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ScienceDirect
Food Chemistry 106 (2008) 269–276
Food Chemistry
www.elsevier.com/locate/foodchem

Chemical composition and immuno-stimulating properties of polysaccharide biological response modifier isolated from *Radix Angelica sinensis*

Xingbin Yang [a,*], Yan Zhao [b], Guoli Li [a], Zhezhi Wang [a], You Lv [a]

[a] *College of Life Sciences, Key Laboratory of Ministry of Education for Medicinal Plant Resource and Pharmaceutical Chemistry, Shaanxi Normal University, Xi'an 710062, China*
[b] *Faculty of Pharmaceutical Sciences, Fourth Military Medical University, Xi'an 710032, China*

Received 20 September 2006; received in revised form 3 March 2007; accepted 31 May 2007

Abstract

To investigate the immunomodulating activity of *Radix Angelica sinensis*, three different *Radix A. sinensis* polysaccharide fractions (APFs, namely APF1, APF2 and APF3) were isolated by fractionation using gel filtration and were identified as the immunomodulators of murine peritoneal macrophages. In the present study, it was found that various APFs induced a significant increase in cellular lysosomal enzyme activity, nitric oxide (NO) formation, reactive oxygen species (ROS) production and tumor necrosis factor-α (TNF-α) secretion in macrophages *in vitro*. Furthermore, APFs dose-dependently stimulated macrophages to produce NO through the up-regulation of inducible NO synthase (iNOS) activity and the maximal effect occurred at a concentration of 500 μg/ml by APF2, followed by APF3 and APF1 in decreasing order. Moreover, the predominant sugars in various APFs were identified as rhamnose, arabonose, glucose, galactose and the ratio of these monosaccharides differed from one polysaccharide fraction to another, which also affected the cellular effector molecule production in macrophages.

Keywords: *Radix Angelica sinensis*; Polysaccharides; Macrophages; Immunoactivity; Effector molecule

1. Introduction

The roots of *Radix Angelica sinensis* (Oliv.) Diels, a well-known oriental herb belonging to the Umbelliferae family, have been used for thousands of years in traditional Chinese medicinal (TCM) prescriptions (Kim et al., 2005; Kim, Bang, Choi, Han, & Kim, 2005; Lao et al., 2004; Lu et al., 2004). They have also been widely used as health foods for women's care in Asia (Tieraona, 2005), and were marketed in Europe and America as a dietary supplement (Deng et al., 2006; Zhao et al., 2003). In recent years, polysaccharides isolated from natural plants have been regarded as an important class of biological response modifiers. Of the plant polysaccharides, the polysaccharides isolated from the roots of *Radix A. sinensis* have drawn the attention of researchers and consumers due to their nutritional and health protective value in gastrointestinal protection (Ye, Koo, Li, Matsui, & Cho, 2001), anti-ulcer (Ye, So, Liu, Shin, & Cho, 2003) and radioprotective action (Mei, Tao, Zhang, Duan, & Chen, 1988). Therefore, the demand for *Radix A. sinensis* is enormous throughout the world and its applied area is being extended.

Polysaccharides isolated from various traditional medicinal plants have been shown to profoundly affect the immune system both *in vivo* and *in vitro*, and therefore have the potential as immunomodulators with a wide application (Jeon, Han, Ahn, & Kim, 1999; Lee & Jeon, 2003; Tzianabos, 2000). Synchronously, it is also well-known that macrophages play a key role in the host defence mechanism

* Corresponding author. Tel.: +86 29 85310266; fax: +86 29 85303736.
E-mail address: xbyang@snnu.edu.cn (X.B. Yang).

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doi:10.1016/j.foodchem.2007.05.085

and many polysaccharides activate immune responses primarily by activation of macrophages, although direct activation of B cells and other immune cells also are implicated (Lee & Jeon, 2005). Activated macrophages release many inflammatory cytokines to exert their biological effects. Nitric oxide (NO), tumor necrosis factor-α (TNF-α) and reactive oxygen species (ROS) as the main effector molecules play critical roles in the non-specific immune defense against tumor and bacterial infection (MacMicking, Xie, & Nathan, 1997; Rojas, Padron, Caveda, Palacios, & Moncada, 1993). However, there are few data published on the effects of immune responses by the polysaccharides from *Radix A. sinensis*. Therefore, the objective of the present investigation was to isolate and purify polysaccharide fractions from *Radix A. sinensis* and further study the effects of the purified polysaccharide fractions on the release of effector molecules such as NO, TNF-α and ROS in macrophages.

