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Structure and properties of a (1→3)-β-D-glucan from ultrasound-degraded exopolysaccharides of a medicinal fungus

Xia Chen, Ka-Chai Siu, Yi-Ching Cheung, Jian-Yong Wu*

Department of Applied Biology & Chemical Technology, State Key Laboratory of Chinese Medicine and Molecular Pharmacology in Shenzhen, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

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ABSTRACT

A high molecular weight (MW) exopolysaccaride (EPS) fraction EPS1 was isolated from the fermentation broth of a medicinal fungus *Cordyceps sinensis* Cs-HK1 and partially degraded by high-intensity ultrasound (US) into a lower MW fraction EPS1U. EPS1U exhibited a single, symmetric peak on size exclusion chromatography with an average MW of 730 kDa by light scattering analysis. It had a much lower intrinsic viscosity (1.7 versus 15.6 dL/g) but a much higher solubility in water (77.5 versus 5.1 g/L) than EPS1. Based on methylation analysis and NMR spectrometry, the structure of EPS1U was deduced as a (1→3)-β-D-glucan with glucose side chains attached to *O*-6 position at the branching points. EPS1U showed a high moisture absorption capability comparable to chitosan and urea, suggesting its potential as a moisturizing agent for food and cosmeceutical application. This is the first report on a high MW (1→3)-β-D-glucan isolated from EPS produced by *Cordyceps sinensis*.

1. Introduction

Edible and medicinal fungi (or mushrooms) provide an abundant and attractive source of natural, bioactive polysaccharides (PS) and PS-protein (PSP) complexes with notable antitumor, immunomodulation and other bioactivities (Stachowiak & Reguła, 2012). Among various bioactive PS from mushrooms, β-glucans with (1→3)-, (1→6)- and (1→4)-glycosidic linkages are most notable of their antitumor and immunomodulatory activities, such as lentinan, schizophyllan and Grifolan (El & Hatti-Kaul, 2013). *Cordyceps sinensis*, generally called Cordyceps or Dong-chong-xia-cao in Chinese, is a highly valued medicinal fungus in traditional Chinese medicine with a number of health promoting effects (Zhou, Gong, Su, Lin, & Tang, 2009). Since natural Cordyceps (in the form of a caterpillar-fungal fruiting body complex) is rare and expensive, mycelial fermentation has become the major source of Cordyceps materials. Exopolysaccharides (EPS) produced by mycelial fermentation of *C. sinensis* present diverse molecular structures and various biological activities (Yan, Wang, & Wu, 2014).

The mycelial culture of a *C. sinensis* fungus Cs-HK1 has been established in our lab and applied to liquid fermentation for production of mycelial biomass and EPS (Leung, Zhao, &Wu, 2006). The crude and partially purified EPS fractions isolated from the Cs-HK1 have shown notable antioxidant, cytoprotective and radioprotective activities in our previous studies (Huang, Siu, Wang, Cheung, & Wu, 2013; Leung, Zhao, Ho, & Wu, 2009; Wong, Wu, & Benzie, 2011). However, the EPS produced by the Cs-HK1 mycelial fermentation had a high molecular weight (MW) over 10,000 kDa with poor solubility and high viscosity in water (Huang et al., 2013). Partial depolymerization of the high-MW EPS may be an effective approach to improve the solution properties and functional activities. In a previous study from our lab, high-intensity ultrasound (US) has been employed for partial and controlled degradation of the high MW EPS produced by Cs-HK1 to improve the water solubility and reduce the solution viscosity of EPS (Wang, Cheung, Leung, & Wu, 2010). However, none of the US-degraded EPS fractions have been purified and structurally characterized.

Microbial PS especially the EPS produced by liquid fermentation have been widely used in food and cosmetic products because of their special biological functions compared with the synthetic polymers (Rehm, 2009). In addition to their bioactive functions, polysaccharides (PS) such as hyaluronic acid are frequently used as moisturizing agents (Sutherland, 1998; Zhang et al., 2013). For example, EPS from *Streptococcus thermophilus* bacteria has been used to maintain the moisture content in cheese (Petersen, Dave, Mcmahon, Oberg, & Broadbent, 2000). Compared with the low MW organic compounds, polymeric moisturizing agents are more stable with various environmental conditions (Osada & Kajiwara, 2000).

