., 2008; Liu et al., 2008) with minor changes. Polysaccharides sample (5 mg) was dissolved in 2 M trifluoroacetic acid (TFA) (4 ml) and hydrolyzed to monosaccharide at 120 °C for 2 h. The hydrolyzed products were evaporated to dryness and derivatized by using the following method. Hydroxylammonium chloride (10 mg), inositol (5 mg, used as the internal reference) and pyridine (0.6 ml) were added to the hydrolyzed sample. Mixture was placed in water bath of 90 °C to react for 30 min. After cooling to room temperature, acetic anhydride (1 ml) was added, and then the reaction system was placed in water bath of 90 °C for 30 min again. The reaction products were analyzed in GC (GC-6890N, Agilent) with a flame ionization detector and a HP-5 capillary column (30 m × 0.32 mm × 0.25 μm). The operation conditions of GC were as follows: flow rate of N_2, H_2 and air was 25 ml/min, 30 ml/min and 400 ml/min, respectively; the temperature of detector and inlet was 280 °C and 250 °C, respectively; the oven temperature program was set changing from 120 °C (standing for 3 min) up to 210 °C (standing for 4 min) at a rate of 3 °C/min. Standard monosaccharides (rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose) were also derivatized and analyzed in GC as references.

2.3. Assay of FTIR spectroscopy

FTIR spectroscopy of polysaccharides was carried out by the potassium bromide pellet method (Maciel et al., 2008) on FTIR spectrometer (model MB154S, Bomen, Canada) in the wave-number range of 500–4000 cm^{-1}.

2.4. Periodic acid oxidation, Smith degradation and GC assay

Reported methods (Ghosh et al., 2008; Li et al., 2008; Rout et al., 2008) were referred with slight modification to do periodic acid oxidation, Smith degradation and GC assay. Sodium metaperiodate ($NaIO_4$) (0.015 M) and sodium iodate ($NaIO_3$) (0.015 M) were mixed at different ratios (5:0, 4:1, 3:2, 2:3, 1:4 and 0:5). Mixed solution, diluted from 0.1 ml to 25 ml, was detected on spectrophotometer at 223 nm. Standard curve of $NaIO_4$ was produced by using concentration of $NaIO_4$ as *X*-axis and absorption of 223 nm as *Y*-axis.

Polysaccharide sample (10 mg) was oxidized in 0.015 M $NaIO_4$ (10 ml). Two hours later, 0.1 ml of reactive mixture was diluted to 25 ml and the 223 nm absorption of dilution was detected. The reaction system was kept in the dark at 4 °C with interval stirring. Absorption of diluted reactive mixture (from 0.1 ml to 25 ml) was monitored at 223 nm every 24 h until the absorption was unchanging. Ethylene glycol (1.0 ml) was added to the reaction system with stirring for 30 min to decompose the excess $NaIO_4$. Consumption of $NaIO_4$ was calculated according to the $NaIO_4$ standard curve and the 223 nm absorption.

Production of formic acid (HCOOH) was determined by titration. The reaction product (1.0 ml) was mixed with phenolphthalein solution (0.5%, 50 μl), and then NaOH (0.5 mM) was added drop by drop until the color of reaction system changed from colorless to purple red. Production of HCOOH was calculated according to the consumption of NaOH.

The reaction mixture was reduced by adding sodium borohydride ($NaBH_4$) (50 mg) for about 20 h, and then was adjusted to pH 5.5–7.0 by using acetic acid (0.1 M). The reaction solution was dialyzed against running water and distilled water each for 24 h. The dialyzed product was dried, hydrolyzed, derivatized and analyzed in GC by using the same method as described in Section 2.2. Standard monosaccharides (rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose), glycerol and erythritol were used as references.

