Carbohydrate Polymers 77 (2009) 365–369

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Chemical properties and immunostimulatory activity of a water-soluble polysaccharide from the clam of *Hyriopsis cumingii* Lea

Zhiyuan Dai, Hong Zhang *, Yangping Zhang, Honghai Wang

Institute of Aquatic Products Processing, Zhejiang Gongshang University, Hangzhou 310035, PR China

ARTICLE INFO

Article history:
Received 17 August 2008
Received in revised form 11 November 2008
Accepted 8 January 2009
Available online 14 January 2009

Keywords:
Hyriopsis cumingii
Polysaccharide
Composition
Immunostimulatory activity

ABSTRACT

A water-soluble polysaccharide HCLPS-1 was isolated from clam of *Hyriopsis cumingii Lea* by hot water, DEAE-cellulose and Sephadex-G200, gel-permeation chromatography and tested for its immunostimulatory activities. Its structural characteristics were investigated by UV, IR, HPLC, NMR spectroscopy, methylation analysis and GLC–MS. HCLPS-1 consisted of glucose and xylose with the molar ratio of 35:1. Based on the data obtained, HCLPS-1 was determined to has a main chain of (1 → 4)-linked β-D-glucopyranosyl residues, with a weight-average molecular weight of about 1.56×10^5 Da. HCLPS-1 not only could significantly promote concanavalin A (Con A), lipopolysaccharide (LPS)-stimulated splenocytes proliferation in concentration-dependent manner *in vitro*, but also increase Con A- and LPS-induced splenocytes proliferation in mice immunized with the sheep red blood cell (SRBC). Meanwhile, HCLPS-1 remarkably promoted 2, 4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity (DTH) reactions. These results suggested that HCLPS-1 could improve both specific and non-specific cellular immune response and might be explored as a potential natural immunomodulator.

1. Introduction

Hyriopsis cumingii Lea, which is the main fresh mussel in the aquatic production, plays a very important role in aquaculture business. It is a kind of freshwater bivalve mollusks, and is widely cultured in the south of China for producing pearls. However after the pearls are taken out of shells, the mass-produced clams become the waste to be dumped. The clams contain abundant protein, lipid, polysaccharides, and the like. It is valuable to exploit them. In the recent years, there are several chemical and bioactive studies on the clam of *H. cumingii*, such as nutrient analysis (Zhou et al., 2006), enzyme-hydrolysis peptide technical study (Yang, Huang, Zhuang, Xu, & Dong, 2003) and inhibition against the lung cancer cell. However, the polysaccharide from this clam and its immunomodulatory activity has not yet been reported. In the present study, we report the isolation, chemical characterization of a water-soluble polysaccharide from the clam of *H. cumingii*, as well as its immunostimulatory activity *in vitro* and *in invo*.

2. Experimental

2.1. Materials

Fresh clam tissue of *H. cumingii* Lea was picked from a commercial pearl farm in Zhejiang China. Sephadex G-75 and 2,4-dinitrofluorobenzene (DNFB) were purchased from Pharmacia Biotech, CA, USA; DEAE-cellulose, concanavalin A (Con A), lipopolysaccharide (LPS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)were from Sigma Chemical Co., St. Louis, MO, USA; RPMI 1640 medium was from Gibco BRL, Grand Island, NY, USA; trifluoroacetic acid (TFA) and Me_2SO were purchased from E. Merck, AG, Germany. Aqueous solutions were prepared with purified water from a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of the highest available quality.

