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Effects of alkali dissociation on the molecular conformation and immunomodulatory activity of longan pulp polysaccharide (LPI)

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ABSTRACT

The effects of alkali dissociation on the molecular conformation and immunomodulatory activity of longan pulp polysaccharide (LPI) were investigated to explore their possible relationship. The molecular conformations of LPI and its degraded derivatives (LPI1 and LPI2) were examined by size exclusion chromatography combined with multi-angle laser light scattering (SEC-MALLS), Congo red test and atomic force microscopy (AFM). The results confirmed the transformation of LPI from compact sphere-like conformation to slightly dissociated sphere-like conformation (LPI1) or single-helix chain (LPI2). Compared with the control, splenocyte proliferation and NK cell cytotoxicity were significantly enhanced by LPI1 and LPI2 ($P < 0.05$), although they were not stimulated by LPI in 100–400 μg/mL ($P > 0.05$). All the polysaccharides could significantly enhance macrophage phagocytosis at 100 or 200 μg/mL ($P < 0.05$). Single-helix chain might play an important role in activating lymphocytes and NK cells, but having weak contribution to macrophage phagocytosis.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is a subtropical fruit which belongs to *Sapindaceae* family. Its pulp has been traditionally used to promote blood metabolism, soothe nerves, relieve insomnia and prevent forgetfulness (Park et al., 2010; Yang et al., 2008). The extensive bioactivities of longan pulp, such as memory-enhancement (Park et al., 2010), antianxiety (Okuyama, Ebihara, Takeuchi, & Yamazaki, 1999), antioxidation and immunomodulation (Su et al., 2010), have been well demonstrated. Specifically, crude polysaccharides from longan pulp have been confirmed to exhibit free radical scavenging, immunity-modulatory and antitumor activities (Zhong, Wang, He, & He, 2010). However, the information on the structure–activity relationship of longan polysaccharide is still not available.

Many studies have indicated that the activities of polysaccharides are most closely related to their chemical compositions, configurations and molecular weights (Demleitner, Kraus, & Franz, 1992; Im et al., 2005; Kralovec et al., 2007; Lo, Jiang, Chao, & Chang, 2007). The immunostimulatory effects of arabinogalactans from *Chlorella pyrenoidosa* not only depend on the molecular weight and chemical structure, but also on the conformation (Yang & Zhang, 2009). Native (1→3)-α-D-glucan exhibits weaker antitumor activity compared with its water-soluble derivatives obtained by carboxymethylation or sulfation, which indicate that its activity can be enhanced by increasing chain stiffness (Peng, Zhang, Zhang, Xu, & Kennedy, 2005). In fact, the previous reports on the relationship between biological activity and conformation are controversial due to the complicated structure of polysaccharides. It is suggested that the bioactivities of polysaccharides depend on their triple-helixes (Falch, Espevik, Ryan, & Stokke, 2000; Kishida, Sone, & Misaki, 1992; Ohno, Miura, Chiba, Adachi, & Yadomae, 1995; Surenjav, Zhang, Xu, Zhang, & Zeng, 2006; Zhang, Li, Xu, & Zeng, 2005) or single-helixes (Bao, Dong, & Fang, 2000; Saitô et al., 1991). Yang, Wu, and Zhang (2002) have implied that the activities of (1→3)-β-D-glucans are related to the polyhydroxy groups on the surface of single-helix. In addition, Kulicke, Lettau, and Thielking (1997) have suggested that helical structure might have a nonessential or negative role for polysaccharide in immunological activity. Therefore, a basic analysis on the conformation of longan polysaccharide is essential to understanding the potential relationship between molecular characteristic and activity.

The molecular mass (distribution) of polysaccharides is generally determined by SEC-MALLS. On the basis of data obtained from SEC-MALLS, the most probable conformation of

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polysaccharides can be delineated (Huang, Huang, Li, & Zhang, 2009; Tao, Zhang, & Zhang, 2009). As a colourant, Congo red has been commonly used to combine with helical polysaccharides (especially single-helical conformation) resulting the red shift of maximum absorption wavelength (λ_{max}) (Hara, Kiho, Tanaka, & Ukai, 1982; Hara, Kiho, & Ukai, 1983; Lee et al., 2010; Liang, Zhang, Miao, & Zhang, 1994; Ogawa, Wanatabe, Tsurugi, & Ono, 1972; Qin, Huang, & Xu, 2002). In addition, AFM particularly permits nondestructive imaging of soft biological surface, and has been widely adopted to explore the molecular and supramolecular structures of biological macromolecules (Wang, Xu, & Zhang, 2008; Xu, Wang, Cai, & Zhang, 2010; Zhang et al., 2010). Our previous study found that fraction LPI accounted for 56.02% of crude polysaccharide extract, but was the fraction with lowest immunomodulatory activity. To improve its activity as a potential immunoadjuvant, LPI was treated by sodium hydroxide, which could depolymerize polysaccharides with compact and high-organized conformation (Falch et al., 2000; Fariña, Siñeriz, Molina, & Perotti, 2001; Saitô et al., 1991; Zhang et al., 2010), to obtain its derivatives. They were then analyzed on molecular conformation. Moreover, their effects on splenocyte proliferation, NK cell cytotoxicity and macrophage phagocytosis were evaluated *in vitro*.