2.5. Methylation and GC–MS assay

Methylation and GC–MS assay were performed by using the reported procedure (Ghosh et al., 2008; Mondal et al., 2008; Ojha et al., 2008) with slight modification. Polysaccharides sample (10 mg) was dissolved in dimethyl sulfoxide (DMSO) (2 ml). Dried NaOH power (50 mg) was added to the polysaccharides solution with interval vibration under the protection of N_2. One hour later, 1.0 ml of methyl iodide was added to the reaction system with interval vibration under the protection of N_2. After 1 h, reaction was stopped by adding 0.5 ml of water. The reaction solution was dialyzed against running water for 48 h and distilled water for 24 h. The dialyzed product was freeze dried to obtain partially methylated polysaccharides. Completely methylated polysaccharide was gained by repeating above procedure three times. Complete methylation was confirmed by the disappearance of O–H absorption band (3700–3100 cm^{-1}) in FTIR spectrum. The completely methylated polysaccharides was hydrolyzed with TFA, reduced with $NaBH_4$ and derivatized with pyridine–acetic anhydride by using the same method as described in Sections 2.2 and 2.4. The derivatized product was analyzed in GC–MS (GC: model CP3800, MS: model Satum2200, Varian) with quartz capillary column (model DB-5MS, 30 m × 0.25 mm × 0.25 μm). Temperature program was set rising from 80 °C (standing for 1 min) up to 210 °C (standing for 1 min) at 8 °C/min then up to 260 °C (standing for 1 min) at 20 °C/min. Range of mass charge ratio (*m*/*z*) was 30–450.

2.6. Assay of NMR spectra

Assay of NMR spectra was done by using the reported deuterium-exchanging method (Ali, Weintraub, & Widmalm, 2008; Leone et al., 2008; Serrato et al., 2008) with minor modification. Polysaccharides sample (30 mg) was dissolved in heavy water (D_2O) and lyophilized three times. The deuterium-exchanged polysaccharides, dissolved in D_2O again, were detected on NMR spectrometer (model Avance DRX-500, Bruker, Germany) using tetra-methyl silane (TMS) as internal standard. ^1H NMR and ^{13}C NMR spectra were all scanned and reported.

2.7. Helix-coil transition assay

Helix-coil transition assay using Congo red dye was acted according to the reported method (Ogawa, Wanatabe, Tsurugi, & Ono, 1972; Rout et al., 2008) with slight change. Polysaccharides sample (10 mg), dissolved in distilled water (2 ml), was mixed with 80 μM Congo red solution (2 ml). NaOH solution of 1 M was added to above-mixed solution drop by drop to make the final concentration of NaOH in the mixed solution being 0.0 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M respectively. Visible spectra of mixture at various concentrations of NaOH were scanned on UV–vis spectrophotometer (model UV-2450, Shimadzu, Japan) at 400–800 nm and the maximum absorption wavelength was recorded. In another reaction system, distilled water, instead of polysaccharides solution, was mixed with Congo red and NaOH solution, and then the spectra of visible were also scanned employed the same method as that used above.

