Determination of Molecular Weight of Agars and Effect of the Molecular Weight on the Glass Transition

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A novel procedure to determine the molecular weight (MW) and MW distributions for various agars is described. The MW values of commercial agars, an agarose, an agaropectin, and hydrolyzed agaroses were determined by size exclusion chromatography−low angle laser light scattering, using 4.0 M guanidine hydrochloride as eluent to avoid gelation. The MW for the commercial agars was between 106 400 and 243 500 with polydispersity between 1.283 and 6.600. The MW of the agarose separated from a commercial agar was lower than that of the agaropectin. To prepare agaroses with different MW values, the obtained agarose was hydrolyzed. The MW of the agarose decreased with hydrolysis time, and the polydispersity, on the contrary, increased. The glass transition temperature (T_g) of agarose with different MW values and that of agaropectin were measured by differential scanning calorimetry. The T_g of the agarose was higher than that of the agaropectin with higher MW. The T_g of agarose increased with MW.

Keywords: *Molecular weight; size exclusion chromatography; laser light scattering; agarose; agaropectin; glass transition*

INTRODUCTION

Agars are widely used as gel-forming agents, thickeners, water-holding agents, and stabilizers in the food industry. They are cell-wall polysaccharides extracted with water from certain members of marine red algae and mainly composed of alternating β(1−4)-D-galactose and α(1−3)-3,6-anhydro-L-galactose repeating units (Araki and Hirase, 1953).

The agars consist of polysaccharides that differ in the level of substitution by sulfate, pyruvate, and methoxyl groups (Duckworth and Yaphe, 1971; Lahaye et al., 1986). They are divided into agarose and agaropectin by the degree of substitution (Hjertén, 1962; Fuse and Suzuki, 1975). It is well-known that the degree of substitution and the molar ratio of D-galactose to 3,6-anhydro-L-galactose relate to gelling ability, which is the most important property of the agar (Fuse and Suzuki, 1975; Bourret et al., 1986). However, the gelling ability is associated not only with the chemical characteristics of the molecule but also with the molecular weight (MW) (Fuse and Goto, 1971; Mitchell, 1980; Mouradi-Givernaud et al., 1992).

The MW values of polysaccharides can be measured by viscosity (Fuse and Suzuki, 1975), ultracentrifugation (Hickson and Polson, 1968), and size exclusion chromatography (SEC) (Greer et al., 1984). However, the MW is often influenced by the detection method. The determined MW values of agar and agarose vary especially with the detection method because of their gel-forming property and high viscosity.

Laser light scattering coupled with SEC has recently become a powerful analytical tool in determining the MW and the MW distribution, because laser light scattering does not depend on the standard used (known MW) and the determining condition. Size exclusion chromatography−low angle laser light scattering (SEC-LALLS) and size exclusion chromatography−multiple angle laser light scattering (SEC-MALLS) have been used with success for numerous polypeptides (Mitsuiki et al., 1998a) and polysaccharides (Hizukuri and Takagi, 1984; Vijayendran and Bone, 1984; Lecacheux et al., 1985; Miya et al., 1986; Capron et al., 1995).

SEC-LALLS was utilized in the determination of the MW of the agarose and agarose-type polysaccharides (Rochas and Lahaye, 1989). However, no comparison of MW data obtained by using SEC-LALLS for them has been done. Moreover, there seem to be no data obtained by using SEC-LALLS on the MW values of hydrolyzed agarose and agaropectin.

In recent years, a growing number of food scientists have increasingly recognized the practical significance of the glass transition as a physicochemical event that can govern food processing, product properties, quality, safety, and stability (Slade and Levine, 1991a). Therefore, the characteristic temperature at which the glass transition occurs, called the glass transition temperature (T_g), has been studied as a function of water content for various food polymers. A number of food scientists have investigated the T_g of polysaccharides such as starch (Slade and Levine, 1984; Zeleznak and Hoseney, 1987; Mizuno et al., 1998) and their component polymers (Scandola et al., 1991; Kalichevsky et al., 1992), gellan gum (Papageorgiou et al., 1994), xanthan gum (Yoshida et al., 1990), sodium alginate (Hatakeyama et al., 1996), cellulose (Salmen and Back, 1977), carboxymethylcellulose (Hatakeyama et al., 1996), pullulan (Scandola et al., 1991), dextran (Scandola et al., 1991), and chitosan (Pizzoli et al., 1991).

