*Nutraceuticals & Food*
*Vol. 8, p. 29~35 (2003)*

**Nutraceuticals and Food**

# Characterization of the Immunologically Active Components of *Glycyrrhiza uralensis* Prepared as Herbal *Kimchi*

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## Abstract

**A crude polysaccharide fraction (GU-3) from the roots of *Glycyrrhiza uralensis* (licorice root), a screened herbal plant used in the preparation of herbal *kimchi*, enhanced Peyer's patch mediated bone marrow cell proliferation and NK cell-mediated tumor cytotoxicity against Yac-1 cells. GU-3 was further purified by DEAE-Sepharose CL-6B yielding fractions designated as GU-3I, and 3IIa~3IIe. GU-3IIa is mainly composed of arabinose, galactose and galacturonic acid, and showed the highest bone marrow cell proliferation activity. In addition, GU-3IIb had arabinose, galactose, rhamnose and galacturonic acid as the component sugars with a small quantity of protein; GU-3IIb also enhanced activity of NK cell-mediated tumor cytotoxicity. After these fractions were further fractionated via gel filtration on Sepharose CL-6B or Sephacryl S-300, two immunological active polysaccharides, GU-3IIa-2 and 3IIb-1 were purified from the respective fractions. GU-3IIa-2 mostly contained neutral sugars (75%) such as arabinose and galactose (molar ratio; 1.0 : 0.7) in addition to a considerable amount of galacturonic acid (20%), whereas GU-3IIb-1 was composed of arabinose, galactose, rhamnose and galacturonic acid (molar ratio; 0.3 : 0.5 : 0.1 : 1.0). Methylation analysis indicated that GU-3IIa-2 was composed mainly of terminal, 4- or 5-linked and 3,4- or 3,5-branched arabinose, 3-linked, 4-linked and 3,6-branched galactose, and terminal and 4-linked galacturonic acid whereas GU-3IIb-1 contained various glycosidic linkages such as terminal and 4- or 5-linked arabinose, 2,4-branched rhamnose, terminal and 4-linked galactose, and terminal and 4-galacturonic acid. Single radial gel diffusion indicated that only GU-3IIa-2 strongly reacted with β-D-glucosyl-Yariv antigen. These results suggest that bone marrow cell proliferating activity and enhancement of NK cell-mediated tumor cytotoxicity of GU-3 are caused by polysaccharides containing a pectic arabinogalactan (GU-3IIa-2) and pectic polysaccharide (GU-3IIb-1).**

**Key words:** *Glycyrrhiza uralensis*, bone marrow cell proliferation, NK cell-mediated tumor cytotoxicity, pectic polysaccharide

## INTRODUCTION

Medicinal plants with pharmacologically active polysaccharides are typically composed of pectic polysaccharides that can be categorized as pectins, pectic arabinogalactans and pectic heteroglycans (1). Pectin is largely composed of $\alpha$-(1→4)-linked polygalacturonan regions and a small ramified region with a rhamnogalacturonan core containing substituted neutral sugar chains such as arabinogalactan and arabinan. Pectic arabinogalactans primarily have arabinogalactan side chains in the ramified region (2). Since most medicinal plants are decocted with water for clinical use, elucidation of the structure, pharmacological activity and structure-activity relationships of water-soluble bioactive pectic polysaccharides will help to evaluate the efficacy of botanical medicines (3).

The root of *Glycyrrhiza uralensis* (licorice root) is a widely used herb in traditional herbal medicines, and has been the subject of numerous investigations into its pharmacologically active constituents. *G. uralensis* is used to treat hepatitis, diabetes, and peptic ulcer (4), it is also used as a corroborant to treat general physical weakness and fatigue (5). Although MeOH-soluble and low-molecular-weight substances such as glycyrrhizin (a saponin composed of one molecular of 18$\beta$-glycyrrhetinic acid and two molecules of glucuronic acid) and glycyrrhetinic acid from *G. uralensis* have been found to exhibit anti-inflammatory, anti-ulcer, anti-allergy, anti-carcinogenic, and immuno-modulatory properties (6,7); and to be inhibitors of protein kinase C (8); little is known about the macromolecules in the roots of *G. uralensis*. However, we previously demonstrated that (9) hot-water soluble fractions and macromolecules rather than only MeOH-soluble and low-molecular-weight substances in *G. uralensis*,

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which are used in preparing herbal *kimchi*, exhibited potent bone marrow cell proliferating activity and increased NK cell-mediated tumor cytotoxicity.

