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ORIGINAL ARTICLE

Chemical modification, characterization and bioactivity of a released exopolysaccharide (r-EPS1) from *Lactobacillus plantarum* 70810

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Abstract In the present study, a released exopolysaccharide (r-EPS1) from *L. plantarum* 70810 was modified by acetylation, phosphorylation and carboxymethylation. Scanning electron micrograph (SEM) and thermogravimetric analysis (TGA) showed the r-EPS1 derivatives had different surface morphology and thermal behavior. Compared with r-EPS1, the derivatives exhibited stronger antioxidant and antitumor activities. The study provided experimental evidences that chemical modification could be an effective way to improve the bioactivity of exopolysaccharide from *L. plantarum* 70810. It is noted that these derivatives could be explored as novel potential antioxidant and antitumor agents.

Keywords Released exopolysaccharides · Antioxidant · Antitumor · *Lactobacillus plantarum* · Chemical modification

Introduction

Free radicals, including oxygen free radicals and non-oxygen free radicals, are continuously produced during our normal metabolism process or generated through the stimulation of exogenous factors and agents [1–3]. Though lower concentrations of free radical can play a crucial role in regular physiological functions, unregulated overproduction of free radicals may induce damages of biomolecules such as proteins and DNA [4, 5]. This has been considered as a potential risk to many diseases such as cardiovascular diseases, diabetes, central nervous system disorders, arthritis, aging and cancer [6, 7]. The deleterious consequences of overproduction of free radicals are sometimes beyond the self-antioxidant defenses and repair systems of organisms thus have resulted in the exploitation of new and efficient antioxidant supplements to attenuate the deleterious effects [2, 8].

Previously reports revealed many natural polysaccharides and their derivatives possess potent antioxidant activities and potential applications as antioxidants [9, 10]. It is worth noting that the derivatives of many polysaccharides exhibited significantly higher antioxidant or antitumor activities than their corresponding native polysaccharides [11–14]. Generally, the bioactivity of polysaccharides was closely related with its molecular weight, functional groups and degree of substitution (DS) [15]. Introduction of suitable ionic groups with appropriate DS can significantly improve the bioactivity of polysaccharides, such as antioxidant, antitumor, antiviral and immunomodulating activities [3, 9]. However, chemical modifications of exopolysaccharide from Lactobacillus have been scarcely reported. Therefore, it is of great interest to investigate alternations of bioactivity of exopolysaccharides from Lactobacillus via molecular modifications.

In the previous study, we have reported the preparation, structure and bioactivity of a high-yield released exopolysaccharide (*i.e.*, r-EPS1) from *Lactobacillus plantarum* 70810 [16]. The r-EPS1 is a branched heteropolysaccharide and mainly consisted of α-linked sugar units such as glucose, galactose and mannose. Preliminary tests *in vitro* indicated the high-yield r-EPS1 exhibited some antioxidant and antitumor activities, but the activities were relatively poor. Therefore, in the present work, the effects of chemical modifications, namely, actylation, phosphorylation and carboxymethylation on the improvements of bioactivity of r-EPS1 were investigated. The structure, surface morphology, thermal behavior, *in vitro* antioxidant and antitumor

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activities of the derivatives and native samples were characterized and compared. The relationships between the structure and bioactivity of different derivatives were also discussed.

Materials and methods

Materials and chemicals

The preparation process of purified exopolysaccharides (r-EPS1) is based on the process described by our previous method [16]. Nitro blue tetrazolium (NBT), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), phenazine methosulfate (PMS), 1,10-phenanthroline, Penicillin (100 U/mL) and streptomycin (100 mg/L) were from Sigma Chemical Co., Ltd. (St. Louis, USA). Dulbecco's modified eagle medium (DMEM) and Bovine serum albumin (BSA) were purchased from Gibco (Gibco BRL, Grand Island, NY, USA). All other reagents were of analytical grade.

Acetylation of r-EPS1

The r-EPS1 powder (200 mg) was dispersed in 10 mL of formamide (FA) and maintained at 60 °C for 30 min, then 5 mL of 5 % (*w*/*v*, dissolved in acetic anhydride) *N*-Bromosuccinimide (NBS) was added. The reaction was carried out at 60 °C for 4 h, before 2 mL deionized water was added to terminate the reaction and the mixture was neutralized with 1 M NaOH solution. The polysaccharide derivative was dialyzed, concentrated, freeze-dried and named as r-EPS1A.

The degree of acetylation (DS_{Ac}) was calculated according to the equation [17]:

$$DS_{Ac} = (\textit{integrals of acetylgroups signals at about } 2.0\ \textit{ppm}) \times 6/(\textit{integrals signals of polysaccharides at } 3.5\text{–}5.5\ \textit{ppm}) \times 3$$

Phosphorylation of r-EPS1

The r-EPS1 powder (200 mg) was dissolved in 10 mL of DMSO at 60 °C for 30 min, followed by the addition of 3.6 g of urea and 1.5 mL H_3PO_4 to start the reaction. The reaction was conducted at 60 °C for 4 h, and terminated by the addition of 2 mL deionized water and then the mixture was neutralized with 1 M NaOH solution. Finally, the polysaccharide derivative was dialyzed, concentrated and freeze-dried. This sample was named as r-EPS1P.

