Isolation of Polypeptide Fractions from Different Parts of Red Ginseng

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ABSTRACT

Chemical studies of nitrogen compounds of *Panax ginseng* seem relatively rare, probably due to the isolation difficulties, subsequently the investigations of biological activities are little. The experimental conditions were established for highly complete extraction of peptides (basic, acidic and neutral) from *Panax ginseng*. This task was achieved by applying the follow isolation procedure : 1, the sequential extraction with water, 0.1% TFA in 20% acetonitril and buffer pH 6.5 (water-pyridine-acetic acid 100:3:900) : 2, fractionation by ultrafiltration : 3, n-butanol extraction : 4, cation- and anion-exchange chromatography : 5, chromato-electrophoresis. The comparison of red ginseng (xylem & pith part) and red ginseng inside-white (xylem & pith part) was also provided. To analyze the peptide mixture the chromato-electrophoresis method of separation was applied. Optimal conditions for peptide mapping of sample were explored.

Our experiments revealed the quantitative difference of peptide between xylem & pith and phloem & cortex parts. We have also found the qualitative difference in the composition of polypeptides between normal red ginseng (xylem & pith part) and red ginseng inside-white (xylem & pith part)

Introduction

Preventive health care becomes more popular than ever. Recently ginseng *(Panax ginseng)* is cultivated in many countries of Europe. For the standardization, ginsenosides are still considered important (1). Traditional quality criteria of Korea ginseng indicate the importance of central part (pith & xylem) which contains little ginsenosides and relatively high level of soluble protein (2). Ginseng peptide studies are very rare (3, 4, 5). Furthermore peptide study of red ginseng is hardly found. Peptide separation from red ginseng is much difficult due to reddening materials than from fresh or white ginseng. The elucidation of peptide composition in relation to ginseng quality factors not only red but also fresh and white ginseng appears to be very important and urgent. We present the preliminary results of different peptide composition between normal red ginseng and inside-white ginseng.

Materials and Methods

Red ginseng samples

Normal red ginseng and inside-white (central part of root does not change the color into red) one were selected from 6 year old red ginseng processed by traditional methods. Roots were separated into the central part (pith and xylem) and the outer part (phloem, cortex and epidermis). All parts were ground to 100 mesh

Extraction

Korea red ginseng powder was mixed with water followed by stirring for 48 hours at 4 ℃. Extracted mixture was centrifuged at 10,000 × g for 20 minutes at 4 ℃. Then supernatant was centrifuged at 32,000 × g for 40 minutes at 4 ℃. The procedure was twice repeated to achieve the transparent sample solution. Precipitates was again extracted with 0.1% TFA (trifloroacetic acid) in 20% of acetonitrile for extraction of basic components after stirring for 24 hours at 4℃. Then this extract was centrifuged and the same conditions was used for water extract.

Residue was extracted three times (to gain the extraction of acidic components) with the buffer, pH 6.5, pyridin - acetic acid - water = 10 : 0.3 : 90, after stirring for 24 hours at 4 ℃. Then the mixture was centrifuged at the same conditions, that was used for water extract (Fig. 1).

Protein quantification

The Bio-Rad Protein Assay, based in the method of Bradford(6), was used for determining the concentration of solubilized protein. The method involves the addition of an acidic dye to protein solution and subsequent optical absorption measurement at 595 nm using a spectrophotometer. To complete the measurements the comparison with calibration curve of BSA was provided.

Ultrafiltration

Amicon and Grace company types from USA, model L 8400, PM 10 and YM 10 were applied with >10 kD MW cut-off and YC 05 was applied with 500 MW cut-off.

Each of the separation procedure (i.e. for every each sample) was provided in "cold room" and 4 ℃ condition was constantly maintained.

Extraction of saponin

The fractions containing peptides with MW<10KD were applied to eliminated saponins. n--Butanol was added to the sample solution in 3 : 1 ratio followed by shaking for 2 hours at 24 ℃. The extraction mixture was kept for 10 hours at 4 ℃. Then water phase was separated from n-butanol phase.