In this study, we further characterized the active components responsible for bone marrow cell proliferating activity and increased NK cell-mediated tumor cytotoxicity by evaluating immunological activities of a crude polysaccharide fraction (GU-3) of *G. uralensis*, and describing its chemical properties and those of its subfractions.

## MATERIALS AND METHODS

### Materials

Roots of *Glycyrrhiza uralensis*, which were cultivated and dried in Chungbuk, Korea, were purchased from Sangsoo-Herbland Co. Ltd. (Chungbuk, Korea). A voucher specimen of the plant was deposited at the department of Kimchi and Food Science, Chongju National College of Science and Technology (herbarium No. 001). DEAE-Sepharose CL-6B, Sepharose CL-6B and Sephacryl S-300 were obtained from Pharmacia, and Sep-pak $C_{18}$ cartridge from Waters Associates. β-D-glucosyl-Yariv antigen was obtained from Biosupplies (Parkville, Australia). RPMI-1640 medium and Hank's balanced salt solution (HBSS) were obtained from Gibco (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Cell Culture Laboratories (Cleveland, OH), and penicillin, streptomycin and amphotericin B from Flow Laboratories (Irvine, Scotland). Alamar Blue™ was obtained from Alamar Bio-Sciences Inc. (Sacramento, CA).

### Animals and cell cultures

Specific pathogen-free female C3H/He, Balb/c mice, 5-7 weeks old, were purchased from Daehan-Biolink Co. Ltd., Chungbuk, Korea. The mice were housed in an environmentally controlled facility at a constant temperature, 24±1°C, and humidity, 55%; and had free access to commercial chew pellet diet (Samyang-Feed Co. Ltd.) and water. Yac-1 cells, an NK-sensitive mouse lymphoma cell line, were maintained in RPMI-1640 supplemented with 7.5% FBS and L-glutamate.

### General methods

Total carbohydrate, uronic acid, and protein were determined by the phenol-$H_2SO_4$ (10), *m*-hydroxydiphenyl (11), and Bradford's (12) method with Bio-Rad dye (Bio-Rad Co.), respectively, using galactose, galacturonic acid, and bovine serum albumin as the respective standards. Component sugars of the polysaccharides were converted into the corresponding alditol acetates after hydrolysis with 2 M TFA for 1.5 h at 121°C (13), and analyzed by GLC according to the procedure of Zhao et al. (14) GLC was carried out on a Hewlett-Packard 6890 II gas chromatograph (Hewlett-Packard, USA) equipped with an SP-2380 capillary column (0.2 μm film, 0.25 mm i.d.×30 m, Supelco). Temperature program was: 60°C for 1 min, 60→220°C (increasing 30°C/min), 220°C for 8 min, 220→250°C (increasing 8°C/min), and held at 250 °C for 15 min. The molar ratios were calculated from the peak areas and response factors using the flame-ionization detector (FID). Single radial gel diffusion using the β-D-glucosyl-Yariv antigen was performed according to the procedure of Holst and Clarke (15).

### Ion-exchange chromatography of GU-3

The GU-3 extract (1 g) was applied to a column (4.0×40 cm) of DEAE-Sepharose CL-6B ($Cl^-$ form) equilibrated with $H_2O$. The column was eluted with $H_2O$ to obtain an unabsorbed fraction (GU-3I; yield, 2.7% of GU-3). The absorbed fractions were eluted by stepwise elution with 0.1, 0.2, 0.3, 0.4, 0.5, 1.0 and 2.0 M NaCl. Five absorbed fractions were obtained as lyophilizates of 0.1, 0.2, 0.3, 0.5 M and 1.0 M NaCl elution after dialysis (yields: GU-3IIa, 5.7%; 3IIb, 18.5%; 3IIc, 3.8%; 3IId, 6.7; 3IIe, 4.1%).