The content of phosphorous (P%) was determined by an inductively coupled plasma-atom emission spectrograph (Optima 2100 *DV*, Perkin Elmer Co., USA). The phosphorous content was determined as 1.51 %. The degree of phosphorylation (DS_P) was estimated according to the following equation [2]:

$$DS_P = 5.22 \times P\%/(1\text{–}2.61 \times P\%)$$

Where P% was the content of phosphorous.

Carboxylmethylation of r-EPS1

The r-EPS1 powder (300 mg) was dissolved in the mixture of 5 mL 20 % NaOH and 12.5 mL isopropanol in an ice bath with stirring for 3 h. Then the carboxylmethylation agent (a mixture of 2.63 g chloroacetic acid, 5 ml of 20 % NaOH and 12.5 ml isopropanol) was added slowly with stirring and the reaction was continued at room temperature for 3 h. After reaction at 60 °C for another 1.5 h, the mixture was cooled to room temperature and neutralized with 0.5 M HCl solution. The polysaccharide derivative was dialyzed, concentrated, freeze-dried and named as r-EPS1C.

Carboxymethyl content was determined by the method of neutralization titration [18]. The degree of carboxymethylation (DS_C) was calculated according to the equation:

$$A = (C_{NaOH} \times V_{NaOH} – C_{HCl} \times V_{HCl})/W$$

$$DS_C = 0.162A/(1\text{–}0.058A)$$

Where W was the weight of sample. C_{NaOH} and C_{HCl} was the concentration of NaOH and HCl, respectively. V_{NaOH} and V_{HCl} was the volume (mL) of NaOH and HCl, respectively.

Molecular weight analysis

The molecular weight (Mw) of r-EPS1 and its derivates was determined by an Agilent 1100 series HPLC (Agilent Technologies, Wilmington, DE). Ten microliter sample was injected into the TSK-GEL G4000SWXL column (300 mm×7.8 mm, Tosoh Co., Tokyo, Japan) and eluted with deionized water (0.8 mL/min). The detection of eluate was couducted by an evaporative light-scattering detector (ELSD). Standard dextrans were used to estimate the Mw of samples.

FT-IR and NMR analysis

The determination of the main functional groups presented in r-EPS1 and its derivates was conducted by a Bruker Tensor-27 FT-IR spectrophotometer (Bruker Co., Ettlingen, Germany). The FT-IR spectra were recorded with KBr pellets from 400 cm^{-1} to 4000 cm^{-1}.

For NMR spectroscopic analyses, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR spectra (300 MHz) were recorded on a Bruker

AVANCE AV-500 spectrometer (Bruker Group, Fällanden, Switzerland) at 300 K. The samples were dissolved in D_2O and H_3PO_4 was used as internal standard for ^{31}P NMR spectra.

Morphological and thermal gravimetric (TG) analysis

The surface morphology of polysaccharide samples was observed by a Hitachi S-3,000 N scanning electron microscopy (Hitachi, Tokyo, Japan). The polysaccharide samples (1 mg/mL) were placed on copper studs with the help of double-sided adhesive and gold-sputtered. Each sample was observed at an accelerating voltage of 10 KV.

The thermal behaviors of r-EPS1 and its derivatives were determined by thermogravimetric analysis (TGA). TGA was performed by a PerkinElmer Pyris 1 TGA (PerkinElmer Co., Wellesley, MA, USA) heating from 30 to 800 °C at a rate of 10 °C/min under a nitrogen flow of 20 mL/min.

Antioxidant activity *in vitro*

Hydroxyl radical scavenging activity

The scavenging activity of hydroxyl radical was assayed according to the method of Li *et al.* [19]. The scavenging activity of hydroxyl radical was calculated using the following equation:

$$\textit{Scavenging activity}\ (\%) = (A_1 - A_0)/(A' - A_0) \times 100\%$$

Where A_1 was the absorbance of sample system, $A_0$was the absorbance of the system replaced sample with deionized water; A' was the absorbance of the system replaced sample and H_2O_2 with deionized water.

DPPH radicals scavenging activity

Measurement of DPPH radicals scavenging activity was based on the method of Qiao *et al.* [20]. The scavenging activity of DPPH radical was determined using the following equation:

$$\textit{Scavenging activity}(\%) = [1 - (A_1 - A')/A_0] \times 100\%$$

Where A_1 was the absorbance of sample, A_0 was the absorbance of the system replaced sample with deionized water, A' was the absorbance of the system under same conditions as A_1 contained deionized water instead of DPPH, respectively.

Reducing power

Reducing power of r-EPS1 and its derivates was measured by the method of Liu *et al.* [21]. The reducing power was calculated using the following equation:

$$\textit{Reducing power} = A_1 - A'$$

Where A_1 was the absorbance of sample, A' was the absorbance of the system under same conditions as A_1 with deionized water instead of $FeCl_3$.

Antitumor *in vitro*

The antitumor activity of r-EPS1 and its derivates was evaluated by using MTT assay and performed according to a method mentioned in our previous report [16]. Briefly, 100 μL of human liver cancer HepG-2 or colon cancer HT-29 cells were incubated on a 96-well plate at a concentration of 2×10^5 cells/mL under 5 % CO_2 at 37 °C. After inoculation for 24 h, the tumor cells were treated with various concentrations of sample (0–600 μg/mL) or fluorouracil (5-FU, 50 μg/mL) for 24 h, respectively. Then, the cells were inoculated for another 4 h after each well was added into 10 μL (5 mg/mL) of MTT. Liquid was removed and 100 μL of DMSO was added. The absorbance was measured at 570 nm. The inhibition ratio was expressed as follows:

$$\textit{Inhibition ratio}\ (\%) = [1 - (A_1 - A')/(A_0 - A')] \times 100\%$$

Where A_1 was the absorbance of samples, A_0 and A' were the absorbance of the system without the addition of samples and cells, respectively.

