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Structural features and immunological activity of a polysaccharide from *Dioscorea opposita* Thunb roots

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Abstract

A new polysaccharide (YP-1) from a variety of Chinese yam, *Dioscorea opposita* Thunb, having health benefits was extracted by water and further successively purified through DEAE-Cellulose A52 and Sephadex G-100 columns. YP-1 contained glucose, mannose and galactose in the molar ratio of 1:0.37:0.11. Its molecular weight was determined to be 42 kDa by high-performance gel-permeation chromatography. Structure features of the purified polysaccharide were investigated by a combination of chemical and instrumental analysis, such as methylation analysis, Smith degradation, GC–MS, ^{13}C and ^{1}H NMR and FT-IR. The results indicated that the polysaccharide has a backbone of (1→3)-linked α-D-glucopyranosyl residues, which occasionally branches at O-6. The branches were mainly composed of (1→2)-linked α-D-mannopyranosyl residues, and terminated with β-D-galactopyranosyl residues. Preliminary tests in vitro revealed that YP-1 could stimulate ConA-induced T lymphocyte proliferation and its branches are extremely important for the expression of the enhancement of the immunological activity.

Keywords: *Dioscorea opposita* Thunb; Polysaccharide; Structure; Immunological activity

1. Introduction

Dioscorea opposita Thunb, a variety of Chinese yam, is a well-known edible and traditional medicinal plant available in most of China. Various parts of the Chinese yam have been used in different prescription for different purposes. To elucidate the medical mechanism of Chinese yam, much research has been carried out on the low molecular weight substances, such as alkaloids, allantoin, diosgenin, dopamine, present in various parts of this plant (Araghiniknam, Chung, Nelson-White, Eskelson, & Watson, 1996; Iwu, Okunji, Akah, Tempesta, & Corley, 1990; Zava, Dollbaum, & Blen, 1998; Zhao & Wang, 2000). In recent years, plant derived nonstarchy polysaccharides have emerged as an important class of bioactive natural products. A wide range of polysaccharides has been reported to exhibit a variety of biological activities (Zhao, Chen, Li, & Kan, 2000), such as anti-tumor (Saima, Das, Sarkar, Sen, & Sur, 2000), immunostimulation (Tzianabos, Wang, & Kasper, 2003), anti-complement (Yamada, 1994), anti-inflammation (Scheppach et al., 2004), anti-coagulation (Hussein, Helmy, & Salem, 1998; Sen et al., 1994), and anti-oxidation (Liu, Ooi, & Chang, 1997). One particular interesting feature of Chinese yam root is that on being cut or squeezed it exuded a significant amount of milk white viscous gum. Several published papers tested this viscous gum for different biological activities (Farombi, Britton, & Emerole, 2000; Hikino et al., 1986). In our early research, a polysaccharide, named as YP-1, was isolated and purified from the root of this plant using water extraction, DEAE-Cellulose A52 column chromatography and Sephadex G-100 size-exclusion chromatography (Zhao, Li, & Chen, 2002). Biological activity tests also showed that YP-1 has a strong effect in immunostimulation and anti-tumor activity in cancer bearing murine models (Zhao, Chen, Li, & Wang, 2003). Since, structure and functions are intimately related, an in-depth study of structure of the polysaccharides present therein would be of interest. Therefore, the aim of this research is to investigate the structural features of this polysaccharide. Moreover, the possible immunomodulatory

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effect of the polysaccharide was studied in vitro by a ConA-induced lymphocyte proliferation test.

2. Experimental

2.1. Materials

In Novermber 1999, 13 kg of fresh roots of *D. opposita* Thunb, cultivated in Chongqing City of People's Republic of China, was harvested and identified at department of Botany, College of horticulture, South west Agricultural University, by comparison with the authenticated species. The fresh roots were air-dried at 70 °C and ground into a powder. The moisture content of the fresh roots of *D. opposita* Thunb was 89±3% (mean±SD). T-series Dextran, DEAE-Cellulose A52, and Sephadex G-100 were purchased from Pharmacia Co. (Sweden). Trifluoroacetic acid (TFA) and monosaccharide standards were from Merck (Germany). Concanavalin A (ConA), polymyxin B and fetal bovine serum were from Sigma Chemical Co. (USA), and Medium RPMI-1640 was purchased from Miltenyi Biotechnology GmbH (Germany). All other chemicals and reagents used were of analytical reagent grade.