2. Materials and Methods

2.1. Materials and reagents

The roots of *Radix A. sinensis* were purchased from Minxian County, Gansu Province, China and identified according to the identification standard of the Pharmacopeia of the People's Republic of China. A gel filtration column of Sephacryl S-400 HR (0.5 cm i.d. × 90 cm) was obtained from Pharmacia Biotech Co. (Shanghai, China). Mannose, rhamnose, glucose, galactose, arabonose, xylose, glucuronic acid, trifluoroacetic acid (TFA), lipopolysaccharide (LPS) and MTT (Thiazolyl blue) were purchased from Sigma (St. Louis, MO, USA). Inositol was purchased from Huamei Biochemistry Co. (Shanghai, China). Triton X-100 was obtained from Amersco Inc. (Solon, OH, USA). N^G-nitro-L-arginine methyl ester (L-NAME) was from Calbiochem-Behring (San Diego, CA, USA). RPMI1640 and fetal bovine serum (FBS) was the product of Gibco (Grand Island, NY, USA). All other chemicals and solvents were of analytical grade.

2.2. Isolation and purification of polysaccharide fractions

The polysaccharide extracts were isolated by hot-water extraction and ethanol precipitation and followed by a further purification with Sephacryl S-400 gel filtration chromatography. In detail, the dried roots of *Radix A. sinensis* (550 g) were defatted with 95% alcohol and then extracted with distilled water (g/ml = 1:10) for 3 h. After each three-hour period of water extraction, the water extracts were collected and the residue was extracted again for three cycles. The combined extracts were pooled, concentrated to 30% of the original volume under a reduced pressure and then centrifuged at 3000 rpm for 15 min. The supernatant was collected and three volume of 95% alcohol was added slowly and stirred to precipitate the polysaccharides, and then the mixture was stored overnight at 4 °C and finally the polysaccharide pellets were obtained by centrifugation at 4000 rpm for 15 min. The polysaccharide pellet was completely dissolved in an appropriate volume of distilled water and intensively dialyzed for 2 days against distilled water (cut-off M_w 8000 Da). The retentate portion was concentrated, deproteinated by freeze-thaw process (BenchTOP, Virtis Co. USA) repeated seven times and then centrifuged to remove insoluble material. Finally, the supernatant was lyophilized to give crude *Radix A. sinensis* polysaccharides with a nearly colorless fluffy shape.

One gram of the crude polysaccharides was dissolved in 0.1 M NaCl and filtered through filter paper (0.45 μm). Then the solution was applied to Sephacryl S-400 gel filtration column chromatography and eluted with 0.1 M NaCl. Eluted fractions (5 ml) were collected and monitored for carbohydrate content based on phenol-sulfuric acid method at 490 nm absorbance. Finally, the eluted fractions were concentrated and lyophilized to yield three white *Radix A. sinensis* polysaccharide fractions (APFs), namely APF1, APF2 and APF3 with different molecular size according to the elution profile.

2.3. Analysis of monosaccharide composition

Neutral monosaccharide composition was analyzed according to the following procedure: the polysaccharide samples (10 mg) were hydrolyzed with 10 ml of 2 M TFA at 110 °C for 6 h to release component monosaccharides. Then the hydrolyzed monosaccharides (inositol as the internal standard) were derivatized by the aldononitrile acetate method (Mawhinney, Feather, Barbero, & Martinez, 1980) and isothermally separated by gas chromatography (GC) in a Agilent 4890D system (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID) and a HP-5 capillary column (15 m × 0.2 mm × 0.33 μm). The operation was performed at a column temperature program from 110 °C to 210 °C at 2 °C/min, holding for 1 min at 210 °C, then increasing to 250 °C at 30 °C/min and finally holding for 10 min at 250 °C. The molar percentage of the component monosaccharides was calculated as follows. The correction factor is shown in the equation: $f_{i/s} = (W_i/W_s)/(A_i/A_s)$, where A_s and A_i are the values of peak areas for inositol and a component monosaccharide of tested samples, respectively. W_s and W_i are the values of weights for inositol and a component monosaccharide of tested samples, respectively. The molar ratio value is shown in the equation: $R_{i/s} = f_{i/s} \times (A_i/A_s)/M$, where A_i/A_s is the ratio value of peak area for the component monosaccharide and inositol. M is the molecular weight of the monosaccharides and $f_{i/s}$ is the correction factor.