* Corresponding author. Tel.: +852 3400 8671; fax: +852 2364 9932.
E-mail addresses: jian-yong.wu@polyu.edu.hk, bcjywu@polyu.edu.hk (J.-Y. Wu).

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Nwodo, Green, and Okoh (2012) have recently reviewed the various functions of bacterial EPS including adhesion, nutrition and water retention. For moisture retention, (1→3)-β-D-glucans are of special interest because of their strong nature to form a helix structure giving high viscosity and gelling properties (Zhang, Li, Wang, Zhang, & Cheung, 2011).

Up to date, there is still no reported work on the moisture absorption and retention capacity of PS from the *C. sinensis* fungus. This work was carried out to characterize the structure and conformation of a (1→3)-β-D-glucan purified from a US-degraded EPS fraction produced by Cs-HK1 mycelial fermentation, and to evaluate its moisture absorption and retention properties.

2. Materials and methods

2.1. Cs-HK1 mycelial fermentation and EPS isolation

The Cs-HK1 fungus was originally isolated from the fruit body of a wild Cordyceps and was preserved in mycelial culture on a solid substrate as reported previously (Leung et al., 2006). For production of mycelial biomass and EPS, the Cs-HK1 mycelial culture was maintained in a liquid medium consisting of (per liter) 40 g glucose, 10 g yeast extract, 5 g peptone, 1 g KH_2PO_4 and 0.5 g $MgSO_4{\cdot}7H_2O$, in Erlenmeyer flasks on a shaking incubator at 150 rpm and 20 °C. In this study, the Cs-HK1 mycelial liquid fermentation for EPS production was carried out in 1-L Erlenmeyer flasks each containing 200 mL of the liquid medium. After 7-days of shaking incubation, the mycelial fermentation broth in the flasks was collected for the recovery of mycelial biomass and EPS.

The Cs-HK1 mycelial broth was centrifuged at 14,000 rpm for 15 min and the supernatant was collected for EPS isolation by ethanol precipitation. Ethanol (95% grade) was added to the broth supernatant with stirring to a final concentration of 40% (v/v). The mixture was kept in stationary condition at room temperature for 12 h, followed by centrifugation at 10,000 rpm for 20 min. The precipitate was collected and lyophilized to give fraction EPS1. The application of a low ethanol concentration (40% instead of 80% in common practice) was to precipitate the higher-MW fraction of EPS produced by the Cs-HK fungus.

2.2. Ultrasonic treatment of EPS1

Power US was generated with a Model VCX 750 processor of 20 kHz frequency and 750 W maximum output power (Sonics & Materials Inc., Newton, USA). A probe horn of 13-mm tip diameter was used in this work. The EPS1 sample was dissolved (dispersed) in de-ionized (DI) water at 10 mg/mL by vigorous stirring at room temperature. For ultrasonic treatment, 100 mL of the EPS1 solution was filled in a 200 mL plastic bottle, and the US probe was inserted into the liquid at a fixed depth of 2 cm. The sample bottle was surrounded with ice-water during the treatment to avoid overheating. The US power was fixed at 70% amplitude (corresponding to intensity of 4.3 W/cm^2 tip surface and power density of 0.31 W/cm^3 liquid). After the ultrasonic treatment for a selected period of time, the EPS solution was dialyzed (MWCO 3000 Da) against DI water at room temperature for 48 h. The retentate in the dialysis bag was freeze-dried to give US-degraded EPS1.

2.3. Purification of EPS1U from EPS1 and MW analysis

The freeze-dried US-degraded EPS1 from above was redissolved in DI water at 50 mg/mL and purified by size exclusion chromatography (SEC) on a Sephacryl S-300 HR column (100 cm × 2.6 cm) (Sigma-Aldrich). The column was eluted with 0.1 M NaCl aqueous solution at 0.6 mL/min and detected by the Anthrone test, yielding a major fraction designated EPS1U.