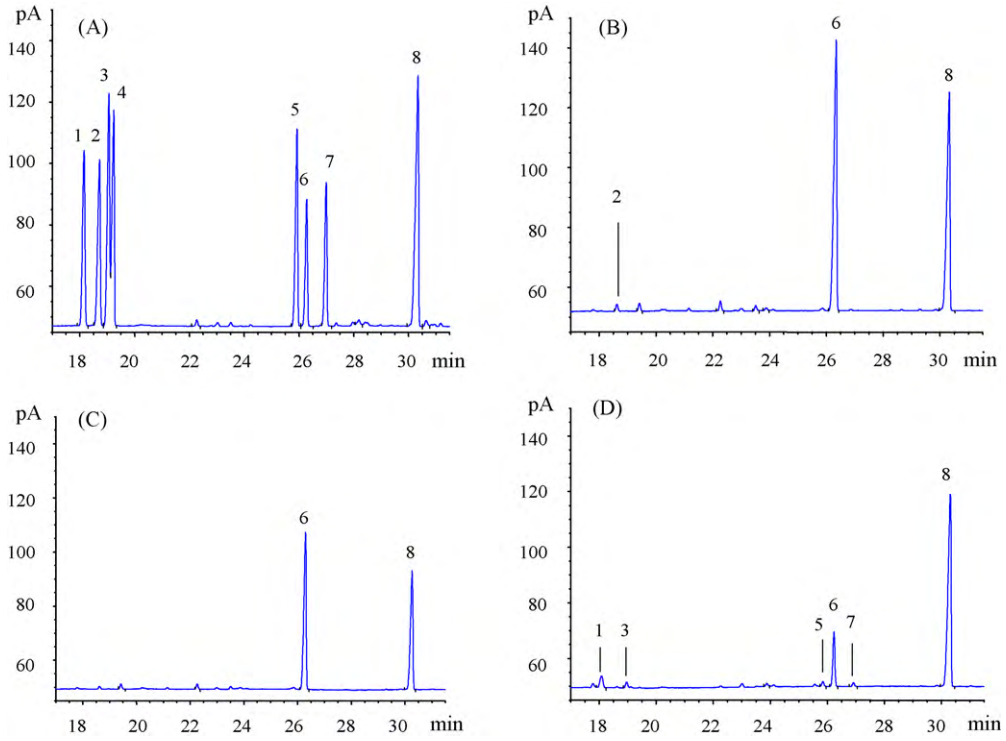

Fig. 1. GC spectra of derivatives from standard monosaccharide (A) and monosaccharide composition of HCPS-1 (B), HCPS-2 (C) and HCPS-3 (D). 1, rhamnose; 2, arabinose; 3, fucose; 4, xylose; 5, mannose; 6, glucose; 7, galactose; 8, inositol.

3. Results and discussions

3.1. Relative molecular weight and monosaccharide compositions of purified HCPS

To determine the relative molecular weight, size-exclusive HPLC was employed and standard glucans were used as references. Results showed that relative molecular weight of HCPS-1, HCPS-2 and HCPS-3 was 432.2 kDa, 457.9 kDa and 503.1 kDa respectively.

GC spectra of derivatives from standard monosaccharide and monosaccharide composition of HCPS-1, HCPS-2 and HCPS-3 were shown in Fig. 1. Table 1 presented the monosaccharide molar percent of HCPS-1, HCPS-2 and HCPS-3. From Fig. 1 and Table 1, we know that glucose was main monosaccharide and other monosaccharides (rhamnose, arabinose, fucose, mannose and galactose) occurred in less percentage in HCPS. HCPS-1 was composed of glucose and arabinose, HCPS-2 was composed only of glucose, and HCPS-3 was composed of rhamnose, fucose, mannose, glucose and galactose. These results indicated that monosaccharide composition of HCPS-3 was more complicated than that of HCPS-1 and HCPS-2. Complexity of monosaccharide composition of HCPS resulted mainly from HCPS-3.

3.2. FTIR spectroscopy of purified HCPS

In FTIR spectra of HCPS-1, HCPS-2 and HCPS-3, two characteristic absorptions of polysaccharides, at about 3700–3100 cm^{-1} and 3000–2800 cm^{-1}, were observed in HCPS-1, HCPS-2 and HCPS-3. In HCPS-1 and HCPS-2, three strong absorption peaks, existed at 1100–1010 cm^{-1}, indicated that sugar rings of HCPS-1 and HCPS-2 were pyranose rings. There was an absorption peak at about 844 cm^{-1}, but no absorption band at about 891 cm^{-1}. These results implied that the glycosyl residues of HCPS-1 and HCPS-2 were linked mainly by α-configuration glycosidic bond (Li et al., 2008; Yang & Li, 2008; Zhang, 1999). As to HCPS-3, strong absorption peak of about 1100–1010 cm^{-1} was existed, but the number of peak (two or three) was not clear. Absorption at about 900–800 cm^{-1} was also not clear. These results mean that sugar ring and linkage mode of HCPS-3 could not be determined only by FTIR spectra. It needs to be analyzed by other methods (Li et al., 2008; Yang & Li, 2008; Zhang, 1999).

3.3. Periodic acid oxidation, Smith degradation and GC spectra of purified HCPS

Glycosidic linkage location of polysaccharides may be preliminarily determined by consumption of $NaIO_4$ and production of HCOOH in periodic acid oxidation (Zhang, 1999). In the periodic acid oxidation, 1 M glycosyl consumed $NaIO_4$ of 1.1405 mol, 1.1028 mol and 1.0846 mol then produced HCOOH of 0.2883 mol, 0.2241 mol and 0.3572 mol for HCPS-1, HCPS-2 and HCPS-3 respectively. These results indicated that the non-reducing terminal residues or 1 → 6 linked glycosidic bonds were existed in HCPS-1, HCPS-2 and HCPS-3. The result, consumption of $NaIO_4$ was more

Table 1
Monosaccharide composition of purified HCPS.