2. Materials and methods

2.1. Plant materials

Fresh fruits of longan (cv. Chu-liang) at the mature stage were provided by Pomology Research Institute of Guangdong Academy of Agricultural Sciences (Guangzhou, China). Fruits were selected based on their uniformity of size and colour, and then dried in an oven at 50 °C for a week. The pulps were stripped manually and stored at −20 °C.

2.2. Preparation of LPI

The extraction of crude LP was performed by the methods described in our previous studies (Yi et al., 2010a, 2010b). 200 mg crude LP was re-dissolved in 40 mL distilled water and centrifuged at 4500 rpm for 15 min. The supernatant was purified using anion-exchange DEAE52-cellulose column chromatography (50 cm × 2.6 cm) equilibrated by distilled water. The column was eluted by distilled water at a flow rate of 0.4 mL/min. 6 mL/tube of eluant was collected. The polysaccharide concentration in the eluant was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Pebers, & Smith, 1956). The eluants containing polysaccharides were combined and concentrated at 55 °C by a vacuum rotary evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China). The concentrated solution was lyophilized to obtain LPI, which was a neutral polysaccharide and mainly composed of glucose and mannose at the molar ratio of 2.27:1.00 by gas chromatography coupled with mass spectrometer.

2.3. Treatment of sodium hydroxide

50 mg LPI was dissolved in 50 mL distilled water, then 50 mL NaOH solution was added in with the final concentration of 0.25 or 0.50 mol/L. After 10 min of alkali dissociation at room temperature, 5 mol/L HCl solution was used to neutralize pH. The mixture was dialyzed (regenerated cellulose bag filter, MWCO8000, Spectrum, USA) against distilled water at 4 °C for 3 days. The remaining solution was concentrated by a vacuum rotary evaporator at 55 °C, and the concentrated solution was then lyophilized to obtain LPI1 or LPI2.

2.4. Molecular characterization analysis

2.4.1. SEC-MALLS measurement

The SEC-MALLS measurement was performed on a SEC column (Shodex SB-804 connected with Shodex SB-802) equipped with a pump (Waters 515 HPLC) at 25 °C. A multi-angle laser light photometer (λ = 633 nm, Wyatt-DAWN HELEOS-II, Wyatt Technology Co., USA) and a differential refractive index detector (RI, Wyatt-Optilab rex) were simultaneously connected. The concentration of polysaccharide solution was 3.0–5.0 mg/mL in distilled water. After passing through 0.2-μm syringe filters (Whatman, England), 200 μL solution was injected. The isocratic mobile phase was 0.1 mol/L sodium nitrate at a flow rate of 0.5 mL/min. The specific refractive index increments (dn/dc) value of the polysaccharides in distilled water, which was determined by OPTILAB DSP differential refractometer (Wyatt Technology Co., USA) at 633 nm and 25 °C, was 0.147 mL/g. Astra software (version 4.90.07 for Windows, Wyatt Technology Corp.) was utilized for data acquisition and further analysis.

2.4.2. SEC measurement

10 mg sample was dissolved in 5 mL distilled water, and then centrifuged at 4500 rpm for 15 min. 1 mL supernatant was injected onto the Sephadex G-100 gel column (20 cm × 1.6 cm) equilibrated by distilled water, and then eluted by distilled water at a flow rate of 0.1 mL/min. 1.5 mL/tube of eluant was collected and detected by phenol–sulfuric acid method (Dubois et al., 1956).

2.4.3. Congo red test

The conformational structure of polysaccharide in aqueous solution was determined by characterizing Congo red–polysaccharide complex (Ogawa et al., 1972). 2 mL LP (0.5 mg/mL) was mixed with 2 mL Congo red (50 μmol/L) and 1 mL NaOH solution (final concentration of NaOH: 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 or 0.50 mol/L). Meanwhile, 2 mL Congo red (50 μmol/L) was mixed with 1 mL NaOH solution as the control. After been kept for 10 min at room temperature, λ_{max} was measured in the range from 400 to 600 nm.