Statistical analysis

Data were expressed as mean±standard deviation and statistical analysis were performed by one way ANOVA of Tukey method (SPSS 16.0), and $P<0.05$ was taken as significant.

Results and discussion

Degree of substitution, yield and molecular weight analysis

The degrees of substitution of r-EPS1A, r-EPS1P and r-EPSC were 0.41, 0.08 and 1.1, respectively. The yields of r-EPS1A, r-EPS1P and r-EPS1C were 55, 52 and 45 %, respectively.

HPLC analysis confirmed the homologeity and molecular weight (Mw) of polysaccharides derivatives. All polysaccharides derivatives appeared as symmetrical sharp peaks in HPLC chromatograms (Fig. 1) which revealed the samples

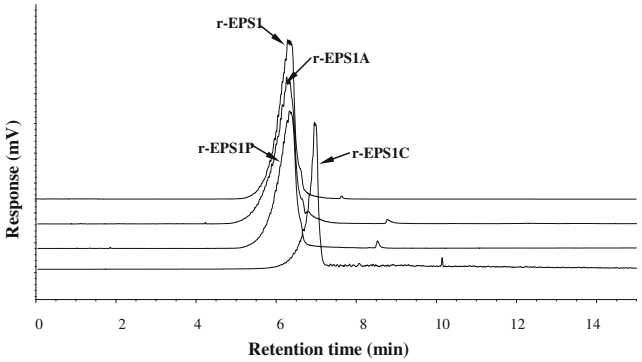

Fig. 1 HPLC chromatograms of r-EPS1 and its derivatives on TSK-GEL G4000SW$_{XL}$ column

were homogeneous. Compared to the native form of r-EPS1 (Mw of 204.6 kDa), Mw of the acetylated derivative was increased to 217.8 kDa, which might be resulted from the incorporation of acetylated groups. r-EPS1P exerted a similar Mw (204.4 kDa) than that of native polysaccharide, whereas Mw of the carboxylmethylated derivative decreased to 114.8 kDa, which was probably due to the β-elimination in an alkaline medium [15]. Many reports [9, 22, 23] showed that the Mw of chemical modification polysaccharides had a slight or major decrement during the violent reaction process. However, the Mw of r-EPS1A in this experiment and the Mw of sulfated lacquer polysaccharides even at a more intense reaction condition were increased [24]. These results suggested the properties of polysaccharide itself (such as glycosidic bonds, branched chains, and so on) might also affect the Mw of polysaccharide derivatives.

FT-IR characterization

FT-IR spectra of the native polysaccharide and its derivatives are shown in Fig. 2. The intense absorption peaks at around 3,392 cm^{-1} (O–H group), 2,937 cm^{-1} (C–H and C–O groups) and 1,058 cm^{-1} (C–O group) were the typical absorption peaks of a polysaccharide [25–27]. For r-EPS1A (Fig. 2b), a new strong absorption peak at around 1,686 cm^{-1} was attributed to the C=O stretching vibration, which indicated the acetyl group were successfully incorporated [28]. This was also confirmed by the enhanced signal at 1,234 cm^{-1} [10]. In the FT-IR spectrum of phosphorylated derivative (Fig. 2c), the peak appeared at 1,368 cm^{-1} could be attributed to P=O stretching. The peaks found at 1,056 and 525 cm^{-1} were assigned to P–OH group [29]. For the spectrum of r-EPS1C (Fig. 2d), two new absorption peaks at 1,600 cm^{-1} and 1,422 cm^{-1} were observed and represented the asymmetrical and symmetrical COO^- stretching vibration, respectively. Additionally, the absorption peak at 1,326 cm^{-1} represented the –CH_2– vibration [9, 12, 24]. The emergence or strengthening of these absorption peaks indicated the incorporation of the carboxymethylated groups.

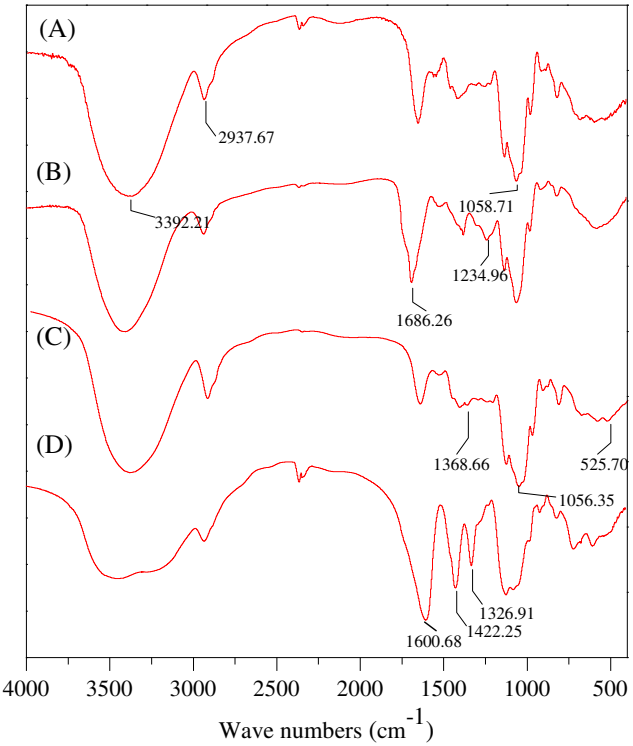

Fig. 2 FTIR spectra of r-EPS1 (**a**), r-EPS1A (**b**), r-EPS1P (**c**) and r-EPS1C (**d**)

