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Structural elucidation of an exopolysaccharide from mycelial fermentation of a *Tolypocladium* sp. fungus isolated from wild *Cordyceps sinensis*

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ABSTRACT

A novel polysaccharide designated EPS-1A with an average molecular weight around 40 kDa was fractionated and purified by anion-exchange and gel-filtration chromatography from the crude exopolysaccharide (EPS) isolated from fermentation broth of Cs-HK1, a *Tolypocladium* sp. fungus isolated from wild *Cordyceps sinensis*. The structural characteristics of EPS-1A were determined with various methods (e.g. GC, GC–MS, FT-IR, ^{1}H NMR and ^{13}C NMR) and through acid hydrolysis, methylation, periodate-oxidation and Smith degradation. The results suggested that EPS-1A was composed of glucose, mannose and galactose at 15.2:3.6:1.0 M ratio. EPS-1A was a slightly branched polysaccharide and its backbone was composed of (1 → 6)-α-D-glucose residues (~77%) and (1 → 6)-α-D-mannose residues (~23%). Branching occurred at O-3 position of (1 → 6)-α-D-mannose residues of the backbone with (1 → 6)-α-D-mannose residues and (1 → 6)-α-D-glucose residues, and terminated with β-D-galactose residues.

1. Introduction

Mushrooms (edible) are favorite foods in our daily diet, many having tonic effects and medicinal properties. Polysaccharides (PS) are the major bioactive constituents of medicinal mushrooms and useful components of health foods and dietary supplements. Because of their strong antitumor and immumodulation activities, a number of PS and PS-protein complexes derived from mushrooms or mycelial cultures have been commercialized as immunomodulation agents and adjuvant drugs for cancer therapy (Ooi & Liu, 2000; Wasser, 2002). Unlike the small-molecule anticancer drugs by directly killing the cancer cells, these PS or PSP from the mushrooms usually have low toxicity and produce the antitumor effects through modulation or activation of the host immune responses. In addition to antitumor and immonomodulation, some of these PS have shown hypoglycemic, hypolipidemic, antioxidant and radical-scavenging activities. The notable health effects and increasing application of mushroom extracts have motivated considerable research efforts on the purification of PS and investigation of their molecular structures, and the structure–activity relationships. Many of the antitumor PS from mushrooms belong to β-D-glucans such as lentinans from *Lentinus edodes* and schizophyllan from *Schizopgyllum commune* and the PS moiety of PSP from *Coriolus versicolor* (Ooi & Liu, 2000; Wasser, 2002; Zhang, Cui, Cheung, & Wang, 2007). Although the structure–activity relationship is not well established, it has been suggested that β-(1 → 3) linkages in the main chains and β-(1 → 6) linkages in the branching points are important to the antitumor action.

Cordyceps sinensis (Berk.) Sacc., generally called Cordyceps or DongChongXiaCao in China, is a special form of mushroom with a fruiting body formed on an insect larva. It is an Ascomycete fungus while most edible and medicinal mushrooms belong to Bacedomycetes. Cordyceps is a precious and highly acclaimed medicinal fungus in traditional Chinese medicine (TCM) with a broad spectrum of health promoting effects on the kidney, lung, liver and immune functions (Zhu, Halpern, & Jones, 1998; Li & Tsim, 2004). Polysaccharides represent a major class of bioactive constituents of Cordyceps contributing to the health effects and pharmacological activities such as anticancer and antioxidation. Since natural Cordyceps has a limited resource not sufficient to meet the demand, mycelial fermentation has become a major and more economical source of Cordyceps materials. Liquid fermentation is a more efficient process than solid-state fermentation for production of mycelial biomass and bioactive compounds, especially exopolysaccharides (EPS) and other exo-biopolymers such as PS-protein complexes (PSP) released into the fermentation medium. Although numerous studies have demonstrated the processes for production of EPS by mycelial fermentation of *Cordyceps* fungi (references cited in Leung, Zhao, Ho, & Wu, 2009), little information is available about the molecular structures of EPS. The PS structures documented so far of various *Cordyceps* species are all isolated from the fruit bodies or mycelium

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biomass (Methacanon, Madla, Kirtikara, & Prasitsil, 2005; Wu, Sun, & Pan, 2006; Yu et al., 2007, 2009).

