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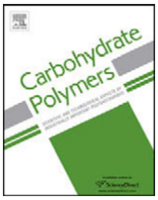

# Isolation and structural elucidation of novel homogenous polysaccharide from *Mactra veneriformis*

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Crude polysaccharide, possessing immune-enhancing and anti-hyperglycemic activities, was extracted from *Mactra vene*riformis by water extraction and alcohol precipitation. Three homogeneous polysaccharides (MVPS-1, MVPS-2 and MVPS-3) were successfully purified from the crude polysaccharide of *M. veneriformis* by DEAE-cellulose column chromatography. The average molecular weights (Mw) of the three polysaccharides were 446, 426 and 452 kDa, respectively. Monosaccharide analysis indicated that the homogeneous polysaccharides contained only D-glucose residue. Fourier-transform infrared spectra (FTIR) revealed the typical characteristics of carbohydrate polymer and the typical peak of α-type glycosidic bond. The MVPS-2 was selected to characterize its absolute chemical structure by analysis of nuclear magnetic resonance (NMR) in this article. According to $^1$H NMR, $^{13}$C NMR and 2D-NMR analysis, MVPS-2 contains two kinds of α-linkages for the glucose residue with a ratio of 6:1. One is α-(1 → 4) and the other is α-(1 → 2) linkage. The H–H correlation spectrometry (COSY) and heteronuclear single-quantum coherence (HSQC) result showed that MVPS-2 was consisted by a repeating structure unit as $[\rightarrow 4\mathrm{Glc}1 \rightarrow 4\mathrm{Glc}1 \rightarrow 4\mathrm{Glc}1 \rightarrow 2\mathrm{Glc}1 \rightarrow 4\mathrm{Glc}1 \rightarrow 4\mathrm{Glc}1 \rightarrow 4\mathrm{Glc}1]_n$.

## 1. Introduction

*Mactra veneriformis*, a typical marine bivalve mollusk, delicious sea food while low-cost, is ubiquitous and abundant in Chinese coastal areas, especially in the coastal shoals of Jiangsu province. Compendium of Materia Medica, a classical Chinese medical book, recorded that flesh of *M. veneriformis* could be used as a traditional Chinese medicine to prevent or treat diabetes. The suffered people might effectively alleviate their bed complication if they frequently take sufficient decoction of the bivalve. The folk clinic reports implied that the flesh of *M. veneriformis* must contain some anti-diabetes substance, and this substance could be availably extracted by boiling water. The modern medical research on *M. veneriformis* exhibits that polysaccharide from it has hypoglycemic activity and is the basic bioactive substance (Wang, Wu, Chang, & Zhang, 2010; Wang, Zhang, Zheng, & Wu, 2010). In our previous work, it was indeed proved that the polysaccharide possessed anti-hyperglycemia and immune enhancing activities (Wang, Wu, et al., 2010; Wang, Zhang, et al., 2010).

However, the purification and chemical characteristics of polysaccharide from *M. veneriformis* (MVPS) had never been reported. It is well known that a good understanding on the structure of the polysaccharide is essential for investigating its bioactivities, especially for its possible interactions with cells or other bio-molecules in vivo (Srivastava & Kulshreshth, 1989). In this research, a crude polysaccharide was extracted by the method of water decoction and alcohol precipitation, and then was deproteinized to prepare a relatively pour polysaccharide fraction (MVPS). The purified MVPS was further applied to a column chromatograph to separate some more pure sub-fractions until a homogenous polysaccharide was got. The apparent molecular weight and monosaccharide composition of the homogenous polysaccharides were analyzed. Furthermore, the representative polysaccharide MVPS-2 was selected to determine its polymer chain structures with Fourier-transform infrared spectra (FTIR), one dimensional (1D) NMR and two dimensional (2D) NMR. This work for the first time provided systematically valuable and fundamental informations on the structure and chain conformation of the polysaccharide from *M. veneriformis*.

## 2. Materials and methods

### 2.1. Materials and reagents

The specimens of bivalve *M. veneriformis*, growing 3 years, were harvested from the Lvsi aquacultural field of Jiangsu province of

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China in June 2007 and identified by Prof. Wan (Institute of Oceanology and Marine Fisheries, Jiangsu). The collected specimens were starved in an aquarium for 24 h to evacuate their gut contents, and then flesh were excavated from the shell and stored at −10 °C condition for further using.

T-series Dextran (T-1000, T-500, T-100, T-40, and T-10) was purchased from Sigma–Aldrich Company. DEAE-cellulose was purchased from Whatman Company. Six standard monosaccharides (Ribose, Arabinose, Xylose, Mannose, Glucose, Galactose) were all D-type and purchased from Sigma Company. Trichloroacetic acid (TCA) and Trifluoroacetic acid (TFA) were from Merck Company. All other chemical reagents were of analytical grade and commercially available, used as received.