We have studied the T_g as a function of water content for various galactans such as agars and carrageenans by using differential scanning calorimetry (DSC) and

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Table 1. List of Commercial Agar Samples Studied and Their MW Values and Polydispersities Measured by Using SEC-LALLS

agar	co.	M_w ($\times 10^3$)[g]	M_n ($\times 10^3$)[g]	M_w/M_n[g]
S6	I[d]	243.5 ± 11.0	162.7 ± 9.1	1.497 ± 0.069
UP16	I	183.9 ± 7.3	124.2 ± 4.4	1.481 ± 0.084
UP37	I	184.3 ± 5.4	118.5 ± 0.8	1.555 ± 0.036
AX200	I	124.7 ± 4.8	68.9 ± 2.6	1.809 ± 0.026
AX30	I	106.4 ± 4.1	16.4 ± 2.9	6.600 ± 0.883
T6[a]	S[e]	155.8 ± 8.8	121.6 ± 7.1	1.283 ± 0.072
T7[b]	S	131.7 ± 7.7	94.5 ± 5.7	1.394 ± 0.024
JS[c]	J[f]	131.4 ± 7.6	85.4 ± 4.0	1.539 ± 0.108

[a] Type VI. [b] Type VII. [c] Junsei agar powder. [d] Ina Food Corp., Nagano, Japan. [e] Sigma Chemical Co., St. Louis, MO. [f] Junsei Chemical Co. Tokyo, Japan. [g] Values are mean ± standard deviation of three experiments.

dynamic mechanical analysis (Mitsuiki et al., 1998b). The results showed that the T_g values of agars leveled off at higher water contents and that the minimum T_g values of agars were much higher than those of the carrageenans and a sample of wheat starch. Nevertheless, no T_g data for agarose, agaropectin, and their hydrolysates have been reported.

Slade and Levine (1991a) reviewed the effect of the MW of food polysaccharide on the T_g at low moisture contents. They found that the T_g of malto-oligosaccharides increased with the MW. However, there seems to have been no study on the effect of the MW on the T_g for agarose.

Therefore, the objectives of this study were to develop the method to determine the MW and the MW distribution of various commercial agars, an agarose, an agaropectin, and hydrolyzed agaroses by SEC-LALLS and to clarify the effect of the MW on T_g of agarose and agaropectin.

MATERIALS AND METHODS

Materials. The agar samples studied were obtained from various companies. Table 1 lists the samples. To investigate the accuracy and reproducibility of this MW determination method, a commercial pullulan with an accurately known MW (P-100, Showa Denko Co., Ltd., Tokyo, Japan) was purchased.

Preparation of Agarose and Agaropectin Samples. Agarose and agaropectin were prepared from an agar named S6, which is one of the most popular agars in the Japanese food industry (Table 1). First, the agaropectin was precipitated from the agar solution by the addition of cetylpyridinium chloride according to the procedure of Hjertén (1962). The precipitate was suspended in 2.0 M NaCl aqueous solution and liquefied by heating. The Na salt of agaropectin was obtained as a precipitate from the solution by the addition of 10 times volumes of ethanol.

The agarose was obtained from the supernatant after removal of the agaropectin precipitate. After being left for several hours at ambient temperature, the supernatant gelled. The gelled supernatant was crushed, washed, then converted to the Na salt, and precipitated with ethanol by the usual way as follows (Hjertén, 1962; Fuse and Suzuki, 1975): the gelled supernatant was suspended in 2.0 M NaCl aqueous solution and liquefied by heating; the Na salt of agarose was obtained as a precipitate from the solution by the addition of 10 times volumes of ethanol.

The Na salts of agarose and agaropectin were lyophilized prior to measurement of MW and T_g values.

Hydrolysis of Agarose. To prepare agaroses with different MW values, the obtained agarose was hydrolyzed in 0.1 or 0.01 N HCl at 60 °C for 0, 30, 60, 120, and 240 min. After neutralization with 0.01 N NaOH, the hydrolysates were dialyzed and precipitated with ethanol. A cellulose dialyzer tubing VT351 (MW cutoff = 3 500, Nacalai Tesque, Inc., Kyoto, Japan) was employed for the dialysis of the hydrolysate with lower MW. The hydrolysates were lyophilized and used for measuring their MW and T_g values.