### Gel filtration of the active fractions

GU-3IIa was fractionated on a column (3.0×90 cm) of Sepharose CL-6B with 0.2 M NaCl to obtain the sub-fraction with the highest bone marrow cell proliferating activity (GU-3IIa-2; yield, 1.7% of GU-3) which eluted in an intermediate volume in addition to GU-3IIa-1 and 3IIa-3, which were less active fractions. GU-3IIb yielded a sub-fraction (3IIb-1; yield 5.6%), with the most potent NK cell-mediated tumor cytotoxicity, eluted in the void volume via gel filtration on Sephacryl S-300 (3.0×90 cm, 0.2 M NaCl).

### Methylation analysis

Each sample was methylated according to the Hakomori method (16). The methylated products were recovered using a Sep-pak $C_{18}$ cartridge by the procedure of Waeghe et al. (17), except that the samples were eluted only with EtOH. Carboxymethyl groups in methylated products were reduced $LiB(C_2H_5)_3D$ in THF (Super-Deuteride, Aldrich) at room temperature for 1 h (18), and the reduced products were recovered on a Sep-pak $C_{18}$ cartridge. The methylated products were hydrolyzed with 2 M TFA at 121°C for 1.5 h and converted into partially methylated alditol acetates. The partially methylated alditol acetates were analyzed by GLC and GLC-EIMS. GLC was performed on a Hewlett-Packard 6890 II gas chromatograph equipped with an SP-2380 capillary column, and EIMS was done on a Hewlett- Packard 6890 II mass spectrometer. Conditions of GLC were as described previously (14), and methylated alditol acetates

were identified by their fragment ions in EIMS and their relative retention times in GLC. Their molar ratios were estimated from the peak areas and the response factors (19).

**Peyer's patch mediated bone marrow cell proliferating activity**

Peyer's patch mediated bone marrow cell proliferating activity was measured in accordance with the procedure of Hong et al. (20). Peyer's patch cells were isolated from the small intestines of C3H/He mice and suspended in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS). About 180 μL of the cell suspension ($2\times10^6$ cells/mL in RPMI 1640-FBS) was cultured with 20 μL of test sample in a 96-well flat bottom microtiter plate for 5 days at 37°C in a humidified atmosphere of 5% $CO_2$-95% air. The resulting culture supernatant (50 μL) was added to 100 μL of bone marrow cell suspension ($2.5\times10^5$ cells/mL) from C3H/He mice, and incubated for 6 days in a humidified atmosphere of 5% $CO_2$-95% air in order to evaluate growth capacity of the bone marrow cells. After 20 μL of Alamar Blue™ solution was added to each well, the cells were then continuously cultured for 5~24 hr to estimate the cell numbers (21). Cells were counted by fluorescence intensity measured during cultivation using Fluoroskan II (Labsystems, Austria) at an excitation wavelength of 544 nm and emission wavelength of 590 nm during cultivation. The bone marrow cell proliferating activity was expressed as the ratio of bone marrow cell growth with Peyer's patch cells incubated with the extract to the growth of control cells, in which Peyer's patch cells were incubated with distilled water instead of the extract.

**NK cell-mediated tumor cytotoxicity**

NK cell-mediated cytotoxicity was determined by the radioactive $^{51}Cr$-release assay as described by Yoo et al. (22). Two Balb/C mice per group were intravenously administered their respective extract fractions (100 μg), and their splenocytes harvested after 1 day. Single splenocyte cell suspensions (100 μL/well) were added to $^{51}Cr$-labelled Yac-1 cells ($1\times10^4$ cells/100 μL/well) to obtain predetermined effector (splenocytes)-to-target (Yac-1) cell ratios (E/T ratio) of 100 : 1, 50 : 1 and 25 : 1 in U-bottomed 96-well plates. The cultures were incubated for 6 hr at 37°C in 5% $CO_2$ air atmosphere. After incubation, the plates were centrifuged for 10 min at $900\times g$. The supernatant (100 μL) of each well was absorbed onto a cotton swab and monitored for radioactivity using a gamma counter. The percentage of cytotoxicity generated by NK cells was calculated from the radioactivity (count $min^{-1}$) according to the following formula.

Cytotoxicity (%) = [(experimental release - spontaneous release)/(maximum release - spontaneous release)] × 100.

**Statistical analysis**

All results are expressed as the mean ± SD. The difference between the control and the treatment in these experiments was tested for statistical significance by Student's *t*-test. A value of $p<0.05$ or $p<0.01$ was considered to be statistically significant.