The MW distribution and homogeneity of EPS1 and EPS1U were analyzed by size exclusion chromatography (SEC) on a Waters e2695 HPLC equipment connected with a Waters 2414 refractive index detector (RI), a Waters 2998 UV detector, and a Wyatt DAWN laser scattering detector. Two columns, Ultrahydrogel™ 2000 and Ultrahydrogel™ 500 column were connected in series, eluted with 0.15 M $NaNO_3$ and 0.05 M NaH_2PO_4 at a flow rate of 0.5 mL/min. The columns and RI detector were maintained at 35 °C.

2.4. Chemical composition analysis

The EPS sample (~3 mg) was hydrolyzed with 2 mL of 2 M trifluoroacetic acid (TFA) at 110 °C for 4 h in a sealed test tube. The acid was removed under reduced pressure by repeated evaporation with MeOH, and then the hydrolysate was converted into the alditol acetate, followed by GC analysis (Bjorndal, Lindberg, & Svensson, 1967). The total carbohydrate content of EPS samples was determined by the Anthrone test, through sulfuric acid hydrolysis of the sample in the presence of anthrone agent (Sigma-Aldrich) at 100 °C. The absorbance of the sample solution was measured at 620 nm and calibrated to total carbohydrate content using glucose as a standard. The protein content of EPS samples was determined by the Lowry method using bovine serum albumin (BSA) as a standard and absorbance measured at 750 nm (Leung et al., 2009).

2.5. Determination of intrinsic viscosity and water solubility

The intrinsic viscosity $[\eta]$ of EPS samples was determined by the serial dilution method. The viscosity of dilute EPS sample solution in water was measured with an Ubbelohde viscometer (0.5–0.6 mm capillary diameter) at 30 ± 0.1 °C. The $[\eta]$ value of each sample was the intercept of line η_{sp}/c versus c (η_{sp} for specific viscosity and c for concentration in g/L) estimated by linear regression (Huang et al., 2013).

The water solubility of EPS1 and EPS1U was determined as described by Wang et al. (2010). In brief, each sample (~100 mg) was suspended in 1 mL of DI water in a micro-centrifuge tube and agitated vigorously with a mini magnetic stir bar at room temperature for 24 h. The liquid was centrifuged at 18,000 rpm for 30 min, and the un-dissolved residue was collected, freeze-dried, and its weight was deducted from the original sample weight to attained the solubility.

2.6. Analysis of EPS1U structure and conformation

For distortionless enhancement by polarization transfer (DEPT) NMR analysis, EPS1U was deuterium-exchanged by dissolving the sample (30 mg) in 0.6 mL of D_2O (99.9% D). For better resolution, EPS1U was also dissolved in DMSO-d_6/D_2O (6:1) mixture solvent (30 mg in 0.6 mL) (Peng, Zhang, Zeng, & Kennedy, 2005). The ^{13}C NMR spectra were recorded on a Brucker AVANCEIII 600 MHz spectrometer with Topspin 3.0 software for data processing. All chemical shifts were derived in reference to Me_4Si.

Methylation was performed to investigate the chain linkage of EPS1U. The vacuum-dried EPS1U (10 mg) was methylated three times as described by Needs and Selvendran (1993) with minor modifications. EPS1U was weighed precisely and dissolved in 3.0 mL DMSO, and 200 mg of powdered NaOH was added to the solution, followed by mixing assisted by sonication in an ultrasonic bath for 10 min. After incubation for 10 min at room temperature with stirring, 1 mL iodomethane was added slowly to the reaction mixture solution. The reaction mixture was kept in darkness for 1 h, followed by addition of 2.0 mL DI water. The methylated EPS1U was extracted with 2 mL chloroform for three times and dried at depressed pressure on a rotary evaporator. Complete methylation was confirmed by the disappearance of the hydroxyl absorption on

the infrared (IR) spectrum. The methylated alditol acetates were prepared and analyzed by GC–MS.

