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Structural characterisation of a novel bioactive polysaccharide from *Ganoderma atrum*

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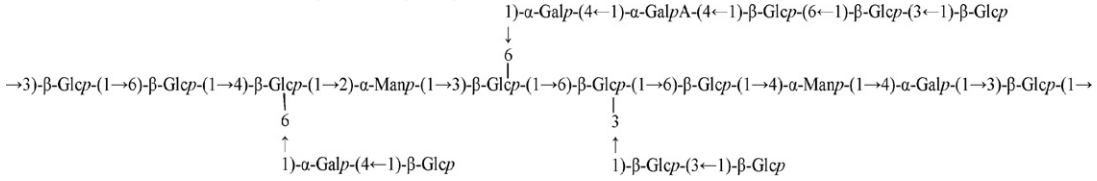
ABSTRACT

The structure of a novel bioactive polysaccharide fraction from *Ganoderma atrum* (PSG-1) was characterised by methylation analysis and 1D/2D nuclear magnetic resonance (NMR) spectroscopy. Sugar analysis revealed that PSG-1 was composed of glucose (Glc), mannose (Man), galactose (Gal) and galacturonic acid (GalA) in molar ratio of 4.91:1:1.28:0.71. Methylation and GC–MS analysis indicated that the main linkage type was 1,3-linked-Glc*p* (21.08%), followed by T-Glc*p* (18.68%), 1,3,6-Glc*p* (12.97%), 1,4-Gal*p* (12.70%), 1,6-Glc*p* (12.33%), 1,2-Man*p* (8.06%), 1,4-Gal*p*A (6.15%), 1,4-Man*p* (4.55%) and 1,4,6-Glc*p* (3.24%). Combined the methylation analysis results with 1D (^{1}H, ^{13}C) and 2D (DQF-COSY, TOCSY, HSQC and HMBC) NMR spectroscopy, a preliminary structure of PSG-1 was proposed as follows:



1. Introduction

The genus *Ganoderma*, well known as "Lingzhi" in Chinese, is one of the most important resources for dietary supplements as well as curing agents in Asian folk medicine. Ancient Chinese medical scholars believed that "Lingzhi" could strengthen body resistance and consolidate the constitution of patients, i.e., "Fuzheng Guben", which is one of the major principles in the therapeutics of traditional Chinese medicine (Cong & Lin, 1981). *Ganoderma* has been shown to possess hepatoprotective (Chen & Yu, 1999; Zhang, Wang, Ni, Teng, & Lin, 2002), antihypertensive (Morigiwa, Kitabatake, Fujimoto, & Ikekawa, 1986), hypocholesterolemic (Komoda, Shimizu, Sonoda, & Sato, 1989; Shiao, 1992), anti-HIV (El-Mekkawy et al., 1998) and anti-tumor and immunomodulatory activities (Bao, Liu, Fang, & Li, 2001; Lin & Zhang, 2004). These beneficial effects have been partly attributed to its variety of chemical components, such as adenosine, lectins, polysaccharides, proteins, sterols and triterpenoids. Because of these considerable activities, *Ganoderma* is called as a therapeutic fungal biofactory (Russell & Paterson, 2006).

In recent years, many polysaccharides isolated from natural sources have been proved to possess excellent bio-activities (Zhang, Cui, Cheung, & Wang, 2006), which have attracted much attention in the field of biochemistry and pharmacology. As the main ingredient of water-extracts, polysaccharide is considered as one of the most important substances to demonstrate the therapeutic function of *Ganoderma*. In previous studies, the significant anti-tumor and immuno-enhancing activities of *Ganoderma* were attributed to the effect of polysaccharide (Chen, Yang, Wong, & Shih, 2010; Huang & Ning, 2010; Miyazaki & Nishijima, 1981; Xu, Chen, Zhong, Chen, & Wang, 2011). For these reasons, great interest has been focused on finding reliable methods to study the structure and the structure–activity relationships of polysaccharides. It has been reported that structural features of (1 → 3)-β-linked glucose residue as main chain and β-glucose residue substituted at *O*-6 position as branch point were important factors in bioactivity, and the degree of substitution on the main chain and the length of side chains might also have the same effects on biological activities (Zhang, Li, Xu, & Zeng, 2005). However, many other chemical

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structures of polysaccharides such as heteroglycan, glycopeptides and mannoglucan were also shown to possess bioactivities (Bao, Wang, Dong, Fang, & Li, 2002; Zhuang et al., 1993).