Choice of Eluent for SEC-LALLS. To select a suitable eluent for the SEC-LALLS, we looked for an eluent in which the agar would dissolve without gelling at the usual polysaccharide concentration (2 mg/mL) for measuring MW by the SEC-LALLS. It is thought that the gel network of the agar was formed by the intra- and intermolecular hydrogen bonds of agar molecules (Arnott et al., 1974). Thus, the solutions of guanidine hydrochloride and urea, which are employed as a disruptor of hydrogen bonds, were examined for use as eluents for SEC-LALLS. The agar S6 was dissolved in boiling distilled water, in 2.0, 4.0 and 8.0 M guanidine hydrochloride, and in 2.0, 4.0 and 8.0 M urea at 2.0 mg/mL. Each agar solution was poured into a tube (10 mm i.d.) and left at 25 °C for 24 h. The applicability as the eluent for SEC-LALLS was determined if the solution gelled.

Determination of MW and MW Distribution. The MW values and the MW distributions of the agar samples, agarose, agaropectin, and hydrolyzed agaroses were measured by SEC-LALLS. The SEC-LALLS measurement was carried out on HPLC equipment consisting of the following components: an 880-PU pump (Japan Spectroscopic Co., Tokyo, Japan), an 851-AS autosampler (Japan Spectroscopic Co.), an Asahipak GFA-7M HQ column (Showa Denko Co., Ltd.), an LS-8000 LALLS photometer (Tosoh Corp., Tokyo, Japan), and an RI-8011 differential refractometer (Tosoh Corp.). The column was thermostated at 40 °C and eluted with a chosen eluent at flow rate of 0.7 mL/min. Samples were dissolved in the same solution at 2.0 mg/mL and injected (200 μL). Commercial pullulan (P-400, Showa Denko Co., Ltd.) was used as the standard for MW determination. Weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity (M_w/M_n) of each sample were calculated using software (GPC-LALLS version 3.04, Tosoh Corp.) based on the previously described theory of SEC-LALLS (Mua and Jackson, 1997). The M_w, M_n, and M_w/M_n were the mean values of three experiments.

Sample Preparation for DSC Analysis. The agar S6, the agarose, the agaropectin, and the hydrolyzed agaroses with various water contents (10−40%) were prepared according to the method of Mitsuiki et al. (1998b). The water content of each sample was determined according to the method described in previous papers (Mizuno et al., 1998; Mitsuiki et al., 1998b).

Measurement of T_g by DSC. The T_g values of various agarose and agaropectin samples were determined according to the method described previously (Mizuno et al., 1998; Mitsuiki et al., 1998b). The method is as follows: heat-flux DSC (DSC120, Seiko Instruments, Chiba, Japan) was used for measurement of the T_g values of the samples. The DSC was calibrated with Ga, In, and Sn. Each sample (~45 mg), prepared with a different water content, was placed into a silver pan (70 μL). The pan was then hermetically sealed and accurately weighed. An empty silver pan was used as reference. The pans were heated in the DSC at 10 °C/min to detect the incremental change in heat flow associated with the glass transition.

It is well-known that the glass transition does not occur at a single temperature but at a range of temperatures. There are many papers showing T_g as a temperature range [e.g., Cherian and Chinachoti (1997)]. However, it is thought that a maximum change in heat flow or rheological property occurs at the midpoint of T_g and that the midpoint is approximately the mean value of the distribution (Brinke et al., 1983; Mitsuiki et al., 1998b). We believe that the midpoint T_g data suffice for comparison of the height of T_g. Hence, we defined T_g as the peak in the derivative of the heat-flow curve accompanying the shift in the heat flow, which is considered by us to provide the most unequivocal T_g (Mizuno et al., 1998; Mitsuiki et al., 1998b).

Samples that did not clearly reveal such a change during the first heating were rapidly cooled and reheated at 10 °C/min to more clearly reveal the change in heat flow.

Table 2. Gel Formation of Agar Solutions Containing Guanidine Hydrochloride or Urea at Different Concentrations

solvent	gel strength
distilled water	+++[a]
2.0 M guanidine hydrochloride	+
4.0 M guanidine hydrochloride	−[b]
8.0 M guanidine hydrochloride	−
2.0 M urea	+
4.0 M urea	+
8.0 M urea	−

[a] Number of +'s is indicative of gel strength of a 2 mg/mL agar solution. [b] No gel forming at an agar concentration of 2 mg/mL.