2.2. Isolation and purification of HCLPS-1

The clam tissue of *H. cumingii* Lea (100 g) was mashed into mincemeat and defatted with 95% EtOH twice at 80 °C for 2 h, and filtered. The residue was further extracted with 750 mL of water for 1 h. The combined aqueous extracts were concentrated in a rotary evaporator under reduced pressure at 50 °C and filtered. Then the filtrate was mixed with 4 vol. of 95% EtOH at 4 °C, followed by centrifugation at 5000 g for 20 min. The precipitate was dissolved in 300 mL of water and deproteinized three times with 60 mL sevage reagent (isoamyl alcohol and chloroform in 1:5 ratio)

* Corresponding author. Address: College of Food Science and Biological Engineering, Zhejiang Gongshang University, No. 149 Jiaogong Road, Hangzhou, Zhejiang 310035, PR China. Tel./fax: +86 571 88905733.
E-mail addresses: dzy@zjgsu.edu.cn (Z. Dai), zhanghong163@hotmail.com (H. Zhang), zhangyanping@zjgsu.edu.cn (Y. Zhang), wanghh1@1yahoo.com.cn (H. Wang).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.01.003

as described by Sevag (Sevag, Lackman, & Smolens, 1983). The resulting aqueous fraction was extensively dialyzed against double-distilled water for 3 days and precipitated by addition of 3 vol. of 95% EtOH. After centrifugation the precipitate was washed with anhydrous EtOH and then vacuum-dried at 40 °C to yield the crude polysaccharide (7.23 g). The crude polysaccharide was dissolved in distilled water, applied to a DEAE-cellulose column (2.0 × 40 cm) and eluted with water. Each fraction of 4 mL was collected at a flow rate of 40 mL/h and monitored by the phenol-sulfuric acid method at 490 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The collected fractions were dialyzed and lyophilized. The product was further chromatographed on a Sephadex-G200 column (1.5 × 40 cm) with water and lyophilized to give white power, HCLPS-1, which was subjected to the subsequent analyzes.

2.3. Gel-permeation chromatography (GPC) analysis

The homogeneity and molecular weight of HCLPS-1 was determined on a Waters HPLC system (Allances 2695, Waters, USA) equipped with a Waters Ultrahydrogel 250 column (7.8 × 300 mm) and a Waters 2410 differential refractometer. A sample solution (20 μL of 0.1%) was injected in each run, with 0.1 mol/L NaCl as the mobile phase at 0.6 mL/min. The HPLC system was precalibrated with pullulan standards (Shodex Standard P-82, Waters).

2.4. Monosaccharide composition analysis

HCLPS-1 (5 mg) was dissolved in 6 mL 2 mol/L TFA and hydrolyzed at 120 °C for 2 h, followed by evaporation and addition of MeOH to the residue. The hydrolyzate was reduced with $NaBH_4$ for 3 h at room temperature. The excess $NaBH_4$ was decomposed with HOAc and removed by repeated evaporation to dryness with the addition of 10% (v/v) HOAc in MeOH. Alditol acetates of the reduced sugars and authentic standards (D-glucose, D-mannose, D-galactose, D-fucose, D-xylose and D-arabinose with myoinositol as the internal standard) were prepared with AC_2O at 100 °C for 1 h and subjected to GLC analysis on an HP 6890 GC (Hewlett-Packard, USA) fitted with a capillary column DB-225 (60 m × 0.25 mm i.d., film thickness 0.25 μm) and a flame-ionization detector (Blakeney, Harris, Henry, & Stone, 1983). High-purity helium was used as the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was kept for 3 min at 190 °C following injection of 1 L samples in a split ratio of 1:10 and then raised at 4 °C min^{-1} to 230 °C. The injector and detector temperatures were 250 °C and 270 °C, respectively.

2.5. Methylation analysis

The HCLPS-1 (20 mg) was methylated three times according to Needs and elvendran (1993). Complete methylation was confirmed by the disappearance of the OH band (3200–3700 cm^{-1}) in the IR spectrum. The methylated products were hydrolyzed, reduced and acetylated as described by Sweet, Shapiro, and Albersheim (1975). The partially methylated alditol acetates were analyzed by gas chromatography–mass spectrometry. GC–MS was done on a TRACE DSQ GC/MS instrument (Thermoelectron Company, USA) with an HP-5 ms capillary column (30 m × 0.32 mm × 0.25 μm). The GC temperature program was isothermal at 50 °C for 2 min, followed by a 15 °C min^{-1} gradient up to 260 °C for 5 min