2.4.4. AFM measurement

Polysaccharide solution (1×10^{-3} mg/mL) was dropped onto freshly cleaved ruby muscovite mica substrate and dried for more than 1.5 h in a desiccator at room temperature. AFM images were obtained in air by an atomic force microscope (Multimode Nanoscope III, Veeco Metrology, Santa Barbara, CA) with tapping mode and etched silicon tips (Veeco RTESP probes, 274–335 kHz and 20–80 N/m). The Nanoscope IIIa software (Version 5.30r3.sr3, Veeco Metrology) was used for image manipulation.

2.5. Immunomodulatory activity analysis

2.5.1. Animals and cells

Specific pathogen-free Kunming mice (male, 20.0 ± 2.0 g, certificate number: SCXK-Yue 2006-0015) were purchased from Laboratory Animal Sciences Center of Southern Medical University (Guangdong, China). The mice were bred on a 12-h-dark/12-h-light cycle at 22 ± 2 °C and allowed free access to standard laboratory rodent diet (Laboratory Animal Sciences Center of Southern Medical University, China) and tap water. 8–12 weeks old mouse was sacrificed by cervical dislocation, and its spleen was removed aseptically and then minced in aseptic phosphate-buffered saline (PBS). The splenic cells were harvested through sterilized meshes (200 meshes) at room temperature. After removing the red blood cells by hemolytic Gey's solution, the remaining cells were washed twice and resuspended in RPMI 1640 complete medium (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco

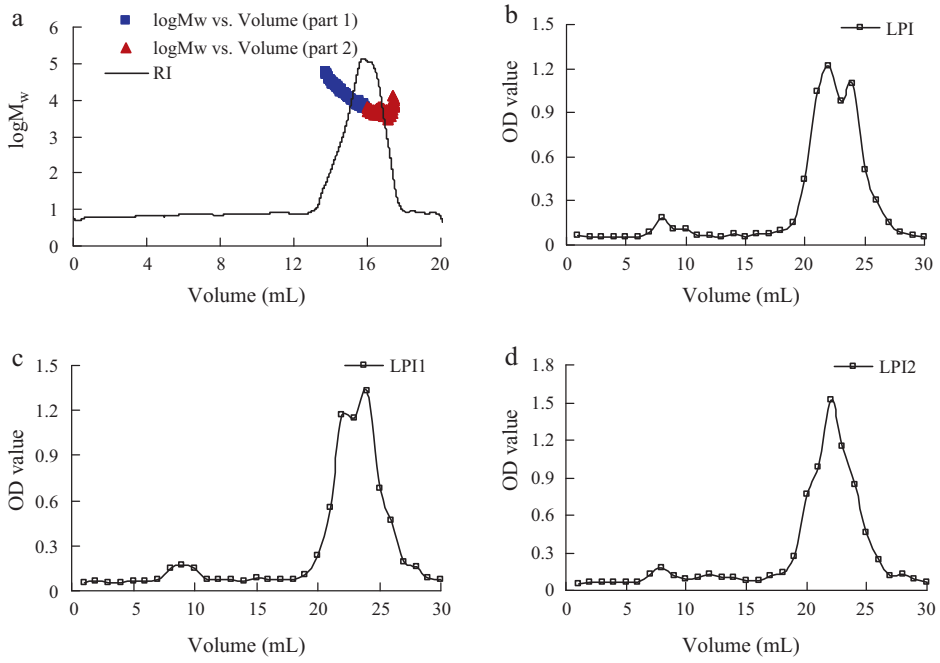

Fig. 1. SEC elution curves of longan polysaccharides. (a) The plot of the molecular mass vs. volume of the SEC-LLS chromatograms for LPI detected by RI in water at 25 °C. The main peak was separated into two parts. SEC chromatograms of LPI (b), LPI1 (c) and LPI2 (d) detected by the phenol–sulfuric acid method.

BRL). The cell concentration was adjusted to 1×10^7 cells/mL. The resident macrophages of mouse were harvested by peritoneal lavage, and its cells were subsequently cultured in complete medium at the concentration of 2×10^6 cells/mL. The experiment procedures were approved by the laboratory animal committees of Guangdong Province. All the treatments to animals were performed in accordance to the Guide for the Care and Use of Laboratory Animals.

YAC-1 lymphoma cell line was provided by Experiment Animal Center of Sun Yat-sen University (Guangdong, China) and used as target cell at the concentration of 5×10^5 cells/mL.