## RESULTS AND DISCUSSION

**Identification of the active fractions**

In a previous study (9), a hot-water extract of *G. uralensis* (GU-0), which is a screened herbal plant used in the preparation of herbal *kimchi*, was fractionated to obtain a MeOH-soluble fraction (GU-1), a supernatant fraction of EtOH precipitation (GU-2), and a crude polysaccharide fraction (GU-3). Of these subfractions, GU-3 exhibited the most potent bone marrow cell proliferation and NK cell-mediated tumor cytotoxicity enhancing activities (9). The active fraction of *G. uralensis*, GU-3, was further fractionated by anion-exchange chromatography on DEAE-Sepharose CL-6B ($Cl^-$ form) yielding an unabsorbed (GU-3I) and 5 absorbed fractions (GU-3IIa to 3IIe) (Fig. 1). GU-3IIa was the most effective stimulator of bone marrow cell proliferating activity (1.8-fold of the control). GU-3IIb had intermediate activity (1.5-fold), whereas GU-3IIc expressed weak activity (1.2-fold) (Table 1). The other fractions (GU-3I, GU-3IId and 3IIe) showed no activity.

Stimulation of NK cell cytotoxicity activity by the fractions obtained from anion-exchange chromatography was tested. The GU-3IIb fraction was the most potent activity enhancer (2.9-fold, 4.2-fold and 4.9-fold of the control) which was greater than GU-3IIa (2.1-fold, 3.1-fold and 3.7-fold) and GU-3IIc (1.6-fold, 2.7-fold and 3.0-fold) at the respective E/T ratios of 25 : 1, 50 : 1 and 100 : 1. GU-3I, GU-3IId and 3IIe fractions had negligible effects on activity (Fig. 2).

Because the main activities of GU-3IIa and 3IIb were different, the active substances were separately identified from GU-3IIa and 3IIb. When GU-3IIa, possessing the bone marrow cell proliferating activity, was fractionated by gel filtration on Sepharose CL-6B, three subfractions (GU-3IIa-1~3IIa-3) were obtained (Fig. 3A). GU-3IIa-2, eluting near the intermediate volume, was the most potent bone marrow cell proliferating activity enhancer (1.9-fold of the control), but GU-3IIa-1 and 3IIa-3 had weak activity (1.3-fold and 1.2-fold) (Table 2). By gel filtration on Sephacryl S-300, GU-3IIb, which was the NK cell-mediated tumor cytotoxicity fraction, yielded two subfractions (GU-3IIb-1 and 3IIb-2) (Fig. 3B), of which GU-3IIb-1 was the most potent NK cell-mediated tumor

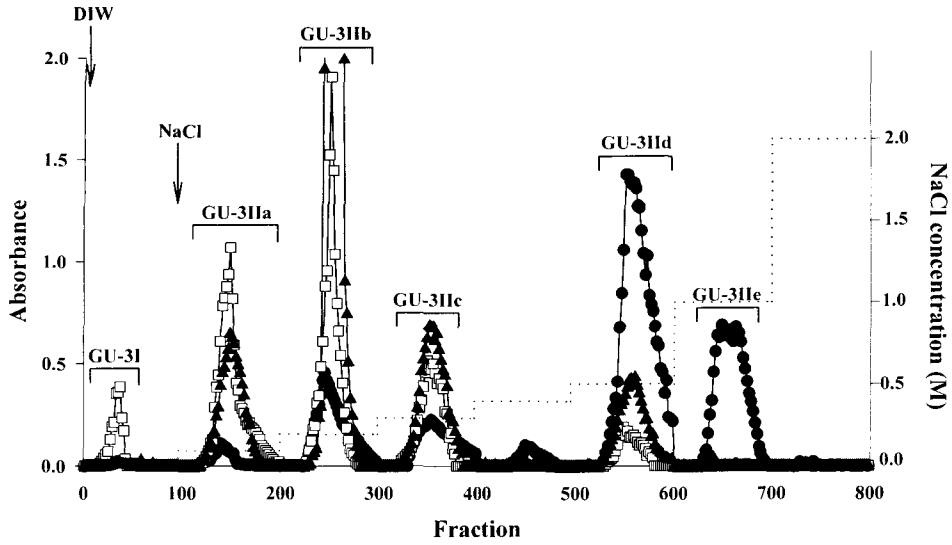


**Fig. 1.** Elution pattern of the crude active fraction (GU-3) on DEAE-Sepharose CL-6B (Cl⁻ form). After the column was eluted with DIW to obtain an unabsorbed fraction, the absorbed fractions were eluted by stepwise elutions with 0.1, 0.2, 0.3, 0.4, 0.5, 1.0 and 2.0 M NaCl. Column size: 4.0×40 cm. ●: UV- absorbance (280 nm), □: Carbohydrate (490 nm), ▲: Uronic acid (520 nm). .....: Concentration of the eluted NaCl.