2.6. UV, IR and NMR analysis

UV–vis absorption spectra were recorded with a Shimadzu MPS-2000 spectrophotometer between 190 and 290 nm. The FT-IR spectra (KBr pellets) were recorded on SPECORD in a range of 400–4000 cm^{-1}. For NMR measurements, HCLPS-1 was dried in a vacuum over P_2O_5 for several days, and then exchanged with deuterium (Dueñas-Chasco et al., 1997) by lyophilizing with D_2O for several times. The deuterium-exchanged polysaccharide (50 mg) was put in a 5-mm NMR tube and dissolved in 0.7 mL 99.96% D_2O. Spectra were recorded with a Bruker AV-500 spectrometer. The ^1H and ^{13}C NMR spectra were recorded at 50 °C. Acetone was used as an internal standard (δ31.12 ppm) for the ^{13}C spectrum. The ^1H NMR spectra were recorded fixing the HOD signal at δ4.68 ppm at 50 °C.

2.7. Experimental animals

Female ICR mice (grade II, 5 weeks old) weighing 20 ± 2 g were purchased from Zhejiang Experimental Animal Center (Certificate No. 22-2001001, Hangzhou, China) and acclimatized for 7 d before use. Rodent laboratory chow and tap water were provided *ad libitum*, and maintained under controlled conditions: temperature 24 ± 1 °C, humidity 50 ± 10%, 12-h light/12-h dark cycle. All the procedures were in strict accordance with the P.R. China legislation on the use and care of laboratory animals, and with the guidelines established by the Institute for Experimental Animals, and were approved by the Committee for Animal Experiments.

2.8. In vitro splenocyte proliferation assay

Spleen collected under aseptic conditions was minced and passed through a sterilized ion mesh (200 mesh) to obtain single spleen cell suspensions. Erythrocytes in the cell mixture were destroyed by the rapid addition of H_2O. Then, the cells were washed twice with phosphate-buffered saline (PBS) and adjusted to a density of 5 × 10^6 cells/mL in the RPMI 1640 complete medium [RPMI 1640 supplemented with 12 mM HEPES (pH 7.1), 0.05 mM 2-mercaptoethanol, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS]. Splenocyte proliferation was assayed as previously described (Wang, Li, Song, & Bi, 2002). Briefly, an aliquot of 100 μL of splenocytes were seeded into 4 wells of a 96-well flat-bottom microtiter plate, thereafter Con A (final concentration 10 μg/ml), or LPS (final concentration 20 μg/ml), or medium with HCLPS-1 (final concentration 0.01, 0.1, 1.0, 10, and 100 μg/mL) were added giving a final volume of 200 μl. After incubation at 37 °C in a humid atmosphere with 5% CO_2 for 44 h, 50 μl of MTT solution (2 mg/ml) were added to each well and incubated for another 4 h. The plate was centrifuged on 1500 rpm for 5 min, and the supernate was discard, and then 150 μL Me_2SO was added per well. Absorbance at 570 nm was measured on an ELISA reader (Bio-TEK USA).

2.9. Splenocyte proliferation assay in vivo

Six-week-old female ICR mice were divided into five groups, each consisting of ten mice. Mice were immunized intraperitoneally (i.p.) with 5% the sheep red blood cell (SRBC) suspension 0.5 mL in saline (Bao, Liu, Fang, & Li, 2001). Beginning on the day of immunization, the immunized mice were administered ip with HCLPS-1 at the doses of 10, 20, 40, 60 mg/kg for 6 days once daily. The control groups received the same volume of saline. Twenty four hours after the last administration, splenocytes were prepared as described above. An aliquot of 100 μL of splenocytes prepared from immunized mouse was seeded into each well of a 96-well plate in the presence of Con A (10.0 μg/mL) or LPS (20.0 μg/mL). The plates were incubated at 37 °C in a humid atmosphere with 5% CO_2. Splenocytes proliferation was assayed as described before. The stimulation index (SI) was calculated based on the following formula: SI, the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