Congo red test was performed to examine the chain conformation of EPS1 and EPS1U as reported by Villares (2013) with minor modifications. The EPS samples were dissolved in 2.0 mL NaOH solution of various concentrations from 0 to 0.5 M. Congo red was added to the solution at 24.4 μM final concentration and maintained at room temperature for 1 h, and the maximum absorbance λ_{max} values was recorded from 200 to 700 nm on a UV–vis spectrophotometer.

2.7. Evaluation of moisture absorption and retention

The moisture-absorption and retention properties of EPS1 and EPS1U were examined gravimetrically and compared with those of chitosan and urea, which are frequently used as hygroscopic and moisturizing agents (Li et al., 2011). All samples (EPS1, EPS1U, chitosan and urea) were ground into fine powder and oven-dried at 100 °C for 4 h. For measurement of moisture absorption, the samples (100 mg each) were placed in a closed humidity chamber maintained by saturated K_2CO_3 at 43% relative humidity (RH) or saturated $(NH_4)_2SO_4$ at 81% (RH) at 25 °C for a selected time intervals. The moisture absorption rate (X_a) was represented by the weight gain: X_a (%) = $100 \times (W_t - W_0)/W_0$, where W_0 was the weight of an oven-dried sample and W_t the weight of the sample after moisture absorption over a specific time period in the humidity chamber (Li et al., 2011; Onoda, Yamaguchi, & Takenaka, 2012).

For measurement of moisture retention, the samples (100 mg each) were first placed in a humidification chamber containing DI water at 25 °C for 24 h and then transferred to a humidity chamber containing saturated K_2CO_3 (43% RH) to dehydrate at 25 °C over a period of 24 h. The moisture retention rate (X_r) was evaluated by weight loss of the sample: X_r (%) = $100 \times W_t/W_0$, where W_0 was the weight of DI water absorbed in the humidification chamber and W_t the water remaining after a specific time in the K_2CO_3 saturated chamber (Zhang, Wang, Han, Zhao, & Yin, 2012).

3. Results and discussion

3.1. Molecular properties and chemical composition of EPS1 and EPS1U

The EPS1 attained by 40% ethanol precipitation of the Cs-HK1 mycelial broth was poorly soluble in water and had a high intrinsic viscosity. During the US treatment, the intrinsic viscosity of EPS1 dropped rapidly from about 16.0 dL/g to about 3.0 dL/g within 10 min and decreased slowly in the remaining period to about 1.0 dL/g after 60 min treatment (Fig. 1). Based on this trend of intrinsic viscosity change, EPS1 was treated by US at the same intensity for 20 min to attain the US-degraded product. The US-degraded EPS1 was further purified by gel filtration to obtain the homogeneous polysaccharide EPS1U. Table 1 provides a summary of the molecular properties and chemical composition of EPS1 and EPS1U. Compared with the original EPS1, EPS1U had a much lower average MW (730 versus 2700 kDa) and a lower intrinsic viscosity (1.7 versus 15.6 dL/g) but a much higher solubility in water (77.5 versus 5.1 g/L).

On the HPSEC spectrum (Fig. 2), EPS1U exhibited a higher and more symmetric peak with a lower average MW (longer elution time) than EPS1. Although the same concentration of EPS1 and EPS1U was applied to the HPSEC analysis, the much higher peak of EPS1U may be attributed to its much lower MW and higher solubility. The more uniform MW distribution of EPS resulting from the US treatment is a common outcome of ultrasonic polymer degradation which occurs mainly to the higher-MW components (Wang et al., 2010). Associated with the dramatic reduction of average MW was a significant drop in the intrinsic viscosity of EPS1U. The solubility of EPS1U in water was also increased dramatically compared with that of EPS1. After 24 h of vigorous mixing in water, EPS1 (5 mg/mL) formed a milky dispersion (Supplemental Fig. 1A), while EPS1U (5 mg/mL) formed a clear solution (Supplemental Fig. 1B).