In China, the genus *Ganoderma* was first classified as six kinds according to the difference in color and luster in "Shen Nong's Herbal Classic", but most of the previous studies were just concentrated on *Ganoderma lucidum* (red) and *Ganoderma tsugae* (red-brown) which were commonly known to possess medicinal and/or nutritional values. However, more and more studies have shown that another member *Ganoderma atrum* (black) could also be used to promote health and longevity (Gao, Chan & Zhou, 2004; Gao et al., 2005). In recent years, our group has paid much attention on this kind of *Ganoderma*, which is popular in Southern China. A polysaccharide from *G. atrum* (PSG-1) was extracted and purified in our laboratory (Chen, Xie, Nie, Li, & Wang, 2008). The antitumor effect of PSG-1 in sarcoma 180-bearing mice has also been investigated (Li et al., 2011). In addition, the effect of PSG-1 against oxidative stress has also been demonstrated by our previous work (Li et al., 2010). However, to our knowledge, there were few reports about the structure of polysaccharide from *G. atrum*. The objective of the current work was thus to study the structural feature of PSG-1 using methylation analysis and 2D NMR spectroscopy including homonuclear $^{1}H/^{1}H$ correlation spectroscopy (DQF-COSY, TOCSY), and heteronuclear $^{13}C/^{1}H$ multiple-quantum correlation experiments (HSQC, HMBC).

2. Materials and methods

2.1. Materials and reagents

The crude polysaccharide of PSG was extracted from fruiting bodies of *G. atrum* which were cultivated in Ganzhou, Jiangxi Province, China. The purified sample of PSG-1 fraction was obtained by gel-filtration chromatography with Superdex-G 200 (2.6×60 cm). Its homogeneity was determined by high performance gel permeation chromatography (HPGPC) on a Waters HPLC system which showed as a single and symmetrically sharp peak on Ultrahydrogel-500 column. The fraction details, chemical characterisation and physicochemical properties have been described (Chen et al., 2008). All reagents used were of analytical grade unless otherwise specified.

2.2. Sugar analysis

The content of neutral saccharide was measured by phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), and the uronic acid content was determined using the modified carbazole and sulphuric acid spectrophotometric method (Radhakrishnamurthy & Berenson, 1963).

Reduction of uronic acid was conducted following a published procedure (Taylor & Conrad, 1972; York, Darvill, Mcneil, Stevenson, & Albersheim, 1986) with slight modifications. The reduction degree was detected by carbazole and sulphuric acid spectrophotometric method (Radhakrishnamurthy & Berenson, 1963) combined with FT-IR spectrum to make sure that the —COOH group (around at 1700 cm^{-1}) disappeared completely. The reduced sample was named as RPSG-1.

Monosaccharide compositions of PSG-1 and RPSG-1 were detected by gas chromatography (GC, Agilent 6890, USA) with a flame ionization detector (FID), and the procedures were described in our previous study (Chen et al., 2008). The type of uronic acid was determined by comparing the monosaccharide composition between PSG-1 and RPSG-1.

2.3. Methylation and GC–MS analysis of RPSG-1

Methylation analysis of RPSG-1 was carried out according to the method by Ciucanu and Kerek (1984) with slight modification (Nie et al., 2011). The methylated sample was detected by FT-IR spectrum to make sure that the peak of —OH band (3200–3700 cm^{-1}) disappeared completely. Then the dried methylated sample was hydrolyzed and derived as partially methylated alditol acetates (PMAA) which were then analyzed by GC–MS system (Agilent technology 7890/7000 QQQ, USA) equipped with a SP-2330 (Supelco, Bellefonte, Pa) capillary column (30 m × 0.25 mm, 0.2 mm film thickness) programmed from 160 to 210 °C at 2 °C/min, and then 210–240 °C at 5 °C/min.

2.4. Nuclear magnetic resonance (NMR) spectroscopy of PSG-1

PSG-1 was kept in a vacuum oven (~80 °C) for about 6 h. Then the dried sample was exchanged with deuterium by lyophilizing with deuterium oxide (D_2O) for 3 times, and sample was finally dissolved in D_2O at room temperature for 3 h before NMR analysis. Both ^{1}H and ^{13}C spectrum were recorded on a Bruker Avance 600 MHz NMR spectrometer (Brucker, Rheinstetten, Germany). The spectrum of ^{1}H, ^{13}C, and homonuclear $^{1}H/^{1}H$ correlation experiments (DQF-COSY, TOCSY), and heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) experiments were conducted at 60 °C.

3. Results and discussion

3.1. Sugar analysis

The neutral saccharide and uronic acid content of PSG-1 were determined to be 73.1% and 6.4%, respectively. GC analysis revealed that PSG-1 was composed of glucose (Glc), mannose (Man), galactose (Gal) and galacturonic acid (GalA) in molar ratio of 4.91:1:1.28:0.71. The considerable proportion of uronic acid in PSG-1 would make it difficult to carry out the methylation experiment (Cui, 2005), so the carboxyl group of PSG-1 should be reduced before methylation analysis.

3.2. Methylation analysis

The individual peaks of the partially methylated alditol acetate (PMAA) from the GC analysis and mass spectroscopy of each peak were identified by their retention time and by comparison with mass spectrum patterns from literature (Carpita & Shea, 1989). The related linkage patterns and corresponding percentage of PSG-1 were shown in Table 1, and the molar ratio of monosaccharide residues was calculated according to the peak areas and response factor of the total ion chromatogram (TIC) in Agilent GC–MS system.