**Table 1.** Bone marrow cell proliferating activity through Peyer's patch of subfractions obtained from the crude polysaccharide fraction (GU-3) on DEAE-Sepharose CL-6B (Cl⁻ form)

| Subfractions | Fluorescence intensity (Mean ± SD)[2)] |
|---|---|
| Control[1)] | 4,850 ± 310 |
| GU-3 | 8,000 ± 500[3)] |
| GU-3I | 5,130 ± 640 |
| GU-3IIa | 8,730 ± 420[3,4)] |
| GU-3IIb | 7,270 ± 410[3,4)] |
| GU-3IIc | 5,650 ± 350[3)] |
| GU-3IId | 5,080 ± 520[4)] |
| GU-3IIe | 4,930 ± 560 |

[1)]For control, saline without sample, and the final concentration of sample was 100 μg/mL.

[2)]The proliferation of bone marrow cells was measured by a fluorometric method, using the Alamar Blue[TM] reduction assay. Each value was expressed as the mean ± SD of quadruplicate assays.

[3)]$p < 0.05$ : Significant difference between the control and subfractions.

[4)]$p < 0.05$ : Significant difference between GU-3 and subfractions.

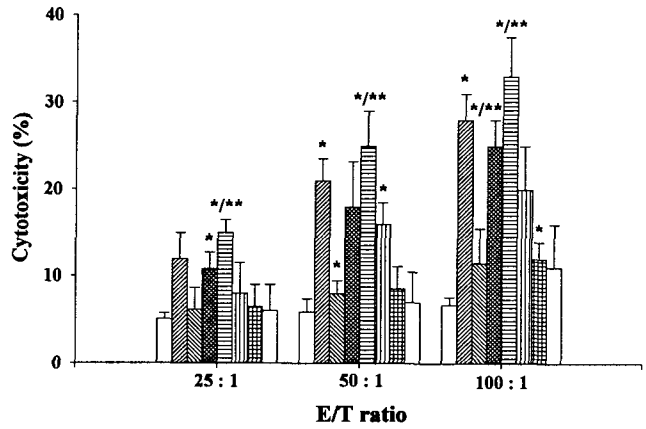


**Fig. 2.** NK cell-mediated tumor cytotoxicity of subfractions obtained from the crude polysaccharide fraction (GU-3) on DEAE-Sepharose CL-6B (Cl⁻ form). Single splenocyte cell suspensions (100 μL/well), which were harvested from mice administered i.v. with each subfraction (100 μg), were added to $^{51}$Cr-labelled ac-1 cells ($1 \times 10^4$/100 μL/well) to obtain effector (splenocytes)-to-target (Yac-1) cell ratios (E/T ratio) of 100 : 1, 50 : 1 and 25 : 1. *$p < 0.05$; significant difference between the control and subfractions, **$p < 0.05$; significant difference between GU-3 and subfractions. □ (1st), Control; ▨, crude polysaccharide fraction from *G. uralensis*. (GU-3); ▧, GU-3I; ▩, GU-3IIa; ▤, GU-3IIb; ▥, GU-3IIc; ▦, GU-3IId; □ (2nd), GU-3IIe.

cytotoxicity enhancer at an E/T ratio of 100 : 1 (4.6-fold of the control) (Table 2).