NMR characterization

The ^{1}H NMR spectra of r-EPS1 and its acetylated product (r-EPS1A) were shown in Fig. 3a and b, respectively. Compared to the native polysaccharide, the new resonance peaks appeared at around 1.9–2.2 ppm indicated the incorporation of the acetylated groups [2, 30]. Similarly, two resonance peaks in the region of 0.9–3.1 ppm of the ^{31}P NMR spectrum of r-EPS1P (Fig. 3c) confirmed the phosphated groups were bound to the polysaccharide chain [31, 32]. The absence of a sharp resonance peak at 2.6 ppm indicated the sample was lack of free H_3PO_3 [32]. In addition, a resonance peak observed at −1.79 ppm suggested the presence of another type of phosphated group in r-EPS1P, which might be attributed to the formation of diphosphate esters [33, 34]. These results indicated the acetylated and phosphorylated groups might locate at different positions of their derivatives [32]. The ^{13}C NMR spectra of r-EPS1 and its carboxylmethylated derivative are shown in Fig. 3d and e, respectively. Carboxylmethylation modification could not seem to change backbone structure of r-EPS1 due to the existence of the anomeric carbon signals (between 100–105 ppm). A new peak appeared at 180.23 ppm were the evidence of carboxymethylated reaction, which was in accordance with the FT-IR results [9, 35]. The decreased signal at 63.46 ppm and the increased signal at 72.68 ppm revealed carboxymethyl substitution occurred at C-6 position [22]. The downfield shift of the C-6 carbon atom linked by the carboxymethylated group was about 9.2 ppm, which was in

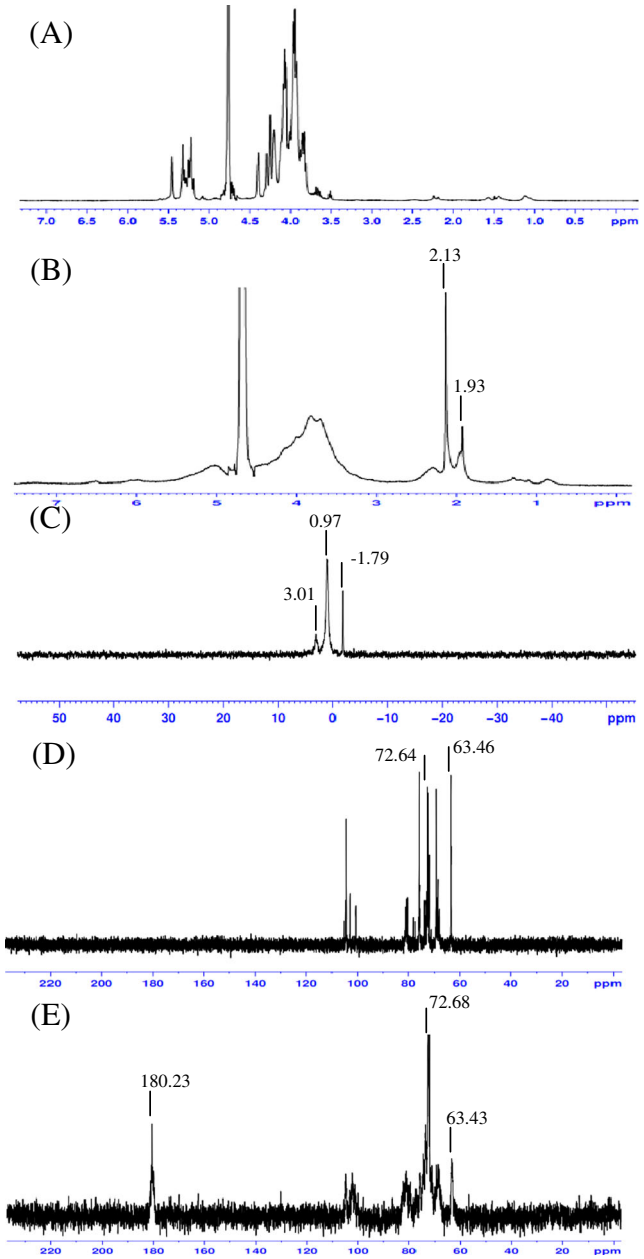

Fig. 3 NMR spectrograms of the r-EPS1 and its derivatives: A, ^{1}H NMR spectrogram of r-EPS1; B, ^{1}H NMR spectrogram of r-EPS1A; C, ^{31}P NMR spectrogram of r-EPS1P; D, ^{13}C NMR spectrogram of r-EPS1; E, ^{13}C NMR spectrogram of r-EPS1C

line with previous description in Yang and Du's report [24]. Due to the overlapping of the signals at 70–82 ppm, and the complex shift of the carbons indirectly connected to the carboxymethylated group, the assignment of chemistry shifts of C2–C5 carbon atoms was difficult [9, 24].

