研　究　報　文

伝統的手すき紙製造で用いられる粘質物質の分析

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Analyses of Mucilaginous Compounds Used in Making Traditional Handmade Paper

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In the case of making Japanese and Korean traditional handmade paper, mucilaginous compounds extracted from roots of *Abelmoschus manihot* (Tororo-aoi), *Hydrangae paniculata* (Noriutsugi) and others with water are added to bast fiber suspensions to improve dispersity of the fibers. Although many studies in terms of chemical structures of mucilaginous compounds and their properties in aqueous solutions have been reported, there are still many unclear research subjects.

In this study, three mucilaginous compounds extracted from roots of Japanese and Korean *Abelmoschus manihot* (JAM and KAM, respectively) and Japanese *Hydrangae paniculata* (JHP) are subjected to several chemical analyses to distinguish these compounds. Neutral sugar and uronic acid composition analysis clearly gave different results among the three mucilage samples. The major metal elements were sodium and calcium for the JAM and JHP samples, while that was potassium for the KAM sample. The three mucilage samples were distinguishable by their pyrolysis-GC patterns obtained by the on-line methylation method. Size exclusion chromatographic analysis attached with a

multi-angle laser light scattering detector (SEC-MALS) of the mucilage solutions in 0.1 M NaCl revealed some differences in molecular mass values and conformations in the solutions among the polysaccharide components in the three mucilage samples. The JAM and KAM samples had similar weight average molecular mass values around 2,300,000–2,500,000, and the JAM and JHP samples had similar random-coil molecular conformations. However, it is unknown at this moment whether or not the obtained differences are applicable to all Japanese and Korean mucilaginous compounds extracted from plant roots of the same species.

Keywords：mucilage, tororo-aoi, SEC-MALS, FT-IR, sugar composition, pyrolysis-GC
分類：Y_5 高分子化学，W_4 サイズ剤，S_0 その他

1. Introduction

When Japanese and Korean traditional handmade papers are made by craftsmen, mucilaginous compounds extracted from roots of *Abelmoschus manihot* (Tororo-aoi), *Hydrangea paniculata* (Noriutsugi), *Ulmus japonica* (Nire) and others with water are added to bast fiber suspensions to improve dispersity of the fibers. Moreover, it is well known that the mucilage addition leads to smooth separation of each handmade sheet from piles of wet sheets. The mucilage extracted from root of *Abelmoschus manihot* in Japanese and Korean traditional handmade-papermaking has been used since the middle of 19 th century, and some other plant mucilaginous compounds had been used before that time.

Many fundamental researches concerning the relationships between chemical structures of the mucilaginous compounds and their functions in the handmade-papermaking have already reported[1–16]. The main components in the mucilaginous compounds having the characteristic roles in handmade-papermaking are polyuronic acids having specific thread-forming properties in aqueous solutions. Neutral sugar and uronic acid compositions of the polysaccharides after purification processes have been analyzed, and rhamnose, arabinose, xylose, galactose, glucose, galacturonic acid, glucuronic acid and others seem to be present in the polysaccharides, although their weight ratios and the sugar compositions themselves were remarkably different among the reports[1–7]. These differences might be caused by different purification processes of the polysaccharide components in the mucilaginous compounds, different analytical methods, different locations or seasons in sampling the plant roots of even the same species, and others. Generally, uronic acid units in polysaccharides have clearly high resistances to acid hydrolysis, and thus sugar compositions of polyuronic acids determined by acid hydrolysis are difficult to evaluate quantitatively; some glycoside bonds of uronic acid units still remain without hydrolysis in the hydrolyzates, thus resulting in inaccurate sugar composition data. Thus, until now no consistent conclusions concerning chemical structures of mucilaginous compounds have been established yet, and there are still many unsolved research subjects in this field. Because more than 20 years have past since the last paper concerning chemical structures of mucilaginous compounds was published, it is worthy to re-examine the chemical structures of the mucilaginous compounds by using new analytical tools.