The proportion of the non-reducing T-D-Glc*p* residue was 18.68%. The majority of branched sugar residue was 1,3,6-D-Glc*p* which accounted for 12.97% of the total sugar residues, followed by 1,4,6-D-Glc*p* (3.24%). These data indicated that the ratio between terminal units and branching point was 1.15, which was consistent with the fact that the number of polysaccharide branching points approximately equals to the number of terminal units. The unsubstituted residues were shown to be 1,3-D-Glc*p*, 1,6-D-Glc*p*, 1,4-D-Gal*p*, 1,2-D-Man*p* and 1,4-D-Man*p* in percentage of 21.08%, 12.33%, 12.70%, 8.06% and 4.55%, respectively. The galacturonic acid was found to be as 1,4-D-Gal*p*A with the amount of 6.15%. The data from the methylation analysis suggested that PSG-1 was mainly composed of →1)-D-Glc*p*-(3→ and →1)-D-Glc*p*-(6→ as the backbone with some →1)-D-Gal*p*-(4→, →1)-D-Man*p*-(2→ and →1)-D-Man*p*-(4→ which could exist in the backbone or side chains.

Table 1
Linkage patterns and corresponding percentage of PSG-1 by methylation analysis.

RT[a] (min)	PMAA	Linkage pattern	Peak area percentage (%)	Molar ratio of monosaccharide residues
14.70	2,3,4,6-Me_4-Glc*p*	T-Glc*p*	18.68	3.4
20.06	2,4,6-Me_3-Glc*p*	1,3-linked-Glc*p*	21.08	3.8
22.24	2,3,4-Me_3-Glc*p*	1,6-linked-Glc*p*	12.33	2.7
28.15	2,4-Me_2-Glc*p*	1,3,6-linked-Glc*p*	12.97	2.4
28.82	2,3-Me_2-Glc*p*	1,4,6-linked-Glc*p*	3.24	0.7
20.31	3,4,6-Me_3-Man*p*	1,2-linked-Man*p*	8.06	1.5
22.51	2,3,6-Me_3-Man*p*	1,4-linked-Man*p*	4.55	0.8
23.09	2,3,6-Me_3-Gal*p*	1,4-linked- Gal*p*	12.70	2.8
23.09	2,3,6-Me_3-Gal*p*A	1,4-linked- Gal*p*A	6.15	1.4

[a] RT, retention time.

The major branching points were at *O*-4 and/or *O*-6 positions of the Glc*p* chain, with β-D-Glc*p* as the terminal residue.

To our knowledge, this was the first report on the structural characteristics of polysaccharide from *G. atrum*. Compared with the previous study of polysaccharide from *G. lucidum*, our results were similar to Liu (Liu, Wang, Pang, Yao, & Gao, 2010) which assigned →3)-Glc*p*-(1→, →4)-Glc*p*-(1→ and →6)-Glc*p*-(1→ in the backbone, and the *O*-4 and/or *O*-6 as the branching points. However, some discrepancies have also been published in the literature. For example, Bao et al. (2002) claimed a polysaccharide from *G. lucidum* (PL-1) with immunocompetence had a backbone consisting of 1,4-α-D-Glc*p* and 1,6-β-D-Gal*p* with branches at *O*-6 of glucose residues and *O*-2 of galactose residues. The discrepancies in these studies cannot be easily resolved because of the differences in species and extraction methods used for sample preparation, and the differences highlighted the uniqueness of PSG-1. The subsequent NMR spectroscopy confirmed the conclusions drawn from methylation analysis and provided us with more details of the structure of PSG-1.

3.3. NMR analysis

The structural features of PSG-1 were further identified by 1D and 2D NMR spectrum. The ^{1}H NMR spectrum (Fig. 1a) showed more than 15 peaks in the anomeric region (4.3–5.8 ppm), but only 9 peaks were found to be significant and used for the analysis. They were designated A, B, C. . .I according to the decreasing order of peak intensity. In the ^{13}C NMR spectrum (Fig. 1b), a small peak at δ 173.31 ppm was a typical signal of uronic acid, which was indicated as 1,4-D-Gal*p*A according to methylation and GC–MS results.

HSQC spectrum (Fig. 2c) showed 9 cross peaks in the anomeric region, which indicated that nine spin systems were present in PSG-1. They were marked as A1, B1, C1. . .I1 in accordance with the ^{1}H spectrum labels. All the ^{1}H and ^{13}C signals of residues from A to I were assigned completely using DQF-COSY, TOCSY and HSQC, and the linkage sites and sequence among residues were identified by HMBC.

