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Immunomodulation and antitumor activities of different-molecular-weight polysaccharides from *Porphyridium cruentum*

Liqin Sun*, Ling Wang, Yan Zhou

Ocean School of Yantai University, Yantai, Shandong 264005, PR China

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ABSTRACT

The extracellular polysaccharides (EPSs) isolated from *Porphyridium cruentum* were degraded by hermetical–microwave and H_2O_2 under ultrasonic waves. Six products were obtained with molecular weights of 6.53, 256, 606, 802.6, 903.3 and 1002 kDa. The antitumor and immunomodulatory activities of different-molecular-weight (MW) polysaccharides were evaluated by the S180-tumor-bearing mouse model *in vivo* and peritoneal macrophage activation *in vitro*. The degraded EPSs all showed clear immunomodulation to different extents. The MW of the EPSs had a notable effect on their activity. The 6.53-kDa fragment had the strongest immunoenhancing activity. Different doses of EPS all inhibited the growth of the implanted S180 tumor. The tumor inhibition index at high, middle and low doses was 53.3%, 47.5% and 40.5%, respectively. In addition, three different concentrations of EPS significantly increased lymphocyte proliferation, which indicated the unique mechanism of the antitumor effect of EPS.

1. Introduction

Since Gerber first identified an active antiviral component in seaweed in 1958, algal polysaccharides have attracted considerable interest because of their special properties and promising applications in many industrial processes (Guzman, Gato, & Lamela, 2003). The unicellular marine microalga *Porphyridium cruentum* can synthesize and secrete sulfated polysaccharides when cells are grown in steady-state culture, and the polysaccharide content can reach 50% of the biomass. The extracellular polysaccharides (EPSs) of *P. cruentum* are highly sulfated acidic heteropolymers, which consist mainly of xylose, galactose and glucose, with a molecular weight (MW) in the range of 2×10^6 to 7×10^6 Da (Geresh, Adin, Yarmolinsky, & Karpasas, 2002). Previous studies have found that *P. cruentum* polysaccharides have antiviral, anti-radiation and antioxidant activities, as well as reducing blood cholesterol level (Gu & Liu, 2002; Huleihel, Ishanu, Tal, & Arad (Malis), 2002; Sun, Wang, Shi, & Ma, 2009; Tehila, Margalit, Dorit, Shlomo, & Arad (Malis), 2005). However, studies on their antitumor and immunomodulatory activities have been rare.

Much evidence has indicated that the biological activities of polysaccharides depend on their structural features such as degree of sulfation, MW, and type of sugar (Lai, Pan, & Li, 2005; Qi et al., 2005; Zhou et al., 2004). We have determined the relationship between the MW of *P. cruentum* polysaccharides and their antioxidant activity (Sun et al., 2009), but the relationship with antitumor and immunomodulatory activities has not been reported.

In the present study, different-MW polysaccharides of *P. cruentum* were prepared by degradation, and in vivo antitumor and in vitro immunomodulatory activities of polysaccharides were evaluated. The relationship between MW and polysaccharide functions was determined.

2. Materials and methods

2.1. Drugs

The EPSs of *P. cruentum* were isolated and prepared in our laboratory. 3-(4, 5-Dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT), concanavalin A (ConA), lipopolysaccharides (LPSs), SDS, and RPMI-1640 culture medium were purchased from Sigma.

2.2. Preparation of different-MW polysaccharides

Oxidative degradation with H_2O_2 under ultrasonic waves was used to prepare the low-MW EPSs (EPS2–EPS6). One hundred milligrams of crude polysaccharide was enclosed in a compression-resistant bottle in a 25-mL reaction volume. The concentration of H_2O_2, ultrasonic time, reactive temperature, and corresponding MW of fragments are listed in Table 1. The solution of degraded polysaccharide was adjusted to pH 7.0 by sodium hydroxide (Ma, Qin, Qiu, & Xu, 2005). The solution was passed through a 0.45-μm filter to eliminate impurities. After dialysis (2.5-cm diameter dialysis sack, MW cutoff 8000), a four-fold volume of 95% (v/v) ethanol

* Corresponding author. Tel.: +86 535 6706299; fax: +86 535 6706299.
E-mail address: sliqin2005@163.com (L.Q. Sun).