Item	Rhamnose (%)	Arabinose (%)	Fucose (%)	Mannose (%)	Glucose (%)	Galactose (%)
HCPS-1	–[a]	2.1209	–	–	97.8791	–
HCPS-2	–	–	–	–	100.0000	–
HCPS-3	13.8037	–	4.5118	7.6987	64.9189	9.0669

[a] Not detected.

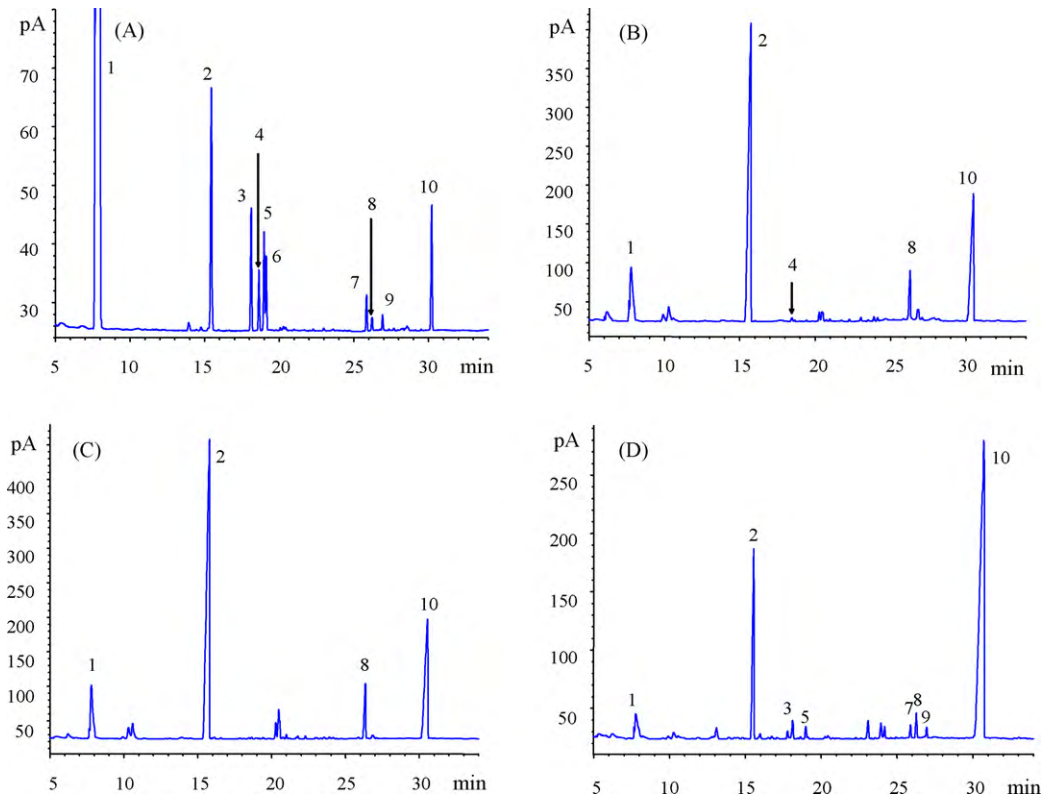

Fig. 2. GC spectra of derivatives from sample references (A), HCPS-1 (B), HCPS-2 (C) and HCPS-3 (D) after periodic acid oxidation and Smith degradation 1, glycerol; 2, erythritol; 3, rhamnose; 4, arabinose; 5, fucose; 6, xylose; 7, mannose; 8, glucose; 9, galactose; 10, inositol.

than double of HCOOH production, means that there were 1 → 2 or 1 → 2,6 or 1 → 4 or 1 → 4,6 linked glycosidic bonds existed in HCPS-1, HCPS-2 and HCPS-3. Value of $NaIO_4$ consumption minus HCOOH production was lesser than 1. These implied that there were 1 → 3 or 1 → 2,3 or 1 → 2,4 or 1 → 3,4 or 1 → 3,6 or 1 → 2,3,6 or 1 → 2,4,6 or 1 → 3,4,6 linked glycosidic bonds existed in HCPS-1, HCPS-2 and HCPS-3. To obtain more information about glycosidic linkage location, the product of periodic acid oxidation was degraded (named Smith degradation) and analyzed on GC (Zhang, 1999).