Cs-HK1 is a *Tolypocladium* sp. fungus isolated from wild or natural Cordyceps in our lab and has been applied to liquid fermentation for production of mycelial biomass and EPS (Leung, Zhang, & Wu, 2006). The crude EPS isolated from the Cs-HK1 fermentation broth by ethanol precipitation was composed of PS-protein complexes with a wide M_W range from less than 5 kDa to more than 200 kDa, and showed notable antioxidant and radical-scavenging activities (Leung et al., 2009; Yan et al., 2009). The aim of present study is to characterize and elucidate the molecular structure of a pure PS fraction from the crude EPS produced by Cs-HK1 mycelial liquid fermentation.

2. Materials and methods

2.1. Cs-HK1 fungus and mycelial fermentation

Cs-HK1 fungus was originally isolated from the fruiting body of a wild *C. sinensis* organism collected on the high plateaus, 4000–4500 m above sea level, in northwest Sichuan province, China. It was identified as a *Tolypocladium* sp. fungus and an anamorph of *C. sinensis* both morphologically and genetically. Cs-HK1 mycelial liquid fermentation were performed in a liquid medium containing (per liter) 40 g glucose, 10 g yeast extract, 5 g peptone, 1 g KH_2PO_4 and 0.5 g $MgSO_4{\cdot}7H_2O$ at 25 °C for an overall period of 5–6 days. After the fermentation, the broth was spun down and the supernatant collected for isolation of EPS. More details of the Cs-HK1 fungus, mycelium culture characteristics and liquid fermentation processes have been reported previously (Leung et al., 2006).

2.2. Isolation and purification of EPS-1A

The Cs-HK1 mycelial fermentation broth (mycelium-free supernatant) was mixed with 4 volumes of 95% ethanol (v/v), and maintained at 4 °C overnight for precipitation. The precipitate was collected (after centrifugation) and freeze-dried, yielding the crude EPS. This crude EPS was composed of PS-protein complexes with ~25% total protein and ~70% total carbohydrate (Leung et al., 2009).

The crude EPS was deproteinized with enzyme (papain) and Sevag reagent (1-butanol/chloroform at 1:4 v/v), decolorized with activated carbon, and dialyzed (with 12–14 kDa membrane) against distilled water as reported previously (Yan et al., 2009). The final EPS solution was concentrated and lyophilized, yielding a purified crude EPS (about 50% the raw crude EPS) for fractionation in chromatography. The purified crude EPS was fractionated by ion-exchange chromatography (IEC) on a DEAE-cellulose-52 column (2.6 × 60 cm) (Whatman) equilibrated with 0.025 M Tris–HCl (pH 7.4). The column was gradient-eluted with aqueous NaCl solution (0–0.3 M), and PS in the eluate was monitored by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The PS fraction eluted out of the IEC with de-ionized water (i.e. at 0 M NaCl) was collected and further purified by gel-filtration chromatography (GFC) on a Sephadex G-100 column (2.6 × 40 cm) (Pharmacia) eluted with de-ionized water, yielding the pure PS fraction EPS-1A.