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Table 1
Polysaccharide oxidative degradation with H_2O_2 under ultrasonic waves.

Samples	Concentration of H_2O_2 (v/v, %)	Ultrasonic temperature (°C)	Ultrasonic time (h)	Average MW ($\times 10^4$ Da)	Sulfate content %
EPS2	8	70	7	25.60 ± 0.26	13.96
EPS3	8	50	6	60.60 ± 0.38	13.52
EPS4	8	50	5	80.26 ± 0.29	12.94
EPS5	8	50	4	90.33 ± 0.41	12.17
EPS6	2	50	4	100.2 ± 0.36	11.63

was added to the solution to precipitate the polysaccharides at 4 °C. The deposited EPS was washed three times with anhydrous ethanol and collected by lyophilization.

The EPS1 was prepared by hermetical-microwave digestion, and the corresponding MW of fragment (EPS1) is listed in Table 2. The hermetical microwave degradation technology was identical with our early work (Sun et al., 2009).

2.3. Analysis of physicochemical properties of polysaccharides

2.3.1. Molecular weight determination

The average MW of degraded polysaccharides was measured by high-performance gel-permeation chromatography (Zhang, 2003) with two types of size-exclusion chromatography columns in series, TSK-G5000PW and TSK-G4000PW (Tosoh Company), on an Agilent 1100 LC device equipped with UV and refractive index detector (RID). Ultrapure water was used as the flow phase at a flow rate of 0.5 mL/min. The standard samples of dextrans (0.2%, w/v; Sigma) were used to calibrate the standard curve. V_t and V_0 were determined by glucose and Blue Dextran 2000 (0.2%, w/v). The relationship between lgM and K_{av} was calculated as shown in equation below.

$$\lg M = -3.2877K_{av} + 6.6358, \quad R^2 = 0.9957$$

2.3.2. Sulfate content determination

Sulfate content was determined by the barium sulfate turbidity method (Zhang, 2003).

2.4. Animals and tumors

Sixty Kunming mice (18–22 g, 6 weeks old, Permit No. SYXK (Lu) 20030020) purchased from Luye Pharma (Yantai, China). were divided randomly into six groups (n = 10). The animals were housed as five per plastic cage with wood chip bedding in an animal room with a 12-h light/dark cycle at room temperature (25 ± 2 °C). Free access to standard laboratory diet was allowed.

S180 tumor cells were maintained in the peritoneal cavity of Kunming mice that were provided by Luye Pharma (Yantai, China). Mouse macrophage cell line RAW264.7 (ATCC No. TIB-71) was purchased from Wuli Biotech Ltd. (Shanghai, China).

2.5. Peritoneal macrophage activation by EPS in vitro

2.5.1. NO emission detection in Raw264.7 cell line

A 100-μL suspension of macrophages that contained 1×10^6 cells/mL was seeded in each well of a 96-well plate. Different concentrations of EPS solution (100 μL) were added to each well and the cells were incubated at 37 °C in 5% CO_2. Fifty microliters of supernatant from each well was transferred into a new 96-well plate, and 50 μL sulfanilamide solution was added to each well at room temperature in the dark for 5–10 min. Fifty microliters of naphthalene ethylenediamine was added to each well and the plate was kept at room temperature in the dark for 10 min. The absorbance was quantified by the ELISA reader (Synergy HT; Bio-TEK, USA) at 540 nm. Blank medium was used as a negative control and LPS (4 μg/mL) was used as a positive control (Liu et al., 2006). Each test was repeated three times. The concentration of NO was calculated based on the following standard curve.