In this study, mucilaginous compounds were extracted from roots of Japanese and Korean *Abelmoschus manihot* (Tororo-aoi) and *Hydrangea paniculata* (Noriutsugi) by water, and these three mucilage samples were subjected to the following analyses; neutral sugar and uronic acid compositions, metal element compositions, ash contents, FT-IR spectroscopy, X-ray diffactometry, pyrolysis-gas chromatography with or without the on-line methylation using tetramethylammonium hydroxide, and size exclusion chromatography attached with a multi-angle laser light scattering detector (SEC-MALS) of mucilage solutions. SEC-MALS analysis of polysaccharide components in the mucilage samples can provide information about their molecular mass values and molecular conformations. It was expected that these analyses gave some information concerning the different polysaccharide structures among the three mucilage sam-

ples used.

2. Experimental

2.1 Materials

Root samples of *Abelmoschus manihot* (Tororo-aoi) harvested in October in Ibaraki, Japan and in Gangwondo, Korea, and that of *Hydrangea paniculata* (Noriutsugi) harvested in October in Hokkaido, Japan were stored at 4℃ before use. These roots were beaten with a hummer, and soaked in de-ionized water at room temperature for one day to extract mucilaginous compounds. The viscous solution was filtered through a filter paper, and the filtrate thus obtained was concentrated by evaporation under reduced pressure below 40℃ followed by freeze-drying. These dried mucilage samples were subjected to the following analyses. Cellouronic acid (β-1, 4-linked polyglucuronic acid sodium salt) prepared from commercial rayon by the TEMPO-mediated oxidation[17] were used as a reference.

2.2 SEC-MALS analysis

SEC elution patterns and the corresponding molecular mass plots of the samples were determined by the SEC-MALS method. A 0.1 M NaCl solution was used as the eluent. Details of the SEC-MALS system used were described in the previous paper[18]. The SEC column consisted of polyhydroxymethacrylate-based gel (OHpak SB-806 M ; Shodex, Japan). Data acquisition and processing were carried out using the ASTRA software (Wyatt Technologies). SEC conditions were as follows : the sample concentration of 0.1% (w/V), injection volume of 100 μL, flow rate of 0.5 mL/min and the column temperature of 40℃. The detector cells of MALS and RI were kept at ambient temperature. The mucilaginous samples were first dissolved in de-ionized water, and then the solutions were adjusted to 0.1 M NaCl by adding designed amounts of NaCl to the solutions. The dn/dc value of 0.149 m*l*/g obtained for carboxymethyl cellulose was applied to the mucilaginous compounds for convenience.

2.3 Other analyses

Pyrolysis-gas chromatograms (Py-GC) of the samples were recorded on a Shimadzu GC-14 B attached with a vertical microfurnace-type pyrolzer[19]. The gas components formed from an approximately 0.5 mg sample pyrolyzed at 450℃ with or without 1 μL 25% tetramethylammonium hydroxide (TMAH)/methanol were directly analyzed by the gas chromatograph having a capillary TC-1 column (0.25 mm × 30 m, GL Sciences, Japan), whose initial temperature, final temperature and program rate were set at 150℃, 300℃ and 5℃/min, respectively. Py-GC patterns obtained were compared with those of birch wood meal, birch holocellulose, cellouronic acid and apple pectin.

About 1 g of a sample was pressed to pellet form using an apparatus for preparing KBr pellets in IR measurements, and relative weight ratios of elements heavier than sodium in the sample were measured by means of an X-ray fluorescence analyzer using an attached determination program (MESA 500, Horiba Co., Japan), where X-ray generated at 15 kV and 500 μA was irradiated on the sample for 100 s in vacuum[20].

Neutral sugar compositions of the samples were determined by the conventional alditol acetate/capillary GC method after acid hydrolysis with 2 M trifluoroacetic acid at 121℃ for 1 h[21-23]. Neutral sugar and uronic acid compositions of the samples were determined by the trimethylsililation/capillary GC method after methanolysis with 1 M HCl/methanol at 80℃ for 15 h[21-23].

Ash content of the samples was determined by the combustion method according to ISO 2144 (1997). About 1 g of a sample was set in a crucible, and incinerated at 900℃ for 1 h in the presence of air, using an electric muffle furnace (EYELA TMF 2000).

FT-IR spectra of the samples were recorded on a Nicolet Magna 860 by the KBr disk technique. X-ray diffraction patterns of the sample pellets (ca. 0.1 g) were obtained by the reflection mode using a Rigaku RINT 2000 with monochromatic CuK_α radiation at 40 kV and 40 mA.