GC spectra of derivatives from sample references, HCPS-1, HCPS-2 and HCPS-3 after periodic acid oxidation and Smith degradation were presented in Fig. 2. From GC spectra, contents of erythritol were more than that of glycerol and monosaccharide in HCPS-1, HCPS-2 and HCPS-3. These indicated that glucose residue of HCPS-1, HCPS-2 and HCPS-3 were linked mainly by 1 → 4 or 1 → 4,6 glycosidic bonds. Glucose was produced in the Smith degradation of HCPS-1, HCPS-2 and HCPS-3, which means that some glycosyls were not oxidated in periodic acid oxidation and 1 → 3 or 1 → 2,3 or 1 → 2,4 or 1 → 3,4 or 1 → 3,6 or 1 → 2,3,6 or 1 → 2,4,6 or 1 → 3,4,6 linked glycosidic bonds were existed in HCPS-1, HCPS-2 and HCPS-3. Other monosaccharides were observed in GC spectra of Smith degradation. These suggested that other glycosyl residues were connected mainly by 1 → 3 or 1 → 2,3 or 1 → 2,4 or 1 → 3,4 or 1 → 3,6 or 1 → 2,3,6 or 1 → 2,4,6 or 1 → 3,4,6 glycosidic bonds.

3.4. Methylation and GC–MS spectra of purified HCPS

FTIR spectra of methylated HCPS-1, HCPS-2 and HCPS-3 were showed in Fig. 3. The absorption band at about 3400 cm^{-1} for O–H stretching vibrations, existed in pre-methylation, could not be observed. Absorption peak at about 3000–2800 cm^{-1} for C–H stretching vibrations was stronger than that in pre-methylation. These suggested that HCPS-1, HCPS-2 and HCPS-3 had been methylated completely (Ciucanu & Kerek, 1984; Zhang, 1999).

Fig. 3 presented the GC/MS spectra of HCPS-1, HCPS-2 and HCPS-3 after methylation. Analyzable methylated sugar residues of HCPS-1, HCPS-2 and HCPS-3 and their retention time, molar ratio and linkage mode were displayed in Table 2. Backbone chains of HCPS-1, HCPS-2 and HCPS-3 were composed of glycose linked by 1 → 4 glycosidic bonds. Branch chains with non-reducing terminal were attached to backbone chain by 1 → 3 and 1 → 6 glycosidic bonds. Other monosaccharide residues could not be detected on GC/MS maybe due to their minor content. In HCPS-1, seven glycosyl residues were connected to form one polysaccharides repeat unit. In HCPS-2, one polysaccharides repeat unit was constituted by nine glycosyl residues. There all were one backbone chain and two branch chains (one linked by 1 → 3 glycosidic bond, another connected by 1 → 6 glycosidic bond) in one repeat unit of HCPS-1 and HCPS-2. As to HCPS-3, there were ten glycosyl residues in one polysaccharides repeat unit, three branch chains (two connected by 1 → 3 glycosidic bond, one linked by 1 → 6 glycosidic bond) were attached to the backbone chain (Ciucanu & Kerek, 1984; Zhang, 1999).

3.5. NMR spectra of purified HCPS

^1H NMR spectra of HCPS-1, HCPS-2 and HCPS-3 were showed in Fig. 4. Chemical shifts, at 5.288 ppm in HCPS-1 and 5.287 ppm in HCPS-2, indicated that sugar rings of HCPS-1 and HCPS-2 were pyranose rings and saccharide residues of HCPS-1 and HCPS-2 were linked by α-configuration glycosidic bond. A group of signals at 3.2–4.0 ppm were produced by C_2–C_6 protons. As to HCPS-3, peaks, appeared at 4.840 ppm, 5.111 ppm and 5.281 ppm, implied that