2.3. General methods for analysis of EPS-1A

Molecular weight was determined by high pressure gel permeation chromatography (HPGPC) with the instruments and conditions as described in detail by Yan et al. (2009). The HPGPC was calibrated (molecular weight versus elution time) with several dextran molecular weight standards ranging from 5–1400 kDa (Sigma, St. Louis, MO, USA). IR spectra were recorded with a Vector 33 Bruker instrument. Optical rotation was measured with a Perkin-Elmer 341 polarimeter at 20 °C. NMR spectroscopy was performed on a Bruker AV400 instrument (^{1}H NMR at 70 °C; ^{13}C NMR at 50 °C). Gas chromatography (GC) was performed on an Agilent 6890 N instrument with a HP-5 fused-silica capillary column (30 m × 0.25 mm × 1 μm) and a flame ionization detector (FID). The column temperature was set at 180 °C in the initial 2 min and then increased to 280 °C at 10 °C/min. The injector and detector heater temperatures were both set at 250 °C. Nitrogen was used as the mobile phase flowing at 0.7 mL/min. GC–MS was performed on an Agilent 6890 N-5973 instrument according to the following temperature program, fixed at 100 °C for the first 3 min, and then heated to 160 °C at 10 °C/min, and further to 210 °C at 3 °C/min and held for 10 min; the injection temperature was fixed at 280 °C. Helium was used as the mobile phase flowing at 1.0 mL/min. The ionization potential was 70 eV and the temperature of ion source was 230 °C.

2.4. Analysis of monosaccharide constituents by GC

EPS-1A (10 mg) was hydrolyzed with 2 M sulfuric acid (5 mL) at 100 °C for 8 h in a sealed tube. Excess sulfuric acid was neutralized with barium carbonate and centrifuged, and the supernatant was evaporated to dryness under reduced pressure, and the solid was repeatedly re-dissolved in methanol and evaporated to dryness for four times. The final solid residual was treated with hydroxylamine hydrochloride (10 mg) and pyridine (1.0 mL) at 90 °C for 30 min, and then with Ac_2O (1.0 mL) at 90 °C for 30 min, and the final product was analyzed by GC. The monosaccharide peaks were identified and quantified by comparison and calibration with the GC profile of monosaccharide standards (Sigma).

2.5. Methylation analysis

EPS-1A (10 mg) was methylated with the method reported by Ciucanu and Kerek (1984). The methylated PS was treated with 90% aqueous formic acid (3 mL) at 100 °C for 10 h in a sealed tube. After removal of the formic acid, the residues were treated with 2 mL of 2 M trifluoroacetic acid (TFA) under the same conditions and the hydrolysate was concentrated to dryness. The methylated sugars were reduced with $NaBH_4$, and acetylated with acetic anhydride. The alditol acetates were analyzed by GC–MS, and the methylated sugar linkages were identified by the retention time and fragmentation pattern (Bjorndal, Lindberg, & Svenndon, 1967; Needs & Selvendran, 1993).

2.6. Periodate-oxidation and Smith degradation

EPS-1A (20 mg) was oxidized with 15 mM $NaIO_4$ (20 mL) at 4 °C in the dark for about 7 days. HIO_4 consumption was determined by spectrophotometric analysis (Aspinall & Ferrier, 1957) and formic acid production by titration with NaOH. The oxidation was stopped by addition of 1,2-ethanediol and the solution was dialyzed against distilled water for 2 days. The dialyzed product was reduced with $NaBH_4$ (40 mg) for 24 h at room temperature, and then the solution pH was adjusted to 5.0 with 0.1 M acetic acid, and dialyzed against water for 24 h and freeze-dried. The final product was hydrolyzed with 2 M sulfuric acid (5 mL) at 100 °C for 8 h in a sealed tube, the constituent nitrile acetates were analyzed by GC with the same conditions as for the above analysis of monosaccharide constituents.

2.7. Partial acid hydrolysis of EPS-1A

EPS-1A (20 mg) was hydrolyzed sequentially with 5, 50 and 250 mM sulfuric acid at 100 °C (5 mL for 5 h at each concentra-

tion). The hydrolysate after each step of hydrolysis was dialyzed (with 3500 Da membrane) against de-ionized water for 48 h, and the dialysate was neutralized with barium carbonate, and the supernatant solution was collected. The three dialysate fractions A, B and C were applied to GC analysis; the final hydrolysate of 250 mM sulfuric acid (fraction D, retained in the dialysis sack) was applied to methylation analysis. All solutions of EPS fractions were concentrated by evaporation under reduced pressure and lyophilized before being applied to further analysis.