3. Results and discussion

3.1 Sugar and metal element composition analyses

Hereafter, the mucilage samples obtained from roots of Japanese and Korean *Abelmoschus manihot* and Japanese *Hydrangae paniculata* are abbreviated to JAM, KAM and JHP samples, respectively. **Fig. 1** shows relative neutral sugar and uronic acid compositions of polysaccharides in the three mucilage samples.

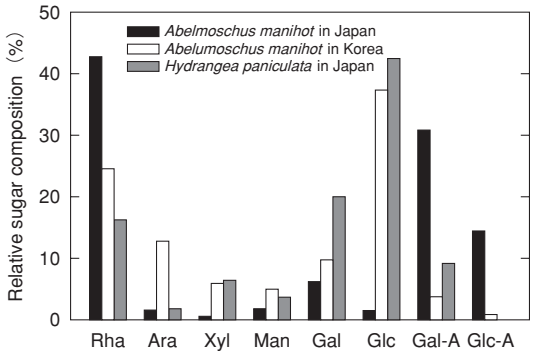

Fig. 1 Relative weight ratio of neutral sugar and uronic acid compositions of polysaccharidres in the mucilage samples.

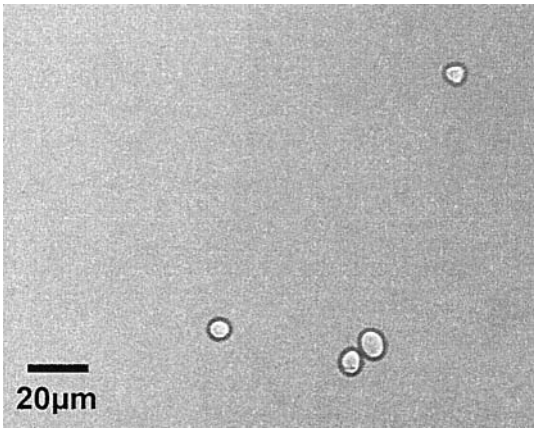

Photo. 1 Optical microphotograph of the mucilage/water solution obtained from root of *Abelmoschus manihot* in Korea.

The JAM sample consisted of rhamnose, galacturonic acid and glucuronic acid with approximate weight ratio of 3：2：1. On the other hand, the KAM and JHP samples had about 40% glucose. However, in the case of the KAM sample, starch granules were clearly observed in its aqueous solution by optical microscopy with cross polarizers (**Photo.1**), and thus most of glucose component present in this KAM sample (Fig. 1) might be due to starch. These starch granules present in the aqueous KAM solution extracted must have passed through the filter paper during the isolation process. In contrast, no such starch granules were observed in aqueous solutions of the JAM or JHP sample；the JHP sample must contain glucose in relatively large quantity as one of the sugar compositions in the polysaccharides. When the glucose content in the KAM sample is regarded to be due to the starch granules, polysaccharides other than starch in the KAM sample consist of rhamnose, arabinose, galactose, xylose and galacturonic acid, and their relative contents decrease in this order. On the other hand, polysaccharides in the JHP sample consisted of glucose, galactose, rhamnose, galacturonic acid, and others. Thus, each mucilage sample has characteristic neutral sugar and uronic acid compositions, although it is not clear at this point whether these differences are applicable to all Japanese and Korean mucilaginous compounds extracted from roots of the same species or just applicable to the particular samples used in this study.

The neutral sugar and uronic acid compositions of the polysaccharides in the mucilage samples in Figure 1 are partly or significantly different from those reported in the previous papers[1–7] in terms of relative weight ratios as well as sugar compositions themselves. These differences are primarily caused by different isolation and purification procedures of mucilage samples. In this experiment, all mucilaginous compounds dissolved in water and filtered were subjected to the sugar composition analysis without purification, because all these extracted compounds must more or less contribute to their characteristic functions in the traditional handmade–papermaking.

Fig. 2 shows relative weight ratios of metal elements in the three mucilage samples. The major metal components present in the two JAM and JHP samples were sodium and calcium, while those of the

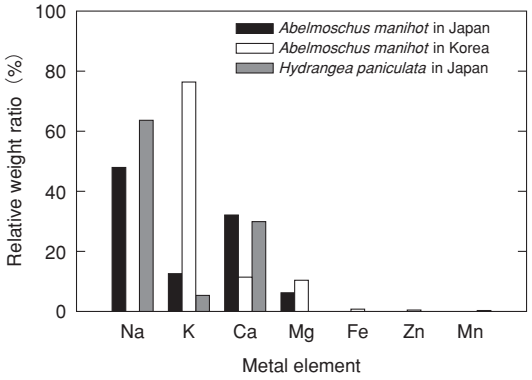

Fig. 2 Relative weight ratio of metal elements in the mucilage samples. Determined by the X–ray fluorescence analysis.