### 2.2. Extraction, isolation and purification

One kilogram of pre-washed flesh materials were cut into pieces by a mincer and then extracted twice by decoction of 3 L boiling water (40 min for each decoction). The whole extracted solution was filtered and centrifuged at 6000 rpm for 20 min, and the supernatant was concentrated to about 0.8 L and then precipitated by the addition of 4 L ethanol at room temperature. After overnight disposal, the precipitates were collected by filtration with 400 mesh fabric. The products were dehydrated by 95% ethanol, and then freeze-dried to give the total crude polysaccharide extract of *M. veneriformis* (C-MVPS) (Wang, Wu, et al., 2010; Wang, Zhang, et al., 2010).

The associated proteins in C-MVPS product were removed using trichloroacetic acid (TCA) deproteinizing method (Damaschun, Damaschun, Gast, & Zirwer, 1999; Rajalingam, Loftis, Xu, & Kumar, 2009). After that, the crude polysaccharide was applied to a column of DEAE-cellulose (2.6 cm × 30 cm) and eluted stepwise with gradient $NaHCO_3$ solution from low to high concentration (0.005, 0.01 and 0.05 M) at flow rate of 0.5 mL/min (Majdoub, Mansour, Chaubet, Roudesli, & Maaroufi, 2009; Moonmangmee et al., 2002). Thirty tubes were used to collect eluent in each gradient and each tube was filled with 10 mL eluent. Meanwhile, 0.2 mL eluent was absorbed from each tube to monitor the polysaccharide content using anthrone–sulfuric acid method at 490 nm. The remainder eluent collected at same gradient and containing polysaccharide were poured together. As a result, three polysaccharide fractions were got and were named MVPS-1, MVPS-2 and MVPS-3, respectively. All fractions were concentrated to 1/5 volume after being repeatedly dialyzed in water to remove small molecular salts. The concentrated fractions were finally freeze-dried to obtain white-powder like samples and stored in sealed container under room temperature.

### 2.3. Homogeneity and apparent molecular weight

The molecular weights of polysaccharide were determined by high performance gel permeation chromatography (HGPC) according to the method of Tuinier et al. (1999) and Capek and Hríbalová (2004). Chromatograms were recorded by a Waters 515 instrument equipped with a Waters 717 Autosampler, a RID-6A detector (SHIMADZU Co.) and two columns (TSK-Gel G4000$PW_{XL}$, 7.8 mm × 30.0 cm, and TSK guard column $PW_{XL}$, 6.0 mm × 4.0 cm, TOSOH Co.) connected in series. The columns were calibrated with T-series Dextran (T-1000, T-500, T-100, T-40, and T-10). Eluant used is phosphate buffer (PBS, pH 7.8–8.0) and the flow rate was 0.4 mL/min. A 20 μL aliquot of sample (100 μg/mL) was injected for each run. Output signals were processed on-line using the HGPC software package.

### 2.4. Monosaccharide composing analysis

The monosaccharide composition of MVPS fractions was quantitatively analyzed by gas chromatography–mass spectrometer (GC–MS) after acetylation (Fan et al., 2009). First, fraction sample was hydrolyzed with 4.0 M of TFA at 120 °C for 6 h in a sealed glass tube. Then the hydrolysate was evaporated to dryness and restored with 0.05 M KOH (0.5 mL) and $KBH_4$ (10 mg). A little of acetic acid in methyl solution (0.1%, v/v) was added to get rid of needless $KBH_4$. Finally, the dried reduzate of acid hydrolysates were acetylated in acetic anhydride and pyridine (1:1, v/v), and converted into acetate derivatives dissolved in trichloromethane. Approximately 1.0 μL supernatant of acetate derivatives was loaded onto HP-5 Capillary column (Agilent) (30 mm × 0.32 mm i.d., 0.25 μm) of the GC–MS. Alditol acetates of authentic mixed standards (glucose, mannose, rhamnose, galactose, xylose and arabinose) were also prepared and subjected to GC–MS analysis separately in the same way. The operation was performed in the following conditions: injection temperature: 240 °C; detector temperature: 240 °C; column temperature programmed: 160 °C holding for 2 min, then increasing to 240 °C at 5 °C/min and finally holding for 5 min at 240 °C. Nitrogen was used as the carrier gas and maintained at 1.0 mL/min.

### 2.5. IR spectroscopy

Infrared spectra of the three polysaccharide fractions were recorded on a FTIR-100 (Thermo Nicolet Co.) infrared spectrophotometer at 4 $cm^{-1}$ resolution (Wang & Zhang, 2009). Samples and potassium bromide were dried at 50 °C in fast infrared dryer for 1 h prior to be pressed into pellets with a sample/KBr ratio of about 2 mg/200 mg. In order to obtain more exact band positions, all the FTIR analysis of polysaccharides were performed in the region 4000–500 $cm^{-1}$.