2.4. Animals, cell isolation and culture

Female specific pathogen-free BALB/c mice (6–8 weeks old, 17–20 g body weight) were obtained from the Experimental Animal Center of Fourth Military Medical

University. Mice were housed in plastic cages with free access to water and food at 20–25 °C under a 12 h light/dark cycle at least 6 days before experiments. The guidelines for the care of the animals were strictly followed throughout the studies.

Macrophages were prepared from BALB/c mice as described previously (Kim, Choi, Lee, & Park, 2004). Briefly, peritoneal macrophages were harvested from 2 to 3 BALB/c mice, which had been injected intraperitoneally with 3 ml of thioglycollate three days before sterile peritoneal lavage with 10 ml of Hank's balanced salt solution. The collected cells were seeded and cultured in RPMI1640 containing 10% heat-inactivated FBS, 100 IU/ml penicillin and 100 μg/ml streptomycin at a density 2×10^6 cells/well. The cells were allowed to adhere for 3 h to a 96-well culture plate at 37 °C in a 5% CO_2 incubator. Then the cultures were washed twice with RPMI1640 to remove nonadherent cells prior to the addition of 1 ml of fresh RPMI1640 containing 10% FBS. The purity of the adherent macrophages was assessed by Giemsa staining (>95%).

2.5. Measurement of nitrite production as an assay of NO release

NO production was determined indirectly by assaying the culture supernatant for accumulated nitrite, the stable end product of NO reacted with molecular oxygen as previously described (Green et al., 1982). Briefly, murine peritoneal exudate was plated into a 96-well plate at 2×10^6 cells/well and adhered macrophages were cultured with various concentrations of APFs or LPS at 37 °C for 36 h. After treatment, 100 μl of isolated supernatants were allowed to react with Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride and 2.5% phosphoric acid) at room temperature for 10 min. Nitrite products were determined by measuring absorbance at 550 nm versus a $NaNO_2$ standard curve using an enzyme-linked immunosorbent assay (ELISA) reader and the results were shown as μM.

2.6. TNF-α bioassay

Adhered macrophages at 2×10^6 cells/well were incubated with either various concentrations of APFs or LPS for 48 h at 37 °C. After incubation, conditioned supernatants were collected for assaying TNF-α release by determining cytotoxicity using a bioassay of TNF-sensitive L929 cells (Ferrari, Fornasiero, & Isetta, 1990). L929 cells (3×10^5 cells, 100 μl) were cultured with serially diluted supernatants (100 μl) in the presence of actinomycin D (1 μg/ml) for 18 h in 96-well microtiter plates. The cells were washed once with PBS and stained with 0.5% crystal violet in methanol for 15 min. The plates were extensively washed with water, and the dye was extracted with methanol. The percentage of L929 cell inhibition was calculated from the absorbance (A) values at 570 nm as follow: $(A_{\text{untreated control}} - A_{\text{treated}})/A_{\text{untreated control}} \times 100\%$.

2.7. Measurement of enzyme activity and ROS release

Cellular lysosomal enzyme activity was measured according to the procedure of Suzuki et al. (1990). Macrophage monolayers in 96-well culture plates (2×10^6 cells/well) were solubilized by adding 25 ml of 0.1% Triton X-100 and incubated for 30 min at room temperature. Then 150 μl of 10 mM *p*-nitrophenyl phosphate was added to per well as a substrate for acid phosphatase, followed by the addition of 0.1 M citrate buffer (50 μl, pH 5.0). After the incubation for 1 h at 37 °C, 0.2 M borate buffer (50 μl, pH 9.8) was added to the mixture to stop the reaction. Optical densities were measured at 405 nm using an enzyme-linked immunosorbent assay (ELISA) reader.

Inducible NO synthase (iNOS) activity was determined using a Diagnostic Reagent Kit from Jiancheng BioEngineering (Nanjing, China) and the assay procedure was based on the instructions of the kit and optical density was measured at 530 nm (wavelength). One activity unit of iNOS was defined as the production of 1 nmol NO per 1×10^6 cells/min and expressed as U/ml. Reactive oxygen species (ROS) activity was assessed by the method of Fenton reaction and Gress colouration according to the instructions provided by ROS detection kit (Nanjing Jiancheng, China) and was expressed as the relative percentage of the control (Tang, Liu, Wang, & Huang, 2005).