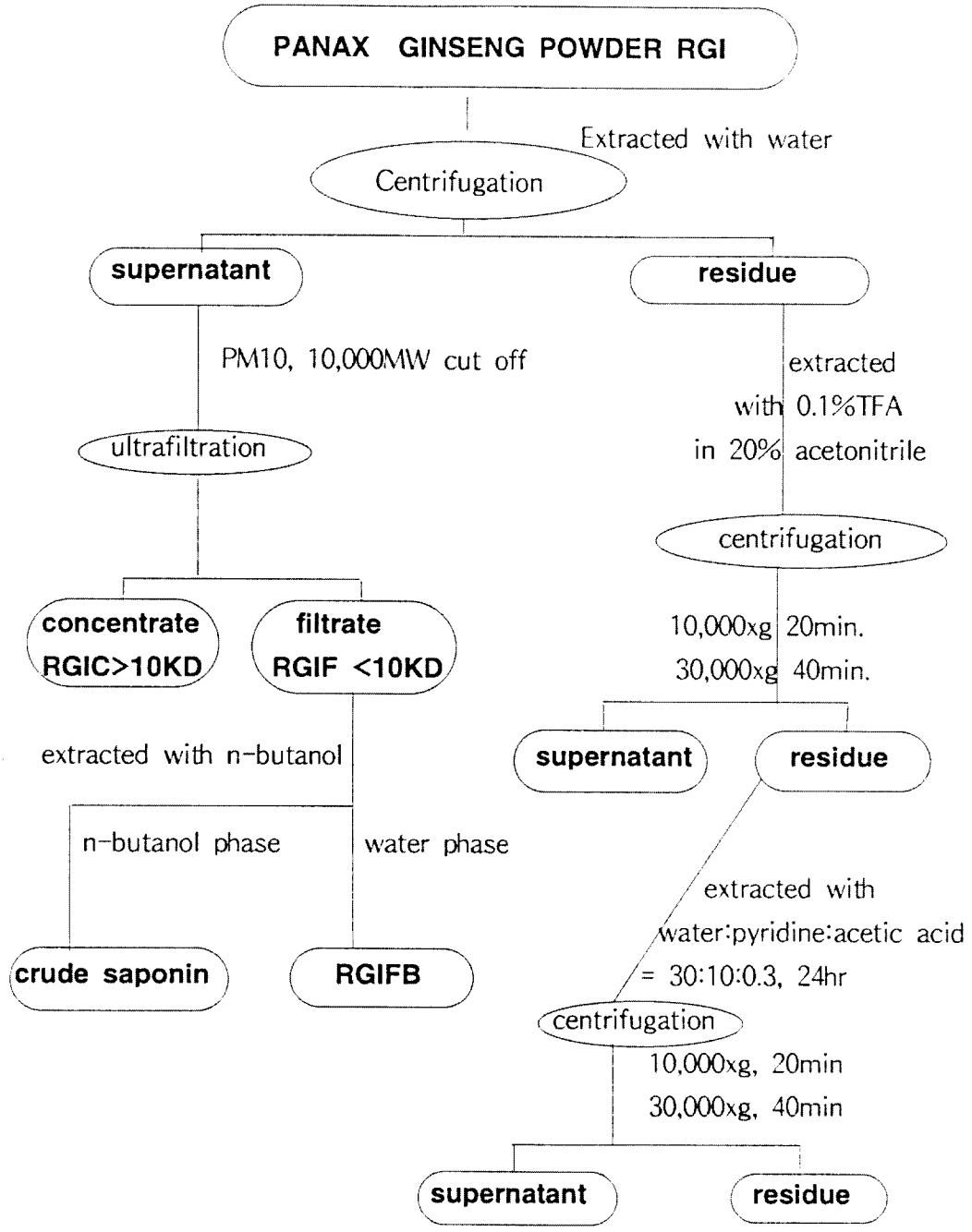

Figure 1. Separation procedures for ginseng extracts

Thin layer chromatography (TLC)

TLC were performed on silica gel plate and on plastic sheets cellulose, layer thickness 0.2 mm (DC-plastickfolien Cellulose, Merck). The spots were determined by spraying with a solution of ninhydrin (0.25% in acetone) on the plate, and heating it in an oven at 100 ℃ for 15 min (7).

Electrophoresis

Electrophoresis of peptide fractions was performed on cellulose plate using Multiphor II Electrophoresis System (Pharmacia Biotech. Sweden). The spots were determined as in TLC. The separation conditions were optimized for the every particular sample.

Ion-exchange chromatography

To separate the peptides both anion- and cation-exchangers were applied. Batch type IEC (ion-exchange chromatography) was used. Cl counterion in Dowex-1 resin was changed with OH ion by washing in 2 N NaOH solution. The counterion was changed in Dowex-50W also (Sigma described procedure).

The anion exchanger and cation exchanger were equilibrated with 0.36 M pyridine, pH 9.3 and with 0.2 M pyridine-acetate, pH 3.1 correspondingly. The samples were dissolved in 70 ml of equilibrium buffer pH 9.3 for anion exchange chromatography and pH 3.1 for cation exchange chromatography. After incubation during 1 hour, resins were separated from the buffer by filtration for the unbound peptides. This process was repeated twice. The bound peptides eluted from resin with 2M pyridine-acetate (pH 5.0) after 1 hour equlibrium. This process was repeated three times until all peptides were eluted.