3. Results and discussion

3.1. Properties and composition of EPS-1A

Two major PS fractions, EPS-1 and EPS-2, were isolated by IEC from the purified crude EPS by deproteinization, decolorization and dialysis (Fig. 1A), and fraction EPS-1 which exhibited a larger peak area in the elution profile was collected for further purification by GFC. As shown in Fig. 1B, only a single peak appeared in the GFC elution profile of EPS-1, and the corresponding PS fraction was collected and designated as EPS-1A. The lyophilized EPS-1A solid exhibited a white color and fibrous morphology. EPS-1A also appeared as a single peak on the HPGPC for molecular weight analysis (data not shown), corresponding to an average molecular weight around 40 kDa. Its purify was further confirmed by a constant optical rotation of $[\alpha]_D^{20} = +6.5$ (measured with polarimeter) maintained at different concentrations of ethanol water solution. The D-configuration of EPS-1A obtained from optical rotation was also confirmed by the following GC analysis of monosaccharides and the later ^{13}C NMR spectral data of EPS-1A. EPS-1A had a total carbohydrate of close to 100% (99.0% by the phenol–sulfuric acid method using glucose as a standard), implying its chemical identity as a polysaccharide instead of a PS-protein complex for the original crude EPS from Cs-HK1 fermentation. The GC analysis of nitrile acetates derived from EPS-1A hydrolysate (Fig. 2) shows three monosaccharide constituents, D-Glc, D-Man and D-Gal, at a molar ratio of 15.2:3.6:1.0.

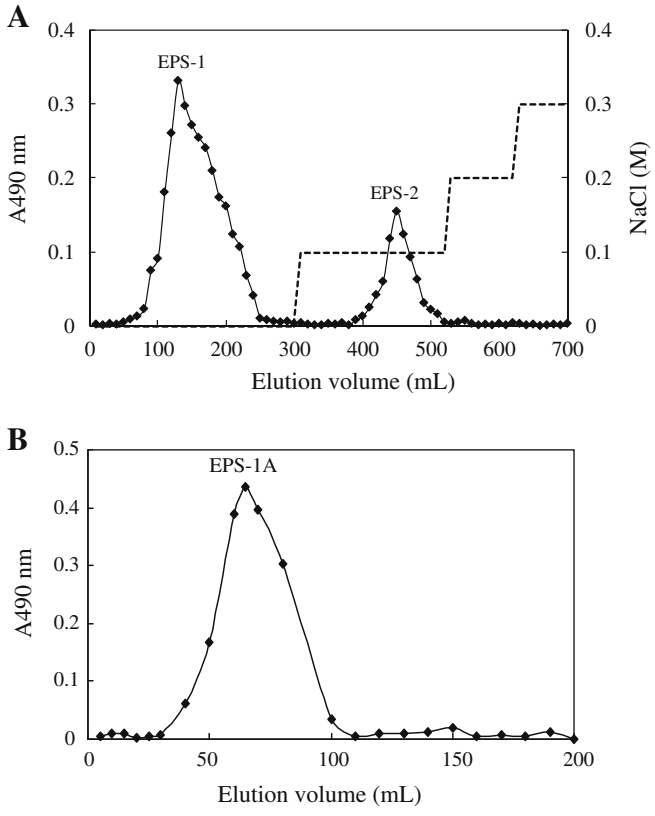

Fig. 1. (A) IEC profile of the purified crude EPS (from a DEAE-cellulose-52 column with the elution scheme shown by the dash line); (B) GFC profile of EPS-1A (from a Sephadex G-100 column eluted with di-ionized water).