2.5.2. Splenic lymphocyte proliferation

The assay of splenic lymphocyte proliferation was implemented according to the method described by Li et al. (2008) and Wang, Wang, Lin, and Lin (2005). 50 μL/well of splenocyte suspension was plated in a 96-well culture plate with or without 5.0 μg/mL concanacalin A (ConA, Sigma, St. Louis, MO, USA). The filter-sterilized samples (final concentration: 0, 50,100 and 200 μg/mL) were added into the cell. After incubation for 68 h (37 °C, 5%CO_2), each well was pulsed with 5 mg/mL final concentration of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma). The plate was further incubated for 4 h, and 100 μL/well acidified isopropyl alcohol was then added and kept for 12 h to dissolve the formazan crystals. After that, the plate was analyzed at 570 nm using a microplate reader (Thermo Labsytems, Helsinki, Finland).

2.5.3. Natural killer cell cytotoxicity

Splenocyte was prepared as the effector cell for splenic natural killer (NK) activity assay as described by Wang et al. (2005). 50 μL/well splenocyte suspension was plated in a 96-well culture plate with 30 μL sample (final concentration: 0, 50,100 and 200 μg/mL). After been incubated for 24 h (37 °C, 5% CO_2), 20 μL of target cell or complete medium was added to be experimental group or effector control. And the well plated with YAC-1 cells only was target control. After incubation for 4 h, each well of the plate was pulsed with 5 mg/mL MTT for 4 h. Then, 100 μL/well acidified isopropyl alcohol was added and kept for 12 h. The plate was finally analyzed at 570 nm. The NK cell cytotoxicity (%) was calculated as: $[OD_T - (OD_{exp} - OD_E)]/OD_T \times 100$, in which OD_{exp}, OD_E and OD_T represented the OD values of experimental group, effector control and target control, respectively.

2.5.4. Macrophage phagocytosis

The purity of macrophages was tested by adherence (Li et al., 2008). 100 μL/well of macrophage suspension was plated in a 96-well culture plate and incubated for 3 h (37 °C, 5% CO_2). The adherent macrophages were washed twice by complete medium and then incubated with samples (final concentration: 0, 50,100 and 200 μg/mL) for 24 h. The stimulated cells were washed twice by PBS, and 100 μL neutral red (0.1%, w/v) was then added. The plate was incubated for 4 h. After the removal of unphagocytized neutral red by PBS, 100 μL cell lysate (the volume ratio of acetic acid to ethanol was 1:1) was added in and kept for 12 h. The OD value of each well was read at 570 nm. The phagocytosis index (%) was calculated as: $(OD_S - OD_C)/OD_C \times 100$, in which OD_S and OD_C represented the OD values of stimulated well and control well, respectively (Wang et al., 2005).

2.6. Statistical analysis

The data were expressed as means ± standard deviations. Significance of difference was evaluated with one-way ANOVA, followed by the Student–Newman–Keuls test by SPSS 11.5 software. *P*-value of 0.05 was used as the threshold for the significance.

3. Results

3.1. Molecular characteristics of longan polysaccharides

3.1.1. Molecular mass

In the SEC-MALLS chromatogram of LPI (Fig. 1a), the RI responded to the concentration of polysaccharides, and a main peak was observed in the chromatogram. The weight-average molar mass of LPI was 1.40×10^4 g/mol, and its polydispersity (M_w/M_n) was 1.36. The plot of molecular mass vs. volume of LPI was not

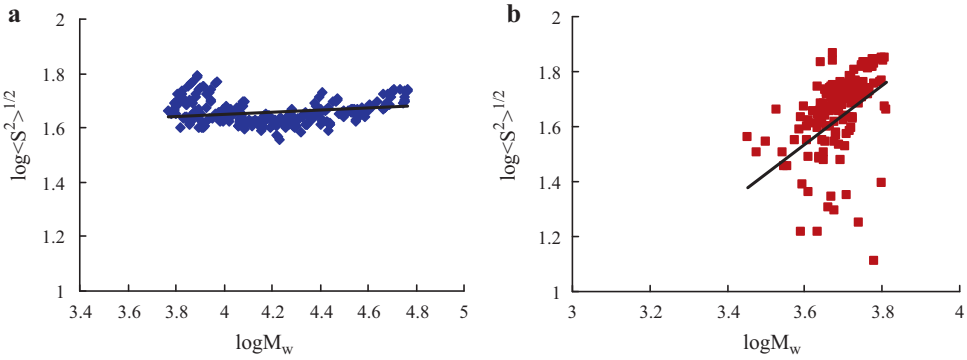

Fig. 2. The plots of $\langle S^2 \rangle_z^{1/2} = kM_w^v$ vs. M_w of LPI in double logarithmic coordinates. The equation $\langle S^2 \rangle_z^{1/2} = kM_w^v$ was established from experimental points in the SEC-LLS chromatogram. Figure (a) responded to the part 1 of Fig. 1a and figure (b) responded to the part 2 of Fig. 1a.

linearly exhibited, which implied that the coexistence of different conformations in LPI. Meanwhile, two overlapped peaks represented two components in LPI (Fig. 1b).