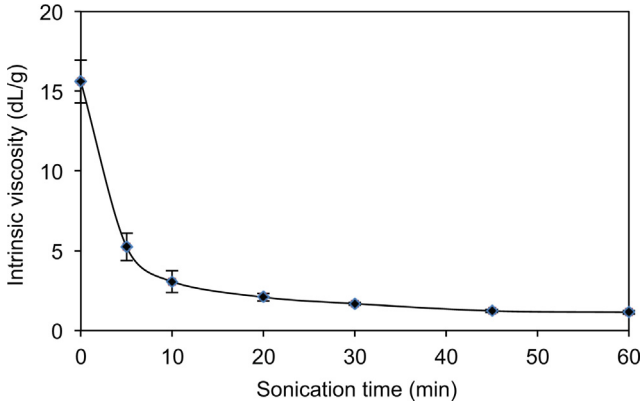

Fig. 1. Intrinsic viscosity of EPS1 solution during ultrasonic treatment at 70% amplitudes. (Error bars for standard deviations from three replicate tests.)

In addition to the physical and molecular properties, the chemical compositions of EPS1 and EPS1U were quite different. EPS1 had relatively high protein content and was either a mixture or complex of PS and proteins, while EPS1U was nearly a pure PS with negligible protein content. As for the molecular constituents, EPS1 was composed of glucose, mannose and galactose at the molar ratio of 81.3:13.8:4.9; EPS1U was composed of glucose (>95%) with minor or negligible mannose and galactose (Table 1). The mannose and galactose constituents were probably weakly bound to EPS1 and removed during the US treatment and the following purification steps.

It is note worthy that EPS1U had a good water solubility with such a high MW of 730 kDa. Previous studies on the EPS produced by the Cs-HK1 mycelial fermentation have been concerned mainly with either the crude EPS precipitated with 3–5 volumes of ethanol (Huang et al., 2013; Leung et al., 2009; Wang et al., 2010) or purified, homogenous PS at relatively low MW below 50 kDa (Wang et al., 2011; Yan, Li, Wang, & Wu, 2010). Even for the PS with known structures such as β-glucans documented so far from edible and medicinal fungi, the MW ranges were mostly below 200–500 kDa (Ahmad, Anjum, Zahoor, Nawaz, & Dilshad, 2012; El & Hatti-Kaul, 2013). By applying a low ethanol concentration of (40% or ~2/3

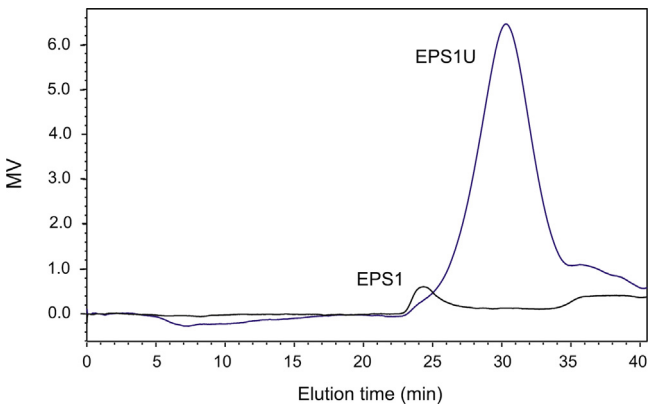

Fig. 2. HPSEC chromatogram of EPS1 and EPS1U.

Table 1
Properties and composition of EPS1 and EPS1U.

Sample	Yield (g/L)	[η] (dL/g)	MW (kDa)	Solubility (g/L)	Carbohydrate (%)	Protein (%)	Glycosyl (mol%)		
							Glc	Man	Gal
EPS1	2.9	15.6 ± 1.3	2700	5.1	77.8 ± 3.2	15.6 ± 0.3	81.3	13.8	4.9
EPS1U	2.1	1.7 ± 0.1	730	77.5	95.2 ± 2.3	0.6 ± 2.0	98.5	1.3	0.2

Table 2
Linkage analysis of EPS1U.