$$y = 0.007x + 0.1269, \quad R^2 = 0.9972$$

2.5.2. Neutral red uptake by Raw264.7 cell line

RAW264.7 cells in logarithmic phase were seeded at 1×10^5 per well in a 96-well plate with RPMI-1640 medium (10% FBS) and incubated at 37 °C in 5% CO_2 for 2 h. The plate was washed three times with PBS and 100 μL new medium was added to each well. RPMI-1640 and 4 μg/mL LPS were used as negative and positive controls, respectively. Cells were cultured with different concentrations of EPS (25–200 μg/mL) for 24 h at 37 °C in 5% CO_2. Supernatants were removed from the plate. One hundred microliters of 0.08% neutral red solution was added to each well and the cells were cultured for a further 20 min. The cell plate was washed three times with PBS and 0.2 mL cell lysis buffer (acetic acid/ethanol; 1:1) was added to each well at room temperature and left overnight. The absorbance at 540 nm was determined using an ELISA reader (Synergy HT; Bio-TEK, USA) (Liu et al., 2006). Six wells were used for each EPS concentration and three independent experiments were performed.

2.5.3. RAW264.7 proliferation assay

Cells were cultured for 24 h with EPS and TCA was added at 50 μL/well at 4 °C. After 1 h, cells were washed five times with distilled water. Sulforhodamine B (100 μL) was added to each well for 10 min, and the plate was washed six times with 1% acetic acid. Tris buffer (150 μL) was added to each well and the plate was vibrated at room temperature for 10 min. The absorbance at 570 nm was detected by ELISA reader (Synergy HT; Bio-TEK, USA).

2.6. Splenocyte proliferation assay

The spleen was removed from a Kunming mouse (6–8 weeks old) under sterile conditions, homogenized and filtered through a 100-μm cell strainer. The cells were washed in Hank's solution and centrifuged at 5000 rpm/min for 5 min. The erythrocytes were removed and the splenocytes were washed in Hank's solution and resuspended in RPMI-1640 medium (2% FBS) at a cell concentration of 8×10^6–10×10^6/mL. A 100-μL cell suspension was seeded into each well of a 96-well plate. Blank medium and 10 μg/mL ConA

Table 2
Degradation conditions of polysaccharide (EPS1) with high-pressure microwave.

Power (W)	250	0	400	550	250	0	400	550	Average MW (kDa)	Sulfate content %
Treatment time (min)	2	2	2	2	2	2	2	0.5	6.553 ± 0.47	14.37

were used as negative and positive controls, respectively. Cells were cultured for 48 h (5% CO_2, 37 °C) and pulsed for the last 4 h with 5 mg/mL MTT (20 μL per well). Acidified 20% SDS (120 μL) was added to each well and the plate was kept overnight. The absorbance was measured at 570 nm in an ELISA reader. The proliferation of splenocyte was defined with proliferation index (PI) showed as follow:

$$\mathrm{PI} = \frac{\text{sample absorbance}}{\text{blank absorbance}}$$

2.7. Antitumor effect of EPS in vivo

2.7.1. Mouse tumor model

A mouse tumor model was established by the tumor inoculation method (Xu, Bian, & Chen, 2002). A cell suspension was collected from peritoneal lavage of a 7-day-old mouse inoculated with S180 tumor. Cells were stained with trypan blue and counted in a hemocytometer to ensure that there were >98% live cells. Sixty Kunming mice were weighed and divided randomly into six groups: normal control, tumor model, cyclophosphamide positive control (CTX) and high-, middle- and low-dose EPS. Cell suspension (0.2 mL; 1×10^7 cells/mL) was implanted subcutaneously into the right frontal groin of the mice in each group except for the normal control group. One day after inoculation, the drugs were administered orally into the stomach for 10 days. The normal control group and tumor model group were treated with physiological saline; the CTX group was treated with 20 mg/kg cyclophosphamide; and 200, 100 and 50 mg/kg per day EPS was given to the high-, middle- and low-dose EPS groups.

