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Preparation of low-molecular weight alginic acid by acid hydrolysis

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Abstract

Three kinds of low-molecular weight alginic acid fractions, Alg.A, B, and C, were prepared from a commercial alginic acid by acid hydrolysis using phosphoric acid. Alg.A was obtained as an insoluble fraction by the filtration of mixture. Alg.B was obtained as a precipitate by pouring the filtered solution into water. Alg.C was obtained as a precipitate by pouring the filtrate into methanol. Measurements with ^{13}C NMR, GPC and WAXS were performed on the prepared fractions for characterization.

Alg.A was composed of rich M and G blocks, and had DP_n and DP_w/DP_n values of 79 and 3.11, respectively. Alg.B was mainly composed of M block, and had DP_n and DP_w/DP_n values of 38 and 2.57, respectively. Alg.C had a random structure including many alternating sequences, and had DP_n and DP_w/DP_n values of 35 and 2.11, respectively. Alginic acid oligomers prepared in this study, Alg.B and C, were improved regarding in solubility in water and the viscosity of their aqueous solution.

Keywords: Alginic acid; Gel permeation chromatography; Degree of polymerization

1. Introduction

Alginic acid is a polyuronide obtainable from brown seaweed, and composed of (1–4) linked β-D-mannuronic acid and α-L-guluronic acid (Fischer & Dörfel, 1955; Nelson & Cretcher, 1929). In alginic acid both residues exist in the form of homopolymer block (M block, G block) or heteropolymer block (MG block) (Haug, Larsen & Smidsød, 1967).

Alginic acid has already established its applications in food, pharmaceutical and medical industries (Kennedy, Griffiths & Atkins, 1984; McNeely & Pettitt, 1973). However, not much has been reported on the chemical modification of alginic acid or its salt. Propylene glycol alginate was produced as the only commercial alginic acid derivative (Kennedy, Griffiths, Philp, Stevenson, Karnbanis & Gray, 1989; Nishide, 1963a,b; Steiner & McNeely, 1951). Although algin acetate and algin sulfate have also been described (Schweiger, 1962a,b), they have not been applied commercially.

Alginic acid is the only polysaccharide which naturally contains carboxyl groups in each constituent residue, and possesses various abilities for functional materials. Considering the potential amount as a natural resource and reproductivity of alginic acid, it is meaningful to develop new possibilities for the chemical source.

As described above, alginic acid has an heterogeneous structure containing both mannuronic and guluronic acids as constituents. For simplicity in chemical modification treatment, it is beneficial to prepare alginic acid oligomer with uniformity in both constitution and molecular weight. In this paper, preparation of alginic acid oligomer by acid hydrolysis was carried out, and the characterization of the product was also performed.

2. Experimental

2.1. Materials

Commercial alginic acid was furnished by Nicca Chemical Co. Ltd. All other chemicals used were extra pure grade reagents in accordance with the Japanese Industrial Standard and used as-received.

2.2. Preparation method

Hydrolysis of alginic acid using phosphoric acid was carried out according to the method for preparation of low-molecular weight cellulose reported by Isogai and Usuda (1991). The scheme of this method is shown in Fig. 1. Raw alginic acid (5 g) was dispersed in 85%

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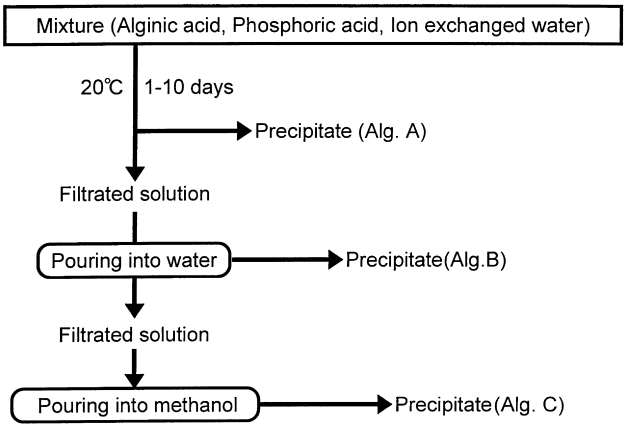

Fig. 1. Preparation scheme of alginic acid oligomer.

phosphoric acid (94 ml) and hydrolyzed heterogeneously at room temperature for 1–10 days. Insoluble fraction (Alg.A) was collected by filtration. The filtrate was poured into water (400 ml) and methanol (2 l) successively and the precipitates (Alg.B, C, respectively) were collected by centrifugation. All fractions collected were washed in water or methanol to remove the phosphoric acid present, prior to lyophilization.