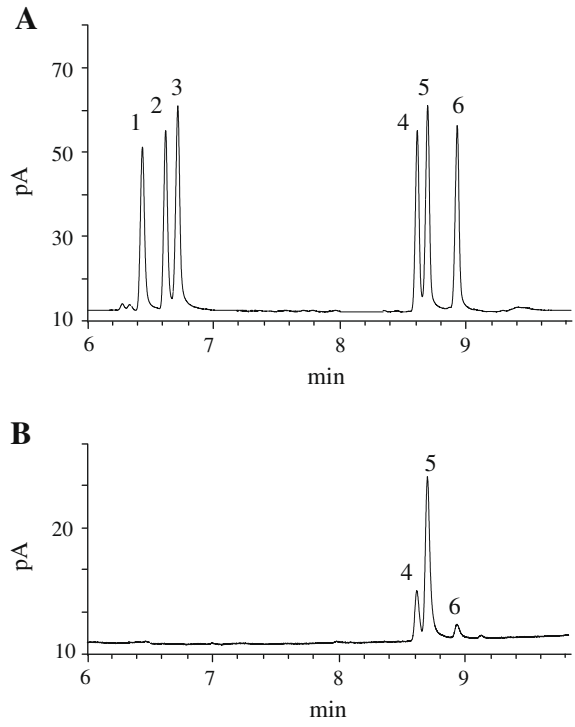

Fig. 2. GC profiles of nitrile acetates of (A) monosaccharide standards and (B) EPS-1A constituents. Peaks: 1, D-Rha (Rt: 6.4 min); 2, D-Ara (Rt: 6.6); 3, D-Xyl (Rt: 6.7); 4, D-Man (Rt: 8.6); 5, D-Glc (Rt: 8.7); 6, D-Gal (Rt: 8.9).

3.2. IR spectrum and elucidation of EPS-1A

Fig. 3 presents the IR spectrum of EPS-1A. The broad and intense stretching at 3398 cm^{-1} is characteristic of hydroxyl groups and the weak stretching at 2933 cm^{-1} is attributed to the C–H bond (Santhiya, Subramanian, & Natarajan, 2002). The absorption bands at 813 and 889 cm^{-1} are suggestive of both α- and β-type glycosidic linkages (Barker, Bourne, Stacey, & Whiffen, 1954), and the bands between 950 and 1200 cm^{-1} are mostly attributed to C–O–C and C–O–H linkages (Kacuráková, Capek, Sasinková, Wellner, & Elbringerová, 2000). The stretching peak at 1060 cm^{-1} is

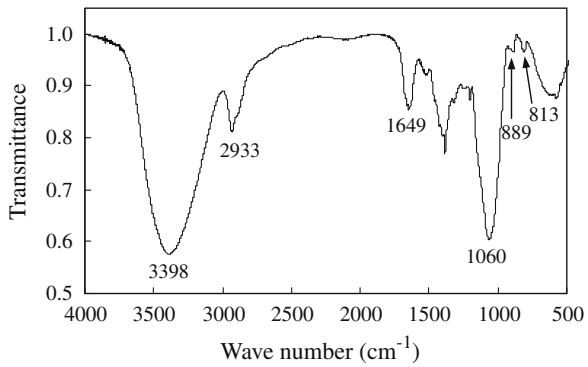

Fig. 3. FT-IR spectrum of EPS-1A.

Table 1
Methylation analysis of EPS-1A and fraction D from partial acid hydrolysis (GC–MS data).

Methylated sugar	t_R (min)[b]	Molar ratio	MS (*m/z*)	Linkage type
EPS-1A				
2,3,4,6-Me_4-Gal	1.08	1.00	45,87,101,117,145,161,205	T-
2,3,4-Me_3-Man	1.15	3.20	45,71,87,101,129,161,189	1, 6-
2,3,4-Me_3-Glc	1.54	14.90	45,71,87,101,189,233	1, 6-
2,4-Me_2-Man	1.62	0.96	71,87,99,101,189	1, 3, 6-
Fraction D				
2,3,4,6-Me_4-Glc[a]	1.00	1.00	45,71,87,101,117,145,161,205	T-
2,3,4,6-Me_4-Man	1.03	trace	71,87,101,117,145,161,205	T-
2,3,4-Me_3-Man	1.19	3.08	45,71,87,101,129,161,189	1, 6-
2,3,4-Me_3-Glc	1.58	8.99	45,71,87,101,189,233	1, 6-
2,4-Me_3-Man	1.63	trace	71,87,99,101,189	1, 3, 6-

[a] 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-gluctiol.
[b] Relative retention time to 1, 5-di-*O*-acetyl-2, 3,4,6-tetra-*O*-methyl-gluctiol.

suggestive of a C–O bond. The band at 1649 cm^{-1} can be attributed to water bound to the PS molecule (Park, 1971).