3.1.2. Molecular conformation

According to the plot of molecular mass vs. volume and the peak values in Fig. 1a, the main peak of LPI could be separated into two parts. Their $\langle S^2 \rangle_z^{1/2} = kM_w^v$ plots, which were constructed on the double logarithmic coordinate, were established from experimental points in the SEC–LLS chromatogram (Fig. 2). Usually, the v values of 0.33, 0.50–0.60 and 1.0 reflect the polymer molecular shape as sphere, random coil and rigid rod, respectively (Huang et al., 2009; Tao et al., 2009). The v value of 0.04 in Fig. 2a indicates a globular conformation with high branching structure, and the v value of 1.05 in Fig. 2b represents a conformation of rigid rod.

The λ_{max} of the LP–Congo red complexes at the NaOH concentration range of 0–0.5 mol/L is shown in Fig. 3. Their λ_{max} gradually decreased with the increasing NaOH concentration, as that of Congo red. At the same NaOH concentration, all the complexes showed red shifts compared with Congo red, and the λ_{max} of LPI–Congo red was obviously higher than that of LPI1–Congo red and LPI2–Congo red.

3.1.3. Molecular morphology

The AFM images of the longan polysaccharides in distilled water, which display the changes of LPI conformation induced by alkali solution, are presented in Fig. 4. The broad distribution of individual spherical particles of LPI was imaged clearly. Its particle diameter was observed in the range of 20–120 nm. The globular conformation was slightly disassociated in the image of LPI1 and completely disappeared in that of LPI2. The changes of molecular morphology were coincided with the results of SEC chromatograms (Fig. 1). Meanwhile, the globular conformation of LPI indicated its high branching structure, which was corresponded to the v value of $\langle S^2 \rangle_z^{1/2} = kM_w^v$.

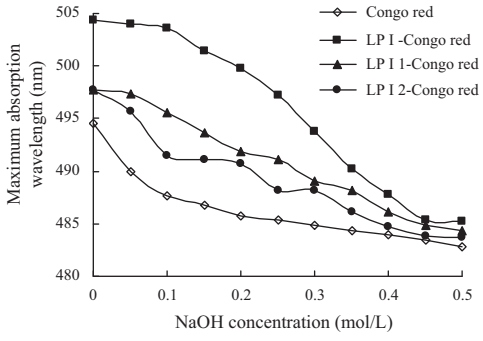

Fig. 3. The maximum absorption wavelengths of longan polysaccharide–Congo red complexes at the NaOH concentration range of 0–0.5 mol/L.

3.2. Immunomodulatory activities of longan polysaccharides

3.2.1. Effects on splenic lymphocyte proliferation

The effects of longan polysaccharides on the proliferation of splenic lymphocytes are shown in Fig. 5. Compared with the control, LPI exhibited no stimulation on lymphocyte proliferation at the doses ranging from 100–400 μg/mL ($P > 0.05$). LPI1 could stimulate the proliferation in the dose range of 200–400 μg/mL ($P < 0.05$). LPI2 also had a significant stimulation on the proliferation in 100–400 μg/mL ($P < 0.05$), and its effect was in a dose-dependent manner. According to the proliferation stimulated by ConA, LPI and LPI1 manifested depressant effects on the proliferating system induced by ConA, and the effects were in a dose-dependent manner. LPI2 significantly stimulated the ConA-induced proliferation at 100 μg/mL ($P < 0.05$), and depressant effects were found at higher dose ($P < 0.05$).

3.2.2. Effects on NK cell cytotoxicity

The effects of longan polysaccharides on the cytotoxicity of NK cells are shown in Fig. 6. Compared with the control, both LPI1 and LPI2 exhibited significant enhancing effects on NK cell cytotoxicity in the dose range of 100–400 μg/mL ($P < 0.05$), but LPI was ineffective in this range ($P > 0.05$). The effects of LPI1 and LPI2 were close, and obviously better than that of LPI. Moreover, they both had the optimal dose of 200 μg/mL.

3.2.3. Effects on macrophage phagocytosis

The effects of longan polysaccharides on the phagocytosis of macrophages are shown in Fig. 7. Compared with the control, both LPI and LPI1 could significantly enhance the phagocytosis at 100 μg/mL ($P < 0.05$), and their effects were not found at higher dose ($P > 0.05$). LPI2 exclusively showed the enhancing effect on phagocytosis at 200 μg/mL ($P < 0.05$). The effect of LPI2 was significantly better than that of LPI and LPI1 at 200 μg/mL ($P < 0.05$), but no significant difference has been found among them at other doses.