2.3. Analysis of the hydrolyzed fractions

^{13}C NMR measurements were performed to determine the composition and the sequential structure of the prepared fractions and raw alginic acid. Their NMR spectra were recorded on a JEOL alpha 500 (125 MHz), using 1H(X)-Tunable probe. The inverse-gated decoupling method was selected as an irradiation mode to eliminate the nuclear Overhauser effect for the quantitative measurement. 60 000 scans were accumulated to obtain a clear spectrum. The hydrolyzates and raw alginic acid were dissolved in deuterium oxide with neutralization by sodium deuteroxide, and used for measurement. Sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 was added as an internal standard.

Gel permeation chromatography (GPC) was performed on hydrolyzed and raw alginic acids to determine their degree of polymerization (DP). TSK-gel G5000PW$_{XS}$ and G3000PW$_{XS}$ (Toso Co. Ltd) connected in series were used as column. Phosphoric acid buffer (pH 7.0) was used as an eluent. Elution speed and column temperature were 0.5 ml/min. and 40°, respectively. The DP_w and DP_n values were obtained from the chromatograms by calibration using five kinds of standard sodium polyacrylates.

X-ray diffraction patterns of the pellet samples were recorded on a RINT 2000, using CuK_α radiation. The conditions for measurements were: reflective method; FT as a scanning mode; 5–40° of scanning range; 0.1° of step angle; and 20 s of sampling at each step.

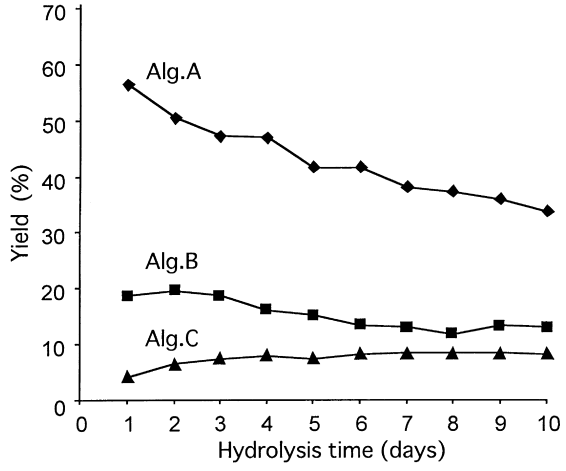

Fig. 2. The yields of hydrolysates.

3. Results and discussion

3.1. Yields of the hydrolyzates

The yields of hydrolyzed fractions at various standing time are shown in Fig. 2. The yield of Alg.A is 56.4% at one-day hydrolysis, which then decreases moderately. The yield of Alg.B gives a maximum around one or two days of hydrolysis, and then decreases. The yield of Alg.C reaches to approximately 8%. Total yield of hydrolyzate decreases rapidly to 79% at one-day hydrolysis. These results suggest the existence of easily hydrolyzable part and non-hydrolyzable part in raw alginic acid. Thus, Alg.A remains as a non-hydrolyzable part, and the easily hydrolyzable part is hydrolyzed in one day. Hydrolyzed fractions were separated by the use of solubilities for water and methanol.

Considering that the final filtrate solution was colored brown, the methanol soluble fraction seemed to lose its uronic skeleton. In this study, this fraction was not collected, because it was thought to be no longer alginic acid oligomer as an objective material, and had difficulties in its isolation from the solution.

