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Pattern-Analysis of *Panax ginseng* Polysaccharide

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Abstract□Total polysaccharide contents in *Panax ginseng* roots were evaluated by a spectrophotometry, utilizing the complex formation of ginseng polysaccharide with alcian blue dye in 50 mM ammonium biphosphate, pH 4.2. The total polysaccharide content in red ginseng was about three times higher than that in fresh ginseng when both were extracted with water, and was increased about two times when red ginseng was extracted with an alkaline solution. The determination of total polysaccharide in various parts of ginseng revealed that main roots contained the component more than fine roots. Fresh ginseng sections stained by the dye showed polysaccharide mainly found in cortex and cambium but not in epidermis. Pattern-analysis on total and acidic polysaccharides from fresh and red ginsengs exhibited that the chemical compositions of the polysaccharides extracted from both ginsengs quite differed from each other.

Key words□*Panax ginseng*, polysaccharide, alcian blue dye, pattern-analysis

Introduction

The polysaccharides from Korean ginseng (*Panax ginseng* C.A. Meyer) have been reported to have the immunological, anti-tumor, anti-complementary and hypoglycemic activities, and to have the inhibitory activity on tumor toxohormone-L induced lipolysis.[1-8] There are at least the 21 kinds of polysaccharides, named panaxans A-U, in white ginseng,[1-4] which are composed of homogeneous glucans (panaxans A-E), heterogeneous glycans (panaxans F-U). Among them panaxans R-U are peptido-glycans, and panaxans Q-U are acidic polysaccharides, particularly panaxan T is a pectin contained 91% of galacturonic acid.

From fresh ginseng, red ginseng and ginseng leaves were also isolated about 15 kinds of acidic polysaccharides with various biological activities.[6-8] Recently it was reported that neutral polysaccharides are responsible for antitumor activity, while acidic polysaccharides for immuno-potentiating activity.[5]

Although various kinds of ginseng polysaccharides were isolated and characterized, the polysaccharide contents in various ginsengs were not compared, and it is unknown that panaxans isolated from white ginseng could be found in fresh and red ginsengs.

This paper describes some comparison studies between fresh, white and red ginsengs in the aspects of their total and acidic polysaccharide contents. For acidic polysaccharide content analysis, uronic acid was assayed by the carbazole method. For analysis of total polysaccharide content in ginseng, we have established a new spectrophotometry using alcian blue dye, which has been used for mucopolysaccharide detection. The ginseng samples were only extracted with water. The water extracts were not treated with ethanol or methanol for partial purification of ginseng polysaccharides, because we recently found that ethanol precipition method using alcohol made denature jam mucopolysaccharides from **Dioscorea** species.[9]

Experimentals

1. Materials

Korean fresh ginseng (four-years old), white ginseng (five-years old) and red ginseng (six-years old) were obtained from Korean Ginseng and Tobacco Research Institute (Daejon 305-345, Korea). Alcian blue and carbazole were purchased from Jassen Chimica, Belgium and Jusei Chem., Japan, respectively.

2. Extraction of ginseng polysaccharides

Five grams of fresh ginseng was homogenized with a waring blender to make 100 m*l* with water. White or red ginseng powder (0.5g) was suspended into 100 m*l* water. Each ginseng preparation was stirred at 4℃ (overnight), 20℃ (overnight), 55℃ (three hrs), or 100℃ (one hr), and then was filtered with a filter paper. Each filtrate was directly applied for assay of polysaccharide.

For extraction of red ginseng powder under an alkaline condition, the powder (0.5g) was suspended into 50 m*l* of 0.1 N NaOH, stirred as described above, neutralized with 0.1 N acetic acid, and filtered. The filtrate was used for assay.

3. Assay of ginseng polysaccharide by alcian blue dye

An aliquot (0.5 m*l*) of water extract of ginseng was taken into a plastic test tube, 5 m*l* of 0.1% alcian blue buffer solution was added to it, and then mixed. After standing at room temperature for two hrs, the complex formed was taken by centrifugation at 3.500 rpm and was dissolved in 5 m*l* of 0.1 N HCl. Absorbance of a blue solution was measured at 620 nm.

4. Assay of uronic acid

Uronic acid for acidic polysaccharide assay was measured by the carbazole method,[10] using glucuronic acid as a standard.

5. Staining fresh ginseng sections by alcian blue dye

Various part sections of fresh ginseng were stained with 0.25% alcian blue dye (in methanol saturated with ammonium biphosphate) for one hr., and then washed with methanol.