2.10. DNFB-induced delayed-type hypersensitivity (DTH) response

Six-week-old female ICR mice were divided into four groups, each consisting of ten mice. Animals were initially sensitized with 50 μL 1% DNFB dissolved in acetone–olive oil (1:1) on the shaved abdominal skin of recipients. Beginning on the day of immunization, the immunized mice were orally administered with HCLPS-1 at the doses of 40, 40, 80 mg/kg for 5 days once daily. The control groups received the same volume of saline. After 5 days, the DTH reaction was elicited by smearing 10 μL 1% DNFB on both sides of the left ear. 24 h later, the DTH response to DNFB was evaluated by measuring weight difference of right and left ear with an analytical balance (Brodmerkel et al., 2005).

2.11. Statistical analysis

The data were expressed as mean ± SD and examined for their statistical significance of difference with one-way ANOVA, followed by Dunnett's test. *P*-values of less than 0.05 were considered to be statistically significant.

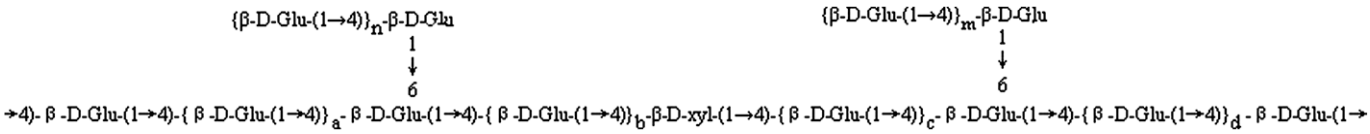
3. Results and discussion

3.1. Isolation, purification and structural analysis of HCLPS-1

The crude polysaccharide was extracted with hot water from the clam of *H. cumingii*. This fraction was separated and sequentially purified through DEAE-cellulose and Sephadex-G200 gel-permeation chromatography, leading to the isolation of a water-soluble purified polysaccharide HCLPS-1, with its yield being 4.6% of the dry material. The fractionation procedure was monitored by carbohydrate content detected by the phenol–sulfuric acid assay.

HCLPS-1 appeared as a white powder. The HPGPC profile (Fig. 1) showed a single and symmetrically sharp peak, indicating that HCLPS-1 was a homogeneous polysaccharide, with a weight-average molecular weight of $\sim 1.56 \times 10^5$ Da. HCLPS-1 was determined to be composed of D-glucose and D-xylose with the molar ratio of 35:1 by GC of the acetylated of monosaccharides.

Methylation analysis of HCLPS-1 by GC–MS revealed three types of glucose and one type of xylose derivatives in a relative molar ratio of 29:4:2:1. The GC–MS results (Table 1) indicated that the backbone chain are mainly (1,4)-linked-β-D-glucosyl (Residue-A), and small amounts of (1,4)-linked-β-D-xylosyl residues (Residue-B), the backbone chain contained non-reducing terminal (1)-β-D-glucosyl and (4)-β-D-glucosyl (Residue-C) groups. The two side chains attached to the C-6 position of Residue-D contained single non-reducing terminal (4)-β-D-glucosyl (Residue-C) groups.



The HCLPS-1 was analyzed by UV, IR and NMR. It had a negative response to the Bradford test and no absorption at 280 or 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid. The bands in the region of 3411 cm^{-1} are due to the hydroxyl stretching vibration of the polysaccharides. The bands in the region of 2917 cm^{-1} are due to C–H stretching vibration, and the bands in the region of 1640 cm^{-1} are due to associated water. In the anomeric region of the ^1H NMR spectrum of HCLPS-1 (Fig. 2), five signals occurred at 5.33, 3.89,3.78,3.58, and 3.357 ppm, which were assigned as Residue-C, Residue-A, Residue-B, and Residue-D, respectively. And accordingly in the anomeric region of the ^{13}C NMR spectrum (Fig. 3), carbon resonances appeared at 103.30,