Methylated sugars	Linkages	mol%
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol	β-D-Glc*p*-(1→	34.9
1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methylhexitol	→3)-β-D-Glc*p*-(1→	35.4
1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methylhexitol	→3,6)-β-D-Glc*p*-(1→	29.7

volume ratio), we were able to isolate the higher MW fraction of crude EPS (EPS1) about 2700 kDa average MW. Upon the ethanol precipitation, the EPS1 formed a large clump floating on the top of liquid (Supplemental Fig. 2) because of the highly hydrated nature. After the US treatment of EPS1, the resultant EPS1U had a lower viscosity and a higher solubility, and could be readily purified through gel-filtration. Similarly, Liu et al. (2014) recently isolated a high MW (1→3)-β-D-glucan about 3750 kDa from hot water extract of *Ganoderma luciduma* fruiting bodies by precipitation with a low ethanol concentration of 20% (v/v).

3.2. Structure elucidation of EPS1U

The methylation analysis results of EPS1U (Table 2) show the linkages of terminal D-glucopyranosyl, (1→3)-linked D-glucopyranosyl, and (1→3, 1→6)-linked D-glucopyranosyl (branch point) moieties about 1:1:1 molar ratio. Because of the very low contents of manose (1.3%) and galactose (0.2%) in EPS1U (Table 1), the peaks of the two residues were barely visible (Supplemental Fig. 3). It can be deduced that EPS1U was a (1→3)-D-glucan with a glucose side chain at *O*-6 in every two repeating units.

Fig. 3A shows the DEPT NMR spectrum of EPS1U. Usually, the shift of anomeric carbon in (1→3)-β-D-glucan is about 103 ppm while the shift of C-1 in (1→3)-α-D-glucan is around 100 ppm (Lehtovaara & Gu, 2011). Thus, the stronger signals at 105.9, 102.2 and 99.3 ppm correlated to C-1 of (1→3, 1→6)-β-D-glucopyranosyl, (1→3)-β-D-glucopyranosyl and terminal D-glucopyranosyl, respectively. The signal at 65.8 ppm was assigned to the substituted C-6 of (1→3, 1→6)-β-D-glucopyranosyl. The weak anomeric signals appearing in the NMR spectrum of EPS1U could be attributed to impurity sugar residues such as galactomannan (Supplemental Fig. 4). The DEPT spectrometry of EPS1U in D_2O was difficult to perform due to its high MW and viscosity even though warming and accumulation time had been applied to improve the recosolution. Improved ^{13}C NMR spectrum was attained with the EPS1U sample dissolved in the mixture solvent of DMSO-d_6/D_2O (Fig. 3B), in which the signal at 103.4 ppm also indicated a β configuration for glucopyranosyl units. Based on the methylation analysis and NMR results, the structure of EPS1U is represented by

$$[3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow]_n$$
$$\begin{array}{c} 6 \\ \uparrow \\ 1 \\ \beta\text{-D-Glc}p \end{array}$$

3.3. Chain conformation of EPS1 and EPS1U

Congo red test was performed to study the chain conformation of EPS1 and EPS1U. It has been estalished that polysaccharide chains in single helix but not other confomation in solution can form a complex with Congo red, resulting in a red shift (Wang et al., 2010). Fig. 4 shows the change of maximum absorbance (λ_{max}) of EPS-Congo red solutions with various alkaline concentrations. The changes in λ_{max} of Congo red complexes with EPS1 and EPS1U followed a simliar trend. All the Congo red and EPS complex soutions at 0 M NaOH had the same λ_{max}, indicating there was no single helix conformation. With 0.2 M or a lower concentration of NaOH, both EPS1-Congo red and EPS1U-Congo red solutions exhibited a red shift solution, indicating the exsistence of single helixes. The EPS1 and EPS1U in water probably formed multiple helixes as observed with another high-MW EPS isolated from the Cs-HK1 fermentation broth (Wang et al., 2010). With NaOH in the low contrnation range, the multiple complexes were split into single helixes by alkinic disruption of the hydrogen bonds. The λ_{max} red shift of EPS1U was