4. Discussion

Polysaccharides in aqueous solution exhibit various conformations, such as sphere-like, random coil, semi-flexible chain, stiff chain, and helical chain including single-helix and triple-helix (Yang & Zhang, 2009; Zhang, Li, Wang, Zhang, & Cheung, 2011). The red shift of λ_{max} of LPI–Congo red complex was similar to that of curdlan–Congo red complex, indicating the existence of

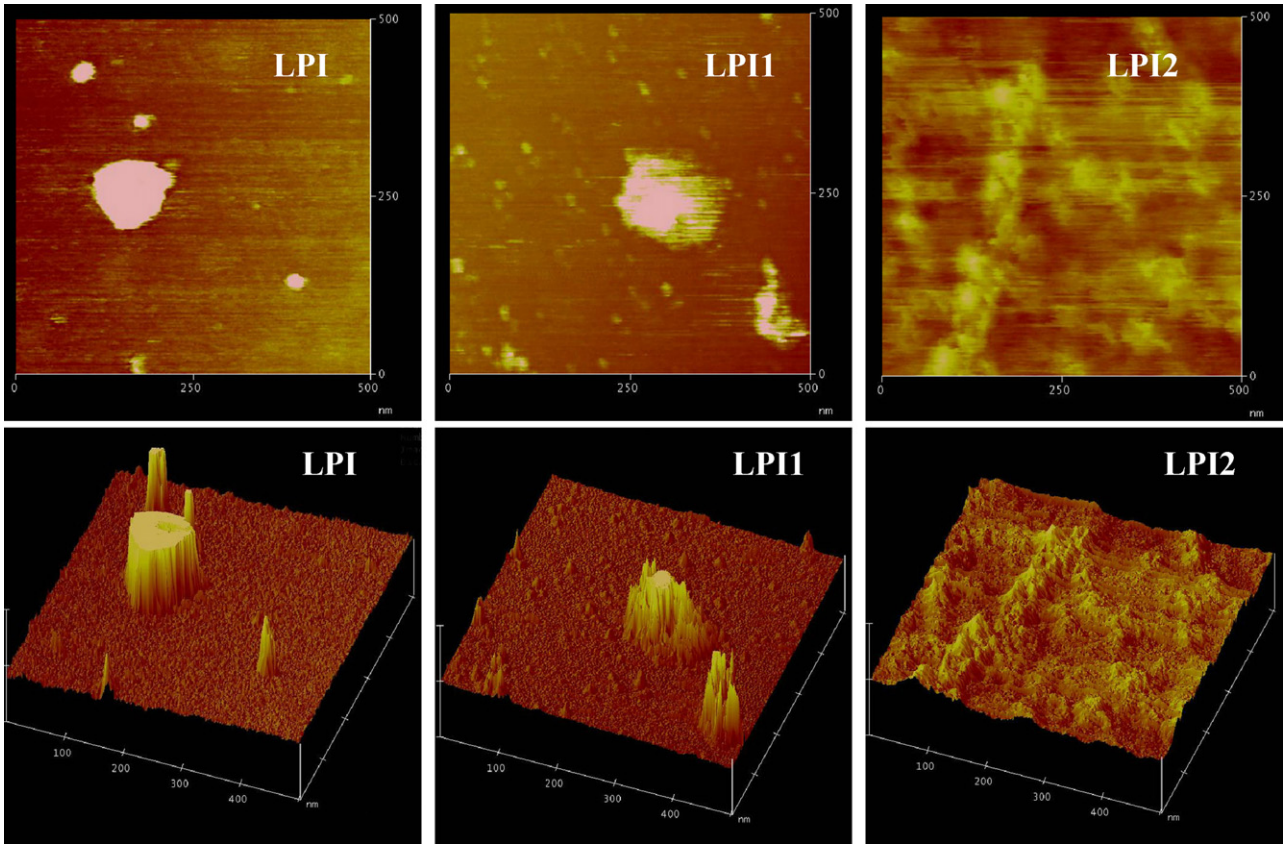

Fig. 4. AFM images of longan polysaccharides in distilled water. 5 μL of sample (concentration: 1×10^{-3} mg/mL) was air dried onto mica and imaged.