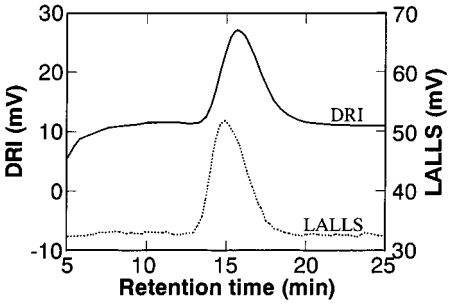

Figure 1. SEC-LALLS and DRI chromatogram for agar S6.

RESULTS AND DISCUSSION

Choice of Eluent for SEC-LALLS. To determine the applicability of 2.0−8.0 M guanidine hydrochloride and 2.0−8.0 M urea as the eluent for SEC-LALLS, we investigated if agar solutions gelled after 24 h. Agar solutions containing ≥4.0 M guanidine hydrochloride and ≥8.0 M urea did not gel (Table 2). This result suggests that guanidine hydrochloride and urea disrupted the intra- and intermolecular hydrogen bonds of agar molecules at the higher concentration and suppressed gelation and coil-to-helix transition of the agar. Because the differential refractive index (DRI) intensity of 8.0 M urea and guanidine hydrochloride is considerably strong, it is thought that the intensity of the agar solutions is liable to increase beyond the detection limit. Therefore, we chose 4.0 M guanidine hydrochloride, which has a relatively low DRI intensity, as the eluent for SEC-LALLS.

MW and MW Distributions of Various Commercial Agars. The MW values and the MW distributions of various commercial agars were determined by SEC-LALLS, for which 4.0 M guanidine hydrochloride was used as the eluent. Figure 1 shows an example of LALLS and DRI chromatogram for an agar (S6). Broad single peaks are observed in both the LALLS and DRI chromatogram for every commercial agar, as shown in the figure. On the other hand, sharp single peaks are observed in both chromatograms for 4.0 M guanidine hydrochloride solution of the pullulan P-100.

The MW and MW distribution of the pullulan and of each agar were calculated from the chromatograms using the software mentioned previously. The calculated M_w, M_n, and M_w/M_n of the pullulan were 107 100 ± 3 400, 104 000 ± 2 100, and 1.029 ± 0.013 (mean value ± standard error of three experiments), respectively. The M_w of the pullulan was determined to be 100 000. Thus, the M_w value agrees fairly well with that obtained by this method. Moreover, the error among three M_w values was ∼3%. Therefore, we believe that the accuracy and reproducibility of present method are satisfactory.

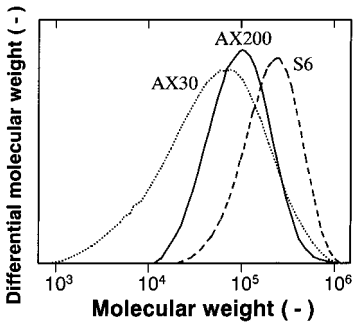

Figure 2. MW distribution of agars S6, AX200, and AX30 determined by using SEC-LALLS. The notation "−" in the unit means that the values are nondimensional numbers.

Table 1 shows the calculated M_w, M_n, and M_w/M_n of commercial agars. Their M_w values, which were generally used as the indicator of the MW, were between 106 400 and 243 500. These values are slightly higher than the MW reported previously by Hickson and Polson (1968), who determined them by sedimentation and diffusion experiments, and by Rochas and Lahaye (1989), who used SEC-LALLS as previously mentioned. For example, Rochas and Lahaye (1989) reported that the M_w values of agar T6 and T7 were 102 000 and 92 000, respectively. Thus, their M_w data agree in the order with our data. However, the M_w values were lower than the present values and were two-thirds of the values obtained in this study (Table 1; T6 = 155 800; T7 = 131 700). The reason for the difference in the M_w between their and our analyses might be due to the temperature conditions in the column.