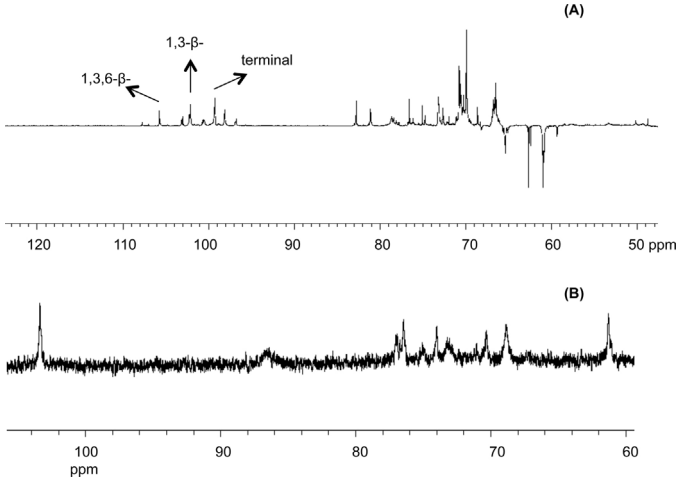

Fig. 3. ^{13}C NMR spectra of EPS1U: (A) distortionless enhancement by polarization transfer (DEPT) spectrum of EPS1U in D_2O; (B) spectrum of EPS1U dissolved in DMSO-d_6/D_2O (6:1).

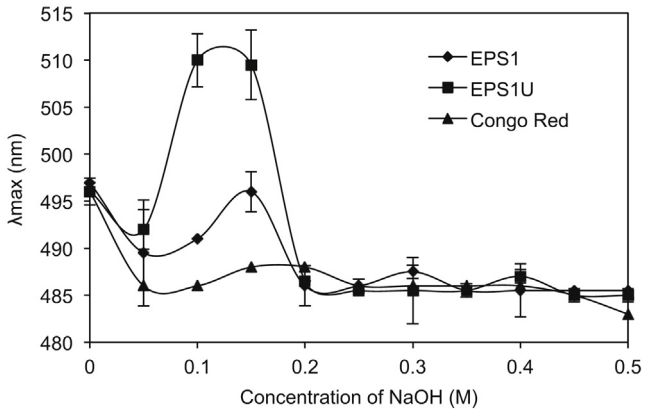

Fig. 4. Change of maximum absorption λ_{max} in solution of Congo red alone, Congo red plus EPS1 and Congo red plus EPS1U at various NaOH concentrations. (Error bars for SD from three replicate tests.)

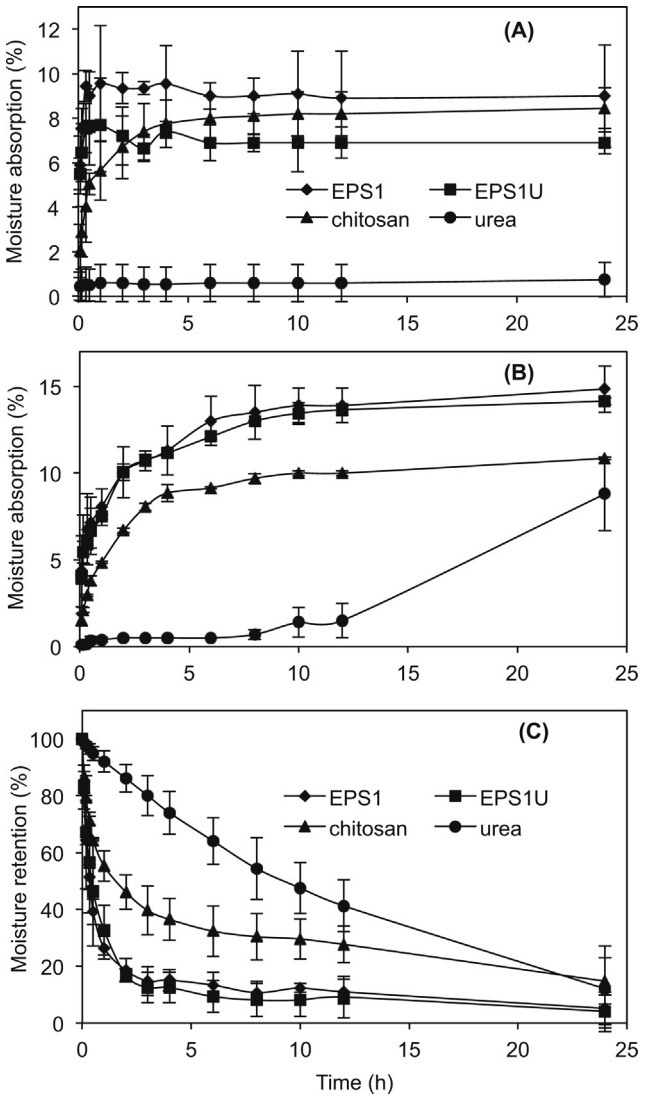

Fig. 5. Moisture absorption rate of EPS1U at 43% RH (A) and 81% RH (B); moisture retention of EPS1U at 43% RH (C). (Error bars for SD from three replicate tests.)