KAM sample were potassium, calcium and magnesium. Especially, the high potassium content of the KAM sample is quite characteristic. Most of these metal elements must be present in the samples as counter ions of uronic acid residues of the mucilaginous polysaccharides. Ash contents of the JAM, KAM and JHP samples were 36.7%, 9.6% and 23.2%, respectively, and these values roughly corresponded to those of relative uronic acid contents in Fig. 1, i.e. 45.5%, 4.6% and 9.3%, respectively. It is possible that divalent calcium ions form intermolecular ionic cross-linkages through uronic acid units of the polysaccharides in aqueous solutions. Thus, the relatively low calcium content of the KAM sample may be related to lower viscosities of KAM solutions, which is empirically well known in traditional handmade-papermaking.

3.2 FT-IR and X-ray analyses

FT-IR spectra of the three mucilage samples are shown in **Fig. 3**, and they had characteristic band patterns in the fingerprint region at 400–1,500 cm^{-1}. The absorption band at 1,600–1,630 cm^{-1} is due to C=O stretching vibration of carboxylate groups of, for instance, uronic acid units. However, because this band is overlapped with that of HOH bending vibration of water molecules adsorbed on the samples, quantitative evaluation is difficult as long as complete drying of the original and KBr disk samples for FT-IR analysis cannot be achieved. The shoulder absorption bands detected around 1,730 cm^{-1} in the KAM and JHP samples indicate the presence of ester bonds, i.e. methyl esters of uronic acid units and/or acetyl esters attached to hydroxyl groups of the polysaccharides in the mucilage samples.

X-ray diffraction patterns of the three mucilage samples are depicted in Fig. 4. All polysaccharide components in these mucilage samples can be regarded to have non-crystalline structures. This is consistent with the result of β-1,4-linked polyglucuronic acid sodium, calcium and aluminum salts, which were prepared from regenerated cellulose by the TEMPO-mediated oxidation[17]. Some sharp diffraction peaks detected in **Fig. 4** are not due to polysaccharide components but probably due to inorganic compounds extracted from the plant roots with water and present in the samples together with the polysaccharide components. Thus, some metal ions detected in Fig. 2 may have originated from the inorganic compounds present in the samples.

3.3 Pyrolysis-GC analysis

Pyrolysis-gas chromatographic (Py-GC) analysis was applied to the three mucilage samples. Generally, Py-

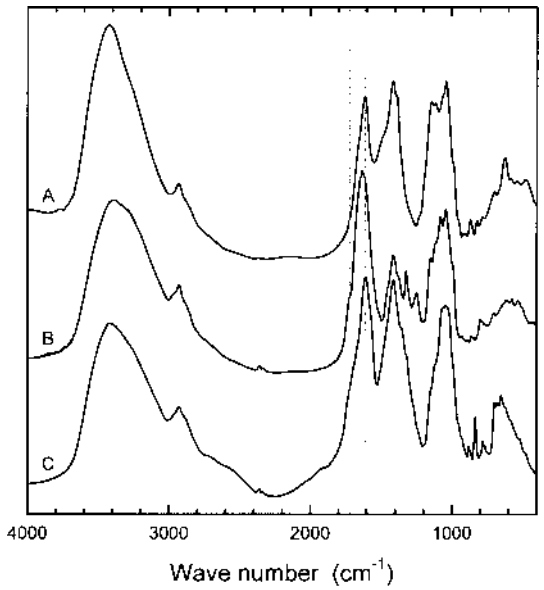

Fig. 3 FT-IR spectra of the mucilage samples obtained from roots of *Abelmoschus manihol* in Japan (A). *Abelmoschus manihot* in Korea (B), and *Hydrangea paniculata* in Japan (C).