2.7.2. Antitumor activity of EPS in S180-tumor-bearing mice

Mice were weighed at 24 h after the final administration and killed. The tumor, spleen and thymus gland were removed and weighed. The indexes for tumor inhibition, spleen and thymus gland were calculated according to the following formulas:

$$\text{Tumor inhibition index} = \left[1 - \frac{\text{Average weight of tumor of the treament group (g)}}{\text{Average weight of tumor of the control group (g)}}\right] \times 100\% \quad (1)$$

$$\text{Spleen index} = \frac{\text{Weight of spleen (mg)}}{\text{Weight of the body (g)}} \quad (2)$$

$$\text{Thymus index} = \frac{\text{Weight of thymus gland (mg)}}{\text{Weight of the body (g)}} \quad (3)$$

2.7.3. Effects of EPS on proliferation of splenocytes from tumor-bearing mice

S180 tumor was implanted into Kunming mice and drugs were administered as described above for 10 days. Twenty-four hours after the final administration, the mice were killed and the spleens were removed. A cell suspension was obtained and the absorbance was determined as described in Section 2.6.

2.8. Statistical analysis

The data were analyzed statistically by ANOVA. Significance of any differences between groups was evaluated using Student's *t*-test. All data in the tables were expressed as mean ± SD.

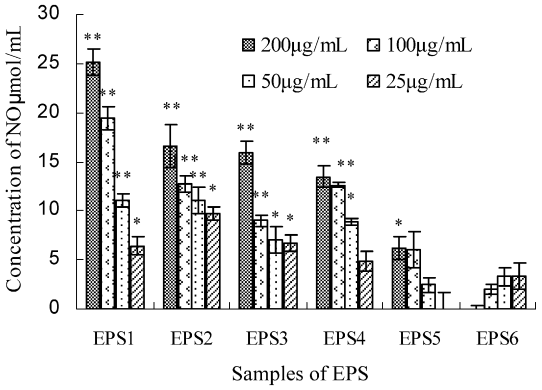

Fig. 1. Effects of EPS on NO production in peritoneal macrophages (mean ± SD, $n = 6$), $^*p < 0.05$; $^{**}p < 0.01$ compared with controls. NO concentration of controls was 4.87 ± 0.71 μmol/mL.

3. Results

3.1. Effects of EPS on immune system in vitro

3.1.1. NO level is elevated by EPS in peritoneal macrophages

EPS from *P. cruentum* with a MW <802.6 kDa significantly increased NO release from macrophages in a dose-dependent manner ($p < 0.05$ for low-dose and $p < 0.01$ for middle- and high-dose EPS) (Fig. 1). At a concentration of 200 μg/mL, EPS1 induced 25.16 μmol/mL NO, which was 5.17 times higher than the control group and 1.87 times higher than for EPS4. However, EPS5 and EPS6 with a MW > 903.3 kDa failed to promote NO release.

3.1.2. Effects of EPS on neutral red uptake

At a concentration range of 25–200 μg/mL, all the EPS groups had a higher absorbance than the control group. Moreover, EPS at 100 μg/mL increased macrophage uptake of neutral red more than the other concentrations. EPS increased neutral red uptake significantly more than LPS did ($p < 0.01$) (Fig. 2). However, as EPS concentration increased, the absorbance decreased, which indicated that there was an optimum concentration of EPS for promoting the greatest uptake of neutral red. The MW of EPS slightly influenced the uptake ability. EPS1 had the highest uptake ability and the absorbance at 100 μg/mL was 0.214, which was 1.66 times higher than that in the control group and 1.14 times higher than in the EPS6 group.