*β-1,3-Glcp (residue **A**)*. According to the methylation analysis, 1,3-Glc*p* was expected to be the most abundant residue. In ^{1}H spectrum of PSG-1, the peak at 4.87 ppm was the most intense in the anomeric region (Fig. 1a). By comparing with previous studies (Laws, Chadha, Chacon-Romero, Marshall, & Maqsood, 2008; Perepelov et al., 2007; Ye, Li, Zhang, & Pan, 2010) in combination with the peak intensity, this peak was tentatively assigned as the anomeric proton of 1,3-Glc*p*. The H-1 chemical shift of residue **A** at δ 4.87 ppm indicated that it was β-linked. A cross peak at δ 4.87/3.66 ppm was easily found in DQF-COSY spectrum (Fig. 2a), implying that the chemical shift of H-2 was δ 3.66 ppm. The other proton signals for H-3, H-4 and H-5 of this residue were assigned according to DQF-COSY spectrum with the same method, and H-6 was confirmed by means of TOCSY (Fig. 2b) combined with DQF-COSY. On the basis of the proton assignments, the chemical shifts of the carbon atoms of residue **A** were readily obtained from the HSQC spectrum (Fig. 2c). Both ^{1}H and ^{13}C chemical shifts were in consistent with previous literatures (Laws et al., 2008; Maity et al., 2011; Perepelov et al., 2007; Ye et al., 2010), and the downfield shifts of the C-1 (δ 103.25 ppm) and C-3 (δ 84.92 ppm) carbon signals indicated that residue **A** was a β-1,3-D-glucopyranose (Glc*p*).

*β-T-Glcp (residue **B**)*. As the second highest peak in ^{1}H spectrum, residue **B** at 4.84 ppm in the anomeric region was tentatively assigned as T-Glc*p* according to the methylation analysis result. The anomeric signal at δ 4.84 ppm indicated that residue **B** was a β-linked residue. The chemical shifts of H-2, H-3, H-4, H-5 and H-6 were obtained from DQF-COSY (Fig. 2a), and confirmed by TOCSY spectrum (Fig. 2b). The carbon signals from C-1 to C-6 of residue **B** were identified from the HSQC spectrum (Fig. 2c). Both carbon and proton chemical shifts were compared with previous studies (Agrawal, 1992; Bhunia et al., 2010; Ge, Zhang & Sun, 2009; Ghosh et al., 2008) to confirm that residue **B** was a β-T-D-Glc*p*.

*α-1,4-Galp (residue **C**)*. The anomeric signal at δ 5.10 ppm indicated that residue **C** was an α-linked residue, which was further corroborated by the C-1 chemical shift at δ 102.32 ppm. Residue **C** was tentatively assigned as 1,4-Gal*p* by comparison with literature values of the anomeric chemical shifts (Das et al., 2009). With the help of DQF-COSY spectrum, the chemical shifts from H-2 to H-6 of residue **C** were assigned, and the results were confirmed by TOCSY spectrum combined with literatures (Chandra, Ghosh, Ojha, & Islam, 2009; Luo et al., 2009; Ojha et al., 2008). The carbon signals from C-1 to C-6 of residue **C** were identified from the HSQC spectrum (Fig. 3c). The downfield shifts of the C-1 (δ 102.32 ppm) and C-4 (δ 76.55 ppm) carbon signals indicated that residue **C** was an α-1,4-D-galactopyranose (Gal*p*).

*α-1,4-GalpA (residue **I**)*. According to the methylation analysis results (Table 1), the content of 1,4-Gal*p*A was approximately 6.15% in PSG-1. The anomeric proton chemical shift of residue **I** at δ 4.97 ppm and the C-1 identified from HSQC at δ 99.77 ppm indicated it α-linked. This residue showed only 5 protons signals and a high chemical shift of H-5 (δ 4.60 ppm) observed from DQF-COSY spectrum, and the resonance at 173.31 ppm in ^{13}C spectrum (Fig. 1b) was attributed to be the —COOH group, so residue **I** was deduced as a galactopyranouronic acid (Gal*p*A). All the ^{1}H and ^{13}C chemical shifts of residue **I** assigned by DQF-COSY and HSQC in this study were consistent with previous studies (Maiti et al., 2008), and the downfield shift of C-4 (δ 79.50 ppm) carbon signals with respect to standard values of methyl glycosides indicated that residue **I** was an α-1,4-D-Gal*p*A.

*β-1,6-Glcp (residue **E**)*. For residue **E**, the anomeric proton signal appeared at δ 4.52 ppm and anomeric carbon signal at δ 103.12 ppm indicated that it was a β-linked residue. The assignment of proton signals from H-2 to H-6 was conducted by DQF-COSY and TOCSY spectrum combined with previous literatures (Das et al., 2010; Mandal et al., 2010). The corresponding carbon chemical shifts were obtained from the cross peaks of H-1-C-1, H-2-C-2,. . .H-6-C-6 in