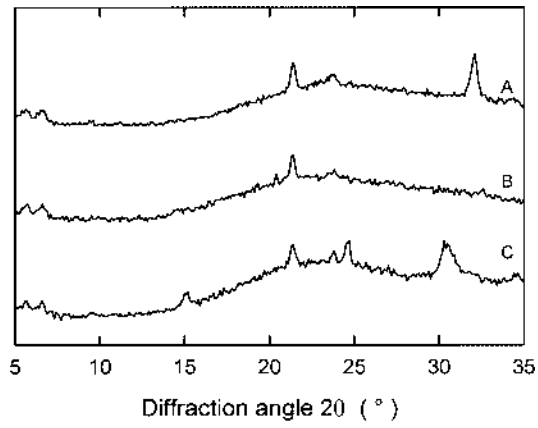

Fig. 4 X-ray diffraction patterns of the mucilage samples obtained from roots of *Abelmoschus manihot* in Japan (A), *Abelmoschus manihot* in Koerea (B), and *Hydrangea paniculata* in Japan (C).

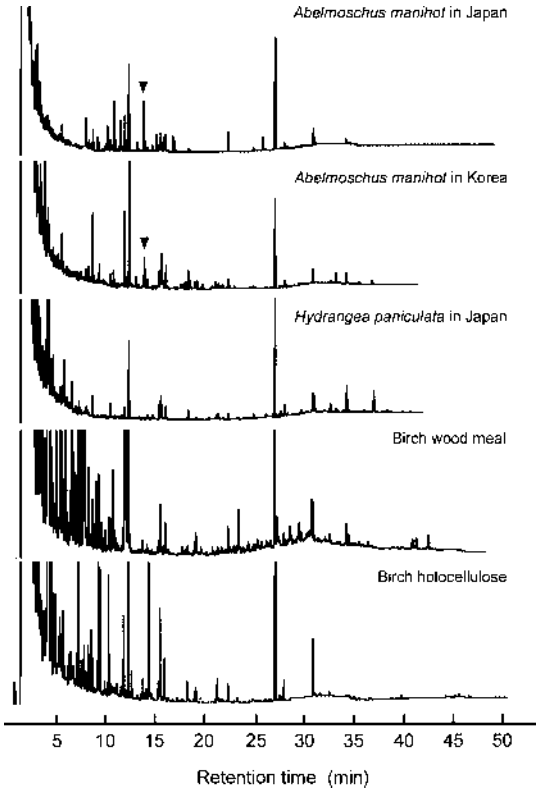

Fig. 5 Pyrolysis–gas chromatograms of the mucilage samples, birch wood meal and birch holocellulose. Measured without the on－line methylation.

GC analysis provides significant information to distinguish or identify organic compounds from peak positions and peak intensities in the obtained Py–GC patterns, just like fingerprint. As shown in Fig. 1, the polysaccharides in the mucilage samples used in the traditional handmade–papermaking contain relatively large amounts of rhamnose as one neutral sugar composition, differing from cellulose and hemicellulose in bast fibers. Thus, differences in Py–GC patterns among the mucilage samples, birch wood meal and birch holocellulose were also studied.

The Py–GC patterns obtained without the on–line methylation are shown in **Fig. 5.** The large peaks at retention times of about 12 and 27 min are due to ghost ones, and appeared in all Py–GC patterns. The peak at about 14 min (pointed by arrows in Fig. 5) was found to be characteristic for the mucilage samples obtained from roots of both Japanese and Korean *Abelmoschus manihot*. Although the origin of this peak is unknown at this point without GC/mass spectroscopic analysis, this characteristic peak may be used for identification of mucilaginous compounds obtained from roots of *Abelmoschus manihot*.

It is well known that the on–line methylation method using tetramethylammonium hydroxide (TMAH) in Py–GC analysis increases thermal stabilities and volatilities of carboxylic compounds formed by pyrolysis[19]. Because peak intensities and number of peaks in the Py–GC patterns are generally improved, consequently, more information can be obtained by the Py–GC analysis combined with the on–line methylation method of, especially, complicated polymeric compounds like wood and paper. Figure 6 depicts the results of Py–GC analysis combined with the on–line methylation using TMAH. The three mucilage samples were distinguishable by the clearly