triple-helix in LPI (Lee et al., 2010; Qin et al., 2002). The increase of λ_{max} of LPI–Congo red complex at the NaOH concentration range of 0–0.05 mol/L responded to the depolymerization of triple-helix to single-helix, and the further decrease was consistent with the change of single-helix to random coil (Fariña et al., 2001; Liang et al., 1994; Zhang et al., 2010). The changes of λ_{max} on LPI1–Congo red and LPI2–Congo red complexes demonstrated the conformation of single-helix in LPI1 and LPI2. In addition, the v value of $\langle S^2 \rangle_z^{1/2} = kM_w^v$ indicated that LPI had the conformations of sphere-like and rigid rod, and its globular conformation was further confirmed by the AFM image. LPI was mainly composed of two components, in which the component with high molecular mass was sphere-like with triple helical and hyperbranched structure, and another one was rigid rod chain. The dissociation of LPI had positive correlation with NaOH concentration. As the derivative of LPI, LPI1 had the slightly dissociated sphere-like conformation, and LPI2 existed as single-helix chain. Specifically, the initial λ_{max} of LPI1–Congo red was close to that of LPI–Congo red at 0.25 mol/L NaOH, but the initial λ_{max} of LPI2–Congo red was obviously higher than that of LPI–Congo red at 0.50 mol/L NaOH. Moreover, the initial λ_{max} of LPI1–Congo red and LPI2–Congo red was close. It implied that the depolymerization of triple-helix to single-helix was irreversible, but the depolymerization of single-helix to random coil was reversible.

To date, the bioactive polysaccharides with sphere-like conformation are less reported. Polysaccharides and polysaccharide-protein complexes from *Pleurotus tuber-regium* have been confirmed on the presence of sphere-like conformation, and they show antitumor activity *in vitro* (Tao & Xu, 2008; Tao & Zhang, 2008; Tao, Zhang, & Cheung, 2006; Tao et al., 2009). Likewise, water-soluble polysaccharides from the mycelia of *Poria cocos* existed as compact random coil in aqueous solution, which is close to globular shape, and also exhibit antitumor activity (Huang, Jin, Zhang, Cheung, & Kennedy, 2007). In the present work, alkali dissociation could improve the immunological activity of LPI on lymphocytes and NK cells, and that could be due to the changed conformation. Generally, triple-helix chain was essential for the immunological activity of polysaccharides (Falch et al., 2000; Kishida et al., 1992; Ohno et al., 1995; Surenjav et al., 2006; Zhang et al., 2005). For example, the stimulation of scleroglucan on monocytes is markedly weakened with the alkali dissociation of triple-helix chain (NaOH concentration >0.25 mol/L) (Falch et al., 2000). Ultrasonication, DMSO or Me_2SO can modify the conformation of lentinan from triple-helix to single-helix with a decreasing inhibition on Sarcoma 180 tumor cells (Surenjav et al., 2006; Zhang et al., 2005). In addition, Bohn and BeMiller (1995) have indicated that the immunopotentiating activities of (1→3)-β-D-glucans depend on the helical conformation and the presence of hydrophilic groups located on the outside surface of the helix. However, the potency of schizophan on the activation of Factor G has been increased over 100-fold by the treatment of NaOH solution, which leads to a complete or partial conversion from the triple-helix to single-helix conformation (Saitô et al., 1991). Bao et al. (2000) have also confirmed that the single-helical polysaccharides from *Ganoderma lucidum* can stimulate the proliferation of T cells. Furthermore, a polysaccharide from *Cordyceps militaris* exists as a random coil conformation, which can up-regulate the functional events mediated by activated macrophages, such as production of NO and expression of cytokines (IL-1β and TNF-α) (Lee et al., 2010). Therefore, conformation is an important factor for the bioactivities of polysaccharides. The enhanced effects of LPI on lymphocyte proliferation and NK cell cytotoxicity induced by alkali dissociation might depend on single-helix chain. The effects of LPI1 and LPI2 on normal and ConA-induced proliferation indicated that they might selectively enhance the proliferation of B cells (Dourado