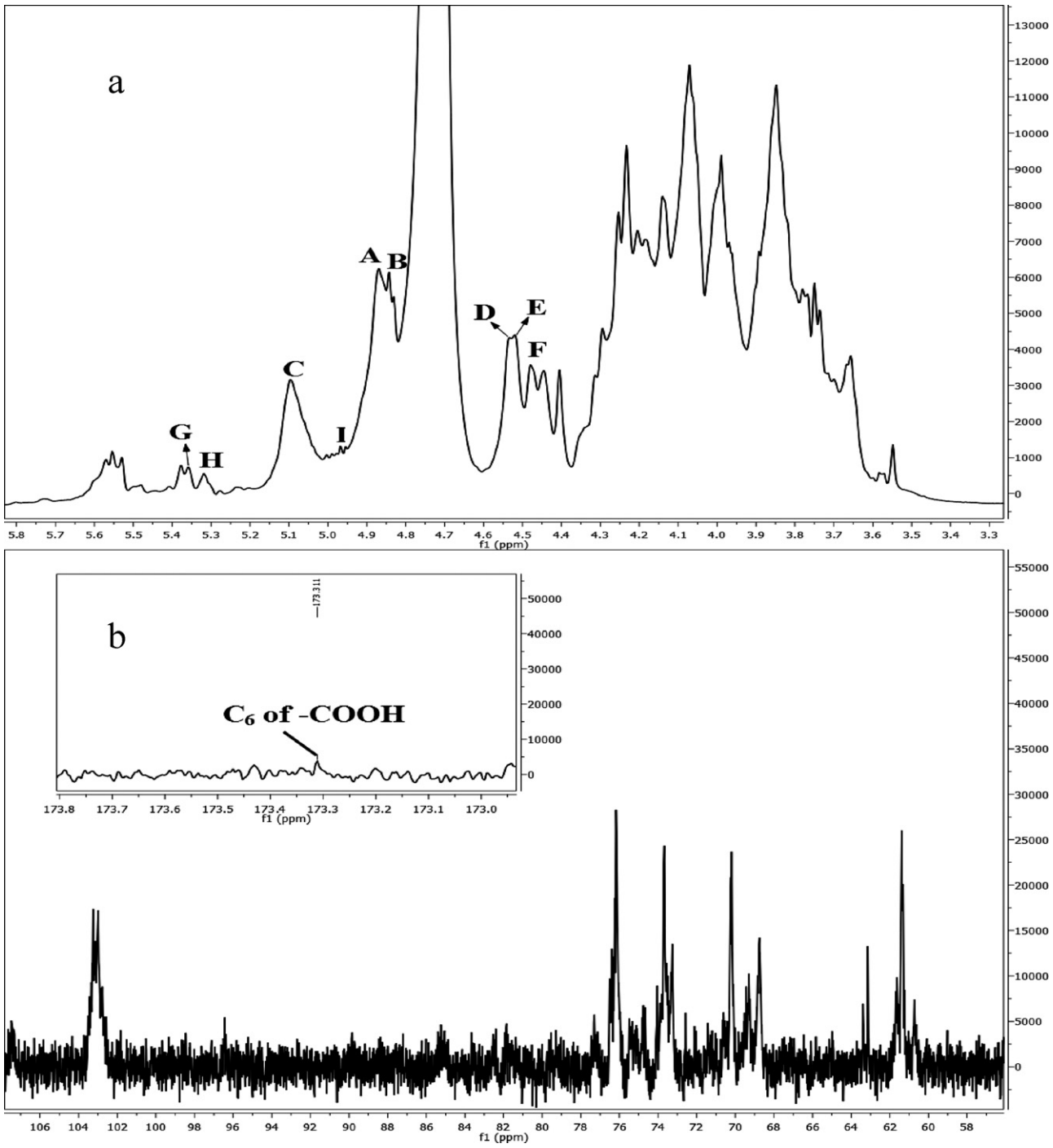

Fig. 1. 1D NMR spectrum of PSG-1 recorded at 60 °C (a) ^{1}H NMR spectrum (600.1 MHz); (b) ^{13}C NMR spectrum (151.0 MHz).

the HSQC spectrum. The downfield shift of C-6 (69.52 ppm) and a small upfield shift for C-5 (73.19 ppm) with respect to standard values indicated that residue **E** was linked at C-6. Based on the above results and previous reports (Das et al., 2010; Mandal et al., 2010), residue **E** was determined as a β-1,6-D-Glc*p*.

*β-1,4,6-Glcp (residue **F**)*. Residue **F** had an anomeric proton signal at δ 4.48 ppm and an anomeric carbon signal at δ 103.42 ppm which indicated it a β-linked moiety. It was tentatively assigned as 1,4,6-Glc*p* by comparison with literature values of the anomeric chemical shifts (Roy, Maiti, Mondal, Das, & Islam, 2008). The proton and carbon signals of residue **F** were assigned in the same way as described above. The downfield shifts of C-4 (δ 79.21 ppm) and C-6 (δ 69.57 ppm) with respect to the values of standard methyl glycosides indicated that residue **F** was linked at C-4 and C-6. Based on above results, residue **F** was deduced as a β-1,4,6-Glc*p*.

The proton and carbon signals of residue **D**, **G** and **H** were assigned in the same way as described above with the help of DQF-COSY and HSQC spectrum along with previous literatures (Ahrazem, Prieto, Leal, Jimenez-Barbero, & Bernabe, 2002; Bhunia et al., 2010; Dey et al., 2010; Ge et al., 2009). Thus, combined the information from the ^{1}H, ^{13}C, HSQC, DQF-COSY and TOCSY spectrums with methylation analysis results, a complete assignment of all the linkage patterns was identified (Table 2).