3.2. Structural analysis of hydrolyzates

^{13}C NMR spectra of raw alginic acid and hydrolyzed fractions are shown in Fig. 3. The peaks in the spectra are assigned according to the previous data for alginate (Grasdalen, Larsen & Smidsød, 1981). The corresponding peaks are almost the same in the chemical shift but different in intensities. Detailed discussions of their spectra are carried out in the region of anomeric carbon and of C2–C5 to characterize their sequential structure and monomer composition.

^{13}C NMR spectra of the anomeric region are shown in Fig. 4. C-1 carbons of mannuronic and guluronic acids show peaks in the range of 102–104 ppm, and the each peak is separated owing to the vicinal residue bonded with the carbon. Four peaks in the anomeric region of the spectra

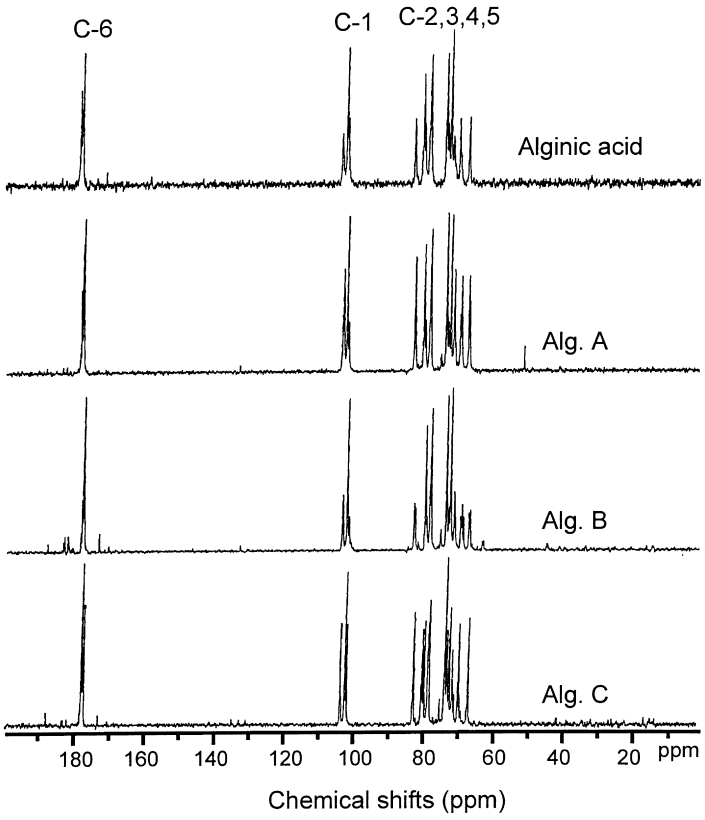

Fig. 3. ^{13}C-NMR spectra of alginic acids and hydrolyzates.

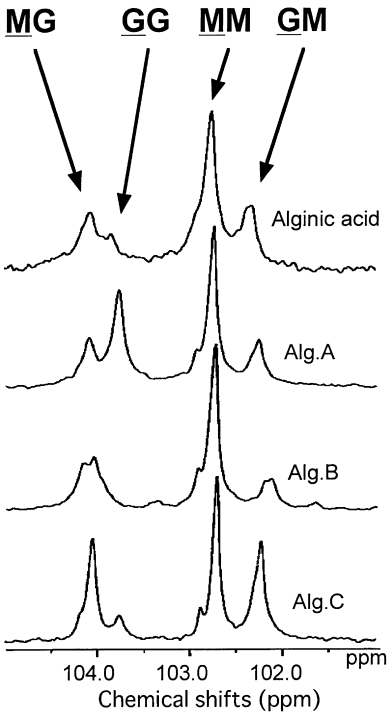

Fig. 4. ^{13}C-NMR spectra of anomer region.