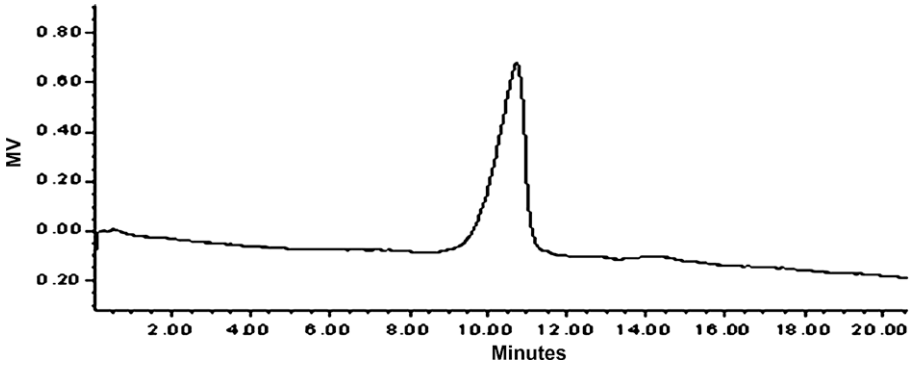


Fig. 1. HPGPC Profile of HCLPS-1.

Table 1
GC-MS data of alditol acetate derivatives from the methylated product of HCLPS-1.

Acetates of sugar[a]	Rt (min)	Molar ratio	Mass fragments (*m/z*)	Mode of linkage
2,3,4,6-*O*-Me_4-Glucose	11.79	3.8	43, 45, 71, 88, 101, 129, 145, 161, 205	Terminal
2,3,6-*O*-Me_3-Glucose	12.57	28.8	43, 45, 85, 87, 113, 129, 131, 173, 233	→4) Glup(→1
2,3-*O*-Me_2-Xylose	12.99	1.0	43, 71, 87, 101, 117, 129, 161, 173, 189	→4) Xylp(→1
2,3-*O*-Me_2-Glucose	13.44	2.5	43, 85, 127, 159, 201, 261, 271	→4,6) Glup(→1

[a] 2,3,4,6-Me-Glu = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucose, etc.

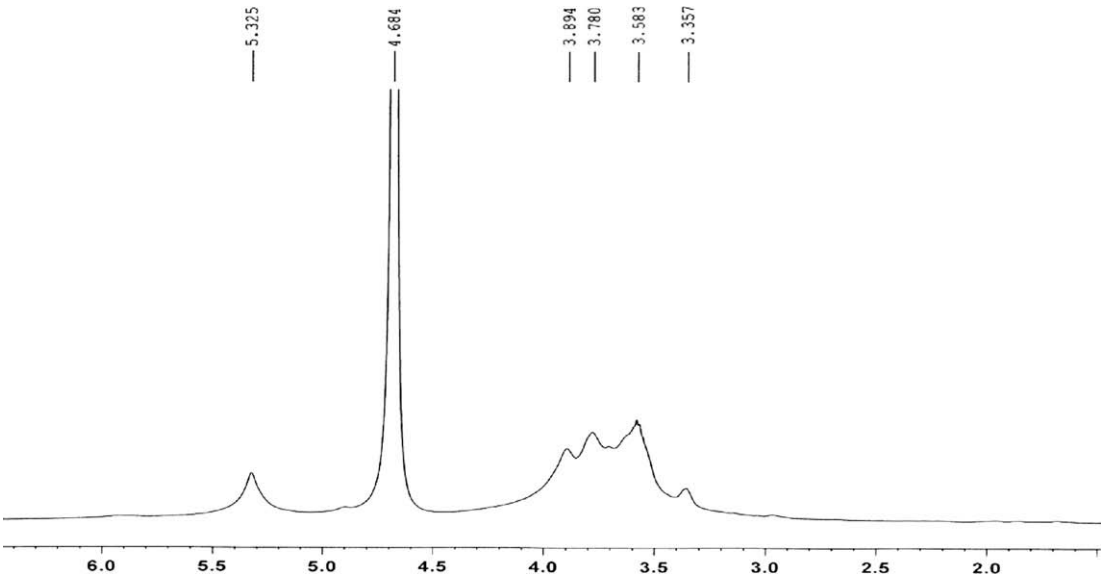


Fig. 2. ^{1}H NMR Profile of HCLPS-1.