Results and Disscussion

1. Optimal pH for the complex formation of alcian blue with total polysaccharide

The pH profiles for the complex formation of alcian blue with total polysaccharide form white gin-

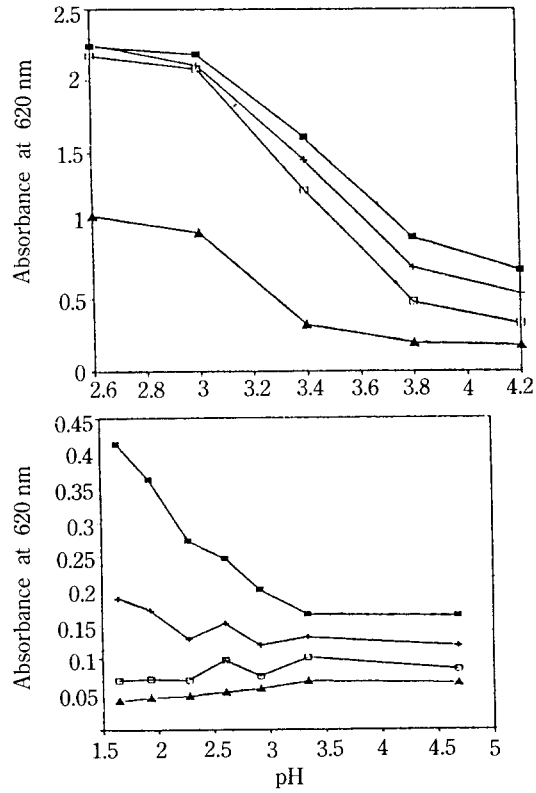

Fig. 1 The pH profiles for the complex formation of alcian blue with ginseng polysaccharide in 0.1 M citric acid/0.2 M Na_2HPO_4 (A) and 0.1 M glycine/NaCl/HCl (B) buffers. One gram of white ginseng powder was added to 10 m*l* of water, and was extracted at 4℃ overnight under stirring. After centrifugation, the supernatant was serially diluted with water. An aliquot (0.5 m*l*) of each sample solution was taken into a plastic test tube, 5 m*l* of 0.1% alcian blue buffer solution was added to it, and then mixed. After standing at room temperature for 2 hrs, the complex formed was taken by centrifugation at 3,500 rpm, and was dissolved in 0.1 N HCl (5 m*l*). Absorbance of a blue solution was measured at 620 nm. Ginseng extract, ■—■; 1/2 diluted, −+−; 1/4 diluted, □—□; 1/8 diluted, ▲—▲. Each data is the mean of duplicate experiments.

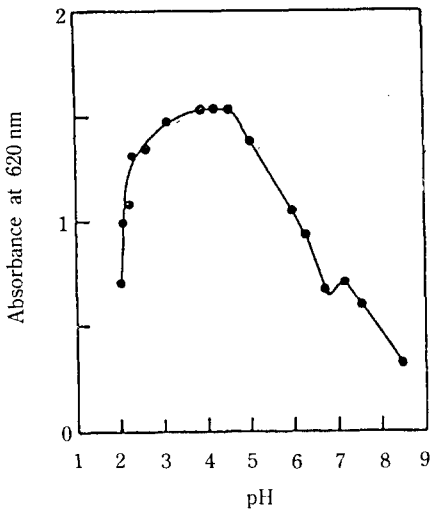

Fig. 2. The pH profile for the complex formation of alcian blue with ginseng polysaccharide in 50 mM sodium phosphate buffer. One gram of white ginseng powder was extracted with 100 m*l* of water at 4℃ overnight. An aliquot (0.5 m*l*) of the water extract was taken for polysaccharide assay as described in the legend of Fig. 1.

Table 1. Solubility of alcian blue in various buffers.

Buffers (pH range)	Absorbance at 620 nm*
0.1 M Citric acid/0.2 M Na_2HPO_4 (2.6~7.6)	1.48 (pH 3.1)
0.1 M Sod. citrate/HCl (1.1~4.9)	0.59 (pH 3.2)
0.1 M Glycine/0.1 M NaCl/HCl (1.6~4.6)	0.09 (pH 3.1)
0.05 M NaH_2PO_4 (4.5)	2.93 (pH 4.5)
0.05 M $NH_4H_2PO_4$ (4.2)	4.42 (pH 4.2)

* 100 mg of alcian blue was stirred at room temperature overnight in 100 m*l* of a buffer with pH indicated in parenthesis, and then was filtered twice using a filter paper. Absorbance of the filtrate was measured at 620 nm.

seng were investigated in three kinds of buffers; (A) citric acid/Na_2HPO_4 (pH range, 2.6~7.6), (b) glycine/NaCl/HCl (pH range, 1.6~4.6), (c) sodium phosphate (pH range, 2.1~8.5).