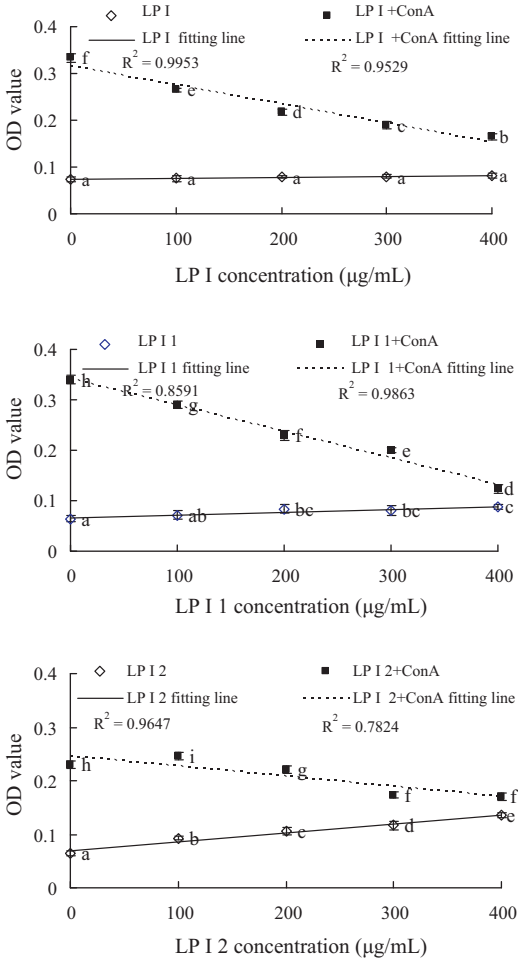

Fig. 5. Effects of longan polysaccharides on the proliferation of splenic lymphocytes. The proliferation was assessed by MTT assay and expressed as means ± standard deviation ($n = 6$). The statistically significant differences among the groups were evaluated with ANOVA, followed by the Student–Newman–Keuls test. Different letters represented the statistical difference at $P < 0.05$.

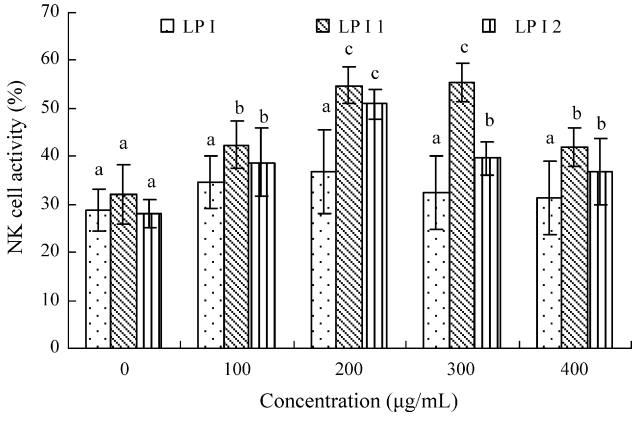

Fig. 6. Effects of longan polysaccharides on the NK cells cytotoxicity. NK cell cytotoxicity was assessed by MTT assay and expressed as means ± standard deviation ($n = 6$). The statistically significant differences among the groups were evaluated with ANOVA, followed by the Student–Newman–Keuls test. Different letters marked on the same sample represented the statistical difference at $P < 0.05$.

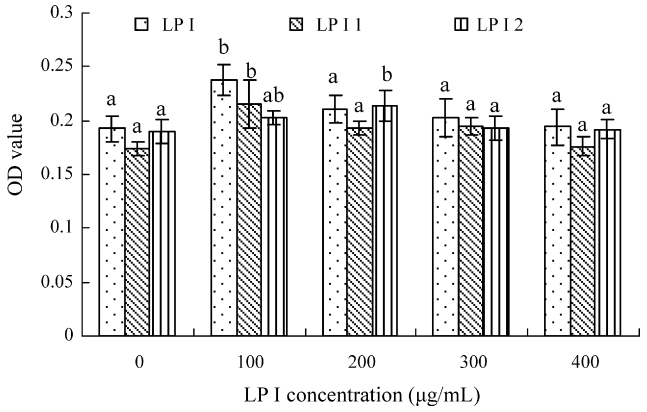

Fig. 7. Effects of longan polysaccharides on the phagocytosis of peritoneal macrophage. The activity was evaluated by the phagocytosis ratio of neutral red, and expressed as means ± standard deviation ($n = 6$). The statistically significant differences among the groups were evaluated with ANOVA, followed by the Student–Newman–Keuls test. Different letters marked on the same sample represented the statistical difference at $P < 0.05$.

et al., 2004; Kim et al., 2005; Zhang, Tang, Zimmerman-Kordmann, Reutter, & Fan, 2002).

The relationship between molecular conformation and immunomodulatory activity was first investigated on longan polysaccharide in our present work. Longan pulp polysaccharide LPI, which contained the conformations of sphere-like and rigid rod, exhibited no immunostimulating effect on splenocytes and NK cells in the dose range of 100–400 μg/mL. As the alkali dissociated derivatives of LPI, LPI1 or LPI2 could significantly enhance splenocyte proliferation and NK cell cytotoxicity due to the presence of slightly dissociated sphere-like conformation or single-helix chain. All of them could significantly enhance macrophage phagocytosis at 100 or 200 μg/mL. The results indicated that single-helix chain importantly contributed to the activation of lymphocyte and NK cell, but not for macrophage. Moreover, the results showed a simple and effective method to improve the immunomodulatory activity of longan polysaccharide as a potential immunoadjuvant.

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