2.2. Extraction and purification of polysaccharide

The dried *D. opposita* Thunb root powder (200 g) was extracted with 85% ethanol (300 ml, ×3) at 70 °C for 4 h to remove the pigments and free sugars, and the supernatant was removed. The residue was extracted with water (800 ml, ×4) at room temperature for 3 h, the solid removed by centrifugation (5000*g*), the supernatant was precipitated with ethanol (1:5, v/v), and the precipitate, collected by centrifugation, was suspended in distilled water, followed by removal of the protein by the Sevag method (Staub, 1965), and exhaustively dialyzed against water, then dried in vacuum to obtain the crude yam polysaccharide (CYP, 5.4 g). The brownish crude product was further washed with 80% aqueous ethanol acidified with HCl (1%, v/v). The polysaccharide was loaded onto a column (2.6×30 cm) of DEAE-Cellulose A52 and irrigated successively with distilled water. The largest, water-eluted fraction, after concentration, was collected and loaded onto a gel filtration column (1.6×70 cm) of Sephadex G-100 and eluted with 0.1 M NaCl, the eluate in 0.1 M NaCl, homogeneous by high-performance gel-permeation chromatography (HPGPC), was concentrated and lyophilized to get a white powder yam polysaccharide (YP-1, 2.7 g).

2.3. Analytical methods

Concentrations were performed under diminished pressure at a bath temperature not exceeding 45 °C. Carbohydrate content was measured by the phenol–sulfuric acid method, using D-glucose as the standard (Dubois, Gilles, Hamiliton, Rebers, & Smith, 1956). Paper chromatography (PC) and gas chromatography (GC) were used for identification and quantification. PC was performed on Xinhua No. 1 paper in the following solvent system: 5:5:1:3 EtOAc–Pyridine–HOAc–H_2O, the sugars were identified by spraying with phthalic acid reagent (0.9 ml of aniline and 1.6 g of phthalic acid were dissolved in 100 ml of water-saturated *n*-butanol) and heating at 100 °C for about 15 min. Total hydrolysis of polysaccharide containing *myo*-inositol as an internal standard was performed with 2 M TFA at 120 °C for 2 h. The sugars in the hydrolysate were converted to their alditol acetates and analyzed by GC on a Shimadzu GC-9A Chromatograph equipped with an OV-225 capillary column (0.22 mm×25 m) (WGA, Dusseldorf, FRG) at a temperature program of 50–230 °C with a rate of 2 °C/min (Albersheim, Nevins, English, & Karr, 1967). Helium carrier gas at 1.2 ml/min, flame ionization detector (FID) at 270 °C. Uronic acid content was determined according to a *m*-hydroxydiphenyl colorimetric method in which neutral sugars do not interfere (Filisetti-Cozzi & Carpita, 1991). Optical rotation of polysaccharide (8 mg/ml) was measured at 20±1 °C using a Perkin–Elmer (Model 341) polarimeter. IR-spectra of polysaccharide (KBr or Nujol pellets) was recorded with a FT-Jh 170 SX spectrophotometer (Nicolet). The homogeneity and molecular weight of the polysaccharide were evaluated and determined by HPGPC method on a Waters (244) instrument with a Shodex sugar KS-804 column (Showa Denkko) using an E-linear (Waters associates) and eluted with triple-distilled water (1.0 ml/min). The column was calibrated using the T-series Dextran standards (T10, T20, T70, T110, T500, and T2000). Elemental composition of polysaccharide was measured using a Vario EL instrument (German Elementar).

