A NEUTRAL POLYSACCHARIDE FROM *Glycyrrhiza inflata*

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A neutral polysaccharide Gi-A1 was isolated from the roots of Glycyrrhiza inflata *Bat. It had a molecular mass of over 2000 kDa and showed* $[\alpha]_D^{20}$ *+ 81.4° (*c *1.05,* H_2O*). Acid hydrolysis and methylation analysis indicated that Gi-A1 was mainly composed of* α*-D-glucose,* α*-L-arabinose, and* α*-D-galactose with a molar ratio of 8.0:1.8:1.0. It can significantly stimulate spleen cell proliferation* in vitro *(P<0.01).*

Key words: *Glycyrrhiza inflata* Bat., root, neutral polysaccharide, immunological activity.

The roots of *Glycyrrhiza* spp. have been the component herb in many kinds of chinese herbal medicine clinically used for the treatment of inflammation, allergy, gastric ulcer, etc. Shimizu and Takada et al [1, 2] have reported that polysaccharides from the roots of *G. uralensis* Fischer and the stolon of *G. glabra var. glandulifera* Reg. et Herd have immunological and anti-complementary activities. Besides them, the root of *G. inflata* Bat. is also a representative Chinese licorice and has been certificated by Chinese Pharmacopoeia. We studied for the first time the polysaccharide composition of *G. inflata* Bat.

We extracted water-soluble polysaccharides (WSPS) from roots of *G. inflata* Bat. and purified several polysaccharides. The present paper describes the acid hydrolysis, methylation analysis, and immunological activity of a neutral polysaccharide Gi-A1 among them.

Dried roots was soaked in 95% ethanol for 24 h. WSPS were obtained by precipitation with alcohol from the aqueous extract of the root residue after refluxing with ethanol and were deproteinnated by the Sevag method [3]. A portion of crude WSPS was dissolved in water and applied to a DEAE chromatograph (700 ×25 mm), eluted with 0–2.0 M NaCl gradient (each gradient was 1.5 liters). The H_2O eluate was purified on a Sepharose CL-6B and Sephadex G-200 chromatograph (500 ×22 mm) to yield 4 fractions: Gi-A1–A4. We report some properties of Gi-A1 in the present paper.

In the HPLC analysis, Gi-A1 was determined as a single peak by size-exclusive chromatography. The molecular weight of Gi-A1 was estimated to be over 2 000 000 Da and it had specific rotation $[\alpha]_D^{20}$ + 81.4° (*c* 1.05, H_2O). There was no protein in Gi-A1 according to the Lowry method [4]. The results of IR spectroscopy showed that there was no characteristic absorption at 1731 cm^{-1} (carboxyl group) and indicated that Gi-A1 was a neutral polysaccharide.

After complete hydrolysis with 2M TFA, GC analysis of the alditol acetates from Gi-A1 showed that it contained glucose, arabinose, and galactose with a molar ratio of 8:1.8:1. The result of methylation of Gi-A1 showed that it was mostly composed of α-1,4-linked D-glucose, α-1,5-linked L-arabinose, and α-1,4-linked D-galactose in a ratio of 8.1:1.62:1 (Table 1). Thus, Gi-A1 was assumed to be an arabinogalactoglucan, having a backbone consisting of α(1→4) linked glucosyl residues, with branches at the O-6, composed of arabinosyl and galactosyl residues and differring from the α-(1,4)-glucan of *G. uralensis* Fisch. previously reported [5, 6]. The results need to be further verified.

Using the stimulation effect on spleen cell proliferation, we showed that Gi-A1 has immunological activities in a dose-dependent manner (Fig. 1). It had a significant immunoenhancing effect on spleen cell proliferation (P<0.01).

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TABLE 1. Glycosyl Linkage Composition of the Gi-A1 Derivatives

Methylation positions	Linkage	Molar ratios	Methylation positions	Linkage	Molar ratios
2,3,5-tri-OMe Ara	Ara(1→	0.85	2,3,4-tri-OMe Glc	→6)Glc(1→	-
2,3-di-OMe Ara	→5)Ara(1→	4.22	2,3-di-OMe Glc	→4,6)Glc(1→	0.45
2,3,4,6-tetra-OMe Glc	Glc(1→	0.65	2,3,4,6-tetra-OMe Gal	Gal(1→	0.24
2,3,6-tri-OMe Glc	→4)Glc(1→	21.09	2,3,6-tri-OMe Gal	→4)Gal(1→	2.61

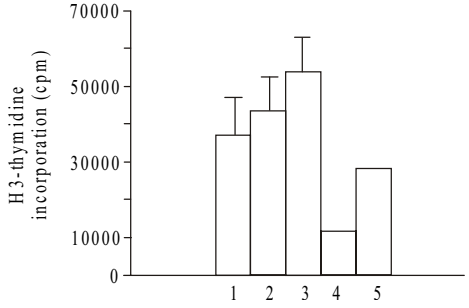

Fig. 1. Immunological activity of Gi-A1: 1 – 25 μg/mL, 2 – 50 μg/mL, 3 – 100 μg/mL, 4 – Control, 5 – LPS (5 μg/mL). Each data point represents the mean ± SD, *n*=6; each has triplicates. Control and LPS indicate absence of sample and positive control, respectively. Sample group *vs* control group is **P<0.01.

EXPERIMENTAL

Plant Material. The roots of *Glycyrrhiza inflata* L. were collected from Hetian, Xinjiang province (China) in October of 2005. The identification of *Glycyrrhiza inflata* Bat. was done by one of the authors (Palida).The voucher specimen was deposited in the Department of Pharmacognosy, Xinjiang Medical University.

General Analysis. The homogeneity and molecular weight of Gi-A1 were evaluated from a calibration curve of the elution volume of standard dextrans (Dextrans T-2000, T-500, T-70, T-40, T-10 and glucose; from Amersham Pharmacia, Sweden) by HPLC performed on an Agilent 1100 series apparatus equipped with a Shodex KS-805 column (Shoko, Japan) and an ELSD detector. Distilled water was used as the solvent and eluent, and the flow rate was kept at 1.0 mL/min. All gel permeation chromatography was done using H_2O as eluent.

Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined with an AVATER-360 spectrometer. GC-MS was performed on a Finnigan Trace GC-MS instrument equipped with a DB-5 column and detected by FID.

Polysaccharide Analyses. The neutral monosaccharide composition was studied by total hydrolysis with 2 M TFA at 110°C for 2 h. The remaining hydrolysates were reduced with $NaBH_4$ (20 mg) for 3 h, then acetylated with Ac_2O (100°C, 1 h). The resulting alditol acetates were analyzed by GC [7]. The sample was reduced by the reported method [8]. The vacuum-dried polysaccharides were methylated using the method reported by [9].

Measurement of Immunomodulating Activity. Mice (6–8 weeks) were sacrificed and their spleens were removed and passed through a sterilized iron sieve to obtain single cell suspensions. The single cell suspension was washed with PBS, and then the red blood cells were lysed with ACK lyses buffer (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM EDTA) for 3 min. The spleen cells were washed and then cultured in U-bottom well plates (2×10^5/well) in a volume of 200 μL per well in the presence of 25, 50, and 100 μg/mL of Gi-A1, negative control and positive control (LPS, 5 μg/mL) groups respectively. After a three-day drug treatment, DNA synthesis was measured by H^3-thymidine (Du Pont) incorporation (1 μCi/well) in the final 6 h of the cultured period. The data were tested for statistical differences using the *T* test.

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