3.3. Structure features of EPS-1A after hydrolysis

The methylation followed by formolysis and acetylation of EPS-1A gave rise to four alditol acetates as detected from GC–MS (Table 1), namely 2,3,4,6-Me_4-Gal, 2,3,4-Me_3-Glc, 2,3,4-Me_3-Man, and 2,4-Me_2-Man in a molar ratio of 1:15:3:1. Based on the results, the linkages of the constituent sugars are identified as the non-reducing-end D-galactopyranosyl (5%), (1 → 6)-linked D-glucopyranosyl (75%), (1 → 6)-linked D-mannopyranosyl (15%), and (1 → 3, 1 → 6)-linked D-mannopyranosyl (branch point) (5%) moieties. Therefore, EPS-1A may be recognized as a slightly branched polysaccharide with the side chains attached to the O-3 position of D-mannopyranosyl residues. The molar ratios obtained here are in close agreement with the monosaccharide composition of EPS-1A obtained from GC analysis of the nitrile acetates (Fig. 2).

The monosaccharide composition of EPS-1A fractions derived from partial acid hydrolysis was determined by GC analysis of the nitrile acetates. Fraction A (derived from 5 mM sulfuric acid hydrolysis of EPS-1A) was composed solely of galactose, which may be the non-reducing-end in the branch structure of EPS-1A. Fraction B (from 50 mM sulfuric acid hydrolysis) was composed of glucose, mannose, and galactose at 4.5:0.9:1.0 M ratio, which may form the branch structure of EPS-1A with mannose attaching to the backbone. Fraction C (from 250 mM sulfuric acid hydrolysis) was composed of glucose and mannose at 5.5:1.0 M ratios, which may also constitute the branch structure of EPS-1A. The results suggest that glucose is most abundant in the EPS-1A structure, ~50% in the backbone and ~20% in the branches and mannose is located at the branching point of the backbone.

Fraction D, the final EPS-1A hydrolysate retained in the dialysis sack after the sequential acid hydrolysis, should have the largest molecular size among the four hydrolyzed fractions and form a major part of the EPS-1A structure. GC–MS analysis of the

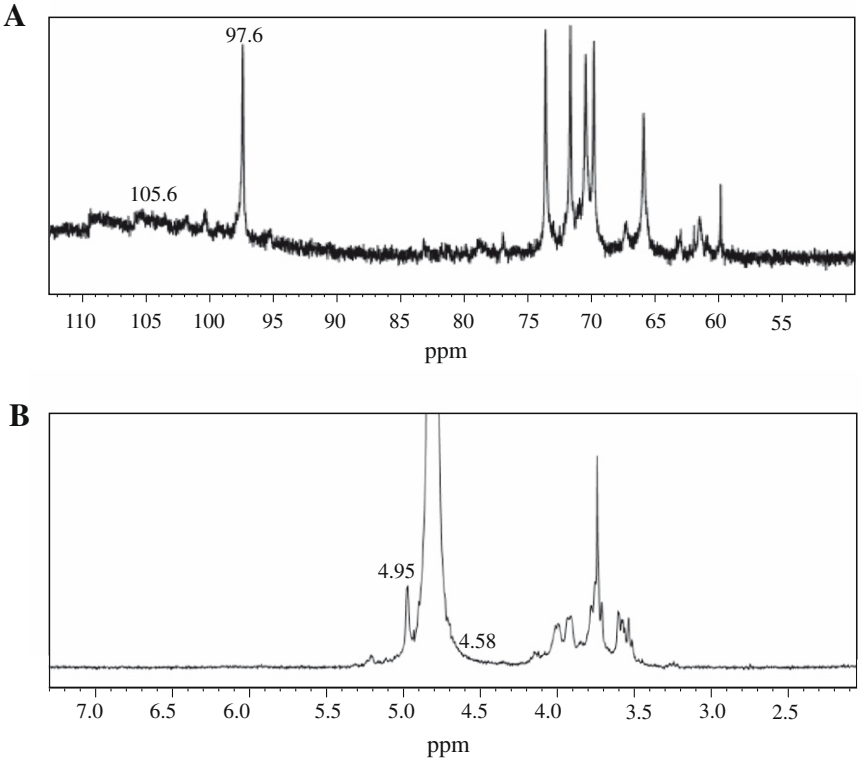

Fig. 4. ^{13}C NMR (A) and ^{1}H NMR spectrum (B) of EPS-1A.