more than that of EPS1, indicating that EPS1U formed more single helixes than EPS1 due probably to the much lower MW of EPS1U. At much higher NaOH concentrations (>0.2 M), the λ_{max} red shifts of EPS1 and EPS1U all disappeared, due proably to the complete disruption of of the helical chain conformation into random coils.

The results of Congo red test sugegsted that high intensity US treatment of EPS1 did not cause conformation change of the polysaccharide chains from multiple to single helixes. It has been suggested that the gel state of polysaccharides such as β-D-glucans in water is mostly associated the triple-helix conformation (Zhang et al., 2011). As aforementioned, the EPS1 formed gels in water with a milky appearance (Supplemental Fig. 1A). The (1→3)-β-D-glucans such as lentinan and curdlan have a strong tendency to form helical structures through the hydrogen bonds, giving rise to high viscosity and gel formation in water. Aqueous alkali can disrupt the hydrogen bonds leading to the transition from multiple to single helix conformation, and eventaully to random coils (Wang et al., 2010).

3.4. Moisture absorption and rentention properties

As shown in Fig. 5A, the moisture absorption rate (X_a) of all samples at 43% relative humidity (RH) increased rapidly in the first 4 h and that of EPS1 increased faster than all other samples. The maximum or saturation moisture absoprion (at 24 h) of EPS1 was also higher (9.0%) than those of EPS1U (6.9%), chitosan (8.5%) and urea (1.3%). At 81% RH (Fig. 5B), the moisture absorption rates of EPS1 and EPS1U were very close throughtout the test period, both being higher than those of chitosan and urea. After exposed to 81% RH for 24 h, the X_a of EPS1, EPS1U, urea and chitosan were 13.9%, 14.0%, 7.3% and 10.8%, respectively. Fig. 5C shows the moisture-retention rates of the samples after the moistrue absorption for 24 h at 43% RH. The moisture absorbed in the EPS1 and EPS1U samples dropped rapidly to below 10% in the first 5 h, while that of chitosan and urea dropped more slowly. After 24 h, the moisture retention rate X_r of EPS1, EPS1U, chitosan and urea were 5.7%, 5.8%, 8.1% and 15.1%, respectively. The results showed that EPS1 and EPS1U had a lower moisture retention capacity than chitosan and urea.

The intermolecular hydrogen bonding of polysacchairde chains is regarded as a major factor for the moisture absorption and retention ability of PS (Chen, Du, & Zeng, 2003). The multiple-helix conformation is favorable for water retention as the water molecules can be retained within the chain networks. According to the Congo red test, EPS1U still maintained the multiple-helix conformation, having the similar moisture absorption and retention capability to EPS1. With a much higher solubility in water, EPS1U should be more desirable than EPS1 for used as a moisturing agent.

4. Conclusions

The US-degraded EPS1 was obtained by US-degradation of the high-MW EPS fraction (EPS1) isolated by ethanol precipitation from mycelial fermentation broth of Cs-HK1 medicinal fungus. A (1→3)-β-D-glucan named EPS1U with a high MW about 730 kDa was purified from the US-degraded EPS1 by gel-filtration chromatography. The EPS1U polymer chain exhibited multiple helix conformation when dissolved in DI water. Compared with EPS1, EPS1U had significantly higher water solubility and lower viscosity. EPS1U as well as EPS1 showed strong moisture absorption capability. With better water solubility and molecular homogeneity, EPS1U had a greater potential as a thickening and moisturizing agent for food and cosmetic applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.02.040.

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