3.1.3. Effects of EPS on macrophage proliferation

EPS significantly increased proliferation of peritoneal macrophages (Fig. 3). In the concentration range 25–200 μg/mL,

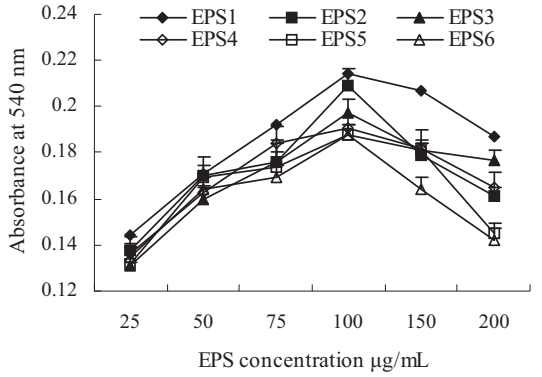

Fig. 2. Effect of EPS on neutral red uptake by mouse macrophages (mean ± SD, $n = 6$), $^*p < 0.05$; $^{**}p < 0.01$ compared with controls. A_{540} of controls was 0.129 ± 0.0041.

Table 3
Effect of EPS on body weight, spleen and thymus indexes in S180-bearing mice (mean ± SD, $n = 10$).

Groups	Dose (mg/kg/d)	Body weight (g)	Spleen index (mg/g)	Thymus index (mg/g)	Tumor weight (g)	Inhibition rate %
Normal control	–	6.08 ± 0.94	6.26 ± 1.48	3.34 ± 0.22		
Model	–	5.42 ± 1.74	6.11 ± 1.33	3.33 ± 0.25	1.157 ± 0.421	
EPS	200	4.85 ± 2.72	7.54 ± 2.12$^{*,\Delta}$	3.33 ± 0.58	0.540 ± 0.238$^{\Delta\Delta}$	53.33%
	100	6.74 ± 1.23	7.91 ± 2.12$^{*,\Delta}$	3.45 ± 0.21	0.608 ± 0.326$^{\Delta\Delta}$	47.45%
	50	5.30 ± 1.19	7.79 ± 1.68$^{*,\Delta}$	3.50 ± 0.54	0.689 ± 0.112$^{\Delta\Delta}$	40.45%
CTX	20	3.80 ± 1.12 $^{**,\Delta\Delta}$	4.83 ± 1.23 $^{*,\Delta}$	2.62 ± 0.82 $^{*,\Delta}$	0.470 ± 0.178 $^{\Delta\Delta}$	59.38%

* $p < 0.05$, compared with normal control.
** $p < 0.01$, compared with normal control.
$^{\Delta}$ $p < 0.05$, compared with mode.
$^{\Delta\Delta}$ $p < 0.01$, compared with mode.

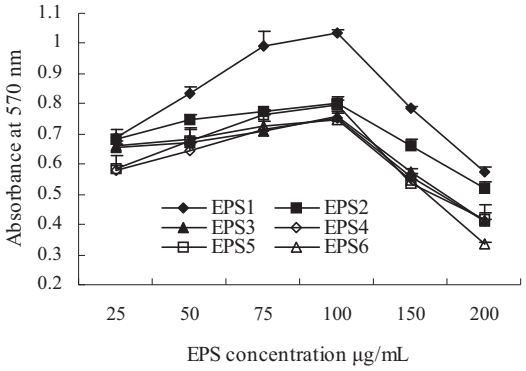

Fig. 3. Effects of EPS on proliferation of mouse peritoneal macrophages (mean ± SD, $n = 6$), $^{*}p < 0.05$; $^{**}p < 0.01$ compared with control. A_{570} of controls was 0.522 ± 0.0153.

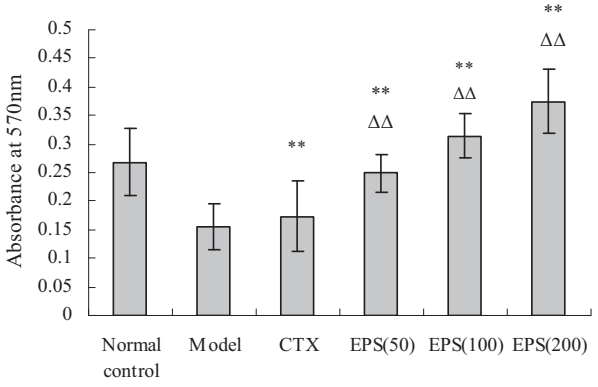

Fig. 5. Effects of EPS on proliferation of lymphocytes in tumor-bearing mice. Values are mean ± SD, $n = 10$. $^{**}p < 0.01$ vs. normal control; $^{\Delta\Delta}p < 0.01$ vs. tumor model control.