SEM observation

Figure 4 illustrates the SEM micrographs of the native sample and its derivatives. The surface of four polysaccharide samples showed significant differences in size and shape. Though both r-EPS1 (Fig. 4a) and r-EPS1A (Fig. 4b) exhibited a blocky appearance with a nonuniform size, the surface of r-EPS1A seemed more smooth and flat than r-EPS1. The r-EPS1P (Fig. 4c) exhibited a smooth surface and regular cubic shape. And the diameter (about 1 μm) was much smaller than that of r-EPS1 and r-EPS1A. Additionally, r-EPS1C (Fig. 4d) showed an irregular flake-like texture. Ma *et al.* [15] reported the acetylated and carboxylated polysaccharides from mushroom *Inonotus obliquus* had an island shape and particle shape appearance, respectively, which was different with our results. This might be due to the dissimilar properties of different polysaccharides from different sources. In addition, the methods to prepare products should also be taken as one of the determinant influence factors to the shape and structure of samples [36].

Thermal properties

Thermal properties of r-EPS1 and its derivatives were determined by TGA. As shown in Fig. 5, the TGA of all samples exhibited two distinct stages. The first stage of weight loss (7.50–14.99 %) in the range of 30 °C–200 °C was due to the loss of adsorbed and bound water. r-EPS1A and r-EPS1C gave the greatest weight loss among all samples, which might be due to their stronger water binding capacity. Singh *et al.* [37] reported polysaccharides with high level of carboxyl group had increased degradation rate in the first phase due to the higher capacity of carboxyl group to bound water molecules. The second stage of weight loss in the range of 200 °C–800 °C was due to the degradation of samples. The differential thermogravimetry (DTG) curves revealed the temperature for the rapidest weight loss of r-EPS1 appeared at 339.45 °C. In general, the introduction of the functional group hampered the package of polysaccharide chain, resulting in the decrease of thermal stability [38]. A lower rapidest degradation temperature (311–339 °C) exhibited by the derivatives implied that they had a more loose packing structure [39].

Antioxidant activity *in vitro*

Hydroxyl free radical scavenging activity

The scavenging activities of r-EPS1 and its derivatives on hydroxyl radicals were estimated and the results are shown in Fig.6 a. The scavenging activities of r-EPS1 and its derivates were dose-dependent and were decreased in descending order of r-EPS1C>r-EPS1A>r-EPS1>r-EPS1P. The scavenging activities of r-EPS1C and r-EPS1A were significantly ($P<0.05$) higher than that of r-EPS1 between 0.25 mg/mL and 4.0 mg/mL. Compared to the scavenging activity of r-EPS1 (31.46 %), the scavenging activity of r-EPS1C and r-EPS1A was improved about 1.5 folds (78.67 %) and 1 folds (58.48 %)

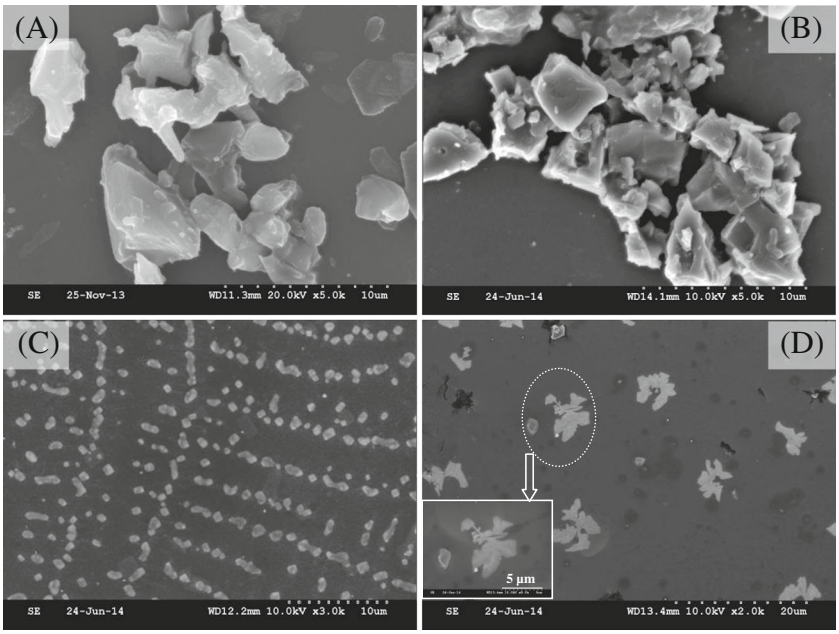

Fig. 4 Scanning electron micrographs of the four polysaccharides (A–D represents r-EPS1 (×5,000), r-EPS1A (×5,000), r-EPS1P (×3,000) and r-EPS1C (×2,000), respectively)

at the concentration of 4.0 mg/mL, respectively. However, the scavenging activity of r-EPS1P was even lower than that of r-EPS1. It has been known scavenging of free radicals was conducted through suppressing against hydroxyl radical generation, or directly clear the generated hydroxyl radical [35]. In the present reaction system, inhibition of the radical generation is related to the chelating of iron ions or rendering them inactive [40, 41]. However, the chelating effect of r-EPS1 and its derivatives on iron ions was not evident (data not given). Therefore, the scavenging activity of hydroxyl radical was probably due to direct clearance or the combination of both two mechanisms. The elucidation of exact mechanism needs an in-depth study.