2.4. Methylation analysis

Methylation of YP-1 was carried out three times by the method of Hakomori as described early (Zhang, 1999). Five milligrams sample was weighted into a glass tube dried overnight over phosphorus pentoxide in vacuum. To the sample, 2 ml of dry dimethyl sulphoxide (DMSO) was added and the tube was immediately sealed with a rubber plug and the air removed with a flow of dried argon. The mixture was stirred, sonicated occasionally when necessary, for 5 h at room temperature until it was fully dispersed. Hakomori reagent (1.5 ml), prepared and stored as described (Mukerjea, Kim, & Robyt, 1996), was added with a dried syringe. The solution was stirred for 1 h at room temperature then cooled in an ice bath and the cold methyl iodide (1 ml) was added dropwise as the methylating agent. The resulting solution was consecutively stirred for 1 h. The excess methyl iodide was removed under an air flow and distilled water (2 ml) was added to the tube. The methylated polysaccharide was extracted with chloroform (1 ml, ×2), and the extract was twice washed with distilled water. After dried over anhydrous sodium sulfate for 24 h, the extract

was evaporated to remove chloroform, and the residue was dried in vacuum resulting in a brown solid. The methylated polysaccharide was examined by IR spectrometry. Complete methylation was confirmed by the lack of a hydroxyl peak. The completely methylated polysaccharide was subjected to 1 ml formic acid, sealed in a nitrogen suffused glass tube, kept at 100 °C for 6 h, formic acid was removed by evaporation, dried in vacuum desiccator, then hydrolyzed in 2 M TFA at 100 °C for 6 h, treated with methanol then evaporated to remove TFA. The remains was mixed with 2 ml water, reduced with sodium borohydride (5 mg) for 5 h, then neutralized with 0.1 M acetic acid and dried in vacuum followed by acetylation with acetic anhydride–pyridine (1:1, 2 ml) at 100 °C for 1 h. The resulting alditol acetates were subjected to GC and GC–MS analysis. Linkages were identified on the basis of relative retention time and fragmentation pattern (Gorin, 1981; Jansson, Kenne, Lindberg, & Lonngren, 1976; Needs & Selvendran, 1993). The quantification for molar ratio for each sugar was calibrated using the peak area and response factor of the FID in GC. A HP 5890 GC interfaced to a HP 5988A mass selective detector was used for mass spectral identification of the GC components at 70 eV ionization energy. The samples were fractionated by HP 5890 GC (Hewlett–Packard, Avondale, PA, USA) with an OV-225 capillary column (0.22 mm × 25 m) (WGA, Dusseldorf, FRG) programmed from 50 to 230 °C at 2 °C/min. Helium carrier gas at 1.2 ml/min, flame ionization detector at 270 °C. The partially methylated alditol acetates were identified on the basis of their published mass spectra (Gorin, 1981; Jansson et al., 1976).

2.5. Periodate oxidation–Smith degradation

For analytical purpose, 10 mg of the polysaccharide was allowed to swell overnight in distilled water (10 ml), dispersed using a blender, and on addition of 30 mM $NaIO_4$ (10 ml), an immediate loss of viscosity occurred. The solution was kept in the dark at 4 °C, 0.4 ml aliquots were withdrawn at 6 h intervals, diluted to 100 ml with distilled water and read in a spectrophometer at 223 nm (Linker, Evans, & Impallomeni, 2001). Complete oxidation, identified with a stable absorbance, was reached in 72 h. Consumption of HIO_4 was measured by a spectrophotometric method (Aspinall & Ferrier, 1957), and formic acid production was determined by titration with 0.086 M NaOH. Glycol (2 drops) was added to consume the excess HIO_4 and end the oxidation. The rest of the periodate product was exhaustively dialyzed against tap water (48 h) and distilled water (24 h), respectively. The nondialysate was concentrated and reduced with sodium borohydride (40 mg, 24 h) at room temperature, and then the pH of the solution was adjusted to 5.0 by adding 0.1 M acetic acid, dialyzed against distilled water (24 h) and the nondialysate was dried in vacuum. The above product was hydrolyzed with 2 M TFA (3 ml) at 100 °C for 12 h. GC analyzed the components of this polyalcohol as the alditol acetate under the same conditions as those used for the monosaccharide composition analysis.