Table 2
^{13}C NMR chemical shifts of EPS-1A (δ, ppm).

Sugar residues	C-1	C-2	C-3	C-4	C-5	C-6
→ 6)-α-D-Glcp(1 →	97.64	70.12	73.34	69.47	71.34	65.46
β-D-Galp(1 →	105.64	73.91	75.11	71.01	75.11	61.41
→ 6)-α-D-Manp(1 →	100.67	70.61	70.61	68.56	71.19	66.83
→ 6)-α-D-Manp(1 → (3 ←	102.11	72.20	76.78	68.83	71.19	66.83

methylated fraction D revealed five fragments (Table 1), 2,3,4,6-Me_4-Glc, 2,3,4,6-Me_4-Man, 2,3,4-Me_3-Man, 2,3,4-Me_3-Glc and 2,4-Me_3-Man. The results suggest that the backbone of EPS-1A is composed of D-glucopyranosyl, D-mannopyransoyl, (1 → 6) D-glucopyranosyl and (1 → 6) D-mannopyransoyl moieties. Together with the GC–MS data of methylated EPS-1A, we conclude that (1 → 6) D-glucopyranosyl and (1 → 6) D-mannopyranosyl in 10:3 M ratio serve as the repeating monosaccharide units of the main chain, and (1 → 3, 6) D-mannose is the branching point in the backbone of EPS-1A structure.

Mannose and glycerin were detected from the periodate-oxidized product of EPS-1A by GC. The presence of mannose suggests that a part of mannose in EPS-1A should be in 1, 3-, 1, 2, 3-, 1, 2, 4-, 1, 3, 4-, 1, 3, 6- or 1, 2, 3, 4-linkage which cannot be oxidized by HIO_4; the absence of glucose and galactose suggests that glucose and galactose are 1-, 1, 6-, 1, 2-, 1, 2, 6-, 1, 4-, or 1, 4, 6-linkage which are liable to oxidation by HIO_4. This and the results of methylation analysis suggest that mannose in the oxidation product of EPS-1A are derived from 1, 3, 6-linked mannose residues, and glycerin from 1, 6-linked glucose, 1, 6-linked mannose and 1-linked galactose. In addition, the HIO_4 consumption and formic acid production during the periodate-oxidation of EPS-1A were 0.722 mol and 0.342 mol per mole of sugar residues, respectively, which are also in agreement with values predicted from the structure elucidated above.

The analytical results from methylation and partial acid hydrolysis of EPS-1A indicate that (1 → 6)-linked-glucose is the most abundant residue in the polysaccharide structure and should be a major part of the backbone structure. Based on the relative amount of (1 → 3, 6)-linked-mannose for the branching point of the backbone, the branch to backbone ratio is estimated at 8% by mole, equivalent to one branching point for every 13 residues on the backbone. The branch structure is composed of non-reducing-end galactose, (1 → 6)-linked-glucose and (1 → 6)-linked-mannose residuals, in accordance with the results from partial acid hydrolysis and Smith degradation.