the absorbance of the EPS-treated groups were significantly higher than that of the control group ($p < 0.01$), among which 100 μg/mL EPS had the most positive effect on proliferation. When EPS concentration was >100 μg/mL, the ability to promote proliferation decreased. Low-MW EPS (<256 kDa) had a greater positive effect on proliferation, while the promotion effect between 606 and 1002 kDa EPS did not have a significant effect on macrophage proliferation ($p > 0.05$).

3.1.4. Effects of EPS on proliferation of splenocytes

EPS from *P. cruentum* significantly promoted the proliferation of splenocytes (Fig. 4). In the concentration range 25–200 μg/mL, EPS had a dose-dependent effect on cell proliferation. Low-MW EPS

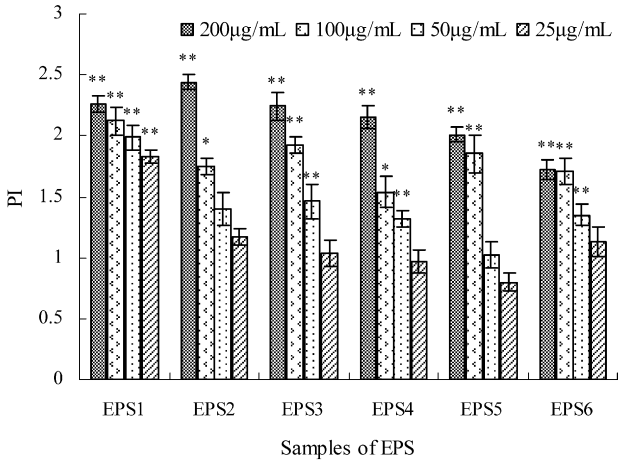

Fig. 4. Effect of EPS on proliferation of splenocytes (mean ± SD, $n = 6$), $^{*}p < 0.05$; $^{**}p < 0.01$ compared with controls. A_{570} of controls was 0.644 ± 0.0487.

at a low concentration had a significant increase effect on mouse splenocyte proliferation. For example, at 25 μg/mL, EPS1 had the greatest PI of 1.83, which was 1.56 and 1.76 times higher than the fragments of EPS2 and EPS3. EPS with a MW > 606 kDa had no effect on the splenocytes at a low concentration (PI < 1.0). At a high dose such as 200 μg/mL, EPS with a MW < 903.3 kDa, at all concentrations, had a PI > 2.0, and there were no significant differences between the effects of the different MWs.

3.2. Anti-tumor effects of EPS in vivo

3.2.1. Inhibition of S180 tumor proliferation by EPS in mice

Compared with the tumor model group, different doses of EPS all inhibited the growth of implanted S180 tumor. The tumor inhibition index at high, middle and low doses was 53.3%, 47.5% and 40.5%, respectively (Table 3). Compared with the normal control group, the body weight, spleen index and thymus index of the tumor model group had no significant changes ($p > 0.05$). The CTX group showed significant inhibition of tumor growth, but also had a significant decrease in body weight ($p < 0.01$). At the same time, the immune system was also inhibited, which was indicated by decreases in the spleen and thymus indexes ($p < 0.05$). EPS had no significant influence on the body weight of tumor-bearing mice and increased the spleen index.

3.2.2. Effect of EPS effect on proliferation of lymphocytes in tumor-bearing mice

Compared with the normal groups, the proliferation of lymphocytes in the tumor-bearing mice and the CTX group decreased significantly ($p < 0.01$). Three different doses of EPS significantly increased lymphocyte proliferation ($p < 0.01$), with a clear dose–effect relationship (Fig. 5). At 200 μg/mL, the proliferative

effect of EPS was 2.41 times higher than that of the tumor model group and 2.15 times higher than in the CTX positive control group.