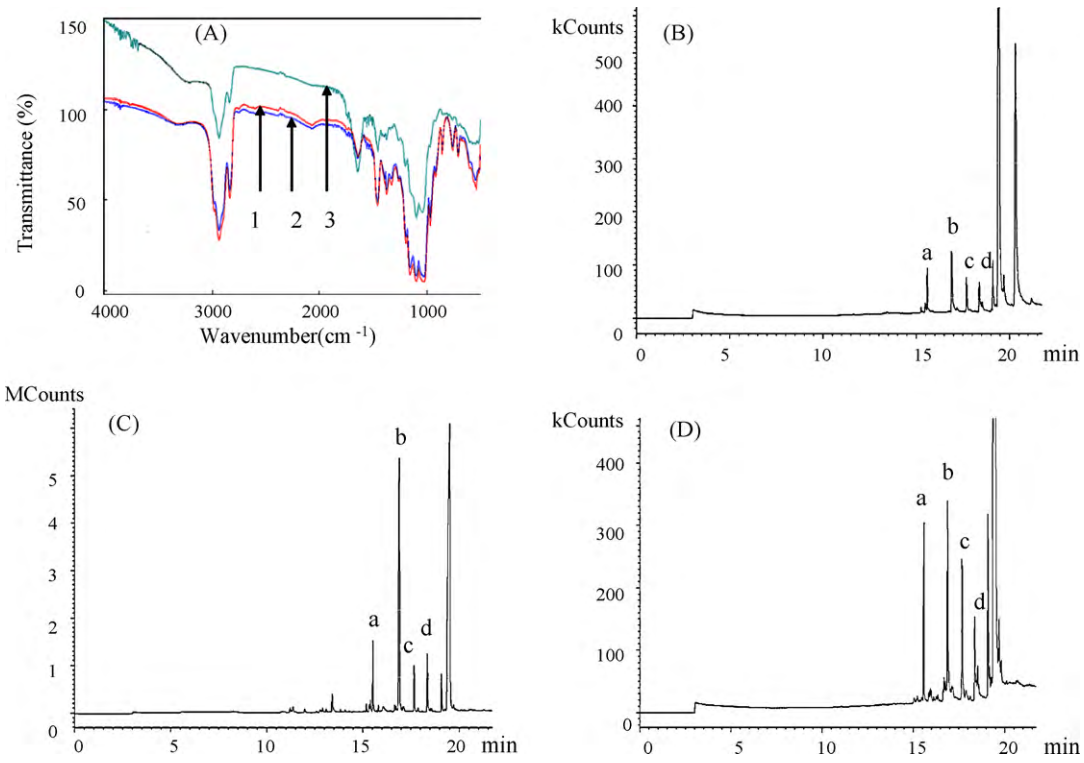

Fig. 3. FTIR spectra (A) and GC/MS spectra of HCPS-1 (B), HCPS-2 (C) and HCPS-3 (D) after methylation reaction 1, HCPS-1; 2, HCPS-2; 3, HCPS-3; a, 2,3,4,6-tetramethyl-glucose; b, 2,3,6-trimethyl-glucose; c, 2,6-dimethyl-glucose; d, 2,3-dimethyl-glucose.

sugar rings of HCPS-3 were pyranose rings and sugar residues of HCPS-3 were connected by α-configuration and β-configuration glycosidic bonds. Signals at 3.2–4.6 ppm were assigned to C_2–C_6 protons, and peaks at 0.6–3.2 ppm, 5.5–6.4 ppm and 6.6–8.4 ppm were created by amino-group, sulfated group and other groups (Zhang, 1999; Ye, Zhang, & Pan, 2007).

Fig. 4 presented ^{13}C NMR spectra of HCPS-1 and HCPS-2. Signals, at 99.986 ppm in HCPS-1 and 99.901 ppm in HCPS-2, suggested that sugar residues of HCPS-1 and HCPS-2 were linked by α-configuration glycosidic bond. Peaks at 77–80 ppm were produced by substituted C_3 and C_4, and signals at 70–77 ppm were assigned to un-substituted C_2, C_3 and C_4. Signals, at 69.408 ppm in HCPS-1 and 69.330 ppm in HCPS-2, were created by substituted C_6. Peaks, at 60.581 ppm in HCPS-1 and 60.444 ppm in HCPS-2, were assigned to un-substituted C_6. These results of NMR spectra were consistent with that of FTIR spectra in Section 3.2 (Zhang, 1999; Ye et al., 2007).

3.6. Helix-coil transition of purified HCPS

The conformational behavior of the polysaccharides, if it possessed triple helix structure, could be evaluated from the shift in the absorption wavelength maximum when Congo red dye was added to polysaccharide at various concentrations of alkali. If the maximal absorption wavelength of mixture increased firstly then decreased with the increasing of NaOH concentration, the polysaccharides possessed the triple helical structure. Otherwise, the polysaccharides did not have the structure of triple helix (Ogawa et al., 1972; Rout et al., 2008). Changes in absorption wavelength maximum of mixture of Congo red and purified HCPS at various concentrations of NaOH were displayed in Fig. 5. Absorption wavelength maximum of mixture decreased gradually with the increasing of NaOH concentration and no characteristic variance of triple helical structure was detected. These results suggested that there were no triple helix structure existed in HCPS-1, HCPS-2 and HCPS-3.