2.8. Statistical analysis

The results were reported as the mean and standard deviation. Statistical differences between the treatment and non-treated groups were assessed by the unpaired Student *t*-test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Influence of APFs on lysosomal enzyme activity in macrophages

Phagocytosis is the first step in the response of macrophages to invading macroorganisms. Lysosomal enzyme and phagocytic activities are crucial aspects of macrophage functional assessments (Jeong, Jeong, Yang, & Song, 2006). In this study, the effects of different polysaccharide fractions isolated from the roots of *Radix A. sinensis* on the cellular lysosomal enzyme activities in murine peritoneal macrophages were investigated and the results are shown in Fig. 1. In preliminary trials, all tested fractions significantly improved the cellular lysosomal enzyme activity of macrophages by 1.5-, 1.3- or 1.4-fold in response to stimulation with 300 μg/ml APF1, APF2 and APF3, respectively ($p < 0.01$ versus control). However, a low dose of LPS (5 μg/ml) as a potent activator of macrophages could yield the same level of response in comparison to APFs (300 μg/ml). From the results it was evident that although cellular lysosomal enzyme

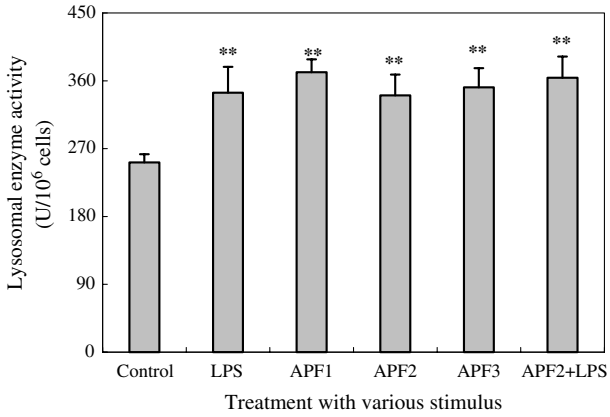

Fig. 1. Cellular lysosomal enzyme activities of murine peritoneal macrophages treated with 300 μg/ml APFs or 5 μg/ml of LPS for 36 h. Saline was used in the negative control and LPS was used as positive group. The macrophage concentration was 2×10^6 cells/well and the lysosomal enzyme activity was calculated as U/l $\times 10^6$ cells. Values are the means of three replicates. $^{**}p < 0.01$, compared with control group.

activities of APFs-treated macrophages were lower than that of LPS-only treatment, APFs also induced strongly macrophage lysosomal enzyme activity. Therefore, we conclude that treatment with APFs might augment innate immune response.

3.2. Effects of APFs on NO production and iNOS activity in macrophages

For further estimating the potential of enhancing innate immune response to APFs stimulation, the concentration dependence of the different polysaccharide fractions on NO production from murine peritoneal macrophages was investigated. As shown in Fig. 2, the three polysaccharide fractions were found to increase NO production by macrophages and the NO release from macrophages was significantly dependent on the employed dosage of APFs. Namely at the concentration range of 0–500 μg/ml, APFs enhanced NO production in a dose-dependent manner and went through a peak at a concentration of 500 μg/ml, and then decreased. The EC_{50} (50% of maximal APFs induction) value was 250 μg/ml. In this study, macrophages treated with APFs produced a high amount of NO at the concentrations as low as 3 μg/ml ($^{*}p < 0.05$) and APF2 proved to be the most potent as an activator of murine macrophage.

However, it was unclear whether the stimulatory effect of APFs on NO production was associated with the up-regulation of iNOS activity. To elucidate the underlying mechanisms of the regulation of NO synthesis by APFs, the change of iNOS activity in APFs-induced macrophages was evaluated. The results from Fig. 3a show that the treatment with APFs also remarkably improved iNOS activity of macrophages in the increasing order of APF1, APF3 and APF2, and the co-stimulated experiment between APF2 and LPS unexpectedly found there did not exist

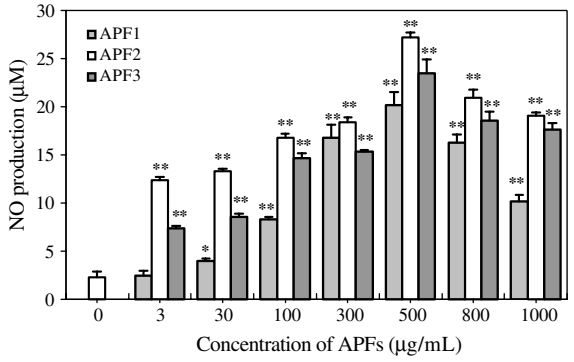

Fig. 2. Concentration dependence of the polysaccharide fractions on NO production by murine peritoneal macrophages. The cells in the normal group were incubated in RPMI1640 medium. The cells in the experimental group were incubated with APF1, APF2, APF3 at the serially diluted concentration of 3, 30, 100, 300, 500, 800, 1000 μg/ml for 36 h, respectively. NO production was determined by measuring the accumulation of nitrite in the medium. Values are the means of three replicates. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group.