### 2.6. NMR spectroscopy

One-dimensional, $^1H$ NMR (500 MHz), $^{13}C$ NMR (125 MHz) and 2D-NMR containing H–H correlation spectrometry (COSY) and heteronuclear single-quantum coherence (HSQC) spectra of the polysaccharide fraction (MVPS-2) were obtained with a Bruker Avance 500 spectrometer (BRUKER Company). Samples containing 15 mg/mL of polysaccharide in $D_2O$ were used. Chemical shifts were reported relative to tetramethylsilane (TMS = 0 ppm). $^1H$ NMR spectra were recorded with the single pulse sequence with 15,720 data points, a spectral width of 3931 Hz, a recycle delay d1 of 1.5 s and a 90° pulse length of 14.8 μs. An exponential window function with a line broadening (1b) of 3.0 Hz was applied before Fourier transformation. Two-dimensional COSY spectra were obtained at 500 MHz using a spectral width of 3100 Hz and a repetition time of 1.5 s. In the F2 and F1 dimensions, 2100 and 267 data points were used. One-dimensional $^{13}C$ NMR spectra were recorded with complete proton decoupling at 125 MHz using a spectral width of 30,303 Hz, 32,767 data points, a recycle delay d1 of 2.0 s, and a 90° pulse length of 9.5 μs. An exponential window function with a line broadening (1b) of 3.0 Hz was applied before Fourier transformation. HSQC experiments were obtained using 2100 points in F2 and 267 points in F1, with spectral widths of 3 and 14,000 Hz, respectively. All of the experiments were done at temperature of 313 K except the COSY experiments at 303 K (Gutiérrez, Martínez, Sanabria, León de Pinto, & Igartuburu, 2005; Ma, Wang, & Zhang, 2008).

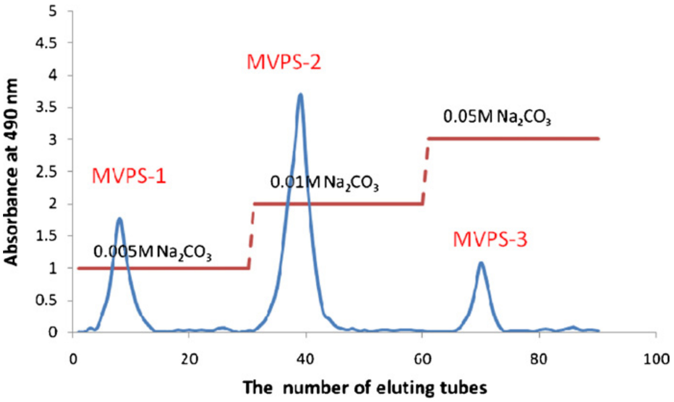

**Fig. 1.** Chromatography of eluted polysaccharide (MVPS) on DEAE-cellulose column (26 mm × 300 mm). MVPS-1 eluted with 0.005 M $Na_2CO_3$; MVPS-2 eluted with 0.01 M $Na_2CO_3$; MVPS-3 eluted with 0.05 M $Na_2CO_3$.

## 3. Results and discussion

### 3.1. Preparation and basic properties

After boiling water extraction and alcohol precipitation, the crude polysaccharide (C-MVPS) was obtained from the flesh of *M. veneriformis*, as described previously (Wang, Wu, et al., 2010; Wang, Zhang, et al., 2010). The yield of C-MVPS in the extraction was about 3.2% and the polysaccharide content in the dried C-MVPS was 58.2%. After purified by method of TCA deproteination, the C-MVPS lose about 49.7% of its total weight, but its polysaccharide content increased to 90.1%. After further following DEAE-cellulose chromatography, this deproteinized product yielded three fractions MVPS-1, MVPS-2 and MVPS-3. All the fractions were eluted as a single symmetric peak (Fig. 1). The yields of the three fractions were 20.5%, 52.3% and 13.8%, respectively. It was also proved that all polysaccharide contents of the fractions were over 99.8%. In addition, all the three polysaccharides were free of protein, as evidenced by a lack of absorbance at 280 nm and a negative response to the Lowry-assay.

### 3.2. Homogeneity and apparent molecular weight analysis

All polysaccharide fractions were tested as a single symmetric peak on the high-performance gel permeation chromatography (Fig. 2), indicating that they are all homogeneous polysaccharide. High-performance gel permeation chromatography (HGPC) was also used to determine the molecular weights (Mw) of the three homogenous polysaccharides with the dextran standards (T-1000, T-500, T-100, T-40, and T-10) used for the calibration curve. The equation of the standard curve was: $\lg \mathrm{Mw} = 10.258 - 0.2602t$ (where Mw represents the molecular weight, while $t$ represents retention time) with a correlation coefficient of 0.9993. The average molecular weight values of the purified polysaccharide MVPS-1, MVPS-2 and MVPS-3 were estimated to be 446, 426 and 452 kDa, respectively.