DPPH free radical scavenging activity

For DPPH free radical, all derivatives exhibited higher antioxidant activities than r-EPS1 *in vitro*. The scavenging activities of samples were dose-dependent and the activities were decreased in the order of r-EPS1P>r-EPS1A>r-EPS1C>r-EPS1 (Fig. 6b). The highest scavenging activity was 54.95 % for r-EPS1P which was almost twice that of r-EPS1 (28.18 %) at 4.0 mg/mL. The scavenging activities of r-EPS1A and r-EPS1C were also significantly ($P<0.05$) higher than that of r-EPS1 within the concentration of 0.5–4.0 mg/mL. The DPPH radical scavenging activity of antioxidants was thought to be attributable to their hydrogen-donating abilities, and it could accept an electron or hydrogen radical to become a stable molecule [20, 22]. Yuan *et al.* [41] reported the phosphorylated κ-carrageenan oligosaccharides possessed higher scavenging activity on DPPH free radicals, which might be ascribed to its stronger hydrogen-donating ability compared to other derivatives. Chen *et al.* [9] also reported the introducing of the acetyl groups could significantly improve the scavenging activity of *Ganoderma atrum* polysaccharide against DPPH free radical, while the opposite effects were observed for the carboxymethyl derivative polysaccharide. The latter was different from the results of the present study. That might be due to the different physicochemical properties of different polysaccharides.

Reducing power

The reducing power is also commonly used for the analysis of antioxidants. In general, the reducing power of all samples was in the order of r-EPS1A>r-EPS1C>r-EPS1P>r-EPS1 (Fig. 6c). In the concentration tested, r-EPS1A showed pronounced advantage compared with r-EPS1. The highest reducing power (0.684) exhibited by r-EPS1A was about one fold higher than that of r-EPS1 (0.352). The reducing power is generally associated with the presence of reductones, which could break the free radical chain by donating a hydrogen atom [23]. The acetyl groups could activate the hydrogen atom of the anomeric carbon, thus resulted in the increased hydrogen-donating ability and reducing power [23, 42]. The improvement of sample solubility after acetylation modification might be another promoting factor [43]. However, the promoting effect of r-EPS1P and r-EPS1C was much lower than that of r-EPS1A. Generally, antioxidant activity of the polysaccharide derivatives was affected by their structural

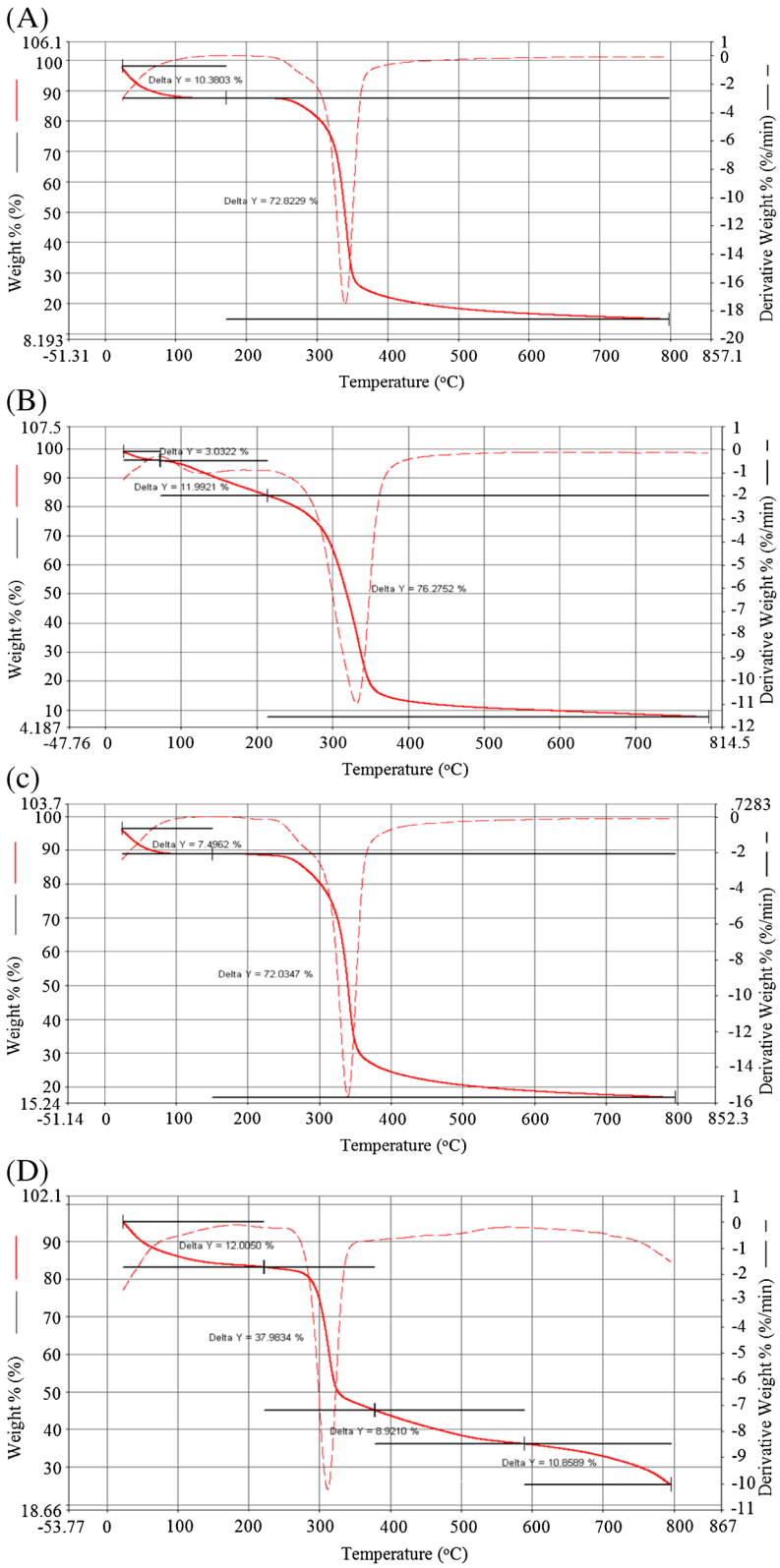

Fig. 5 TGA and DTG curves for r-EPS1 and its derivatives: A, r-EPS1; B, r-EPS1A; C, r-EPS1P; D, r-EPS1C