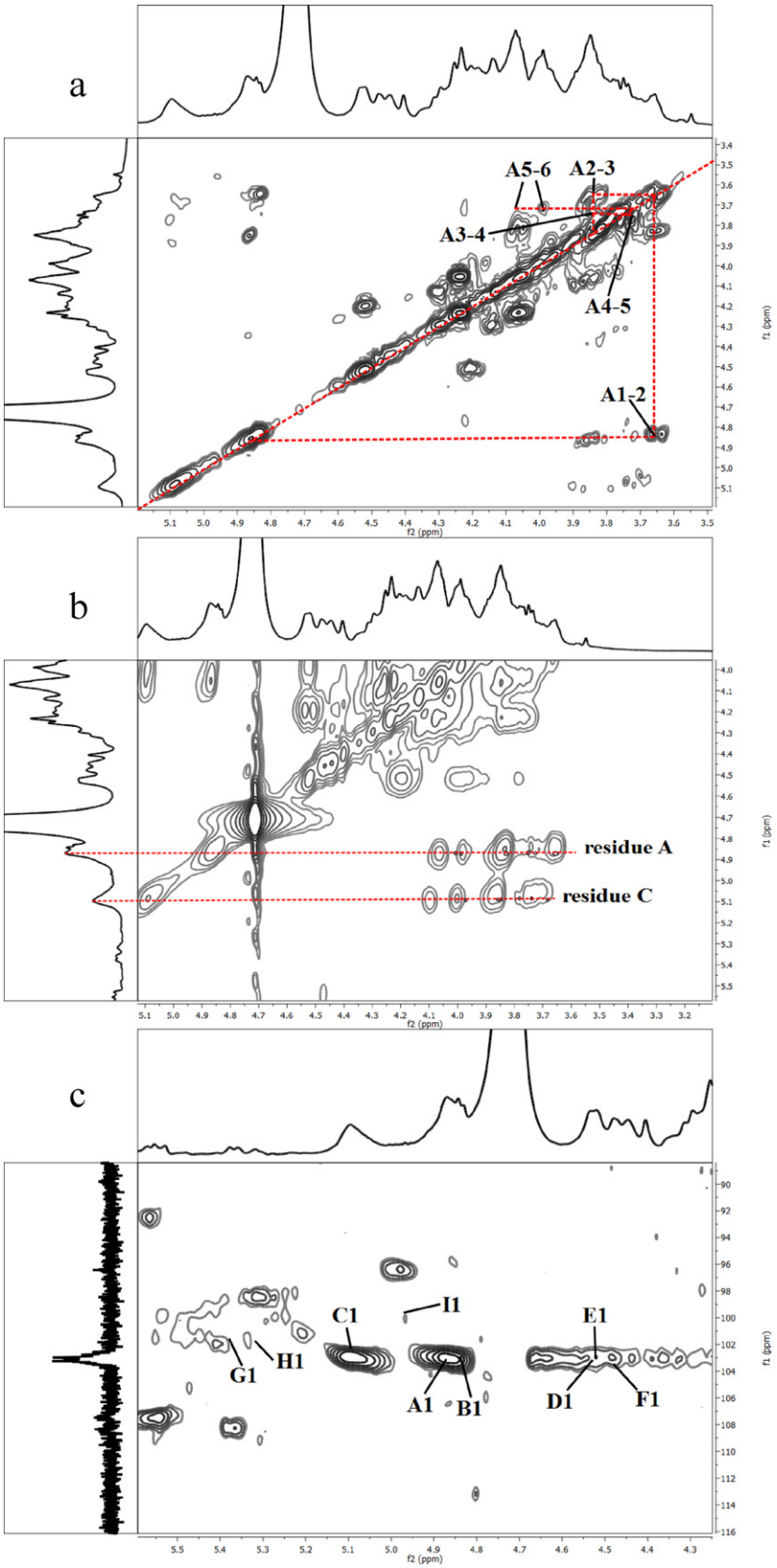

Fig. 2. $^{1}H/^{1}H$ and $^{1}H/^{13}C$ correlation spectrum of PSG-1 (a) $^{1}H/^{1}H$ DQF-COSY correlation spectrum (the assignments are the correlations of protons of β-1,3-D-Glc*p* (residue **A**)); (b) $^{1}H/^{1}H$ TOCSY correlation spectrum (the assignments are the correlations of protons residue **A** and residue **C**); (c) part of $^{1}H/^{13}C$ HSQC correlation spectrum (anomeric region).

Table 2
^{1}H and ^{13}C NMR chemical shifts of PSG-1 recorded in D_2O at 60 °C.