4. Discussion

Stimulation of macrophage responses is one of the most important mechanisms of all known polysaccharides with immunological competence. Several pathways are usually involved in this process: (1) promoting the proliferation of macrophages; (2) improving phagocytic activity; (3) increasing NO and reactive oxygen species (ROS) production; and (4) inducing or regulating the secretion of cytokines and chemokines (Ruan, Su, Dai, & Wu, 2005; Wang and Gong, 2001). In the present study, we proved that all different concentrations of EPS significantly stimulated the ability of macrophages to proliferate, take up neutral red, and produce NO (Figs. 1–3), which indicates that EPS can enhance the immune response via macrophage stimulation.

The spleen is one of the most important immune organs and is the source of lymphocytes. We demonstrated that different concentrations of EPS could stimulate the immune system by promoting proliferation of lymphocytes. At 100 μg/mL, EPS1 had the greatest PI of 2.12 (Fig. 4).

Besides, we showed that the low-MW EPSs had better immunomodulatory effects than those of higher MW. EPSs with a MW of 6553 and 25.6×10^4 Da were best able to stimulate the immune system. Zhou et al. (2004) had reported that λ-carrageenans with MWs of 15 and 9.3 kDa had the best immunomodulatory effects. Lai et al. (2005) had also reported that low-MW chitosan oligosaccharides had best physiological effects, such as decreasing cholesterol and reinforcing the immune system, which was consistent with our results. The enhanced immune response to lower-MW EPSs has two possible explanations. First, during the experiment, we found that low-MW polysaccharides had better water solubility than high-MW polysaccharides, and the more broken chains, the higher the water solubility. Second, the increased number of sulfate groups (from 11.63 to 14.37%) (Table 1) from the broken chains of polysaccharides may increase their biological activities (Qi et al., 2005; Tehila et al., 2005). However, further research is needed to elucidate the relationship between extent of polymer degradation and sulfate content, and whether increased sulfate level contributes to increased biological activities of EPSs from *P. cruentum*.

Date published have confirmed that many kinds of polysaccharides, such as lentinan, *Spirulina maxima* polysaccharide, and λ-carrageenan from *Chondrus ocellatus* exhibit significant antitumor activities and low side-effects (Chen, Lin, Deng, & Kuang, 2008; Zhou, Sheng, Yao, & Wang, 2006; Zhu, Zhang, Wang, Li, & Zhang, 2007). Although the antitumor effects of the polysaccharides may involve mechanisms such as changing the biochemical character of the cell membrane, inducing tumor cell differentiation and apoptosis, and regulating cell signaling pathways, immunomodulation is believed generally to be the most important mechanism (Zhou et al., 2004).

Our results showed that the polysaccharide from *P. cruentum* significantly inhibited implanted S180 solid tumors in mice. At high concentrations (200 mg/kg/day), the tumor inhibition of EPS (53.3%) was even higher than that of *Strongylocentrotus nudus* egg polysaccharide (46.9%) and *Spirulina* polysaccharide (30.73%) (Liu, 2006; Yu et al., 2008). Our preliminary data in vitro showed that EPSs did not exhibit the ability to kill multiple tumor strains obviously (data not shown), which indicated that the antitumor effect was not mediated by a direct cytotoxic mechanism. However, the date in vivo showed that EPS had no obvious effect on mouse body weight, but enhanced the spleen and thymus indexes, and especially the proliferation of splenocytes. From above discussion, we conclude that the immunomodulatory activity is one of the important features of the antitumor activity of EPS from *P. cruentum*.

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References

Chen, T. Q., Lin, K. L., Deng, T., & Kuang, H. R. (2008). Anti-tumor effect of lentinan on S180 sarcomas in mouse. *Chinese Journal of Anatomy*, *31*, 508–810.

Huleihel, M., Ishanu, V., Tal, J., & Arad (Malis), S. (2002). Activity of *Porphyridium* sp. polysaccharide against herpes simplex viruses in vitro and vivo. *Journal of Biochemical & Biophysical Methods*, *50*, 189–192.