### Characterization of the active fractions

The bone marrow cell proliferating fraction, GU-3IIa-2, mainly contained neutral sugar (75%) with considerable amounts of uronic acid (20%) and a small amount of protein (1.5%) (Table 3); whereas GU-3IIb-1, the most potent enhancer of NK cell-mediated tumor cytotoxicity, was about 55% uronic acid, 25% neutral sugar and 15% protein (Table 3). Component sugar analysis showed that GU-3IIa-2 consisted mainly of arabinose and galactose (molar ratio of 1.0 : 0.7), and GU-3IIb-1 was composed mostly of arabinose, galactose, rhamnose and galacturonic acid (molar ratio of 0.3 : 0.5 : 0.1 : 1.0) (Table 3). Methylation analysis indicated that GU-3IIa-2 was composed mainly of terminal, 4- or 5-linked and 3,4- or 3,5-branched arabinose, and 3-linked, 4-linked and 3,6-branched galactose as well as terminal and 4-galacturonic acid (Table 4). GU-3IIb-1 contained a variety of glycosidic link-

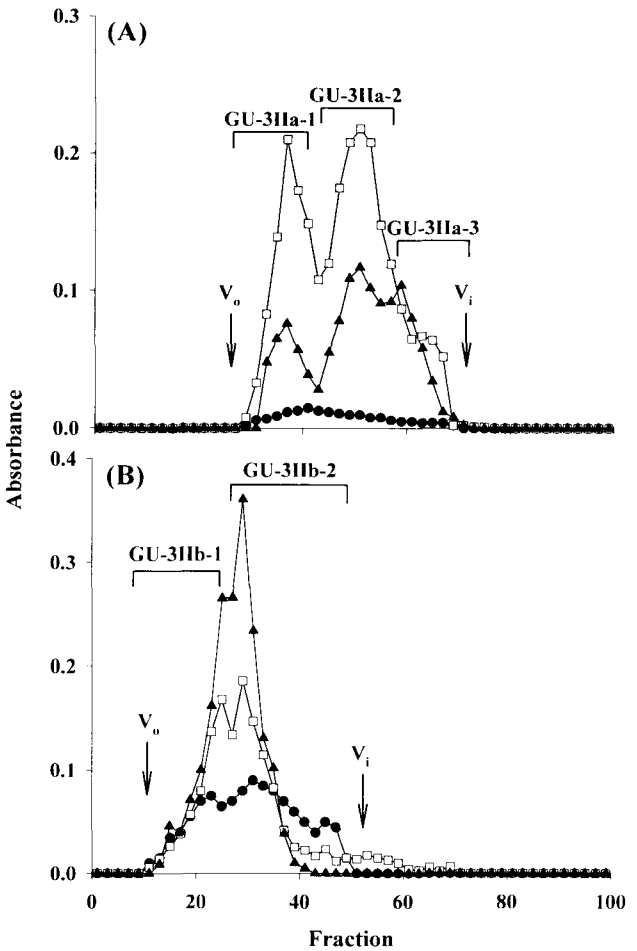


**Fig. 3.** Gel filtration patterns of A) GU-3IIa on Sepharose CL-6B and B) GU-3IIb on Sephacryl S-300. The symbols are $V_o$, void volume; $V_i$, inner volume. Both columns were eluted with 0.2 M NaCl on a 3.0×90 cm column. ●: UV-absorbance (280 nm), ○: Carbohydrate (490 nm), ▲: Uronic acid (520 nm).

ages including: terminal, 4- or 5-linked arabinose, 2-linked and 2,4-branched rhamnose, terminal and 4-linked galactose, and terminal and 4-galacturonic acid (Table 4). GU-3IIa-2 strongly reacted with β-D-glucosyl-Yariv antigen, suggesting the presence of an arabino-3,6-galactan moiety whereas GU-3IIb-1 reacted little to the antigen (Fig. 4).

This study focused on the identification of active macromolecules, rich in carbohydrate. At least two active carbohydrate-rich macromolecules (GU-3IIa-2 and 3IIb-

**Table 2.** Immunological activities of subfractions obtained from GU-3IIa on Sepharose CL-6B and GU-3IIb on Sephacryl S-300

| Subfraction | Fluorescence intensity[2)] | Cytotoxicity (%)[3)] |
|---|---|---|
| Control[1)] | 5,270±250 | 7.5±0.8 |
| GU-3IIa | 9,220±360[4)] | - |
| GU-3IIa-1 | 6,870±410[4)] | - |
| GU-3IIa-2 | 10,010±530[4,5)] | - |
| GU-3IIa-3 | 6,150±570 | - |
| GU-3IIb | - | 32.4±4.5[4)] |
| GU-3IIb-1 | - | 34.2±4.1[4,5)] |
| GU-3IIb-2 | - | 18.7±4.0 |