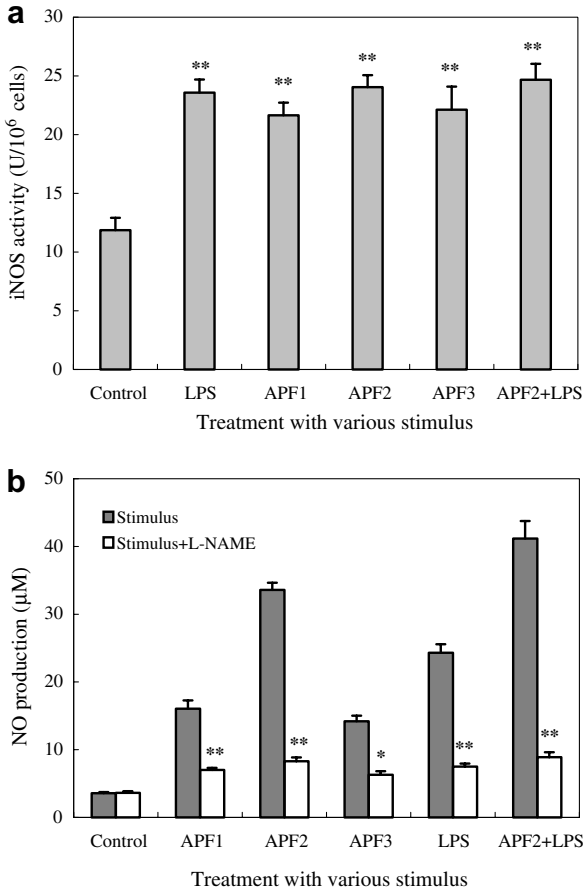

Fig. 3. Effects of APFs on NO production and *i*NOS activity in mouse peritoneal macrophages. (a) Cells were treated with various APFs (300 μg/ml) or LPS (5 μg/ml) for 36 h and iNOS activity were determined as described in Methods. $^{**}p < 0.01$ indicates significant different versus untreated group. (b) Cells were treated with 5 μg/ml LPS or 300 μg/ml APFs as stimulus for 36 h in the absence (■) or presence (□) of L-NAME (500 mM, iNOS inhibitor). $^{*}p < 0.05$, $^{*}p < 0.01$, compared to only stimulus-treated groups.

the synergistical effect although all the APFs in combination with LPS were able to increase iNOS activity in macrophages in comparison with the LPS-only treatment. This increased effect on iNOS activity showed that the augmentation of NO formation in macrophages by APFs was the result of the improvement of iNOS activity. Furthermore, the effect of L-NAME, an inhibitor of iNOS activity, on APFs-stimulated NO production was also evaluated after the cells were incubated with APFs (300 μg/ml) or LPS (5 μg/ml) as stimulus in the presence and absence of L-NAME. As a result, the enhancing effects of APFs or LPS on NO production were markedly inhibited by L-NAME (Fig. 3b), indicating that NO production was associated with the up-regulation of iNOS activity. This was further evident that APFs exhibited a potential inductive effect on iNOS activity in macrophages, resulting in the enhancement of NO release.

3.3. APFs-induced ROS production in macrophages

To understand whether the release of intercellular ROS, a potent macrophage-derived effector molecule, was also involved in the immunostimulatory activity of APFs-activated macrophages, we detected the levels of intercellular ROS in macrophages after treating the cells with APFs for 36 h. As shown in Fig. 4, treatment with 300 μg/ml APFs caused a sharp increase in intracellular ROS level compared to that of the control ($p < 0.01$) and the highest activity was exhibited by APF2 which represented a 5.3-fold enhancement versus the control, followed by APF3 and APF1 in decreasing order. At the same time, no synergistical effect of ROS generation by the co-stimulation of APF2 combined with LPS was observed in macrophages (Fig. 4). The results revealed that APFs could effectively enhance the intracellular ROS production in macrophages.