### 3.3. Monosaccharide composition

The monosaccharide composition of the polysaccharide fractions, obtained after deproteinization and ion-exchange chromatography, was analyzed by GC–MS after being hydrolyzed and acetylated. Result is shown in Fig. 3. Only one peak appeared at 34.76 min on the recording graphs of MVPS-1, MVPS-2 and MVPS-3. The peaks of all monosaccharide derivatives from the three polysaccharides were sharp and symmetrical. Furthermore, the monosaccharide peaks of all fractions were identified as D-glucose when compared with the standard monosaccharide. In order to accurately confirm this result, we also examine the monosaccharide composition of the three polysaccharides with high-performance liquid chromatography and thin-layer chromatography. The results accorded with the GC–MS test in this article but were not given here. Results indicated that all the three polysaccharides contained only one type of monosaccharide residues and they surely belong to glucosan homologs.

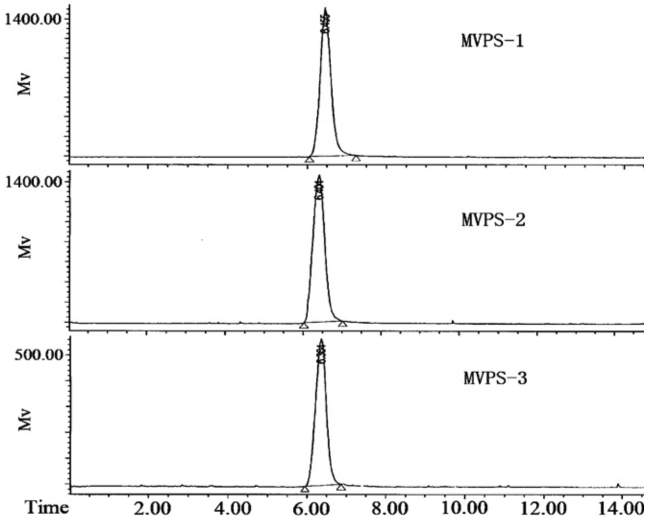

**Fig. 2.** The homogeneity evaluation of MVPS-1, MVPS-2 and MVPS-3 by HPLC.

### 3.4. IR spectroscopy analysis

FTIR spectra presented in Fig. 4 were used to provide more structural information for the three polysaccharides. The different absorption bands of the FTIR analysis were assigned as previously described in literatures (Melin, Perromat, & Déléris, 2000; Ray, 2006; Zhang et al., 2001). For the three samples, a broad band centered at 3393 $cm^{-1}$ assigned to hydrogen-bonded hydroxyl groups. An intense band centered at 2928 $cm^{-1}$ was due to the –CH stretching and was a characteristic absorption of polysaccharide. The absorption band centered at 1646 $cm^{-1}$ was caused by the –OH flexural vibrations of the polysaccharide. The group of bands that extended from 1485 $cm^{-1}$ to 1350 $cm^{-1}$ assigned to –CH ($O{-}CH_2$) flexural vibrations. The intense band extending between

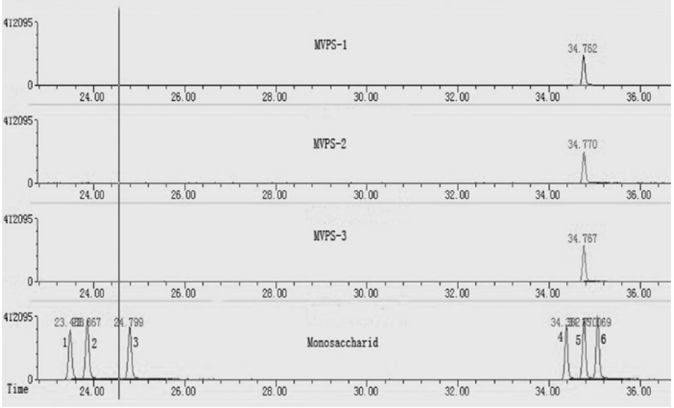

**Fig. 3.** The monosaccharides determination of MVPS-1, MVPS-2 and MVPS-3 by GC–MS chromatogram compared with mixed standard (1 for D-ribose, 2 for D-arabinose, 3 for D-xylose, 4 for D-mannose, 5 for D-glucose and 6 for D-galactose).