Residue		Proton or carbon						
		1	2	3	4	5	6a	6b
β-T-Glc*p* (**B**)	H	4.84	3.62	3.81	3.68	3.76	3.87	4.01
	C	102.99	73.48	76.23	70.31	75.82	63.18	
β-1,3-Glc*p* (**A**)	H	4.87	3.66	3.84	3.75	3.73	3.99	4.07
	C	103.25	73.06	**84.92**	70.18	75.65	61.48	
α-1,4-Gal*p* (**C**)	H	5.10	3.74	3.84	3.97	3.85	3.68	3.77
	C	102.32	70.22	69.35	**76.55**	73.19	61.21	
β-1,3,6-Glc*p* (**D**)	H	4.53	3.49	3.63	3.57	3.61	3.84	4.14
	C	103.17	73.19	**83.34**	70.43	75.81	**69.28**	
β-1,6-Glc*p* (**E**)	H	4.52	3.54	3.67	3.58	3.65	3.86	4.20
	C	103.12	73.65	76.11	71.03	73.19	**69.52**	
β-1,4,6-Glc*p* (**F**)	H	4.48	3.49	3.67	3.80	3.87	3.78	4.09
	C	103.43	73.76	76.23	**79.21**	72.59	**69.57**	
α-1,2-Man*p* (**G**)	H	5.38	4.13	3.87	3.76	3.85	3.74	4.06
	C	101.52	**79.42**	71.42	69.55	70.86	62.79	
α-1,4-Man*p* (**H**)	H	5.32	4.18	3.67	3.82	3.75	3.84	4.02
	C	101.78	69.25	73.21	**80.00**	75.98	63.44	
α-1,4-Gal*p*A (**I**)	H	4.97	3.56	4.12	4.29	4.60		
	C	99.77	71.05	73.39	**79.50**	71.84	**173.31**	

The bold values mean the chemical shifts of carbons which have shifted to low field because of the linked position of residues. For example, residue A is β-1,3-Glcp, so the chemical shift of C-3 is 84.92 ppm which has been used the bold value.

Linkage sites and sequence among residues. The sequences of sugar residues were determined from a long-range HMBC experiment, and the following connectivities were observed: The cross peaks of both anomeric protons and carbons of each sugar residue of PSG-1 were examined from the HMBC experiment, and both intra- and inter-residual connectivities were observed (Fig. 3). Cross peaks were found between H-1 of residue **A** (δ 4.87 ppm) and C-3 of residue **D** (**A** H-1/**D** C-3); C-1 of residue **A** (δ 103.25 ppm) and H-3 of residue **D** (**A** C-1/**D** H-3), H-6 of residue **E** (**A** C-1/**E** H-6), along with intra-residual coupling between H-1 of residue **A** with its own C-3 (**A** H-1/**A** C-3). Similar cross peaks were also found between H-1 of residue **D** (δ 4.53 ppm) and C-6 of residue **E** (**D** H-1/**E** C-6); C-1 of residue **D** (δ 103.17 ppm) and H-6 of residue **E** (**D** C-1/**E** H-6), along with intra-residual cross couplings between H-1 of residue **D**