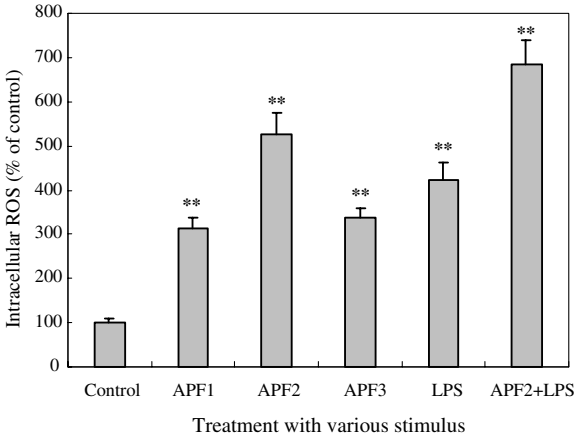

Fig. 4. Intracellular ROS production from murine peritoneal macrophages by APFs. The macrophages (2×10^6 cells/well) were incubated with 300 μg/ml of APFs or 5 μg/ml of LPS and ROS production was determined as described in Methods. $^{*}p < 0.01$, compared to control group.

3.4. TNF-α secretion from macrophages induced by APFs

To further confirm the APFs-mediated activation of macrophages, we also assessed the effect of APFs on TNF-α secretion by macrophages. In this study, adhered cells were cultivated in the presence or absence of APFs and the supernatants were collected at 48 h, and the amounts of TNF-α was measured by a bioassay method. As shown in Fig. 5, the treatment of L929 cells with the supernatants from APFs-stimulated macrophages caused a significant loss of cell viability within the studied concentration range (50–300 μg/ml), indicating that tested APFs dose-dependently increased TNF-α release from macrophages resulting in the augmentation of TNF-α cytotoxicity against L929 cells ($p < 0.05$). Compared with APF1 and APF3, APF2 exerted the highest activity and this result was in agreement with that of APFs-induced NO and ROS production in macrophages. Based on the investigation, it was evident that APFs were the activator of macrophages, and NO, ROS and TNF-α production were strongly modulated by APFs.

3.5. Composition and characterization of APFs

In this study, the isolated crude polysaccharides were further purified by Sephacryl S-400 gel permeation chromatography into the three fractions, namely APF1, APF2 and APF3 (Fig. 7). The yield of crude polysaccharide extracts was 9.8% of the plant raw material, and the yields of the purified polysaccharide fractions were 14.3% for APF1, 61.5% for APF2 and 21.2% for APF3 of the parent crude polysaccharide extract, respectively (Table 1). Furthermore, the co-extracted proteins in the polysaccha-

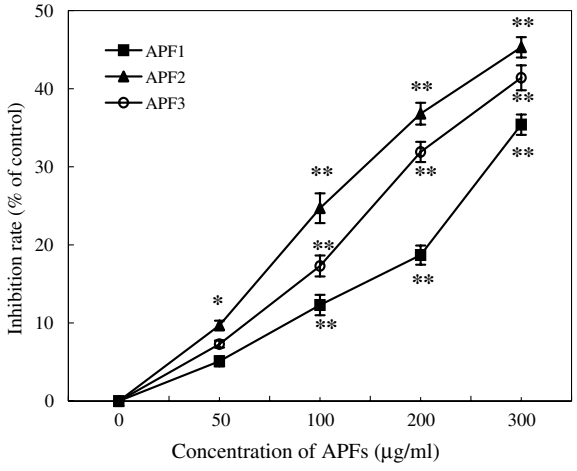

Fig. 5. Inhibition rate of L929 cells treated with the supernatants from APFs-stimulated macrophages. Macrophages were cultured with various concentration of APF1, APF2 and APF3 for 48 h, respectively and TNF-α release from macrophages was measured by a bioassay using L929 cells. Inhibition rate of L929 cells was expressed and calculated as relative percentage to the control incubated without APFs. Values are the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{*}p < 0.01$, compared to control group.

Table 1
Monosaccharide composition, protein content and yield of the crude polysaccharide extract and purified fractions obtained from *Radix A.sinensis*

Sample	Sugar content (mol.%)[a]						Protein (wt%)[a]	Yield (wt%)
	Rhamnose	Arabonose	Xylose	Mannose	Glucose	Galactose		
Crude	9.3	25.3	Tr[b]	6.7	37.2	21.5	2.7	9.8[c]
APF1	7.3	16.5	ND[b]	ND	56.7	19.5	ND	14.3[d]
APF2	4.1	21.8	ND	15.1	37.6	21.3	ND	61.5[d]
APF3	3.7	16.8	Tr	11.0	41.0	27.5	2.4	21.2[d]