Table 2
GC/MS results of methylated sugar residues of purified HCPS.

Methylated sugar residue	Retention time (min)	Molar ratio	Mode of linkage
HCPS-1			
2,3,4,6-Tetramethyl-glucitol	15.607	2	Glc-(1→
2,3,6-Trimethyl-glucitol	16.886	3	→4)-Glc-(1→
2,6-Dimethyl-glucitol	17.653	1	→3,4)-Glc-(1→
2,3-Dimethyl-glucitol	18.408	1	→4,6)-Glc-(1→
HCPS-2			
2,3,4,6-Tetramethyl-glucitol	15.538	2	Glc-(1→
2,3,6-Trimethyl-glucitol	16.820	5	→4)-Glc-(1→
2,6-Dimethyl-glucitol	17.632	1	→3,4)-Glc-(1→
2,3-Dimethyl-glucitol	18.305	1	→4,6)-Glc-(1→
HCPS-3			
2,3,4,6-Tetramethyl-glucitol	15.568	3	Glc-(1→
2,3,6-Trimethyl-glucitol	16.884	4	→4)-Glc-(1→
2,6-Dimethyl-glucitol	17.688	2	→3,4)-Glc-(1→
2,3-Dimethyl-glucitol	18.350	1	→4,6)-Glc-(1→