2.6. Mild hydrolysis of polysaccharide

The polysaccharide (100 mg) was dissolved in 0.05 M TFA (30 ml), and kept at 100 °C for 1 h. After TFA was removed by evaporation, the remains was dissolved in water and dialyzed against distilled water (1000 ml, ×4). The dialyzable fraction was concentrated, hydrolyzed and its monosaccharide composition was analyzed by GC as the alditol acetate after hydrolysis. The nondialysate was precipitated with ethanol and washed with methanol up to disappearance of low molecular weight saccharides in washing solution, which was monitored by PC. The product was dissolved in distilled water and purified by molecular-sieve chromatography on Sephadex G-100 column to give a pure fragment HYP-1 as a single and symmetric peak eluted with distilled water. HYP-1 was subjected to monosaccharide composition analysis and methylation analysis as described above.

2.7. ^{13}C and ^{1}H NMR spectrum

Thirty milligrams of the polysaccharide was dissolved in D_2O (0.5 ml). The ^{13}C NMR and ^{1}H NMR spectra were recorded with 5 mm tubes at room temperature with a Brucker AM-400 NMR spectrometer. All the chemical shifts were in relative to Me_4Si.

2.8. Immunobiological activity assay

Male Kunming mice (8–12 weeks old) were purchased from Experimental Animal Center of Lanzhou Biological Products Institute. All mice were kept at the animal facilities under specific pathogen-free condition until used. Sterile food and water were supplied.

Spleen cells of mice were obtained by gently teasing the organ in RPMI-1640 medium supplemented with penicillin (100 IU/ml), streptomycin (50 μg/ml), 2-mercaptoethanol (0.05 M), polymyxin B (50 μg/ml) and 10% of fetal bovine serum. To isolate mononuclear cells, 5 ml aliquots of the spleen cell suspension were layered onto 2.5 ml aliquots of a polysucrose–sodium ditrizoate solution and centrifuged at 110*g* for 20 min at room temperature. Mononuclear cells were gently removed from the interface between medium and histopaque and transferred to a sterile container and washed with RPMI-1640. At last, the cells were resuspended in 5 ml RPMI-1640 medium, and subjected to a cell count.

To evaluate the immnuobiological activity of the polysaccharide, mononuclear cell suspension was piped on 96-well plates (200 μl/well) and cultured for 42 h at 37 °C in a humid saturated atmosphere containing 5% CO_2. Plated cells were stimulated with 5 μg/ml of concanavalin A

(control), and 50, 150, or 250 μg/ml of the polysaccharide, respectively. ^{3}H-TdR (37 kBq, 25 μl) was added to each well, and the plates were further incubated for another 4 h at the same condition, and then the resolver (100 μl/well) was added. The proliferation of cells was determined by the ^{3}H-TdR-incorporation method (Leung, Fung, & Choy, 1997).

The data were represented as mean ± SD of triple results. Student's *t*-test for unpaired observations between control and tested samples was carried out to identify statistical differences.

3. Results and discussion

3.1. Isolation and purification of polysaccharide

The crude polysaccharide CYP obtained from the water extract of ethanol-insoluble *D. opposita* Thunb roots contained 84% of neutral carbohydrate and 3.4% of protein in 2.7% yield. After fractionation on a DEAE-Cellulose A52 anion-exchange column, a fraction obtained in water eluate, representing 63.1% of the loaded material, was further purified by gel filtration and gave a homogeneous yam polysaccharide (YP-1) in 0.1 M NaCl eluate confirmed by HPSEC, representing 78.9% of the loaded material. The analytical characteristics of YP-1 are given in Table 1. As seen, YP-1 consisted of glucose, mannose and galactose with a molar ratio of 1:0.37:0.11. As determined by *m*-hydroxydiphenyl colorimetric method and GC, the polysaccharide did not contain uronic acid. Elemental analysis also indicated it as a neutral polysaccharide. The relatively high positive value of optical rotation (+188.4°) suggested the dominating presence of α-form glycosidic linkages in YP-1. On HPGPC, YP-1 gave a single, sharp and symmetric peak. Based on Dextran series calibration, the average molecular weight was determined to be 42 kDa.