[1)]For control, saline without sample, and the final and administered concentration of sample was 100 μg/mL and 100 μg.
[2)]The proliferation of bone marrow cells was measured by a fluorometric method, using the Alamar Blue™ reduction assay. Each value was expressed as the mean±SD of quadruplicate assays.
[3)]NK cell-mediated tumor cytotoxicity was determined by the 6 h $^{51}$Cr-release assay using splenocytes (effector) and $^{51}$Cr-labelled Yac-1 cells (target) on the 1 days after each sample treatment. Cytotoxicity = [(experimental release radioactivity - spontaneous release radioactivity)/(maximum release radioactivity - spontaneous release radioactivity)]×100. Effector-to-target cell ratio (E/T ratio) was 100 : 1. Each value was expressed as the mean±SD of quadruplicate assays.
[4)]$p<0.05$ : significant difference between the control and subfractions.
[5)]$p<0.05$ : significant difference between GU-3IIa or GU-3IIb and each subfraction.

**Table 3.** Physicochemical properties of subfractions obtained from GU-3IIa on Sepharose CL-6B and GU-3IIb on Sephacryl S-300

| | 3IIa | 3IIa-1 | 3IIa-2 | 3IIa-3 | 3IIb | 3IIb-1 | 3IIb-2 |
|---|---|---|---|---|---|---|---|
| Content (%) | | | | | | | |
| Carbohydrate[1)] | 80.7 | 81.3 | 75.2 | 60.3 | 48.3 | 24.8 | 25.9 |
| Uronic acid[2)] | 15.3 | 10.9 | 20.4 | 32.4 | 50.7 | 55.4 | 62.0 |
| Protein[3)] | 1.9 | 1.5 | 1.5 | 0.5 | 10.9 | 14.6 | 18.0 |
| Component sugar (mol.%) | | | | | | | |
| Arabinose | 29.3 | 26.7 | 35.1 | 14.5 | 10.4 | 13.5 | 7.7 |
| Xylose | 5.4 | 6.5 | 4.3 | 7.4 | 1.2 | 1.2 | 0.9 |
| Rhamnose | 2.3 | 2.6 | 2.8 | 1.4 | 4.8 | 6.0 | 2.9 |
| Fucose | 1.5 | 1.4 | 1.6 | 1.8 | 1.9 | 1.3 | 1.1 |
| Mannose | 5.4 | 7.9 | 5.9 | 7.9 | 3.4 | 2.7 | 2.7 |
| Galactose | 26.9 | 30.7 | 24.5 | 30.2 | 24.6 | 21.8 | 20.6 |
| Glucose | 9.5 | 15.1 | 7.8 | 1.2 | 3.4 | 2.1 | 8.6 |
| Galacturonic acid | 13.0 | 7.6 | 14.6 | 28.4 | 40.8 | 42.5 | 42.7 |
| Glucuronic acid | 6.7 | 1.5 | 3.4 | 7.2 | 9.5 | 8.9 | 12.8 |

[1] Carbohydrate content: Phenol-sulfuric acid method (as galactose).
[2] Uronic acid content: *m*-hydroxydiphenyl method (as galacturonic acid).
[3)]Protein content: Bradford method (as BSA).

**Table 4.** Comparison of methylation analysis of the immunological active polysaccharides, GU-3IIa-2 and 3IIb-1 from roots of *Glycyrrhiza uralensis*