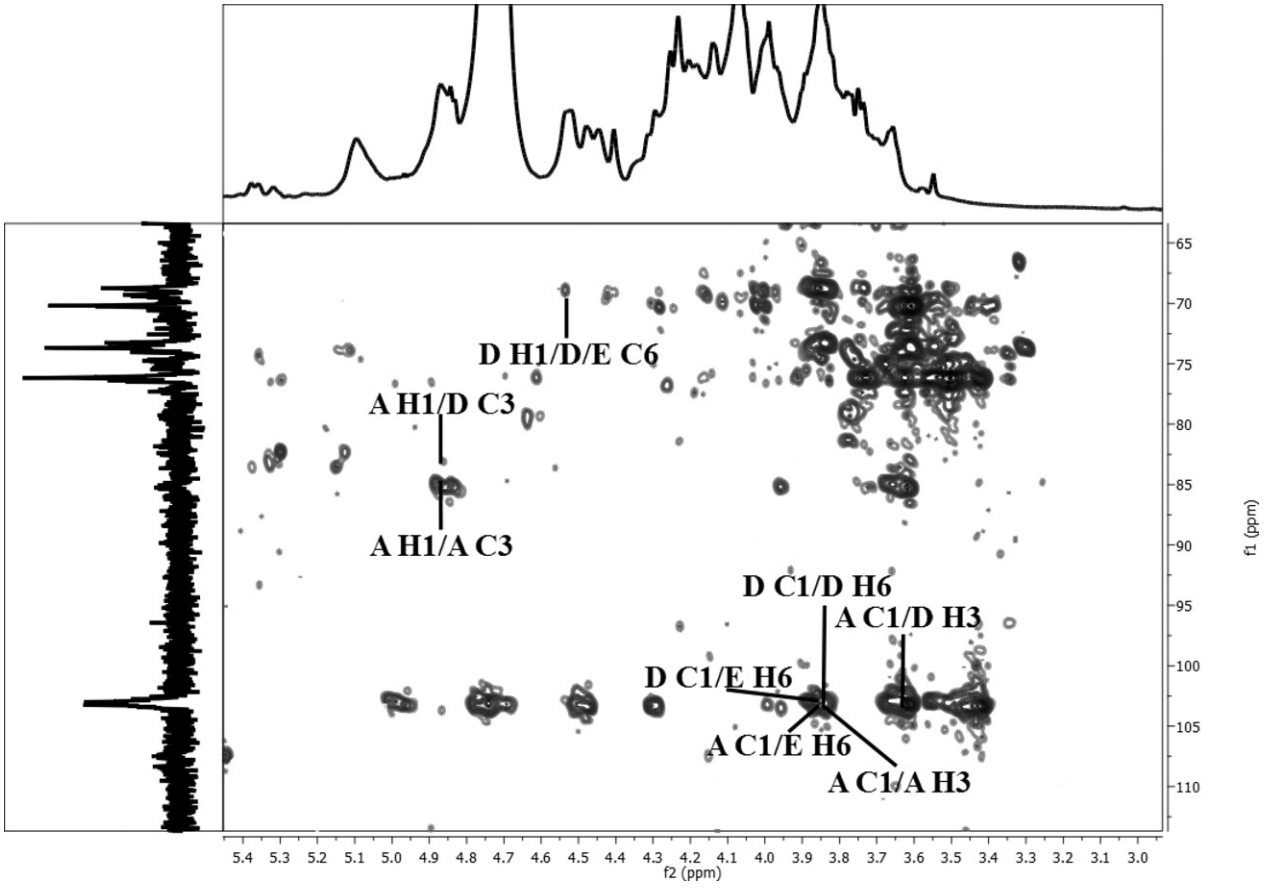

Fig. 3. $^{1}H/^{13}C$ HMBC correlation spectrum of PSG-1.

Table 3
Significant $^3J_{H,C}$ connectivities observed in HMBC spectrum for the anomeric protons/carbons of the sugar residues of PSG-1.

Sugar residue	Sugar linkage	H-1/C-1 δ_H/δ_C	δ_H/δ_C	Observed connectivities Residue	Atom
A	β-1,3-Glc*p*	4.87	84.92	A	C-3
			83.34	D	C-3
		103.25	3.84	A	H-3
			3.63	D	H-3
			3.86	E	H-6
B	β-T-Glc*p*	4.84	84.92	A	C-3
			76.55	C	C-4
		102.99	3.84	A	H-3
			3.97	C	H-4
C	α-1,4-Gal*p*	5.10	84.92	A	C-3
			69.28	D	C-6
			69.57	F	C-6
		102.32	3.84	A	H-3
			3.84	D	H-6
D	β-1,3,6-Glc*p*	4.53	69.28	D	C-6
			69.52	E	C-6
		103.17	3.84	D	H-6
			3.86	E	H-6
E	β-1,6-Glc*p*	4.52	79.21	F	C-4
			80.00	H	C-4
			79.50	I	C-4
		103.12	3.80	F	H-4
			3.82	H	H-4
			4.29	I	H-4
F	β-1,4,6-Glc*p*	4.48	79.42	G	C-2
G	α-1,2-Man*p*	5.38	83.34	D	C-3
		101.52	3.63	D	H-3
H	α-1,4-Man*p*	5.32	76.55	C	C-4
I	α-1,4-Gal*p*A	4.97	76.55	C	C-4

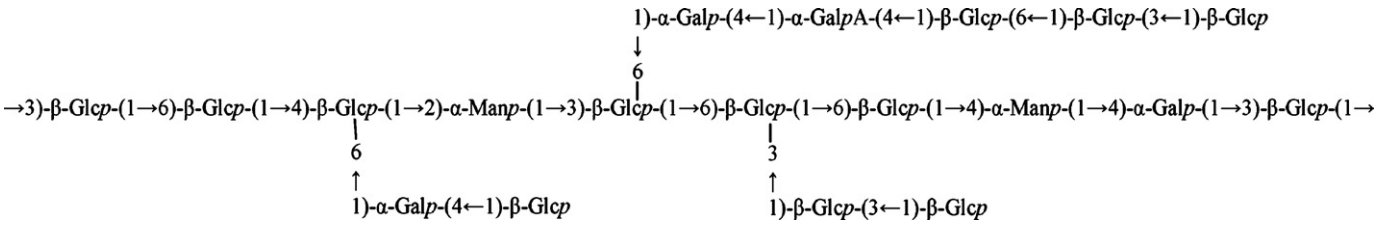

Fig. 4. Possible molecular structure of repeating unit of PSG-1.

and C-6 of its own (**D** H-1/**D** C-6). With the same method, the cross peaks of other residues were found and summarized in Table 3.

4. Conclusions

In summary, PSG-1 had been proved as a heteropolysaccharide comprised a backbone of 1,3-linked and 1,6-linked β-Glc*p* residues substituted at *O*-3 and *O*-6 position as the branch points. The residues of α-1,4-Gal*p*, α-1,2-Man*p* and α-1,4-Man*p* were also found in the backbone. Side chains were terminated by β-Glc*p*, with the composition of α-1,4-Gal*p*, α-1,4-Gal*p*A, β-1,3-Glc*p* and β-1,6-Glc*p*. Based on all these chemical and spectroscopic data, a possible molecular structure of repeating unit of PSG-1 was established as shown in Fig. 4.

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