3.2. Structural features of the polysaccharide

The interglycosidic linkages between monosaccharide residues of YP-1 were investigated by using the Hakomori methylation procedure. Three successive methylations were performed on YP-1 to improve the yield of fully methylated polysaccharide. After hydrolysis and alditol acetate derivatization, partially methylated alditol acetates were analyzed by GC and GC–MS (Bjorndal, Lindberg, & Svensson, 1967). Five compounds were identified on the OV-225 column. All of the peaks were homogeneous. A relatively good agreement was also found between molar ratios of methylated alditol acetates and that of their parent sugars measured by direct analysis (see Table 2). Combined with the corresponding MS spectra, they were identified as terminal, 1,3-, and 1,3,6-linked glucose, terminal galactose, and 1,2-linked mannose, respectively. Methylation analysis (Table 2) of YP-1 revealed its slightly branched nature. Around 10% of glucose was branched. It was also found that all of the galactose residues are present as terminal residues, and the number of them is equal to that of the branched glucose residues, which suggested that all galactose residues located at the end of the branches and all sugar residues appeared to have been completely methylated. The proportion of terminal glucose units was very low indicating a degree of polymerization about 260, in accordance with the mean molecular weight (42 kDa) of the polysaccharide estimated by HPSEC. The remainder glucose residues, as 1,3-linked, certainly constitute the backbone of YP-1.

Table 1
Properties and sugar composition of the polysaccharide

Characteristics		Characteristics	
Total sugar (wt%)	100	Carbon (wt%)	43.02
Glc (mol%)	67.9	Hydrogen (wt%)	6.10
Man (mol%)	24.9	Oxygen (wt%)	50.88
Gal (mol%)	7.2	Nitrogen (wt%)	0
Uronic acid (wt%)[a]	0	Protein (wt%)[b]	0
$[\alpha]_D$	188.4	Mr (kDa)[c]	42

[a] Determined by *m*-hydroxydiphenyl colorimetric method.
[b] Indicated by absence of the absorbance at 280 nm.
[c] Molecular weight determined by HPGPC.

Table 2
The results of methylation analysis of YP-1

Peak no.	Methylated sugar	Molar ratio	T_R[a]	Linkage type
1	2,3,4,6-Me_4-Glc*p*	Trace	1.00	α-Glc*p*-(1→
2	2,3,4,6-Me_4-Gal*p*	2	1.19	β-Gal*p*-(1→
3	2,4,6-Me_3-Glc*p*	17	1.82	→3)-α-Glc*p*-(1→
4	3,4,6-Me_3-Man*p*	7	1.84	→2)-α-Man*p*-(1→
5	2,4-Me_2-Glc*p*	2	4.21	→3,6)-α-Glc*p*-(1→

[a] Retention time compared with that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.

The FT-IR spectrum of YP-1 is shown in Fig. 1. The band in the region of 3430 cm^{-1} is due to the hydroxyl stretching vibration of polysaccharide. The bands in the region of 2932 cm^{-1} are due to C–H stretching vibration and the bands in the region of 1646–1693 cm^{-1} are due to associated water. Absorptions at 924.71 and 808.87 cm^{-1} were typical for D-glucose in pyranose form and mannose, respectively. YP-1 exhibited the absorption at 842.66 cm^{-1}, suggesting an α-dominating configuration, which is in agreement with the data of optical rotation analysis. The absorptions at 1046.23, 1084.68 and 1154.62 cm^{-1} indicated a pyranose form of sugars.