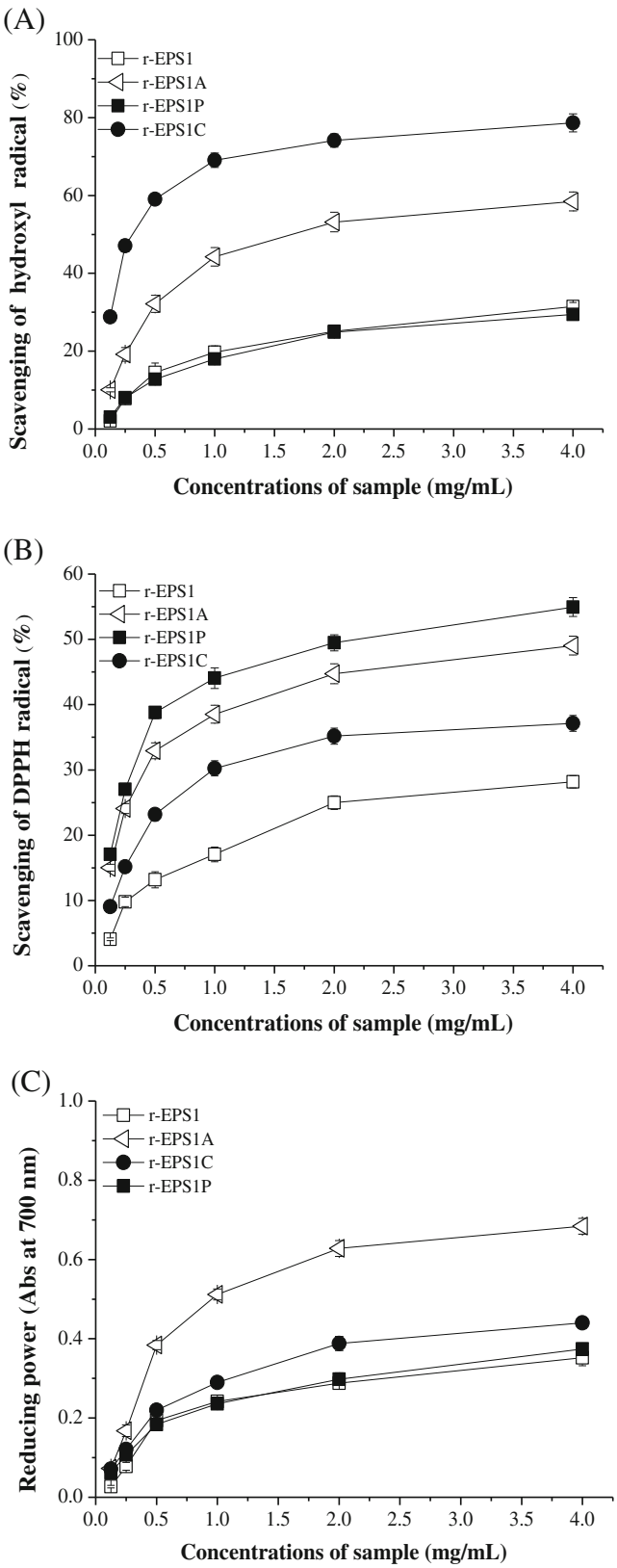

Fig. 6 Scavenging effects of r-EPS1 and its derivatives on hydroxyl radical (**a**), DPPH radical (**b**) and reducing power (**c**)