Figure 2 shows examples of the MW distribution of three commercial agars (S6, AX200, and AX30). As can be seen from Table 1 and Figure 2, there was a significant difference in the MW distribution among the agars. Their M_w/M_n were between 1.283 and 6.600. These values are in good agreement with the values determined by Rochas and Lahaye (1989). They reported that the M_w/M_n of agar T6 and T7 were 1.32 and 1.41, respectively. Therefore, the values agree very well with our data (Table 1; T6 = 1.283; T7 = 1.394).

Because 0.1 M $NaNO_3$ aqueous solution was employed as an eluent in the method of Rochas and Lahaye (1989), the sample solution must be injected hot (90−95 °C) to avoid gelation of the sample solution. Therefore, this method is obviously tedious. Moreover, because the column for SEC was thermostated at 45 °C during the determination, there was a temperature gradient of the sample in the column. Thus, the accuracy and the reproducibility of the method are questionable. On the other hand, it is thought that this novel method is a simpler and more stable method than their method. As mentioned above, we confirm the accuracy and reproducibility of this method for MW standard pullulan. Therefore, though there is a difference in MW and MW distribution between their method and this new method, we believe that the accuracy of MW and MW distribution data obtained by using this method is still high.

MW and MW Distribution of Agarose and Agaropectin. The MW values and the polydispersities of agarose and agaropectin separated from agar S6 were also determined by SEC-LALLS. The M_w and M_n of the agaropectin were much higher than those of both the agarose and agar S6 (Table 3). On the other hand, there was no significant difference in M_w/M_n between the agarose and the agaropectin.

Table 3. MW Values and Polydispersities of Agarose and Agaropectin Separated from Agar S6

sample	M_w ($\times 10^3$)[a]	M_n ($\times 10^3$)[a]	M_w/M_n[a]
agarose	167.6 ± 7.8	115.7 ± 7.7	1.448 ± 0.031
agaropectin	405.2 ± 18.7	306.1 ± 16.4	1.324 ± 0.014
agar S6	243.5 ± 11.0	162.7 ± 9.1	1.497 ± 0.069

[a] Values are mean ± standard deviation of three experiments.

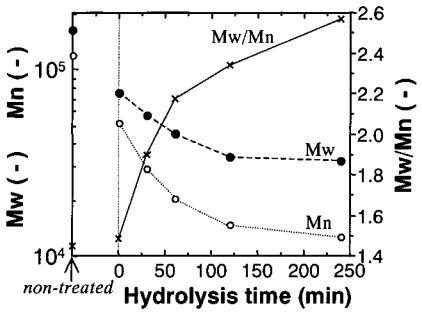

Figure 3. Variation in M_w, M_n, and M_w/M_n for an agarose with time of hydrolysis in 0.01 N HCl at 60 °C. The agarose was fractionated from agar S6. The notation "−" in the unit means that the values are nondimensional numbers.

Table 4. MW Values and Polydispersities of Agarose and Hydrolyzed Agarose in Different Solutions

hydrolysis solution	M_w ($\times 10^3$)	M_n ($\times 10^3$)	M_w/M_n
0.1 N HCl[a]	9.5	3.5	2.714
0.01 N HCl[a]	32.7	12.7	2.572
nontreated	167.6	115.7	1.448

[a] Hydrolyzed in the solution for 240 min at 60 °C.

There seem to have been no studies on comparison of the MW values of agarose and agaropectin by SEC-LALLS. The previous papers reported that the MW of agarose, which was determined by sedimentation, diffusion, and viscosity analyses, was much higher than that of agaropectin (Hickson and Polson, 1968; Fuse and Suzuki, 1975). Those results disagree with our results (Table 3). It was assumed in such MW determination methods that the MW of agaropectin was calculated according to the practical correlation equation between its MW and viscosity or diffusion coefficient for the agarose. There is a significant difference in the level of substitution by sulfate, pyruvate, and methoxyl groups between the agarose and agaropectin (Duckworth and Yaphe, 1971; Lahaye et al., 1986). It is, therefore, questionable that the same correlation equation for the agarose would apply to agaropectin. Thus, we believe that the MW of agaropectin is higher than that of the agarose, at least when separated from the agar S6 used in this study. Further experiments are necessary to determine the MW values of agarose and agaropectin samples separated from many agar samples.