The ^{13}C NMR spectrum of YP-1 is showed in Fig. 2. Based on the data available in the literature, the resonances in the region of 101–106 ppm were attributed to the anomeric carbon atoms of mannopyranose (Man*p*) and glucopyranose (Glc*p*), respectively. The resonance due to C-1 of Man*p* residues substituted at O-3 was observed at 102.5 ppm. The signals at 101.9 and 101.7 ppm correspond to C-1 of terminal and backbone Glc*p* residues linked together by substitution at O-3. The peak at 105.9 ppm

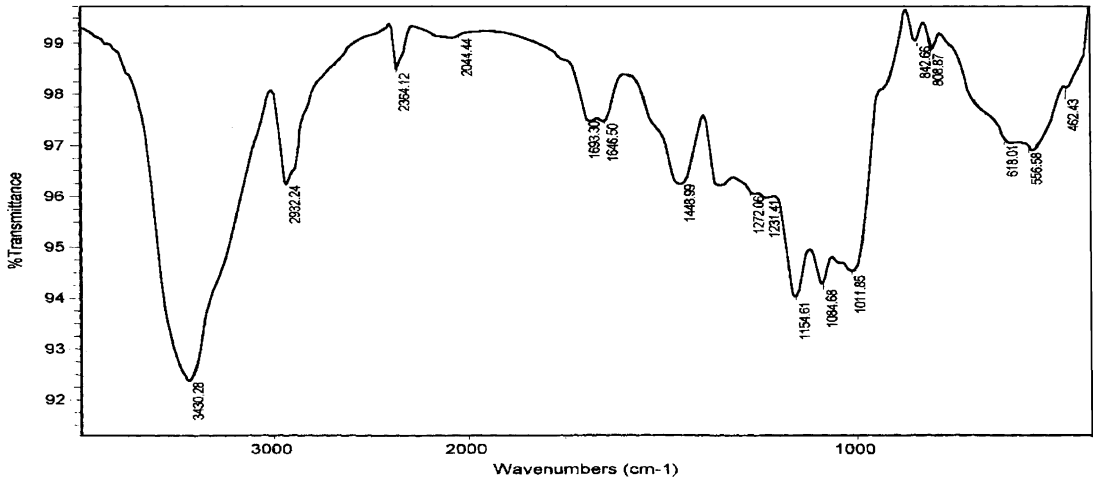

Fig. 1. IR spectrum of YP-1.

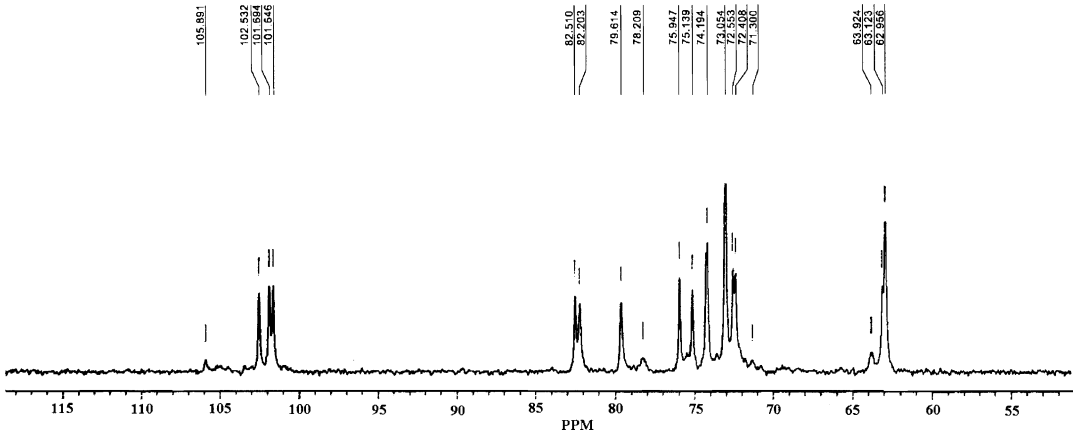

Fig. 2. ^{13}C NMR spectrum of YP-1.

corresponded to C-1 of β-D-galactopyranose (Gal*p*) residues (Agrawal, 1992). Others signals were assigned in Table 3. In the low field region, no typical signals were observed for the carboxyl group of the uronic acid units, which is in agreement with the result from *m*-hydroxydiphenyl colorimetric method.