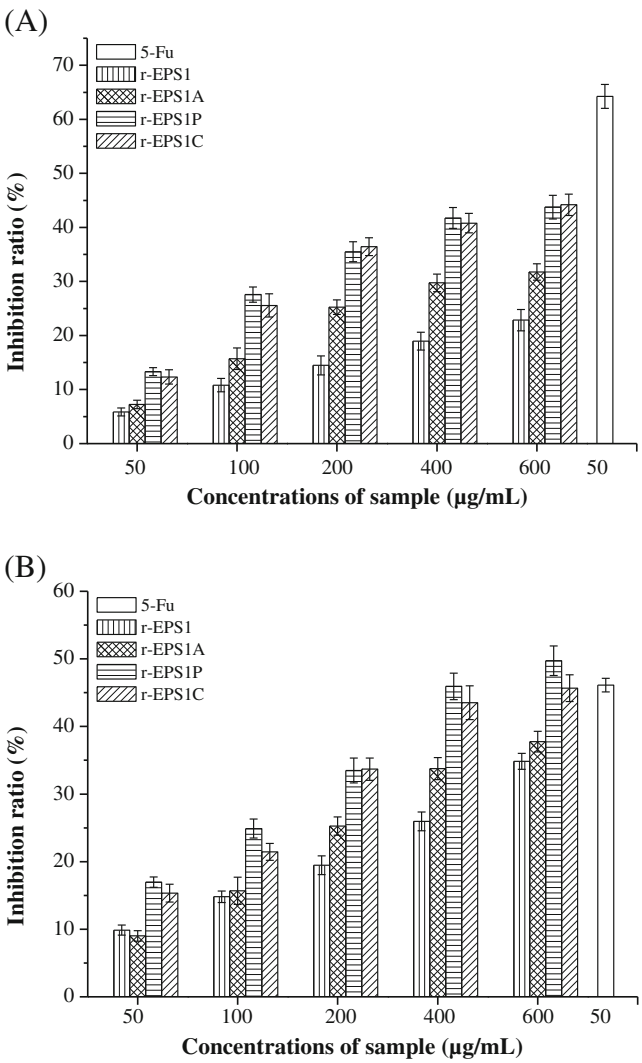

Fig. 7 Antitumor effects of r-EPS1 and its derivatives on HepG-2 (**a**) and HT-29 cells (**b**)

parameters, such as degree of substitution (DS), molecular weight and functional groups, *etc.* [15]. The mechanism of different derivatives on reducing power needs further investigations.

Antitumor activity *in vitro*

Liver cancer is one of the most common tumors and has been ranked as the third cause of mortality attributed to cancer diseases worldwide [44]. Colorectal cancer is also an important cause of death in the western world [45]. Therefore, increasing attention has been paid to investigation of effective agents for mitigate the development of these diseases and elimination of the growth of these tumor cells. In this study, we investigated the inhibition effects of r-EPS1 and its derivatives on human liver cancer HepG-2 and colon cancer HT-29

cells. Preliminary experiments confirmed r-EPS1 and its derivatives had no inhibition effect on the growth of human normal cells (human embryo kidney 293 cells). However, all samples exhibited dose-dependent inhibition effects on HepG-2 and HT-29 cells, and the derivatives exerted stronger inhibition effects than that of the native polysaccharide (Fig.7). At the concentration of 600 μg/mL, the inhibition ratios of r-EPS1, r-EPS1A, r-EPS1P and r-EPS1C against HepG-2 cells were 22.84, 31.75, 43.75 and 44.19 %, respectively. Corresponding to HT-29 cells, the inhibition ratios were 34.84, 37.75, 49.72 and 45.67 %, respectively. Obviously, r-EPS1P and r-EPS1C showed significantly higher inhibition effects than r-EPS1 and r-EPS1A. The highest inhibition ratios of r-EPS1P and r-EPS1C on HT-29 cells were even comparable to that of 5-FU (50 μg/mL, 46.11 %). Liu *et al.* [2] reported the phosphorylated polysaccharide had higher antitumor activity than that of the acetylated polysaccharide, which might be due to the phosphate group capable of binding with receptors of immune cells with high affinity, and thus activated immune response effectively. Tao *et al.* [12] also reported the carboxymethylated polysaccharide-protein complexes exhibited more potent antitumor activities than the original samples *in vitro*. These reports are in accordance with our results. This suggested that the introduction of ionic groups to polysaccharides could lead to the improvement of antitumor activities.

Correlation of structure to bioactivity

The bioactivity results *in vitro* revealed that the acetyl, phosphate and carboxymethyl groups could significantly enhance the antioxidant and antitumor activities of r-EPS1. However, promotion extents of bioactivity were varied depend on the derivative groups, which indicated the introduced groups played an important role on the bioactivity of derivatives. As a whole, the acetylated and carboxymethylated derivatives exhibited stronger antioxidant activity, whereas the phosphorylated and carboxymethylated derivatives showed a better promoting effect on antitumor activity.

It has been reported that the antioxidant activity of polysaccharide derivatives might be related to their electron-donating or hydrogen-donating ability [2, 42]. Though the functional groups such as –COOH, CH_3CO–, −SH and –H_2PO_3 were generally recognized as good electron or hydrogen donors, variations were observed for antioxidant activity of these derivatives [2, 10, 42]. Therefore, the antioxidant activity of these derivatives might also be influenced by other structural features or physical characters such as DS and chain conformation. The low DS of phosphorylated derivative might be an adverse factor for its antioxidant activity, and the relatively extended chain conformation of carboxylmethylated derivative was beneficial for improving its antioxidant and antitumor activity [46]. The increased water solubility of carboxylmethylated derivative might be another factor facilitating polysaccharides to exhibit potent antitumor activity [35]. In addition, the high antitumor activity exhibited by phosphorylated derivative might be attributed to its high affinity to immune cells [47]. The research on structure-activity relationship of chemical derivatives could provide with theoretical supports for their development and utilization. In order to better clarify the correlation of structure to bioactivity of polysaccharide derivatives from r-EPS1 of *L. plantarum* 70810, further study is required for shedding light on the inner mechanism between the structural features and functional properties.

Conclusions

In the present study, a released exopolysaccharide (r-EPS1) from *L. plantarum* 70810 was modified by acetylation, phosphorylation and carboxymethylation. Compared with r-EPS1, the derivatives exhibited different surface morphologies and thermal behaviors. We also evaluated their antioxidant and antitumor activities *in vitro*. The results indicated the antioxidant and antitumor activities of r-EPS1 can be greatly enhanced after being modified. The study provided experimental evidences that chemical modification could be an effective way to improve the bioactivity of exopolysaccharide from *L. plantarum* 70810. It is noted that these derivatives could be explored as novel potential antioxidant and antitumor agents after further research being conducted on their safety and activity *in vivo*.

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