Fig. 1 shows the profiles in the buffers A and B, and Fig. 2 the profile in the buffer C. The complex formation in the buffers A and B increased under more acidic conditions (Fig. 1), while the formation in the buffer C gave optimal pH in the range of 3.8 and 4.5. Furthermore the sensitivity for the complex formation in the buffer C was about two and four times higher than in the buffers A and B, respectively. These results suggested that the complex formation of total polysaccharide with alcian blue was depend on pH and ionic strength of buffer. So, the solubility of alcian blue was measured in various buffers.

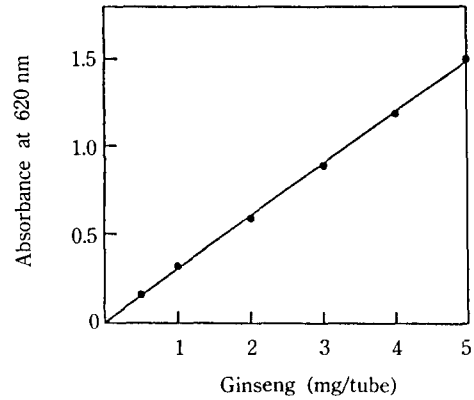

Fig. 3. A calibration curve for total polysaccharide. 0.1% alcian blue in 50 mM ammonium biphosphate (pH 4.2) was use for the complex formation with ginseng polysaccharide which was extracted with water at 4℃ extract for one assay.

Table 1 shows that the solubility of alcian blue in $NH_4H_2PO_4$ (pH 4.2) was greater than that in NaH_2PO_4 buffer (pH 4.5). Therefore, the ammonium biphosphate buffer (pH 4.2) was chosen for analyzing total polysaccharide. Fig. 3 shows a calibration curve for total polysaccharide under the condition of 50 mM ammonium biphosphate solution, pH 4.2. The curve is linear in the polysaccharide amounts corresponding to 0.5~5 mg white ginseng powder/0.5 ml test solution.

2. Total polysaccharide contents in Korean fresh, white and red ginsengs

We define one unit of total polysaccharide as optical density at 620 nm to be one under the standard condition. Table 2 shows the total polysaccharide contents in Korean fresh, white, and red ginsengs, comparing with those of the two kinds of

Table 2. Total polysaccharide contents in *Panax ginseng, Dioscorea batatas,* and *D. japonica.*

Materials[a]	Contents(units/g dry weight)[b]
Panax ginseng[c]	
Fresh	152.7
White	126.1
Red	451.4
Dioscorea batatas[d]	
Fresh	109.5
Processed[e]	26.1
Dioscorea japonica[d]	
Fresh	111.0
Processed[e]	28.9

[a] Extracted at 4℃ overnight.
[b] One unit of total polysaccharide was defined as optical density at 620 nm to be one after the complex was dissociated by 0.1 N HCl.
[c] Optimal pH for assay: pH 4.2 (50 mM $NH_4H_2PO_4$).
[d] Optimal pH for assay: pH 7.4 (50 mM Tris HCl).[9]
[e] Steamed and dried.[9]

Table 3. Total polysaccharide contents in various parts of *Panas ginseng*

Parts[a]	Polysaccharide contents[b]	
	Fresh ginseng	Red ginseng
Rhizome	129.5 (69.2%)	619.6 (128.6%)
Main root	187.1 (100%)	481.8 (100%)[c]
Lateral root	126.6 (67.7%)	368.4 (76.5%)
Fine root	73.8 (39.4%)	361.3 (75%)
Epidermis	–	252.7 (52.4%)

[a] Extracted at 4℃ overnight.
[b] Units per one gram dry weight.
[c] Grade: Yang sam(良蔘)

yams. The total polysaccharide content in red ginseng was about three-times higher than that in fresh or white ginseng, whereas the polysaccharide contents in both fresh yams were about three times higher than that in both processed (steamed and dried) yams. Surprisingly, the total polysaccharide content in red ginseng was about four-times higher than that in fresh yam which is known to be rich in mucopolysaccharide.

3. Total polysaccharide contents in various parts of *Panax ginseng*

Total polysacchairde contents in various parts of Korean fresh and red ginsengs were determined and summarized in Table 3. The order of the polysaccharide content was main root>rhizome, lateral root>fine root in fresh ginseng, and was rhizome >main root>lateral root>fine root>epidermis in red ginseng. Total polysaccharide was richer in main root than in fine root, whereas ginseng saponin is richer in fine root than in main root as well known.

The results was supported by fresh ginseng section staining which showed polysaccharide mainly found in cortex and cambium but not in epidermis (data not shown).