[a] Protein content was calculated as wt%, quantities of the neutral sugars was given in mol.%.
[b] Tr indicates trace amount; ND indicates not detected.
[c] Value is expressed as wt% of the plant raw material.
[d] Value is expressed as wt% of the parent crude extract.

rides were checked according to the Lowry method using bovine serum albumin (BSA) as the standard (Lowry, Rosebrough, Farr, & Randall, 1951) and the results revealed that APF3 contained 2.7% protein whereas APF1 and APF2 were not contaminated with proteins. In the study on immunomodulatory activity, it was unexpectedly found that APF3 with proteins easily produced the flocculent precipitate and a decrease in bioactivity was observed, indicating that APF3 possessed lower stability in comparison to APF1 and APF2. Therefore, the present study also was set up to investigate the effect of co-extracted proteins on NO production from macrophages. As shown in Fig. 6, although the treatment with pronase did not destroy APF3 induced macrophage-activation, pronase-treated APF3 induced a low amount of NO compared to untreated APF3 ($p < 0.01$), where protein-bound APF3 unexpectedly possessed a higher activity than APF2 ($p < 0.05$). In order to obtain a stable bioactivity and confirm that the active component of APF3 was a polysaccharide, the proteins in APF3 were destroyed with pronase in all the presented experiments.

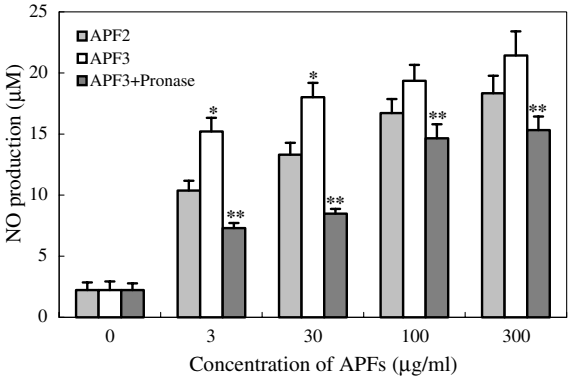

Fig. 6. Effects of bound-protein in APF3 on NO production in macrophages. Proteins contained in APF3 were digested with pronase and the remaining polysaccharides were lyophilized after extensive dialysis. The indicated concentration of APF3 without pronase treatment (■) or with pronase treatment (□) and indicated concentration of APF2 were dissolved in PBS (pH 7.2), and added to the cultures of macrophages for 36 h. The amounts of nitric oxide were measured using a Griess reagent. Date represent means ± SD of three independent samples and $^{*}p < 0.05$, compared to APF2; $^{*}p < 0.01$, compared to untreated group.

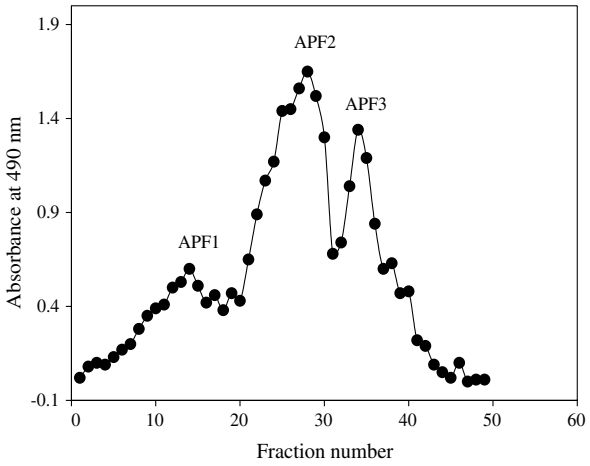

Fig. 7. Gel filtration chromatogram of the crude polysaccharides on Sephacryl S-400 column (5 × 90 cm). The crude polysaccharides were dissolved in 0.1 M NaCl solution and applied to the column. The eluted solution was collected and the carbohydrate content of collected fractions was monitored by phenol-sulfuric acid method, respectively.