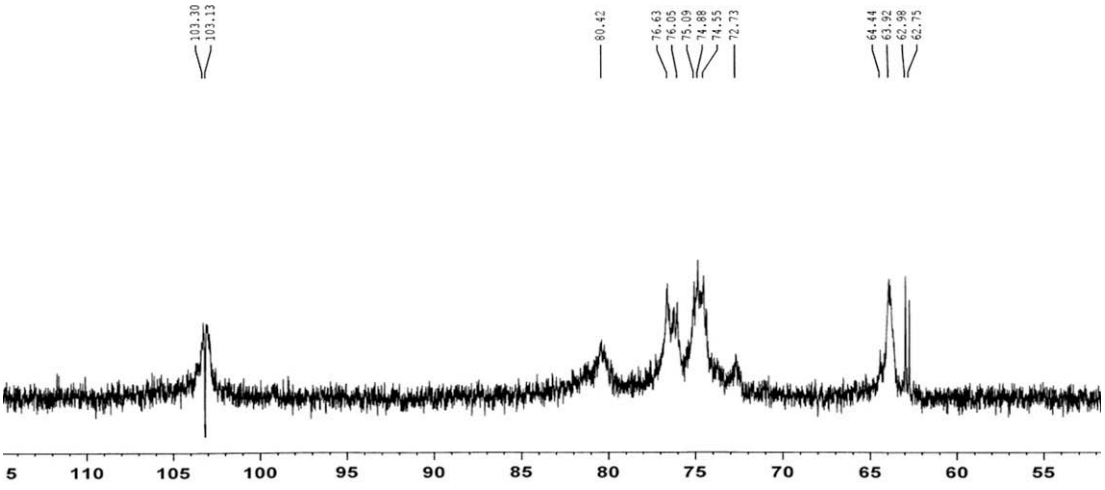


Fig. 3. ^{13}C NMR Profile of HCLPS-1.

103.13, 80.42, 76.63–72.73 and 64.44–62.75 ppm. All the results confirmed the presence of four sugar residues and their configurations: Residue-A, Residue-B, Residue-C and Residue-D are forms of β-configuration. In the high magnetic field, the 62.75 ppm signal should come from C-6 resonance of Residue-D. C-4 chemical shifts of Residue-A and Residue-B, Residue-C occurred at 76.63–72.73 ppm. The 103.30 and 103.13 ppm signal should come from terminal resonance of Residue-C. All the NMR chemical shifts were compared with the literature values (Cui et al., 2007; Hua, Zhang, Fu, Chen, & Chan, 2004; Ishurd et al., 2004; Pramanik, Mondal, Chakraborty, Rout, & Islam, 2005; Sun et al., 2008).

3.2. Immunostimulatory activity in vitro and in invo

The lymphocyte-mediated immunity plays an important role in the cellular and humoral immune responses. The capacity to elicit an effective T- and B-lymphocyte immunity can be shown by the stimulation of lymphocyte proliferation response. It is generally known that Con A stimulates T cells and LPS stimulates B cell proliferation (Han et al, 1998). The proliferation assay showed that HCLPS-1 not only significantly promoted Con A and LPS-stimulated splenocyte proliferation *in vitro* in a concentration-dependent manner (Table 2), but increased Con A- and LPS-induced splenocyte proliferation in the SRBC-immunized mice in a dose-depen-

Table 2
Effects of HCLPS-1 on Con A- and LPS-stimulated mice splenocyte proliferation *in vitro*[a].