Variation in MW and MW Distribution of Agarose with Hydrolysis Time. The agarose fractionated from agar S6 was hydrolyzed in 0.01 N HCl at 60 °C for 0−240 min. The variation in M_w, M_n, and M_w/M_n with hydrolysis time is shown in Figure 3. The M_w and M_n of the agarose gradually decreased with hydrolysis time, and those of the agarose hydrolyzed for 240 min were 32 700 and 12 700, respectively (Table 4). It was found that the extent of the decrease in the M_n with hydrolysis time was larger than that in M_w. As a result, the M_w/M_n gradually increased with hydrolysis time (Figure 3). It was, therefore, proved that the agarose molecules were becoming heterogeneous in their MW with the extent of hydrolysis. This tendency agrees with the heat degradation of the agarose in a previous paper (Hickson and Polson, 1968).

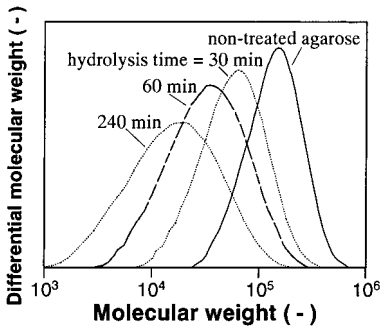

Figure 4. MW distribution for the agarose fractionated from agar S6 and for the agarose hydrolyzed in 0.01 N HCl for 30, 60, and 240 min, which were determined by SEC-LALLS. The notation "−" in the unit means that the values are nondimensional numbers.

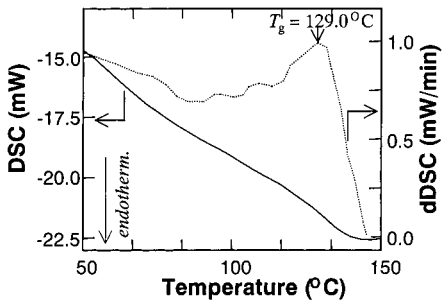

Figure 5. DSC thermogram of the agaropectin fractionated from agar S6 (water content = 10.4%).

The agarose was also hydrolyzed in 0.1 N HCl at 60 °C for 240 min. Most of the hydrolyzed agarose passed through the ordinary cellulose membrane (MW cutoff = ∼12 000). Thus, a cellulose dialyzer tubing VT351 (MW cutoff = 3 500) was employed for the dialysis. The M_w and M_n of the hydrolyzed agarose were much smaller than those hydrolyzed in 0.01 N HCl, as expected (Table 4). On the other hand, there was no significant difference in M_w/M_n between them. It could be thought that the hydrolyzed agarose had apparently become homogeneous, because the hydrolysate with the MW <3 500 was removed.

We could prepare the agaroses with different MW values. Thus, we hereafter investigated the relationship between the MW and T_g for the agaroses.

Glass Transition Properties of Agaropectin and Agaroses with Different MW Values. The agaroses hydrolyzed in 0.01 N HCl at 60 °C for 0 and 240 min were used as the hydrolyzed agaroses with higher MW (M_w = 76 700, see Figure 3) and intermediate MW (M_w = 32 700, see Table 4), respectively. The agarose hydrolyzed in 0.1 N HCl at 60 °C for 240 min was used as the hydrolyzed agaroses with lower MW (M_w = 9 500, see Table 4). The T_g values of hydrolyzed samples, nontreated agarose, and nontreated agaropectin were measured by using DSC.

Figure 5 shows the typical DSC thermogram for the agaropectin with a water content of 10.4%. A clear shift in heat flow and a peak in the derivative of the heat flow (dDSC), which were considered to be associated with the glass transition (Mitsuiki et al., 1998b), were observed around 130 °C. Thus, it was estimated that the T_g of the agaropectin containing 10.4% water was 129.0 °C from the temperature of the maximum in the

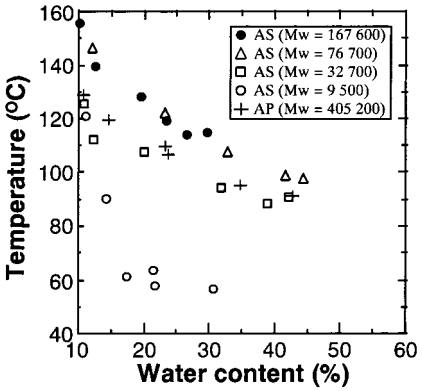

Figure 6. Variation in T_g with water content for agaropectin (AP) and agarose (AS) with different MW values.

dDSC. The T_g values of the agaropectin and agaroses with water contents ranging from 10 to 45% were measured in a similar manner and are plotted as a function of water content in Figure 6. It was found that T_g decreased with increasing water content, indicating that water acts as a plasticizer for all measured samples, as it does for other polysaccharides (Zeleznak and Hoseney, 1987; Scandola et al., 1991; Slade and Levine, 1991a; Kalichevsky et al., 1992; Hatakeyama et al., 1996; Mizuno et al., 1998).