In the proton spectrum (Fig. 3), the three signals in the region of 5.51–5.56 ppm were assigned to anomeric protons of α-D-Glc*p* and α-D-Man*p* residues, and signal at 4.95 ppm was easily assigned to anomeric protons of β-D-Gal*p* residues.

The periodate-oxidated product of the polysaccharide was hydrolyzed and tested by GC. There were only two fractions, glucose and glycerin with a molar ratio of 1:0.5, were found on GC spectrum. The presence of glucose indicating a part of glucose is in 1,3-, 1,2,3-, 1,2,4-, 1,3,4-, 1,3,6-, or 1,2,3,4-linkage, which cannot be oxidized by HIO_4. As galactose and mannose were not found in the hydrolyzed product, it could be inferred that galactose and mannose are in linkages that can be oxidized, namely 1-, 1,6-, 1,2-, 1,2,6-, 1,4-, or 1,4,6-linkage. With results from methylation analysis, it could be concluded that glucose was derived from 1,3- and 1,3,6-linked glucose residues and glycerin was derived from 1,2-linked mannose, 1-linked galactose and 1-linked glucose.

On mild acid hydrolysis, the major dialyzable fraction contained galactose and mannose, in an approximate molar ratio of 1:4, indicating that they were present at outer chains. The nondialyzable fraction was further purified on Sephadex G-100 column to give a pure fragment HYP-1 with a molecular weight of 30 kDa. HYP-1 was confirmed as a 1,3-linked glycan. It was thus concluded that YP-1 is a heteropolysaccharide, having a backbone composed of

Table 3
Assignment of ^{13}C NMR chemical shifts of YP-1

Sugar residues	Chemical shifts, δ (ppm)					
	C_1	C_2	C_3	C_4	C_5	C_6
→3)-α-D-Glc*p*-(1→	101.9	73.1	82.2	72.6	75.1	62.9
→3)-α-D-Glc*p*-(1→	101.7	73.1	82.5	72.4	75.9	62.9
→2)-α-D-Man*p*-(1→	102.5	78.2	74.2	71.3	72.4	63.8
β-D-Gal*p*-(1→	105.9	73.7	75.9	71.3	75.1	63.8

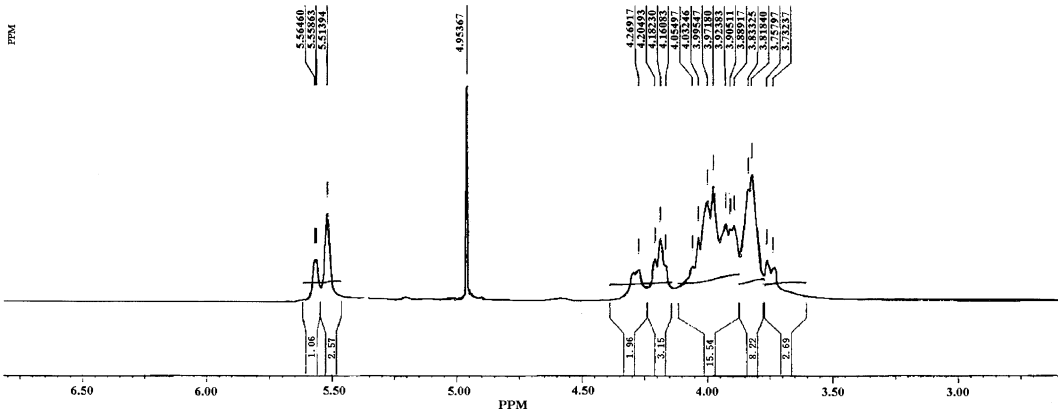

Fig. 3. ^{1}H NMR spectrum of YP-1.