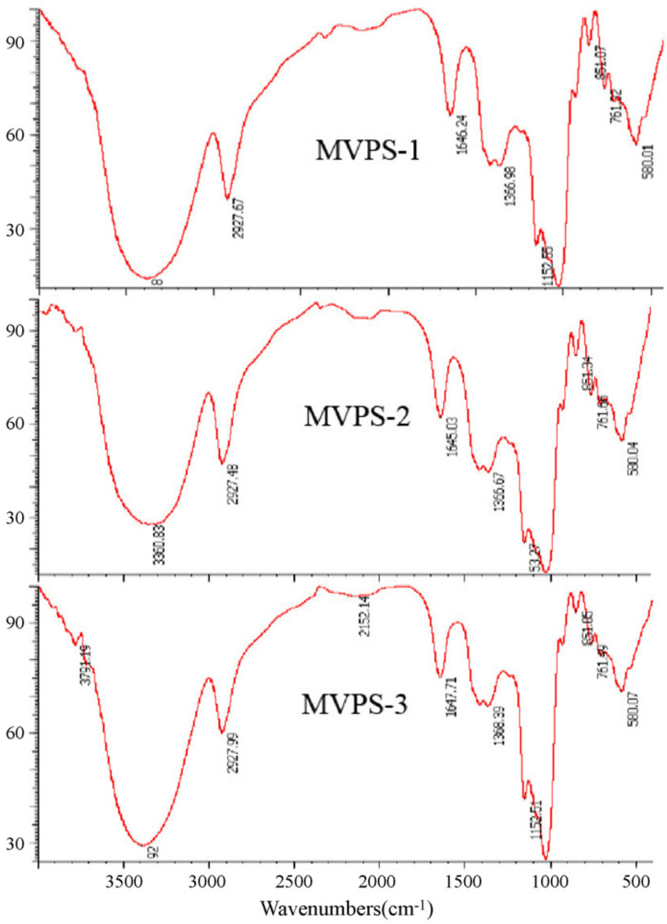

**Fig. 4.** The infrared spectrum of MVPS-1, MVPS-2 and MVPS-3.

1200 and 1020 cm$^{-1}$ corresponded to C–O stretching vibrations. The absorption band centered at 930 cm$^{-1}$ was caused by the C–O (O–$CH_2$) stretching vibrations. The absorption band centered at 851 cm$^{-1}$ was due to the α-type glycosidic bond. All the absorption bands listed above were characteristic FTIR peaks of carbohydrate polymer and primarily indicated the polysaccharide nature of MVPS.

### 3.5. $^{1}$H NMR and $^{13}$C NMR

All of the polysaccharide fractions were analyzed by $^{1}$H NMR and $^{13}$C NMR spectroscopy. However, only MVPS-2 was presented here because this polysaccharide accounted for the majority of the total extracts and had remarkable bioactivity. $^{1}$H NMR and $^{13}$C NMR spectra of MVPS-2 were shown in Fig. 5. Two obvious chemical shift signals of anomeric protons were found at $\delta$5.40 ppm and $\delta$4.98 ppm in $^{1}$H NMR spectrum. These results indicated that the polysaccharide contain two residues (named A and B). Combined with the result of monosaccharide analysis, the A and B residues may reflect two different α-type glycosidic bonds of glucose. Molar proportion of the two residues (A and B) was about 6:1 when estimated by the ratio of peak area of the integration of the H-1 signal for A and B residues. The corresponding H chemical shifts of residues A and B for other positions were packed in the range of 3.3–3.9 ppm and even difficult to discriminate. Similar results refer to some previous studies (Dai, Zhang, Zhang, & Wang, 2009; Snyder, Gibson, Heiss, Key, & Azadi, 2006).

Based on component analysis, FTIR analysis, and on literature data (Chattopadhyay, Adhikari, Lerouge, & Ray, 2007; Ge, Zhang, & Sun, 2009; Wu, Wu, Zhou, & Pan, 2009), the $^{13}$C NMR (shown in Fig. 5) chemical shifts in the area of anomeric carbon atoms also suggested two kinds of α-linkages for the glucose residues. The C-1 signals of the residues were detectable probably at $\delta$102.5 ppm and $\delta$101.0 ppm. The shifts at $\delta$79.8 ppm could be assigned to the substituted carbon atom. Additionally, the peak at $\delta$63.2 ppm could be assigned to C-6 of the glucose residues. Because of the complexity of the $^{13}$C NMR spectrum of MVPS-2, the remaining more or less broad peaks were difficult to be assigned to the respective glucose residues. The absence of carbon atom signal in the range of $\delta$170–180 ppm proved that MVPS-2 contains no uronic acid or glycoprotein.