| Glycosyl residue | Deduced glycosidic linkage | mol % | |
|---|---|---|---|
| | | GU-3IIa-2 | GU-3IIb-1 |
| Arabinose | terminal (*f*) | 30.5 | 13.9 |
| | 4 or 5 | 5.6 | 4.4 |
| | 3,4 or 3,5 | 4.8 | 1.5 |
| Xylose | terminal (*f*) | 0.6 | - |
| | 4 or 5 | 1.8 | 1.2 |
| Rhamnose | terminal | 0.8 | 1.5 |
| | 2 | 0.4 | 2.4 |
| | 2,4 | - | 3.7 |
| Fucose | terminal | 0.2 | 1.2 |
| | 3 | 0.5 | - |
| Mannose | terminal | 0.9 | - |
| | 2 | 1.5 | 1.0 |
| | 2,4 | - | 1.7 |
| Galactose | terminal | 2.2 | 4.4 |
| | 3 | 4.6 | 0.5 |
| | 4 | 5.9 | 5.7 |
| | 6 | 3.1 | 0.8 |
| | 3,6 | 13.8 | 0.7 |
| | 3,4,6 | 0.6 | 0.2 |
| Glucose | terminal | 2.4 | 0.5 |
| | 3 | 1.4 | - |
| | 4 | 1.1 | 2.3 |
| Galacturonic acid | terminal | 3.5 | 11.6 |
| | 4 | 10.3 | 27.9 |
| | 2,4 | 1.6 | 2.9 |
| Glucuronic acid | terminal | 1.9 | 8.8 |
| | 4 | - | 1.2 |

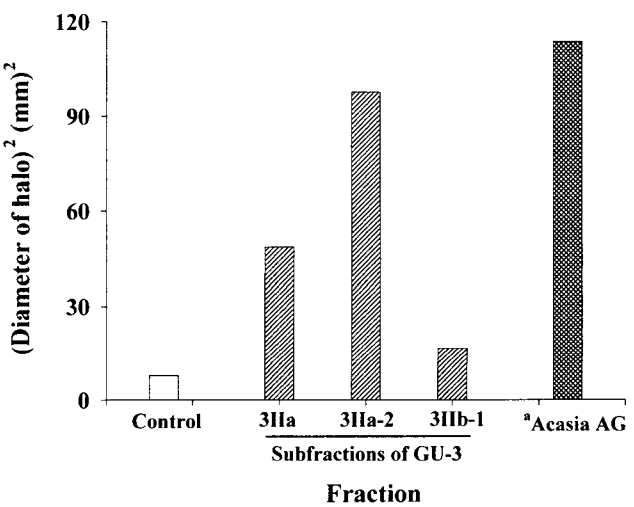


**Fig. 4.** Reactivity of the immunological active fractions (GU-3 IIa-2 and GU-3IIb-1) to μ β-D-glucosyl-Yariv antigen. [a]Acacia AG; acacia arabino-3,6-galactan (positive control). □, Control (distilled water); ▨, the active fractions; ▩, positive control.

1) were obtained. The results suggest that GU-3IIa-2 is a pectic arabinogalactan-type polysaccharide. Arabinogalactans are polysaccharides rich in galactosyl and arabinosyl residues, and in some situations they occur in covalent association with protein as proteoglycans (arabinogalactan-proteins). Arabiongalactans are typically found in higher plants and in liverworts, and are components of cell membranes, extracellular matrices and in gum exudates (23). Clarke et al. (23) classified plant arabinogalactans and arabinogalactan-proteins into type I, type II and other type according to the structure of the arabinogalactan portion. Type I arabinogalactans are arabino-4-galactans which have a (1→4)-β-D-galactan backbone with arabinosyl oligosaccharide side chains. Type II arabinogalactans are arabino-3,6-galactans which have a highly branched (1→3)-β-D-galactan backbone possessing (1→6)-β-D-galactosyl and/or arabinosyl side chains attached at position 6 to some of the galactosyl residues in the backbone (24). Other arabinogalactan types are polysaccharides with arabinogalactan side chains such as a pectic arabinogalactan from *Angelica acutiloba* Kitagawa (25). Because GU-3IIa-2 strongly reacted with the β-D-glucosyl-Yariv antigen, which can recognize type II arabinogalactan (23), and is rich in terminal arabinose, 3-linked and 3,6-branched galactose, and terminal and 4-linked galacturonic acid, it is reasonable to assume that it is pectic arabinogalactan. Further detailed characterization of GU-3IIb-1 revealed this fraction be mostly comprised of pectic polysaccharides, which contained a galacturonan region [poly- or oligomerized $\alpha$-(1→4)-galacturonic acid] and a "ramified" region (rhamnogalacturonan core with side chains), because they contained terminal and 4-linked galacturonic acid as well as 2,4-branched rhamnose.

However, studies on the structure and structure-activity relationship of the active polysaccharides must await further study.

## ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education & Human Resources Development (College Financial Support Grant). We express our sincere gratitude for the support.

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(Received January 3, 2003; Accepted March 8, 2003)