Electrophoresis-chromatography

To control peptide separation by electrochromatography with cellulose plates was used electrode buffer pH 3.5 (acetic acid:pyridine:water-10:1:190) and TLC solvent system was n-butanol:pyridine:water:acetic acid-100:100:100: 18.

Results and discussion

Solvent extraction

Central parts of normal red ginseng (RGI) and red ginseng inside white (RGW) were extracted in sequence with three solvents for neutral, basic and acidic fractions of proteins (Table 1).

Total soluble protein was slightly higher in normal red ginseng (RGI) than the inside white one (RGW). In protein fractions, neutral protein was higher in RGW than RGI, but the content of basic and acidic protein is higher in RGI. The percentage of acidic fraction to total was much higher (12%)

Table 1. Soluble protein contents of central part of red ginseng

protein	solvent	protein content (mg/gdw)		% total	
		RGI	RGW	RGI	RGW
neutral	water 100	0.823	0.927	51.5	65.0
basic	TFA 0.1 in aceton 20, water 80	0.200	0.145	12.5	10.2
acidic	acetic acid 0.3, pyridine 10, water 90	0.576	0.353	36.0	24.8
total				100	100

RGI : normal red ginseng RGW : inside-white red ginseng

in RGI than RGW suggesting that acidic protein might be important in bioactivities. In the case of water extraction, the surface of the sample after centrifuge at 10,000 × g during 20 minutes (at 4 ℃) was covered with a thin condensed white film. After the film was removed the centrifuge process was repeated under other conditions. Finally the obtained solution became transparent.

Effect of saponin on peptide isolation by ultrafiltration and TLC

Separation for M.W. less than 10KD peptides from water extract took 8 hours, resulting in concentrates (RGIC and RGWC, containing>10KD) and filtrates (RGIF and RGWF containing<10KD). RGIF and RGWF were washed with butanol to eliminate saponin and applied to ultrafiltration for cutting of peptides less than 500D M.W. This process took about 10 hours. The longer time of ultrafiltration of 500D without saponin than 10 KD with saponin may indicate that saponin does not affect ultrafiltration.

The comparison of TLC for RGFB (saponin eliminated) and RGF (without elimination) and also for RGWF and RGWFB had shown that the composition of ninhydrin-positive compounds were unchangeable indicating that n-butanol extraction process does not eliminate any peptides.

Electrophoresis-chromatography

The different conditions for the TLC of peptide fractions were tested. A good solvent system for use with cellulose plates in peptide separations was n-butanol: pyridine : water: acetic acid (100:100:100:18) (7)

The different conditions for electrophoresis of peptide fractions were tested. A good electrode buffer for use with cellulose plates in peptide separations was acetic acid:pyridine:water (10:1:190). Two dimentional separation by electrophoresis-chromatography showed desirable result.

Fractions RGIFB (MW 500-10kD, cationic), RGWFB (MW 500-10KD, cationic) revealed one healthy ginseng specific peptide (Fig. 2) and three ginseng specific peptides (Fig. 3).

Fractions of anion exchanger revealed clear diffrent between healthy (Fig. 4) and inside white ginseng (Fig.5). Number of peptides appeared to be small. According to this method peptides with various molecular weight can be isolated.

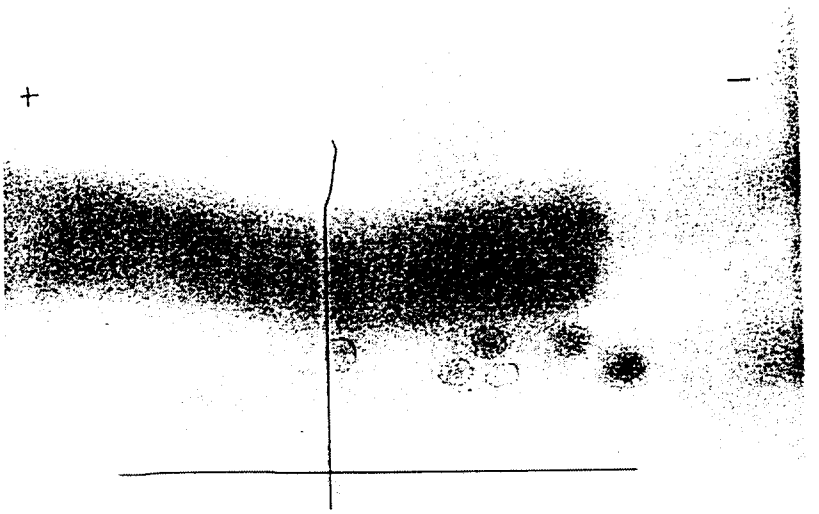

Figure 2. Electro-chromatography of peptide fractions : RGIFB (cationic) Plate : DC-plastikfolien celluose, layer thickness 0.1mm, 20x20cm.
Electrode buffer : pH 3.5 acetic acid : pyridine : water (10:1:190)v/v.
Separation conditions : max 1,000V : 30mA, 30min.
TLC solvent system : n-butanol : pyridine : water:acetic acid(100:100:100:18)
Development time : 21 hrs.
Detections : Ninhydrin spray