### 3.6. 2D-NMR analysis of MVPS

The assignments of $^{1}$H and $^{13}$C resonances and the linkages of glucose residues were obtained from $^{1}$H–$^{1}$H and $^{1}$H–$^{13}$C two-dimensional correlation NMR experiments (Ikuta et al., 1997; Ma et al., 2008; Snyder et al., 2006). The results from COSY (Fig. 6), based on stepwise magnetization transfers from the anomeric protons, could help assign the chemical shifts of other protons. The single-bond correlations between the protons and the corresponding carbons obtained from HSQC spectra (Fig. 6) of

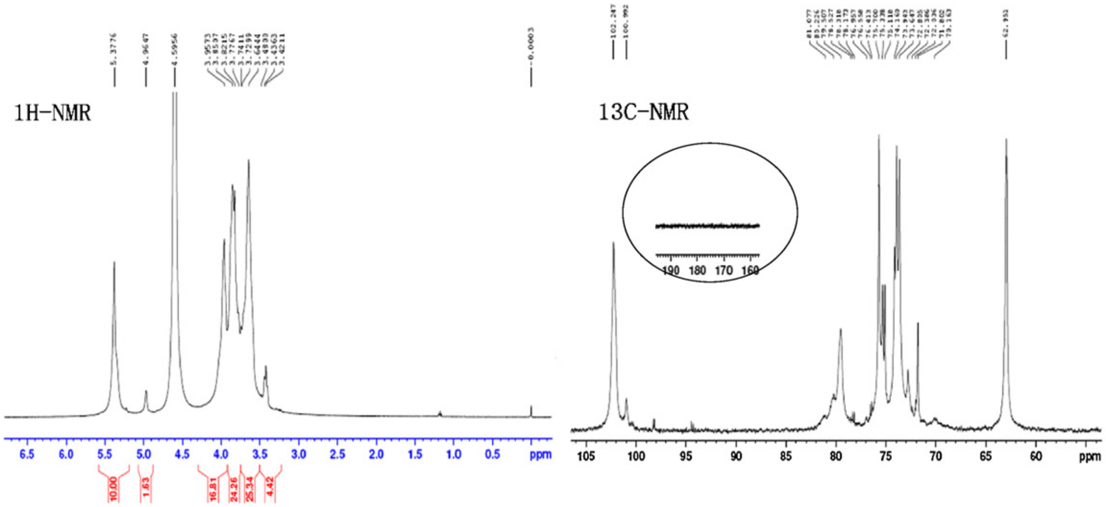

**Fig. 5.** The $^{1}$H NMR and $^{13}$C NMR spectrogram of MVPS-2.

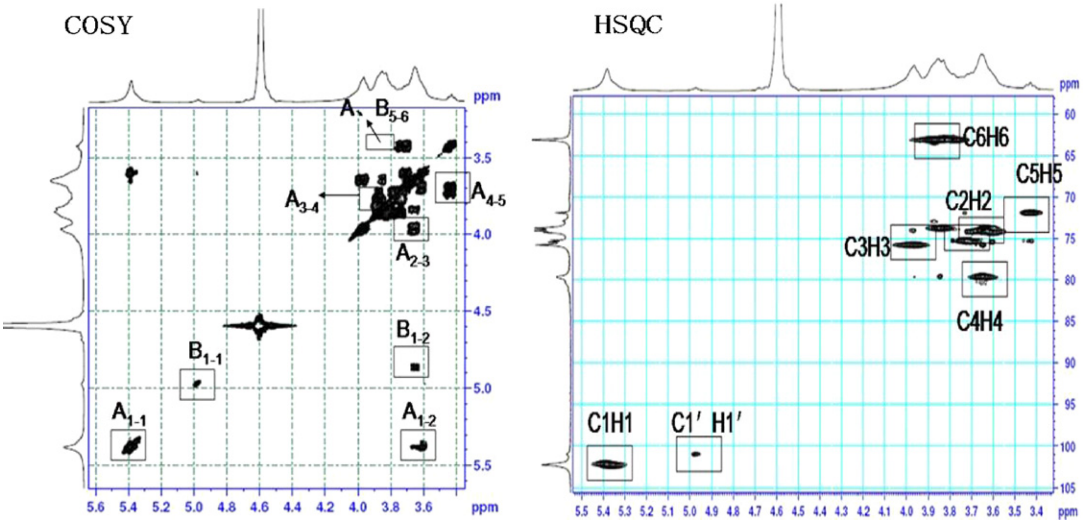

**Fig. 6.** The H–H correlation spectrometry (COSY) and heteronuclear single quantum coherence (HSQC) spectrograms of MVPS-2.