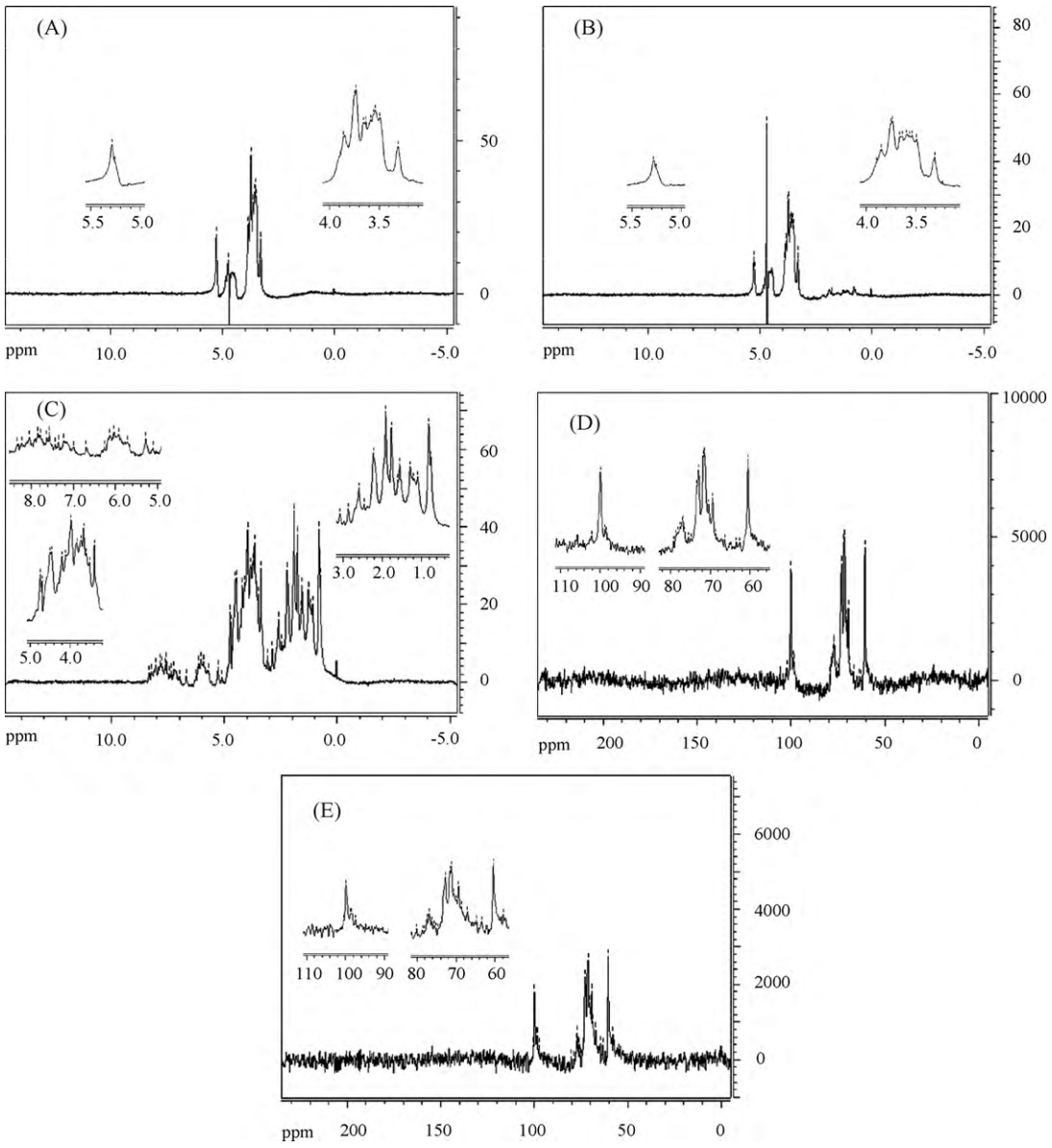

Fig. 4. NMR spectra of HCPS-1, HCPS-2 and HCPS-3 (A) ^{1}H NMR spectrum of HCPS-1; (B) ^{1}H NMR spectrum of HCPS-2; (C) ^{1}H NMR spectrum of HCPS-3; (D) ^{13}C NMR spectrum of HCPS-1; (E) ^{13}C NMR spectrum of HCPS-2.

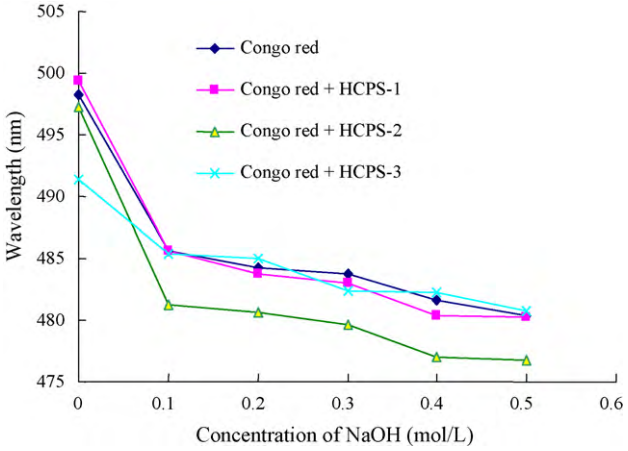

Fig. 5. Changes in absorption wavelength maximum of mixture of Congo red and purified HCPS at various concentrations of NaOH.

4. Conclusion

In this work, the structural characterizations of purified HCPS (HCPS-1, HCPS-2 and HCPS-3) were studied preliminarily. Results showed that sugar rings of HCPS-1, HCPS-2 and HCPS-3 were pyranose rings. Glycosyl residues of HCPS-1 and HCPS-2 were linked mainly by α-configuration glycosidic bond and sugar residues of HCPS-3 were connected by α-configuration and β-configuration glycosidic bonds. Backbone chains of HCPS-1, HCPS-2 and HCPS-3 were composed of glucose linked by 1 → 4 glycosidic bonds. Their branch chains with non-reducing terminal were attached to backbone chain by 1 → 3 and 1 → 6 glycosidic bonds. Number of glycosyl residues, connected to form one polysaccharides repeat unit, was 7, 9 and 10 for HCPS-1, HCPS-2 and HCPS-3 respectively. There were one backbone chain and two branch chains (one linked by 1 → 3 glycosidic bond, another connected by 1 → 6 glycosidic bond) in one polysaccharide repeat unit of HCPS-1 and HCPS-2. As to HCPS-3, three branch chains (two connected by 1 → 3 glycosidic bond, one linked by 1 → 6 glycosidic bond) were pasted to the backbone

chain in one polysaccharides repeat unit. There were no triple helix structure existed in HCPS-1, HCPS-2 and HCPS-3. As HCPS-3 was quite different from HCPS-1 and HCPS-2, further works on their structure–activity relationships are in progress.

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