Concentration (μg/ml)	RPMI 1640 (A_{570})	Con A (A_{570})	LPS (A_{570})
0.0	0.164 ± 0.005	0.207 ± 0.000	0.212 ± 0.014
0.01	0.167 ± 0.001	0.378 ± 0.022*	0.248 ± 0.013*
0.1	0.170 ± 0.005	0.373 ± 0.008**	0.253 ± 0.004*
1.0	0.172 ± 0.002	0.385 ± 0.003**	0.272 ± 0.004*
10.0	0.187 ± 0.001*	0.404 ± 0.012**	0.273 ± 0.003*
100.0	0.210 ± 0.003**	0.439 ± 0.008**	0.312 ± 0.001**

[a] The data are represented as mean ± SD from three independent experiments. Significant differences with 0 μg/ml were designated as $^{*}P < .05$ and $^{**}P < .01$.

Table 3
Effects of HCLPS-1 on Con A- and LPS-stimulated splenocyte proliferation the immunized mice[a].

Groups	Dose (mg/kg)	Con A	LPS
Control	–	1.523 ± 0.369	1.346 ± 0.210
HCLPS-1	10	1.964 ± 0.344	1.438 ± 0.295
	20	2.140 ± 0.421*	1.705 ± 0.261
	40	2.184 ± 0.369*	1.737 ± 0.270*
	60	2.295 ± 0.210**	1.773 ± 0.127**

[a] Mice were immunized i.p. with 0.5 ml of 5% sheep red blood cell (SRBC) suspension on day 0, and administered with HCPLS-1 at the doses of 10, 20, 40, 60 mg/kg for 6 days once daily. The control groups received the same volume of saline. After 6 days, splenocytes were prepared, and cultured with Con A or LPS for 48 h. Splenocyte proliferation was measured by the MTT method as described in the text, and shown as a stimulation index. The values are presented as means ± SD ($n = 5$). Significant differences with control group are designated as $^{*}P < .05$ and $^{**}P < .01$.

Table 4
Effect of HCLPS-1 on DNFB-induced delayed-type hypersensitivity reaction in mice[a].

Group	Dose (mg/kg/d)	Number of mouse	ΔT (mg)
Control	0	10	8.59±1.36
HCLPS-1	40	10	10.11±1.99
	60	10	10.50±1.72*
	80	10	13.43±2.43**

[a] Mice were initially sensitized with DNFB on days 0, and administered with HCLPS-1 at the doses of 40, 60, 80 mg/kg for 5 days once daily. The control groups received the same volume of saline. 5 days later, DTH reaction was elicited in ear by challenge with DNFB. The ear swelling (ΔT) was calculated as the difference between the weights of untreated and DNFB-treated ear punches 24 h after challenge. The values are presented as means ± SD ($n = 10$). Significant differences compared to control group are designated as $^{*}P < .05$ and $^{**}P < .01$.

dent manner (Table 3). The results indicated that HCLPS-1 could significantly induce the activation potential of T and B cells in mice *in vitro* and *in vivo*.

The DTH reaction is a cell-mediated pathologic response involved with T cell activation and the production of many cytokines (Black, 1999). The effect of HCLPS-1 on DNFB-induced DTH reactions in mice was also measured, and the results were shown in Table 4. HCLPS-1 could significantly enhance the ear swelling in DNFB-induced mice at the doses of 60 and 80 mg/kg ($P < .05$ or $P < .01$). The results further confirmed that HCLPS-1 could promote the specific cellular immune response.

In conclusion, the water-soluble polysaccharide HCLPS-1 from clam of *H. cumingii* could improve both specific and non-specific cellular immune response, and be explored as a potential natural immunomodulator. The further studies are needed to elucidate the structure, function, their relationships and mechanisms responsible for its immunological activities.

Acknowledgement

This work was supported by grant from Science and Technology Project of Zhejiang province of China (2006C22041).

References

Bao, X., Liu, C., Fang, J., & Li, X. (2001). Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. *Carbohydrate Research, 332*, 67–74.