MVPS-2 in $D_2O$ enabled all the $^{13}C$ to be assigned. The chemical shifts of all anomeric resonances and $^{13}C$ chemical shifts for the carbons indicated that the peaks at $\delta$5.40 ppm for proton and the carbon peak at $\delta$102.5 ppm could be assigned to the anomeric proton and carbon of residue A, the proton $\delta$4.98 ppm to the carbon at $\delta$100.99 ppm for residue B. The results of $^1H$ and $^{13}C$ chemical shifts were summarized in Table 1. According to the result, we could know that residue A was substituted at C-4 position and residue B was substituted at C-2 position. Thus, residue A and B belong to α-(1 → 4) glucosidic bond linked and α-(1 → 2) glucosidic bond linked D-glucose, respectively. Overall, the mainly chain structure of MVPS-2 might be [→4Glc1 → 4Glc1 → 4Glc1 → 2Glc1 → 4Glc1 → 4Glc1 → 4Glc1]$_n$. The chemical structure of MVPS-2 is shown in Fig. 7.

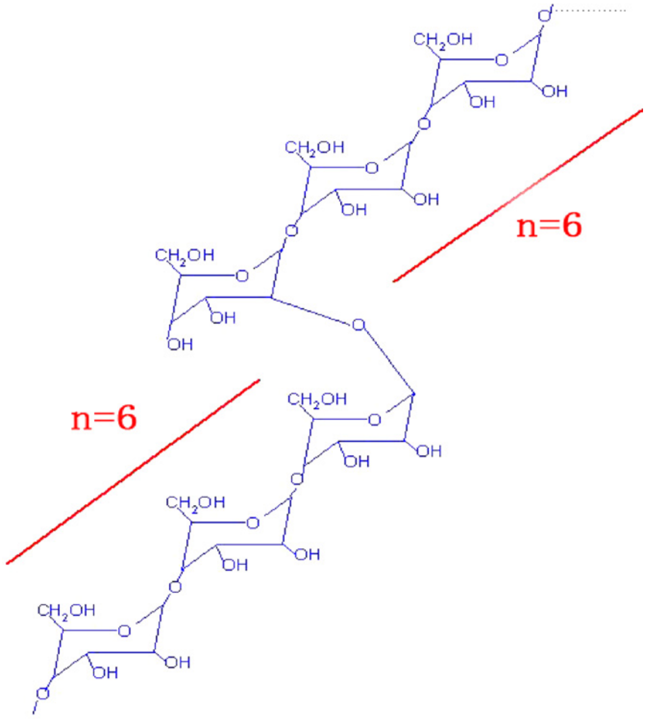

**Fig. 7.** The chemical structure of MVP-2.

**Table 1**
Hydrogen (H) and carbon (C) chemical shifts of two residues (A and B) from MVPS-2 in NMR.

| NMR | Chemical shift (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| H-A residue | 5.37 | 3.64 | 3.96 | 3.72 | 3.43 | 3.82 |
| C-A residue | 102.50 | 74.10 | 75.70 | 79.60 | 72.0 | 62.90 |
| H-B residue | 4.96 | 3.62 | na | na | na | 3.85 |
| C-B residue | 100.99 | 79.81 | na | na | na | 62.70 |

na – not detected.

## 4. Conclusions

Our research team had reported the bioactive studies of MVPS at earlier time. Results indicated that the polysaccharide from *M. veneriformis* had effective anti-hyperglycemia and immune enhancing activities. Based on this consideration, many efforts had been made to isolate the polysaccharide and elucidate the possible relationship between structure and biological functions. This article mainly related to the isolation and structure elucidation of the novel polysaccharides. For that reason, three polysaccharides (MVPS-1, MVPS-2 and MVPS-3), possessing over 420 kDa of apparent molecular weight, were isolated and purified, and was further proved only containing α-D-glucose in monosaccharide analysis. In addition, MVPS-2 was selected to clarify its absolute structure because of its highest yield in the preparation. The $^1H$ NMR and $^{13}C$ NMR analyses indicated that MVPS-2 contains two kinds of α-glucosidic bond-linked glucose residues. The 2D-NMR analysis deduced that MVPS-2 had a repeating unit to compose its main chain as [→4Glc1 → 4Glc1 → 4Glc1 → 2Glc1 → 4Glc1 → 4Glc1 → 4Glc1]$_n$. Although the MVPS-2 has a novel structure, it is still hard to explain the relationships between the structure and biological functions because of lacking datum of mechanism study. Thus, the next studies on MVPS will be focused on mechanism of action, chemical synthesis and structure modification, and so on.

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## References

Capek, P., & Hríbalová, V. (2004). Water-soluble polysaccharides from *Salvia officinalis* L. possessing immunomodulatory activity. *Phytochemistry*, *65*, 1983–1992.

Chattopadhyay, K., Adhikari, U., Lerouge, P., & Ray, B. (2007). Polysaccharides from *Caulerpa racemosa*: Purification and structural features. *Carbohydrate Polymers*, *68*, 407–415.