Geresh, S., Adin, I., Yarmolinsky, E., & Karpasas, M. (2002). Characterization of the extracellular polysaccharides of *Porphyridium* sp.: molecular weight determination and rheological properties. *Carbohydrate Polymers*, *50*, 183–189.

Gu, N. Y., & Liu, Y. F. (2002). Anti-irradiation activities of extracellular polysaccharide of *Porphyridium* sp. *Marine Sciences*, *26*, 53–56.

Guzman, S., Gato, A., & Lamela, M. (2003). Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. *Phytotherapy Research*, *17*, 665–667.

Lai, S. L., Pan, Z. Y., & Li, X. F. (2005). Study on the degradability of chitosan under microwave irradiation. *Journal of Shanxi University of Science & Technology*, *23*, 38–40.

Liu, C. H. (2006). *Studies on an active polysaccharide SEP from Strongylocentrotus Nudus eggs*. Beijing: China Pharmaceutical University.

Liu, C. H., Ye, L., Lin, Q. X., Xi, T., Xing, Y. Y., & Gao, Y. (2006). Studies on preparation and anti-tumor activity of the polysaccharide from the eggs of *Strongylocentrotus nudus* (SEP). *Pharmaceutical Biotechnology*, *13*, 429–432.

Ma, X. J., Qin, Y. Z., Qiu, Y. M., & Xu, S. Y. (2005). Preparation of low molecular weight *Eucheuma striatum* sulfated polysaccharides by oxidative degradation with H_2O_2 under the ultrasonic wave. *Chinese Journal of Marine Drugs*, *24*, 10–13.

Qi, H. M., Zhang, Q. B., Zhang, T. T., Chen, R., Zhang, H., Niu, X. Z., et al. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) *in vitro*. *International Journal of Biological Macromolecules*, *37*, 195–199.

Ruan, Z., Su, J., Dai, H. Z., & Wu, M. C. (2005). Characterization and immunomodulating activities of polysaccharide from *Lentinus edodes*. *International Immunopharmacology*, *5*, 811–820.

Sun, L. Q., Wang, C. H., Shi, Q. J., & Ma, C. H. (2009). Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. *International Journal of Biological Macromolecules*, *45*, 42–47.

Tehila, T. S., Margalit, B., Dorit, V. M., Shlomo, G., & Arad (Malis), S. (2005). Antioxidant activity of the polysaccharide of the red microalga *Porphyridium* sp. *Journal of Applied Phycology*, *7*, 215–222.

Wang, J., & Gong, X. G. (2001). Advances in the studies on the antitumor activity and immunomodulating action of polysaccharides. *Chinese Journal of Biochemical Pharmaceutics*, *22*, 52–54.

Xu, S. Y., Bian, R., & Chen, X. (2002). *Methodology of pharmacology experiment*. Beijing: People's Medical Publishing House.

Yu, L. Y., Zhang, L., Ge, W., Tang, C., Jia, Y. H., Guo, C. S., et al. (2008). Inhibitory effects of high dosage compound PSP from *Spirulina Platensis* on sarcoma180 of mice. *Journal of Liaoning University of TCM*, *10*, 179–181.

Zhang, W. J. (2003). *Saccharide complex biochemical research technology*. Hangzhou: Zhejiang University Press.

Zhou, G. F., Sun, Y. P., Xin, H., Zhang, Y. N., Li, Z. E., & Xu, Z. H. (2004). In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. *Pharmacological Research*, *50*, 47–53.

Zhou, G. F., Sheng, W. X., Yao, W. D., & Wang, C. H. (2006). Effect of low molecular (-carrageenan from Chondrus ocellatus on antitumor H-22 activity of 5-Fu. *Pharmacological Research*, *53*, 129-124.

Zhu, J. H., Zhang, W., Wang, M., Li, Z. J., & Zhang, C. W. (2007). Effect of polysaccharides from *Spirulina maxim*a on the growth of human tumor cell lines. *Marine Sciences*, *31*, 1–5.