Black, C.A. (1999). Delayed type hypersensitivity: Current theories with an historic perspective. Dermatology Online Journal *5*, 7 http://dermatology.cdlib.org/DOJvol5num1/reviews/black.html.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharides analysis. *Carbohydrate Research, 113*, 291–299.

Brodmerkel, C. M., Huber, R., Covington, M., Diamond, S., Hall, L., Collins, R., et al. (2005). Discovery and pharmacological characterization of a novel rodent-active CCR2 antagonist, INCB3344. *Journal of Immunology, 175*, 5370–5378.

Cui, F. J., Tao, W. Y., Xu, Z. H., Guo, W. J., Xu, H. Y., Ao, Z. H., et al. (2007). Structural analysis of anti-tumor heteropolysaccharide GFPS1b from the cultured mycelia of *Grifola frondosa* GF9801. *Bioresource Technology, 98*, 395–401.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Dueñas-Chasco, M. T., Rodríguez-Carvajal, M. A., Mateo, P. T., Franco-Rodríguez, G., Espartero, J., Irastorza-Iribas, A., et al. (1997). Structural analysis of the exopolysaccharide produced by *Pediococcus damnosus*. *Carbohydrate Research, 303*, 453–458.

Han, S. B., Kim, Y. H., Lee, C. W., Park, S. M., Lee, H. Y., Ahn, K. S., et al. (1998). Characteristic immunostimulation by angelan isolated from Angelica. *International Immunopharmacology, 40*, 39–48.

Hua, Y. F., Zhang, M., Fu, C. X., Chen, Z. H., & Chan, G. Y. S. (2004). Structural characterization of a 2-*O*-acetylglucomannan from *Dendrobium officinale* stem. *Carbohydrate Research, 339*, 2219–2224.

Ishurd, O., Kermagi, A., Zgheel, F., FleXa, M., Elmabruk, M., Wu, Y. L., et al. (2004). Structural aspects of water-soluble galactomannans isolated from the seeds of *Retama raetam*. *Carbohydrate Polymer, 58*, 41–44.

Needs, P. W., & elvendran, R. R. (1993). Avoiding oxidative degradation during sodium hydroxide/methyl iodine-mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydrate Research, 245*, 1–10.

Pramanik, M., Mondal, S., Chakraborty, I., Rout, D., & Islam, S. S. (2005). Structural investigation of a polysaccharide (Fr. II) isolated from the aqueous extract of an edible mushroom, *Pleurotus sajor-caju*. *Carbohydrate Research, 340*, 629–636.

Sevag, M. G., Lackman, D. B., & Smolens, J. (1983). The isolation the components of streptococcal nucleoproteins in nucleoproteins in serologic allyactive form. *Journal of Biological chemistry, 124*, 425–436.

Sun, Y. X., Wang, S. S., Li, T. B., Li, X., Jiao, L. L., & Zhang, L. P. (2008). Purification, structure and immunobiological activity of a new water-soluble polysaccharide from the mycelium of *Polyporus albicans* (Imaz.) Teng. *Bioresource Technology, 99*, 900–904.

Sweet, D. P., Shapiro, R. H., & Albersheim, P. (1975). Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydrate Research, 40*, 217–225.

Wang, Y. P., Li, X. Y., Song, C. Q., & Bi, Z. (2002). Effect of astragaloside IV on T, B lymphocyte proliferation and peritoneal macrophage function in mice. *Acta Pharmacologica Sinica, 23*, 263–266.

Yang, W. G., Huang, X. C., Zhuang, R. Y., Xu, D. L., & Dong, M. M. (2003). Study of enzyme-hydrolysis technical of *Hyriopsis cumingii* meat. *Food and Fermentation Industries (Chinese), 1*, 90–93.

Zhou, H. Y., Liu, Z. H., Liu, C. C., Li, Y. S., Li, J. L., & Su, Y. C. (2006). Analysis of nutritional components of *Hyriopsis cumingii* meat and mantle after the pearl was harvested. *Science and Technology of Food Industry (Chinese), 11*, 170–173.