are corresponding to MG, GG, MM and GM in the order from lower-magnetic field side. ^{13}C NMR measurements were performed with quantitative method in order to estimate the sequential structure, so that the intensities of the peaks can indicate the frequency of the diad sequence in the fractions. NMR spectrum of raw alginic acid shows MM rich and GG poor composition. This demonstrates that the alginic acid used in this study has a mannuronic acid rich composition. The frequency of the GG sequence relatively increases in Alg.A as compared with raw alginic acid, which suggests that the G block have unhydrolyzable nature, and the other sequential parts are degraded and dissolved. This nature of G block was already mentioned in a previous publication using oxalic acid (Haug, Larsen & Smidsød, 1966). The similar nature is found again by using phosphoric acid. Small amount of relative frequencies of GM and MG suggests that the homopolymer block (M block, G block) is long in Alg.A. In the spectrum of Alg.B, the peak of GG sequence at 103.08 ppm diminishes while the amount of MM sequence at 102.78 is significantly large. Therefore the Alg.B is composed of long homopolymer block of mannuronic acid. The monomer composition of Alg.C is, similar to Alg.B, rich in MM and poor in GG sequence. However the amounts of GM and MG sequence in Alg.C is more than those of Alg.B, which suggests that the length of the M block in Alg.C is shorter than that in Alg.B.

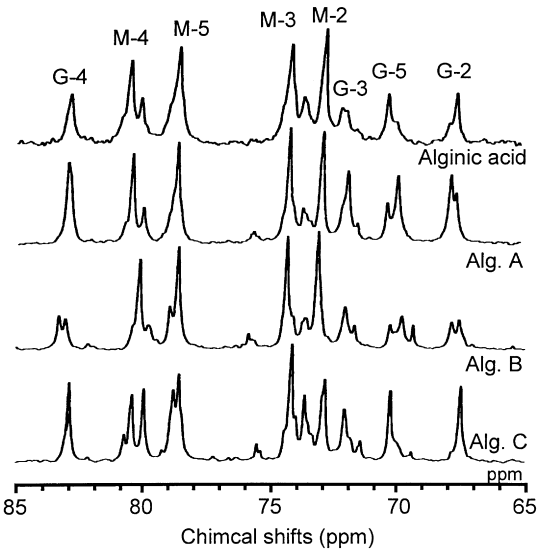

Fig. 5. ^{13}C-NMR spectra of raw and hydrolyzed alginic acids in C2-C5 region.

Spectra of C-2,3,4 and C-5 are shown in Fig. 5. The peaks at 80.5 and 80.0 ppm indicate M-4 carbon which is bonded with mannuronic acid and guluronic acid, respectively. In raw alginic acid, Alg.A and Alg.B, the vicinal residues bonded with M-4 are mainly mannuronic acid; therefore mannuronic acid may form homopolymeric structure. In Alg.C, both mannuronic and guluronic acids bond with the objective carbons in a similar frequency. Thus the sequential structure of this fraction is thought to be alternating.

The peak of G-4 appears at 83.0 ppm. Amount ratios of mannuronic acid and guluronic acid (M/G ratio) in the fractions was calculated by the integration of the peak of G-4 and the doublet of M-4. Results are shown in Table 1. The M/G ratio of alginic acid used in this study is 2.11, on the contrary, the M/G ratio of Alg. A is 1.14. This suggests that the sequence containing guluronic acid is hardly hydrolyzed, and remains in precipitate. Alg. B and Alg. C would contain more mannuronic acid, as their M/G ratios are similar.

The peaks of M-5 and G-5 are at 78.7 ppm and in the region of 69.4–70.3 ppm, respectively. The doublet or triplet of these peaks may depend on the kind of vicinal residue, but not assigned. As the overlap of these peaks with others is relatively less, similar evaluation was performed on these peaks. The results are shown in Table 1. These correspond to the results from C-4. These results support the evaluation from the spectra of C-1 region.

Table 1
M/G ratio of raw and hyrolyzed alginic acids

	M/G ratio	
	C-4	C-5
Alginic acid	2.11	2.20
Alg.A	1.14	1.35
Alg.B	1.91	1.60
Alg.C	1.87	1.92

Table 2
Molecular weight of hydrolyzates

		Standing time (days)		
		2	5	10
Alg.A	M_n	16 400	13 900	11 700
	M_w	53 900	43 000	31 800
	M_w/M_n	3.28	3.11	2.71
Alg.B	M_n	8600	6600	5300
	M_w	24 200	17 000	13 700
	M_w/M_n	2.82	2.57	2.59
Alg.C	M_n	8100	6200	4900
	M_w	19 200	13 100	10 000
	M_w/M_n	2.38	2.11	2.04