Dai, Z., Zhang, H., Zhang, Y., & Wang, H. (2009). Chemical properties and immunostimulatory activity of a water-soluble polysaccharide from the clam of *Hyriopsis cumingii* Lea. *Carbohydrate Polymers*, *77*, 365–369.

Damaschun, G., Damaschun, H., Gast, K., & Zirwer, D. (1999). Proteins can adopt totally different folded conformations. *Journal of Molecular Biology*, *291*, 715–725.

Fan, Y., He, X. J., Zhou, S., Luo, A., He, T., & Chun, Z. (2009). Composition analysis and antioxidant activity of polysaccharide from *Dendrobium denneanum*. *International Journal of Biological Macromolecules*, *45*, 169–173.

Ge, Q., Zhang, A., & Sun, P. (2009). Structural investigation of a novel water-soluble heteropolysaccharide from the fruiting bodies of *Phellinus baumii* Pilát. *Food Chemistry*, *114*, 391–395.

Gutiérrez, de-G. O., Martínez, M., Sanabria, L., León de Pinto, G., & Igartuburu, J. M. (2005). 1D- and 2D-NMR spectroscopy studies of the polysaccharide gum from *Spondias purpurea* var. *lutea*. *Food Hydrocolloids*, *19*, 37–43.

Ikuta, K., Shibata, N., Kanehiko, H., Kobayashi, H., Suzuki, S., & Okawa, Y. (1997). NMR assignment of the galactomannan of *Candida lipolytica*. *FEBS Letters*, *414*, 338–342.

Ma, Z., Wang, J., & Zhang, L. (2008). Structure and chain conformation of β-glucan isolation from *Auricularia auricular-judae*. *Biopolymers*, *89*, 614–622.

Majdoub, H., Mansour, M. B., Chaubet, F., Roudesli, M. S., & Maaroufi, R. M. (2009). Anticoagulant activity of a sulfated polysaccharide from the green alga. *Biochimica et Biophysica Acta*, *1790*, 1377–1381.

Melin, A. M., Perromat, A., & Déléris, G. (2000). Pharmacologic application of Fourier transform IR spectroscopy: *In vivo* toxicity of carbon tetrachloride on rat liver. *Biopolymers*, *7*, 160–168.

Moonmangmee, S., Toyama, H., Adachi, O., Theeragool, G., Lotong, N., & Matsushita, K. (2002). Purification and characterization of a polysaccharide involved in the pellicle produced by a thermotolerant *Acetobacter* strain. *Bioscience, Biotechnology, and Biochemistry*, *66*, 777–783.

Rajalingam, D., Loftis, C., Xu, J. J., & Kumar, T. K. (2009). Trichloroacetic acid-induced protein precipitation involves the reversible association of a stable partially structured intermediate. *Protein Science*, *18*, 980–993.

Ray, B. (2006). Polysaccharides from *Enteromorpha compressa*: Isolation, purification and structural features. *Carbohydrate Polymers*, *66*, 408–416.

Snyder, D. S., Gibson, D., Heiss, C., Key, W., & Azadi, P. (2006). Structure of a capsular polysaccharide isolated from *Salmonella enteritidis*. *Carbohydrate Research*, *341*, 2388–2397.

Srivastava, R., & Kulshreshth, D. K. (1989). Bioactive polysaccharides from plants. *Phytochemistry*, *28*, 2877–2883.

Tuinier, R., Zoon, P., Olieman, C., Stuart, M. A., Fleer, G. J., & de Kruif, C. G. (1999). Isolation and physical characterization of an exocellular polysaccharide. *Biopolymers*, *49*, 1–9.

Wang, J., & Zhang, L. (2009). Structure and chain conformation of five water-soluble derivatives of a β-D-glucan isolated from *Ganoderma lucidum*. *Carbohydrate Research*, *344*, 105–112.

Wang, L. C., Wu, H., Chang, N., & Zhang, K. (2010). Anti-hyperglycemic effect of the polysaccharide fraction isolated from *Mactra veneriformis*. *Frontier of Chemical Engineering*, doi:10.1007/s11705-010-0002-2 (published online)

Wang, L., Zhang, K., Zheng, W., & Wu, H. (2010). Hypoglycemia, protecting liver and immune enhancing effects of the flesh extracts of *Mactra quadrangularis*. *Journal of Nanjing University of TCM*, *26*, 283–285.

Wu, M, Wu, Y., Zhou, J., & Pan, Y. (2009). Structural characterization of a water-soluble polysaccharide with high branches from the leaves of *Taxus chinensis* var. *mairei*. *Food Chemistry*, *113*, 1020–1024.

Zhang, L., Zhang, M., Dong, J., Guo, J., Song, Y., & Cheung, P. C. (2001). Chemical structure and chain conformation of the water-insoluble glucan isolated from *Pleurotus tuber-regium*. *Biopolymers*, *59*, 457–464.