3.4. Structure characteristics of EPS-1A from NMR

Fig. 4 shows the NMR spectra of EPS-1A. On the ^{13}C NMR spectrum (Fig. 4A), the six strong signals between 98–65 ppm are attributed to C-1, -2, -3, -4, -5, and -6 of D-glucopyranosyl unit of the main → 6)-α-D-Glcp-(1 → linkage. The weak resonances between 99 and 106 ppm can be attributed to the anomeric carbon atoms of mannopyranose (Manp) and galactopyranose (Galp), respectively; the peak at 105.6 ppm is attributed to C-1 in β-D-galactopyranose (Galp) residues. In the ^{1}H NMR spectrum (Fig. 4B), the resonance at 4.95 ppm may be attributed to α(1 → 6) glucosyl residues of the main chain (Seymour, 1979b), and the H-1 signals at 4.58 ppm conforms to the β form of D-galactopyranosyl residues. The chemical shifts from 3.4 to 4.2 ppm are attributed to protons of carbons C-2 to C-6 of the glycosidic ring of EPS-1A (Chauveau, Talaga, Wieruszeski, Strecker, & Chavant, 1996). The ^{1}H/^{13}C NMR chemical shifts of monosaccharide residues were assigned according to documented spectral data in literature (Agrawal, 1992; Gorin, 1981; Zhang, 1999; Yu et al., 2007). The ^{13}C NMR chemical shifts are summarized in Table 2.

3.5. PS structures found in Cordyceps fungal species

Polysaccharides of different monosaccharide constituents and molecular structures have been isolated from *C. sinensis* and other *Cordyceps* fungal species. Kiho, Ookubo, Usui, Ukai, and Hirano (1999) characterized a pure PS CS-F10 with an average molecular weight of 15 kDa isolated from the mycelium hot water extract of a *C. sinensis* fungus, as a galactoglucomann composed of 43:33:24 galactose:glucose:mannose. It had a structure of terminal α-D-glucopyranosyl residues, and (1 → 5)-linked β-D-galactofuranosyl residues, and (1 → 2)-linked and branched α-D-mannopyranosyl residues. The CS-F10 PS exhibited significant hypoglycemic activity in normal, streptozotocin (STZ)-induced diabetic and epinephrine-induced hyperglycemic mice. Wu et al. (2006) isolated a PS from the mycelium hot water (buffered with phosphate) extract of a *C. sinensis* fungus having an average M_W of 185 kDa and an α-D-glucan structure with glycosyl linkages. Yu et al. (2009) reported a PS purified from the alkaline extract of cultivated *C. militaris* fruiting bodies with an average M_W of 17 kDa and a structure of a (1 → 4)-α-D-mannose backbone with branches at O-3 position of (1 → 4)-α-D-glucose, (1 → 6)-β-D-galactose and β-D-galactose. It exhibited moderate hydroxyl radical-scavenging activity. The variety of PS chemical structures found in the previous studies may be attributed in part to the different source materials and the isolation, and fractionation protocols. Up to now, however, little knowledge has been gained of the structure and activity relationships of these PS owning to the high molecular weight and complex structure.

4. Conclusion

A water-soluble PS fraction EPS-1A has been purified from the crude EPS produced by *C. sinensis* (Cs-HK1) fermentation and identified as a hetero-polysaccharide molecule with (1 → 6)-α-D-glucose being the major structural component. The structure of EPS-1A is somewhat similar to dextrans which also contain a large number of consecutive α-(1 → 6)-linkages in the main chain, accounting for more than 50% of the total linkages. Based on the analytical results, we conclude that EPS-1A has a backbone of (1 → 6)-α-D-glucose residues and (1 → 6)-α-D-mannose residues and branch chains composed of an (1 → 6)-α-D-mannose residue and five (1 → 6)-α-D-glucose residues, and β-D-galactose residues. The complete structure of EPS-1A is presented in Fig. 5. It is a novel PS from a medicinal fungus which has not been documented before.

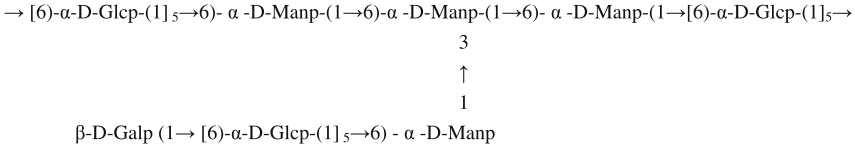

Fig. 5. Complete structure (repeating unit) of EPS-1A.

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