1,3-linked glucose, with branches attached to O-6 of some residues. The branches mainly composed 1,2-linked mannose with a terminal of β-D-Gal*p*. Together with the absence of 3-linked glucose in methylation analysis, it was proposed that a similar branch should be attached to the reducing end of the backbone.

On periodate oxidation, HIO_4 consumption and formic acid production of the polysaccharide were 0.401 mol/mol sugar residue and 0.08 mol/mol sugar residue, respectively, which was in agreement with the theoretically calculated values (0.433 mol/mol for HIO_4 and 0.10 mol/mol for formic acid) on the basis of the structural features described above.

On the basis of the aforementioned results, it can be concluded that YP-1 is composed of a repeating unit having the possible structure as shown in Fig. 4.

3.3. Immunological activity of YP-1

As observed in Fig. 5, when ConA was added as mitogen for spleen T lymphocytes and the polysaccharides, including CYP, YP-1 and HYP-1 at different concentration, CYP and YP-1 could significantly increase spleen T lymphocytes proliferation ($P<0.05$) while HYP-1 has nothing to do with the proliferation in vitro. For crude polysaccharide CYP, the higher the dose, the higher increase in T lymphocytes proliferation was observed. For purified polysaccharide YP-1, the dose of 150 μg/ml was more effective than the doses of 50 and 250 μg/ml. To clarify the different pattern of CYP and YP-1, possible contamination of the CYP by endotoxin was checked by cultivation in the presence of polymyxin B (25 μg/ml) which inhibits, dose dependently, the biological effects of endotoxin, including its mitogenic activity (Jacobs & Morrison, 1977). The negative polymyxin B test indicated the absence of endotoxin contamination in CYP. So, it could be assumed that other substance with mitogenic activity must be existing in CYP, which needs a further investigation. In addition, after mild acid hydrolysis of YP-1, the absence of increase in T lymphocytes proliferation stimulation was observed for HYP-1, which suggested that the branches are extremely important in biological activities of YP-1.

4. Conclusion

The results of the present investigation show that the water-soluble polysaccharide of *D. opposita* Thunb is a heteropolysaccharide and is slightly branched. Preliminary immunopharmacological tests suggested that YP-1 could increase the ConA-induced T lymphocytes proliferation in vitro and crude polysaccharide even has a stronger immunostimulatory effect. Further, studies are necessary to focus on detailed pharmacological effects of YP-1 and the other secreted substances possibly co-existing in crude polysaccharide. The pharmacological results obtained may help in elucidating the use of roots of *D. opposita* Thunb in Chinese traditional medicine.

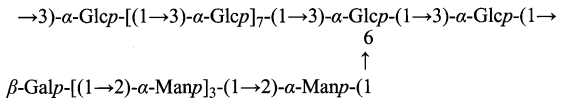

Fig. 4. Predicted structure of YP-1.

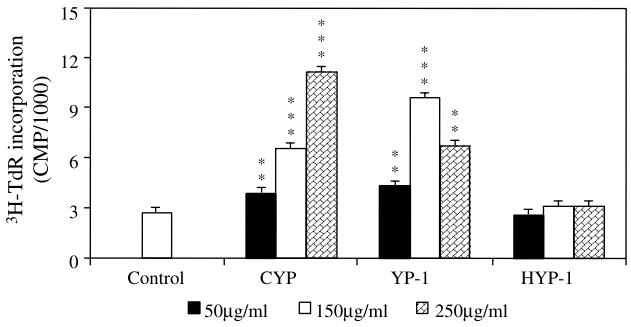

Fig. 5. Effects of CYP, YP-1 and HYP-1 on proliferation of ConA-induced T lymphocyte in vitro. $^{**}P<0.05$, $^{***}P<0.01$, significant different from the control group.

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