3.3. Average molecular weights of hydrolyzates

Number average molecular weight (M_n) and weight average molecular weight (M_w) were calculated based on the polyacrylic polymer standard using GPC. The results are shown in Table 2. Molecular weights of all fractions decreased with increase of hydrolyzation time. Alg.B and Alg.C exhibit similar molecular weight. It is interesting that Alg.B and Alg.C provide similar molecular weight, although their solubilities for water are different. The difference in sequential structures, found in ^{13}C NMR

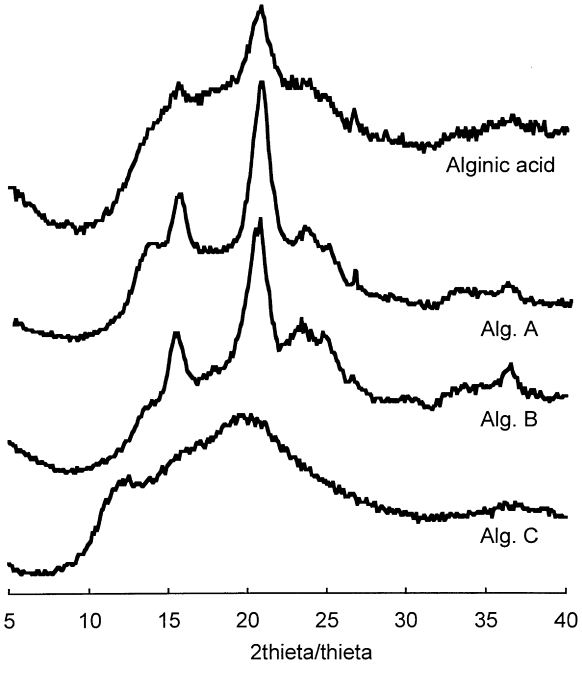

Fig. 6. WAXS profile of raw and hydrolyzed alginic acids.

measurements, may cause the different solubilities of these fractions.

3.4. Crystallinity of hydrolyzates

X-ray diffraction patterns of the hydrolyzates are shown in Fig. 6. In the diffraction pattern of raw alginic acid, there are peaks around 16 and 21° attributable to crystal structures and broad pattern from 15 to 25° attributable to amorphous structures. Alg.A and Alg.B provide smaller broad patterns, as compared with raw alginic acid. This means that Alg.A and Alg.B contain a relatively high amount of crystalline structure. Broad pattern shown in the profile of Alg.C indicates its amorphous structure. From the results of ^{13}C NMR measurements, Alg.A and Alg.B consists of an homopolymeric block, and Alg.C has an alternating structure. Taking account of these results, the difference in crystallinity is supposed to reflect the difference in the sequential structure of each fraction.

4. Conclusion

Three fractions of alginic acid, Alg.A, Alg.B and Alg.C, were prepared by hydrolysis using phosphoric acid and by fractional precipitation. Characterization of these fractions was performed with ^{13}C NMR, GPC and WAXS. Alginic acid used in this study contains a relatively high amount of mannuronic acid. Alg.A is rich in GG sequence and crystal structure, and has DP of 80. The yield of this fraction is 60% at one-day hydrolysis and decreases moderately.

Considering this high yield in the amount of GG sequence than the other hydrolyzates, GG rich structure of this fraction is hardly hydrolyzable in nature. By the using their solubility difference in water and methanol, the hydrolyzable part of alginic acid was successively separated into two fractions. Alg.B, precipitate in water, mainly consists of mannuronic acid, and has a crystal structure. Alg.C, precipitate in methanol, forms an amorphous structure and has a shorter homopolymer block than Alg.B. Both fractions have DP of 30–40.

Alg.B and Alg.C were oligomers with uniformity in their constitution and molecular weight, obtained by phosphoric acid hydrolysis. Their characteristics may be of an advantage in the special use of alginic acid, for example in further chemical modification, although their yields are low.

Acknowledgements

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