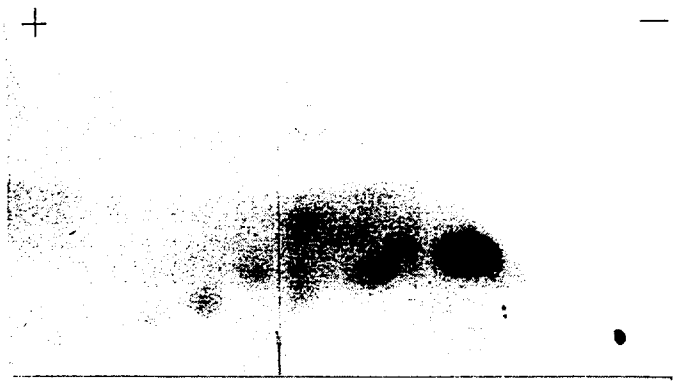

Figure 3. Electro-chromatography of peptide fractions : RGIWFB (cationic)
Plate : DC-plastikfolien celluose, layer thickness 0.1mm, 20x20cm.
Electrode buffer : pH 3.5 acetic acid:pyridine:water (10:1:190) v/v.
Separation conditions : max 1,000V : 29mA, 35min.
TLC solvent system : n-butanol : pyridine:water:acetic acid (100:100:100:18)
Development time : 21 hrs.
Detections : Ninhydrin spray

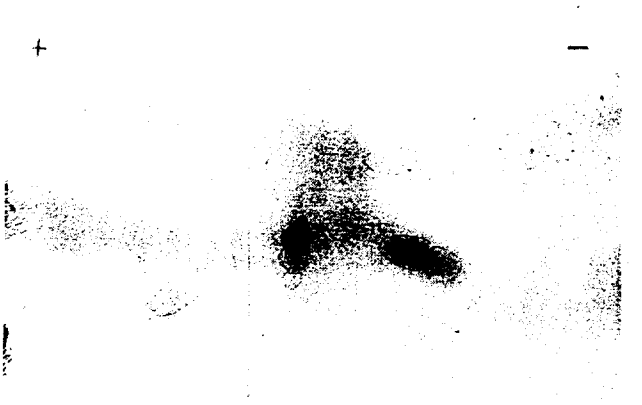

Figure 4. Electro-chromatography of peptide fractions : RGIFB (anionic) Plate : DC-plastikfolien celluose, layer thickness 0.1mm, 20x20cm.
Electrode buffer : pH 3.5 acetic acid:pyridine:water (10:1:190) v/v.
Separation conditions : max 1,000V : 28mA, 35min.
TLC solvent system : n-butanol : pyridine:water:acetic acid (100:100:100:18)
Development time : 21 hrs.
Detections : Ninhydrin spray

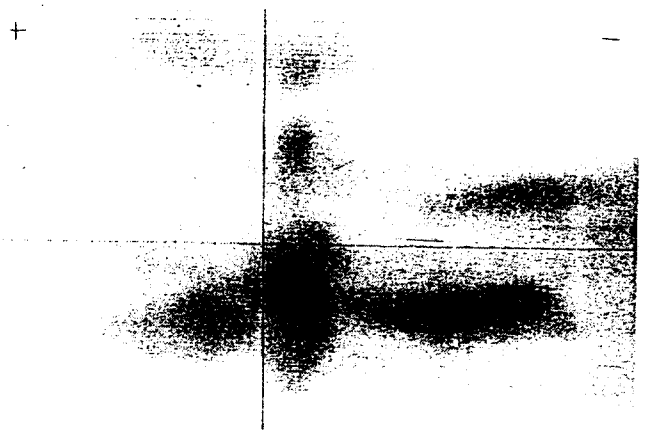

Figure 5. Electro-chromatography of peptide fractions : RGIFB (anionic) Plate : DC-plastikfolien celluose, layer thickness 0.1mm, 20x20cm.
Electrode buffer : pH 3.5 acetic acid:pyridine:water (10:1:190) v/v.
Separation conditions : max 1,000V : 28mA, 42min.
TLC solvent system : n-butanol:pyridine:water:acetic acid (100:100:100:18)
Development time : 23 hrs.
Detections : Ninhydrin spray

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