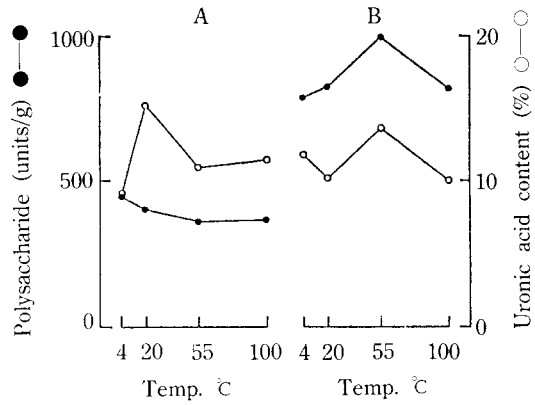

Fig. 4. Extraction of total and acidic polysaccharides from red ginseng as a function of temperature under neutral and alkaline conditions. Each 0.5g of red ginseng powder was extracted with 100 m*l* of water (A), or 50 ml of 0.1 N NaOH (B) at 4℃ (overnight), 20℃ (overnight), 55℃ (3 hrs), or 100℃ (1 hr), and the alkaline extract was neutralized with 50 m*l* of 0.1 N acetic acid before assay. Assay methods: alcian blue, ●—●; carbazole for uronic acid, ○—○.

4. Extraction of red ginseng polysaccharide under various conditions

Extraction of the polysaccharides from Korean red ginseng was determined a function of temperature under neutral and alkaline conditions. The polysaccharides extracted were assayed by two methods: one is the alcian blue dye method for total polysaccharide content analysis, and the other is the assay of uronic acid by the carbazole method for acidic polysaccharide content analysis.

Fig. 4 shows that total polysaccharides were extracted about two-times under the alkaline condition

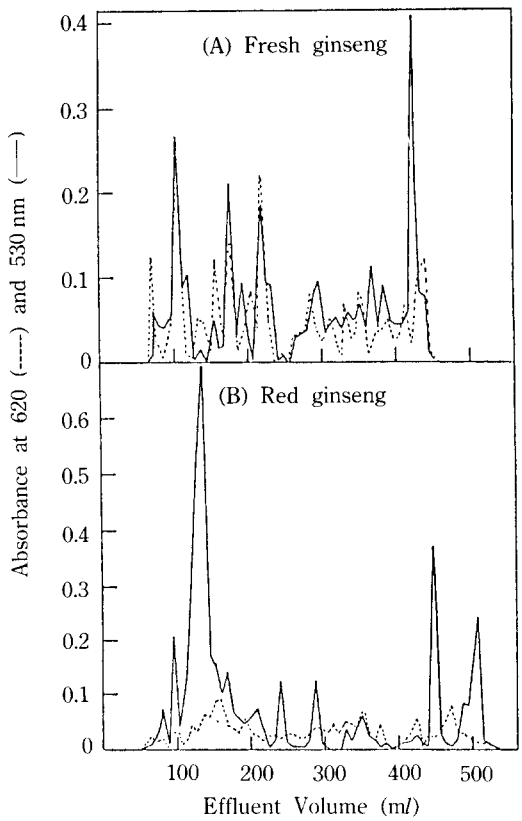

Fig. 5. Gel-filtration of polysaccharides from fresh (A) and red (B) ginsengs on a Sephacryl S-500 column using 5% ethanol as an eluting solvent. Water extract dialysate (4 ml) was applied to the column (1.0×80 cm). Assay methods: ··· alcian blue at 620 nm; –– carbazole at 530 nm.

more than under the neutral condition, while the uronic acid contents did not differ from each other under both the conditions. Under the neutral condition, the total polysaccharides were well extracted at 4℃, while the acidic polysaccharide was well extracted at 20℃. On the other hand, under the alkaline condition, both the total and acidic polysacchairdes were well extracted at 55℃, and the heat treament at 100℃ seemed to destroy both the polysaccharides.

5. Pattern-analysis of polysaccharides from fresh and red ginsengs

Pattern-analysis of polysaccharides from fresh and red ginsengs was carried out by gel-filtration on a Sephacryl S-500 column (Fig. 5). The effluents from the column were monitered by both the alcian blue dye and carbazole methods. The gel-filtration profile of the polysaccharides from fresh ginseng shows about 20 peaks, and the alcian blue peaks are mostly overlapped with the uronic acid peaks.

On the other hand, the gel-filtration profile of the polysaccharides from red ginseng shows that the alcian blue peaks are not overlapped with the uronic acid peaks except a few ones, and that the heights of the uronic acid peaks are very greater than those of the alcian blue peaks. Futhermore the elution positions of the uronic acid peaks of red ginseng polysaccharide are mostly different from those of fresh ginseng polysaccharide. These results and others indicated that the compositions of the polysaccharides extracted from fresh and red ginsengs quite differ from each other.

Acknowledgement

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