Moreover, composition analysis of polysaccharides is an important step in controlling quality standard and gives basic information on the polysaccharides. GC analysis with derivatization is an acknowledged method for the quantification of neutral sugars. In this study, APFs were hydrolyzed with TFA and then GC analysis of hydrolysates was performed by precolumn-derivatization techniques using aldononitrile acetate to identify the component monosaccharides released from the polysaccharide (Table 1). The results of GC quantitative analysis with good linearity ($r > 0.99$) revealed that each polysaccharide fraction contained similar neutral monosaccharide composition of rhamnose, arabonose, glucose, galactose and the ratio of these monosaccharides greatly differed from one polysaccharide fraction to another. Among APF1, APF2 and APF3, it was found that mannose was only found in APF2 and APF3, but not present in APF1. Arabonose was a predominant sugar in APF1, but as a minor sugar for APF2 and APF3. Additionally, glucose was a major sugar in APF2 and APF3, but it was present as a minor sugar in APF1. Concretely, the crude polysaccharide extracts consisted of rhamnose, arabonose, mannose, glucose and galactose with the molar ratio of 1.00:2.72:0.72:4.00:2.32; APF1 consisted of

rhamnose, arabonose, glucose and galactose with the molar ratio of 1.00:2.27:7.80:2.69; APF2 and APF3 consisted of rhamnose, arabonose, mannose, glucose and galactose with the molar ratio of 1.00:5.29:3.66:9.11:5.17 and 1.00:4.54:2.98:11.09:7.45, respectively. Furthermore, total uronic acid content was measured by *m*-hydroxydiphenyl method using glucuronic acid as the standard (Filisetti-Cozzi & Carpita, 1991), and the results showed that the good linearity was obtained by regression analysis between A (absorbency) and C (μg/ml) and regression equation is as follow: $A = 0.015569C - 0.0026$ $(r = 0.9999)$ at 10.0–60.0 μg/ml. APF2 contained the highest uronic acid content (39.2%) in comparison with APF1 (28.4%) and APF3 (34.6%).

4. Discussion

Food constituents possess not only the nutritional value but also the physiological effects by modulating the immune, nervous and endocrine systems. Therefore, the herbal medicines have been frequently used in the treatment of several diseases such as autoimmune diseases, inflammations and allergies, which are difficult to be cured by western medicines. In the present investigation, we isolated three different polysaccharide fractions from *Radix A. sinensis* and they were identified as the immunomodulators of macrophages. Macrophage is one of the research foci of the immunology community and exhibits cytotoxicity by phagocytosis, direct cellular cytotoxicity through cell-to-cell contact and the secretion of cytotoxic cytokines (IL-1, TNF-α) and ROS such as O_2^-, H_2O_2 and NO (Mateo, Reichner, & Albina, 1996; Remer, Reimer, Brcic, & Jungi, 2005). In this work, APFs induced a significant increase in cellular lysosomal enzyme activity, NO formation, ROS production, and TNF-α secretion in macrophages and the maximal effect occurred by APF2, followed by APF3 and APF1 in decreasing order.

Human health is related to immune responses, but an inappropriate prolongation of inflammation will cause diseases (Kuo et al., 1998). Therefore, the use of immunomodulators must be cautious and discreet. In this study, we found that treatments of macrophages with APFs showed significant difference compared with the untreated macrophages ($p < 0.05$), but cellular lysosomal enzyme activity, NO formation, ROS production, and TNF-α secretion in APFs-only treated macrophages were relatively low compared with an LPS-only treatment, indicating that APFs themselves were milder immunostimulants. However, in this study all the various APFs in combination with LPS were able to increase the cytokine response (TNF-α) and NO and ROS production from macrophages in comparison with the LPS-only treatment. This finding suggested that APFs from *Radix A. sinensis* might be immunity enhancers and play important roles against bacterial infection and in anti-tumor activities (Chen, Hsu, Lin, Lai, & Wu, 2006). However, *Radix A. sinensis* polysaccharides had no direct cytotoxicity to tumor cells, but the cultural supernatant of macrophages treated with APFs could kill L929 cells and APFs could also promote the releases of NO and ROS by macrophages, indicating that *Radix A. sinensis* polysaccharides may indirectly play the role of anti-tumors through the releases of effector molecules such as TNF-α, NO and ROS by macrophages. Therefore, APFs-mediated activation of macrophages and production of various effector molecules may contribute to its anti-tumor activity.

5. Conclusion

The study presented here has demonstrated that the component sugar within the APFs differed from one polysaccharide fraction to another, which also affected the cellular effector molecule production in murine peritoneal macrophages. In addition, APFs-stimulated macrophages to produce NO through the induction of iNOS activity and could be used for milder immunostimulants. Such information would facilitate the use of *Radix A. sinensis* polysaccharides in food, pharmaceutical and other technical applications, which would contribute towards the sustainable use of plant resources.

Acknowledgements

This work was supported by the science and research foundations of Shaanxi Province and Shaanxi Normal University, China (No. SF200224; No. 2002K10-G2).

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