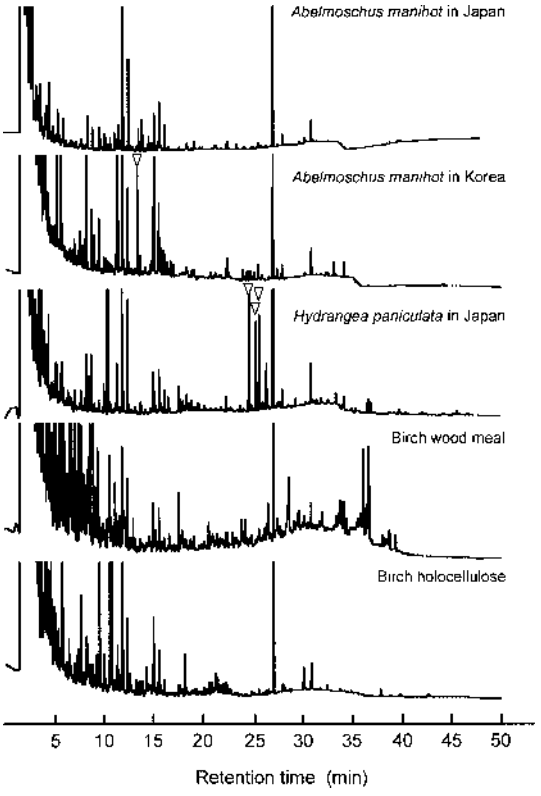

Fig. 6 Pyrolysis–gas chromatograms of the mucilage samples, birch wood meal and birch holocellulose. Measured with the on–line methylation using TMAH.

separated peaks pointed with arrows in **Fig. 6**, and no such peaks were observed in the Py–GC pattern of birch wood meal or birch holocellulose. These peaks may be due to some carboxylic compounds present as minor components in these mucilage samples. Thus, the Py–GC method combined with the on–line methylation allows to distinguish or identify these mucilaginous compounds on the basis of their chromatograms. On the other hand, however, the rhamnose component present in the mucilage samples could not give any characteristic peaks in the Py–GC patterns obtained under the adopted conditions.

3.4 SEC–MALS analysis

SEC–MALS analysis was applied to the mucilage samples to evaluate molecular mass values of the polysaccharide components and their conformations in aqueous solutions. The 0.1 M NaCl was used as the eluent for the SEC analysis to eliminate the effect of carboxylate groups in the polysaccharides on their conformations. When sample solutions for the SEC analysis were prepared by direct dissolution of the freeze–dried mucilage samples in 0.1 M NaCl, clear coagulations or agglomerations were observed, and the polysaccharide molecules in the mucilage samples could not be separated properly according to their occupied volumes by the SEC column. Thus, the freeze–dried mucilage samples were first dissolved in water followed by adjustment to 0.1 M NaCl for the SEC analysis through the addition of NaCl to the solutions.

The obtained SEC elution patterns and their molecular–mass plots are depicted in **Fig. 7.** All SEC elution patterns had peaks around the elution volume of 6.3–6.5 m*l*, which are close to the exclusion limit (i.e. the void volume). However, the molecular–mass plots of every sample decreased with increasing the elution volume, indicating that the polysaccharide molecules in the mucilage samples are properly separated according to their occupied volumes by the SEC column. Again it must be pointed out from the results in Fig. 1 and Photo. 1 that some starch molecules other than the original mucilaginous polysaccharides are present in the KAM sample, and the following discussions con-