The water content dependence of the T_g for the hydrolyzed agarose with higher MW (M_w = 76 700) was quite similar to that of the nontreated agarose (M_w = 167 600) (Figure 6). On the other hand, the T_g of the hydrolyzed agarose with intermediate MW (M_w = 32 700) was lower than those of both the nontreated and higher MW samples at all water contents. It seems that T_g values of the nontreated sample and the hydrolyzed samples with higher and intermediate MW leveled off at higher water content (>25%) and that their minimum values were well above ambient temperature. Such a water content dependence for the T_g was different from those of most food polysaccharides and was similar to those for "water-sensitive" polymers such as a lignin (Kelly et al., 1987) and agars (Mitsuiki et al., 1998b). This small difference in the water content dependence between T_g values of agars confirmed the accuracy in our previous paper (Mitsuiki et al., 1998b) and that of an agarose in this study. We therefore believe that T_g values in this study are accurate as well.

On the other hand, the water content dependence of T_g for the hydrolyzed agarose with lower MW (M_w = 9 500) was considerably different from those of the agaroses with higher MW (Figure 6). The T_g of the lower MW agarose greatly decreased as the water content increased. As a result, the T_g values of the hydrolyzed agarose with lower MW were much lower than those of the agaroses with higher MW and of agaropectin, especially at higher water content. Such a water content dependence for the T_g was similar to those of most food polysaccharides. Therefore, it is thought that the anomalous water content dependence of the T_g for agar and agaroses is lost with decreasing MW.

From these results, it was considered that the T_g of the agarose increased with increasing MW and then leveled off with further increases in MW.

For a homologous series of amorphous linear synthetic polymers, it has been well-known that the T_g increases with increasing MW, due to decreasing free volume contributed by chain ends, up to a plateau limit for the region of entanglement coupling in rubber-like viscoelastic random networks, and then levels off with further increases in MW (Slade and Levine, 1991b). Moreover, it is understood that the T_g values for each synthetic polymer reveal three distinguishable intersecting linear regions in the plot of log MW versus T_g: (1) a steeply rising region for nonentangling small o1igomers; (2) an intermediate region for nonentangling low polymers; and (3) the horizontal plateau region for entangling high polymers (Slade and Levine, 1991b). Thus, the relationship between T_g and MW for the synthetic polymers agrees fairly well with the relationship between them for agaroses (Figure 6). It was, therefore, assumed that the T_g of the agarose also increases with increasing MW, due to decreasing free volume up to a plateau limit for the region of entanglement coupling in rubber-like viscoelastic random networks, and then levels off with further increases in MW. It was estimated that there was a plateau limit in M_w for the agarose between 32 700 and 76 700 (Figure 6).

On the other hand, despite the much higher MW (M_w = 405 200), the T_g of the agaropectin was lower than those of both the nontreated agarose and the hydrolyzed agarose with higher MW, over the entire range of water content (Figure 6). The water content dependence of the T_g for the agaropectin was rather similar to that for the hydrolyzed agarose with intermediate MW. Thus, it could be thought that the T_g of the agaropectin was lower than that of agarose with the same MW. In our previous paper (Mitsuiki et al., 1998b), we assumed that the T_g values of galactans such as agar and carrageenan decrease with increasing sulfate group content. Therefore, we believe that the difference in the water content dependence of T_g between agarose and agaropectin is explained by the difference in their sulfate group content.

It is well-known that the content of sulfate groups, in addition to MW, shows a good correlation with gel strength of agars and agar sulfates (Fuse and Suzuki, 1975). From the above data, it is thought that the content of sulfate groups and the MW influence somewhat the T_g for the agar and its component polymers. We think that an investigation of the correlation between the T_g curves and the sulfate group content or MW is necessary.

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