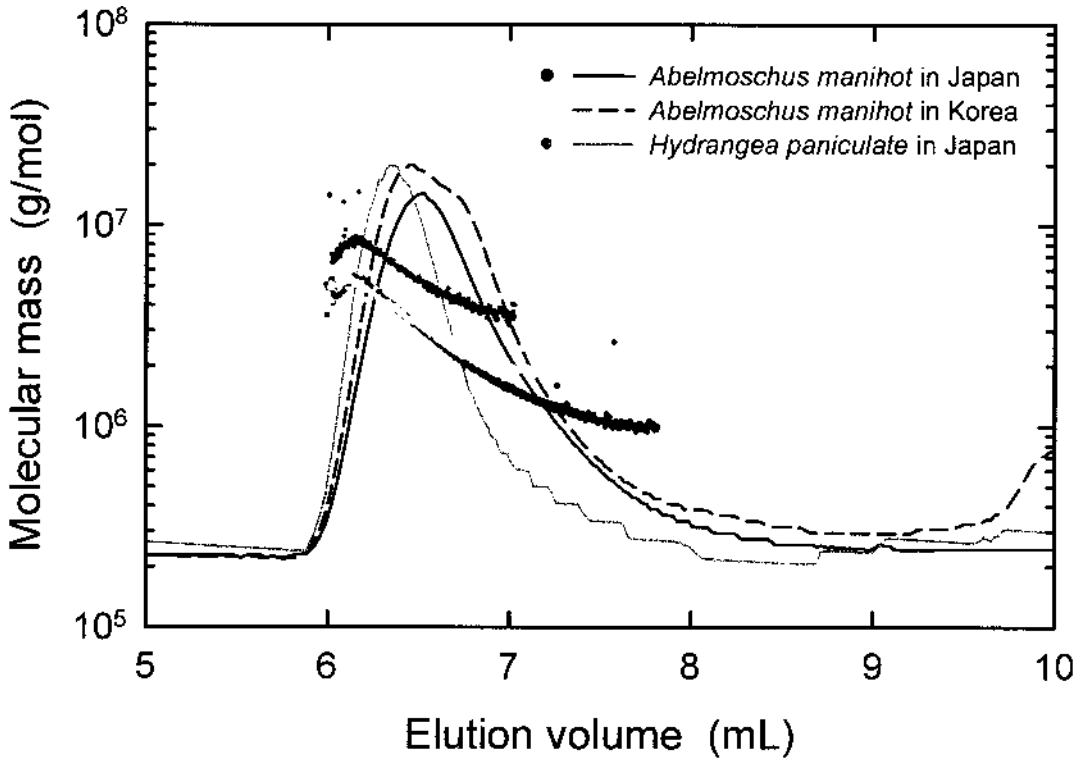

Fig. 7 SEC elution patterns and the correponding molecular mass plots of polysaccharides in the mucilage samples dissolved in 0.1 M NaCl.

Table 1 Weight and number average molecular mass values (Mw and Mn, respectively) of polysaccharides in the mucilage samples*

	Mw	Mn	Mw/Mn
Abelmoschus manihot in Japan	2,370,000	1,970,000	1.2
Abelmoschus manihot in Korea	2,510,000	2,510,000	1.0
Hydrangea paniculata in Japan	4,760,000	3,930,000	1.2

*Calculated by the SEC-MALS method using dn/dc of 0.149 m*l*/g

cerning the KAM sample must be influenced more or less by the starch component.

Weight and number average molecular mass values (Mw and Mn, respectively) of the polysaccharide components calculated by the SEC-MALS method using the same dn/dc value of 0.149 for convenience are listed in **Table 1.** Because the polysaccharides in the three mucilage samples consist of different neutral sugar and uronic acid compositions, the absolute molecular mass values do not make any sense. However, approximate sizes of polysaccharide molecules in the mucilage samples dissolved in 0.1 M NaCl can be evaluated and compared among the three samples. It was found from the results in Table 1 that the polysaccharides in the mucilage samples have quite high molecular mass values. Some of the molecules in this fraction may form some super molecular structures owing to, for instance, intermolecular ionic cross-linkages in aqueous solutions. Furthermore, it is characteristic that the average molecular mass values of the polysaccharides in the JHP sample are about twice as much as those of the JAM and KAM samples (Table 1). In any cases, such quite high molecular mass values of the polysaccharide components in the mucilage samples may consequently bring about their characteristic functions in the traditional handmade-paper-

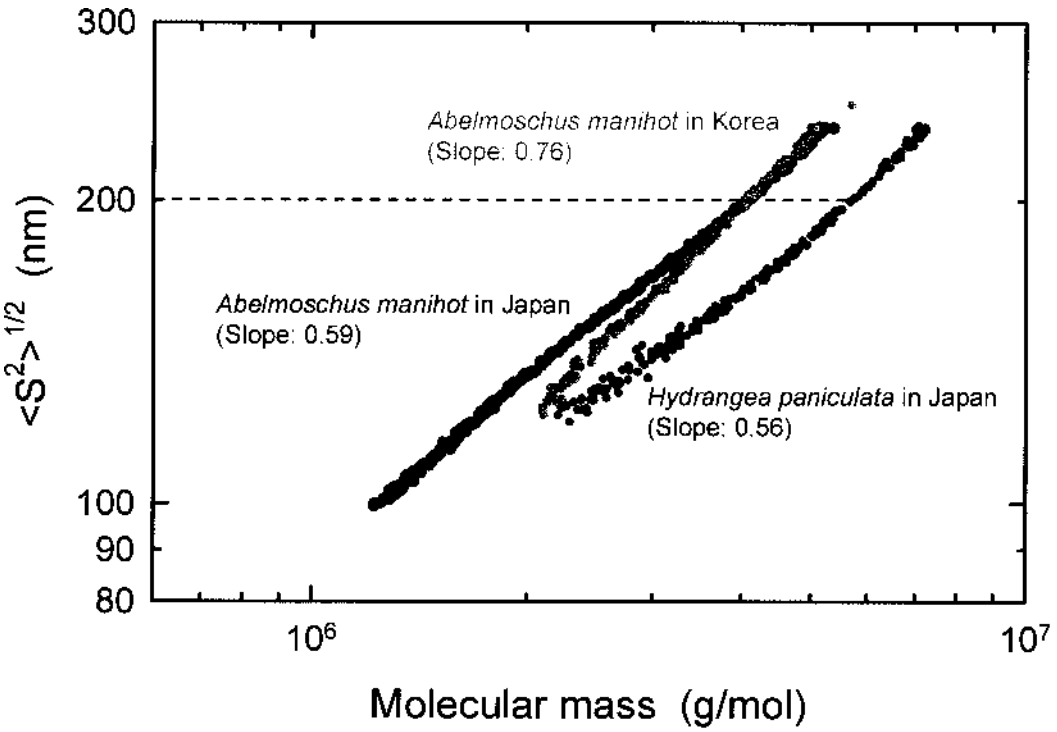

Fig. 8 Radius-of-gyration or conformation plots of polysaccharides in the mucilage samples dissolved in 0.1 M NaCl.

making process.

The molecular mass values of the JHP sample are clearly higher than those of the other two samples at the same elution volume in the whole elution volume range, indicating that the polysaccharides in the JHP sample have more dense structures than those in the JAM and KAM samples. Details about the conformation of the polysaccharides in the mucilage samples are further studied on the basis of their radius-of-gyration or conformation plots in **Fig. 8.** Because the polysaccharide component in the JHP sample had a clearly higher molecular mass value at the same radius-of-gyration than those of the other two, the former polysaccharides may have some branched structures or intermolecular cross-linkages formed between uronic acid units through divalent ions. The slope values of the plots in Fig. 8 are 0.56–0.59 for the JAM and JHP samples, indicating that the polysaccharides in these samples have random coil conformations in 0.1 M NaCl. On the other hand, the slope value for the KAM sample is 0.76, and thus the polysaccharides in this sample have more extended random coil conformations.

Several analytical experiments carried out in this study revealed clear structural differences among the three mucilage samples. However, because the examined sample number is limited, it is unknown at this moment whether these differences are applicable to all Japanese and Korean mucilaginous compounds extracted from roots of the same species or only applicable to the particular samples used in this study. Further studies are, therefore, required for obtaining more general information about the structural differences of mucilage samples as well as for making clear the relationships between chemical structures of mucilaginous compounds and their functions in the traditional handmade-papermaking.

4. Conclusions

Three mucilaginous compounds extracted from roots of Japanese and Korean *Abelmoschus manihot* (JAM and KAM, respectively) and Japanese *Hydrangae paniculata* (JHP) by water are subjected to several chemical analyses, and the following results were obtained;

(1) Sugar compositions are clearly different among the three mucilage samples. The orders of the major sugar components in the JAM, KAM and JHP samples are rhamnose＞galacturonic acid＞glucuronic acid, glucose＞rhamnose＞arabinose and glucose＞ galactose＞rhamnose, respectively. The high glucose component of the KAM sample is, however, mostly due to starch granules present in the sample.

(2) The order of the major metal elements in the JAM and JHP samples is sodium＞calcium, while that for the KAM sample is potassium≫calcium ＝magnesium.

(3) Clear differences in FT-IR spectra were observed among the three mucilage samples, whereas polysaccharides in all mucilage samples had non-crystalline structures.

(4) The three mucilage samples are distinguishable by their pyrolysis-GC patterns obtained by the on-line methylation method.

(5) SEC-MALS analysis of the mucilage solutions in 0.1 M NaCl revealed some differences in molecular mass values and conformations in the solutions among the three mucilage samples. The JAM and KAM samples have similar molecular mass values, while the JAM and JHP samples have similar molecular conformations in the solution.

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