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Structure characterization of alginate and conformational behaviors of various alkali-metal alginates in solution

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Summary

The M/G ratio, i. e., number-ratio of D-mannuronate (M) to L-gluronate (G), and diad (MM, GG, MG, etc.) and triad (MMM, MMG, MGM, etc.) frequencies were determined with 100 MHz ^{13}C-NMR spectra for two alginate samples, M-rich and G-rich samples. The M/G ratios obtained were in accord with those determined from chemical analysis on hydrolyzed products fairly well. Molecular chain dimensions in solution were investigated by viscosity method for five alkali-metal alginates carrying Li, Na, K, Rb and Cs, and the results were discussed based on ion radius and electronegativity of alkali-metals. Finally, ion-exchange characteristics of these alginates by Ca^{++} ion were interpreted with M/G ratio and electronegativity.

Introduction

Alginic acid is known (1-3) as a linear polysaccharide of (1→4)linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues arranged in a non-regular, blockwise fashion along the chain. That is, a chain is composed (1) of M-block including only M residues, G-block including only G residues, and MG-heteroblock including G and M residues. The chain conformations of M-block and G-block were suggested (4,5) to be "flat-ribbon" and "buckled-ribbon" forms, respectively. Both conformations are drastically different each other, hence, the M/G ratio, indicating the number-ratio of M-residues to G-residues in the chain, is an important measure to discuss the reactivity of the chain such as the reaction toward Ca^{++} ion. Gelation of alginate is responsible to "egg-box" formation between G-blocks via Ca ion (6-8), and solubility of alginic acid in acid is controlled by the MG-heteroblocks (9).

The M/G ratio was first determined by means of column chromatography (10) by using hydrolyzed product of alginate. Along with the recent progress in ^{13}C-NMR spectroscopy, Grasdalen et al.(11, 12) first succeeded in to determine not only M/G ratio but also diad (MM, MG, GM, and GG) and triad (MMG, MGM, MGG, GMM, GMG, GGM, MMM, GGG) frequencies in alginate chain.

In an application field of alginates, it was reported that various alkali-metal alginates are extremely effective (13) as a water/ethanol separation membrane: Water selectivity in separation was as high as 10^4 independent of the kind of alkali-metal, and permeation velocity was increased with increasing ion radius of the alkali-metal from 10^2 g/m^{2}h for Li to 10^3 for Cs (feed: Water/ethanol=10/90 in wt., 60°C, and 80 Pa.). Such behavior should originate from chain conformations in casting solution and mobility of chains in the membrane.

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In this work, first, we determine the M/G ratio, diad and triad frequencies in sodium alginate chain from ^{13}C-NMR spectra by using M-rich and G-rich samples, whereby the obtained M/G ratios were compared with those determined from analysis on hydrolyzed products. ^{13}C spin-lattice relaxation times of the carbons were also measured at 100 MHz and 50 C for M-rich sample. Secondly, molecular chain dimensions of various alkali-metal alginates carrying Li, Na, K, Rb, and Cs in aqueous solution were evaluated by using the viscosity numbers at c = 1.0 g/1000 ml. Finally, we discuss ion-exchange characteristics of these alginates by calcium ion based on the M/G ratio and kind of alkali-metal.

Experimental

Materials

Two kinds of commercial sodium alginates (Lot.42810) were purchased from Kimitsu Chemicals. One is a M-rich and the other is a G-rich samples. These samples were purified by the following procedure: Samples were dissolved in distilled water at a polymer concentration of 3 g/1000 ml. The solutions were dialyzed for 1 week by using cellulose tublar membranes (cut-off molecular weight: 12000-14000) in deionized water, and then further dialyzed for 1 week in distilled water. The dialyzed products were precipitated with adding ethanol, washed with methanol and then ether, and finally freez-dried to obtain alginic acid. Hereafter, the two samples are designated as Alg-M_2G_1 and Alg-M_1G_2.

For preparation of alkali-metal alginates, equivalent amount of 0.1N-alkali-metal hydroxide (LiOH, NaOH, KOH, RbOH or CsOH) aqueous solution was added to the weighed amount of alginic acid. The molecular weights of sodium alginates were determined from limiting viscosity number [η] in 1N-NaCl aqueous solution at 30°C by using the equation (14): $[\eta] = 3.62 \times 10^{-4} P^{1.2}$, in which P is the degrees of polymerization, and c (polymer concentration) is given in g/1000 ml unit.

Determination of M/G ratio by chemical method

Haug's method (10, 15) was followed to obtain M/G ratio. 50 mg sodium alginate was mixed with 0.5 ml 80% sulfuric acid in an ice bath, then the mixture was kept at 20°C for 18 hr, and to which 6.5 ml water was added in the ice bath, whereby 2N-H_2SO_4 solution was obtained. The solution was then hydrolyzed for 5 hr in boiling water bath equipped with a reflux, the product solution was neutralized with a slightly excess of $CaCO_3$, and the precipitate was filtered off and washed with water until about twice the original volume of hydrolysate has passed through the filter.

The hydrolysate was adjusted to pH 8 by adding NaOH. The column chromatography was carried out on Dowex 1x8 anion-exchange resin in 20cm x 2cm column. The elution of the column was carried out with acetic acid in a linear gradient from 0.5N to 2N, and fractions of 10 ml each were collected. During the elution, first guluronic acid, and secondly mannuronic acid fractions were obtained. The amount of uronic acid in each of the two groups was determined from ultra-violet absorbance by means of orcinol method (16).

Structure characterization of sodium alginate by ^{13}C-NMR

0.1g sodium alginate was dissolved in 2ml D_2O at 40°C, and placed in a 10mm ϕ NMR-test tube. 100 MHz ^{13}C-NMR spectra were recorded with JEOL JNH-GX400 spectrometer using 9.4T, 45° pulse, pulse repetition time of 2s, and 27,000-30,000 scans, at 70°C. The spin-lattice relaxation time, T_1 was measured by means of inversion recovery method by using recycle time 25s at 100 MHz and 50°C.

Measurements of viscosity numbers of alkali-metal alginates

Viscosity numbers of lithium, sodium, potassium, rubidium, and caesium alginates in aqueous solution were measured at polymer concentrations c = 0.48 – 1.6g/1000ml and 30°C, with an Ostwald viscometer.

Ion exchange characteristics of alkali-metal alginates by divalent cation Ca^{++}

0.1g/1000ml alkali-metal alginates were titrated with 0.1M $CaCl_2$ aqueous solution, whereby the electric conductivity and pH were measured as functions of titration volume with Horiba DS-8M conductivity meter and Horiba F-11 pH meter, respectively. The weight (W_{ex}) of exchanged residues is obtained from conductometric titration. So, the exchange ratio(wt%) of alkali-metal by Ca^{++} is obtained from W_{ex}/W, where, W denotes the weight of sample. Furthermore, the selectivity coefficient K defined by Haug (17) was also calculated from the titration curve.

Results and Discussion

In Figure 1, the UV absorbances at 666.5nm for eluents of hydrolyzed alginates are plotted against the volume of fractional extraction. The M/G ratio estimated from the ratio of M and G peak areas for Alg-M_2G_1 (M-rich sample) and Alg-M_1G_2 (G-rich sample) are shown in Table 1, and compared with those obtained from ^{13}C-NMR spectra. The table also includes molecular weights of samples determined from the limiting viscosity numbers.

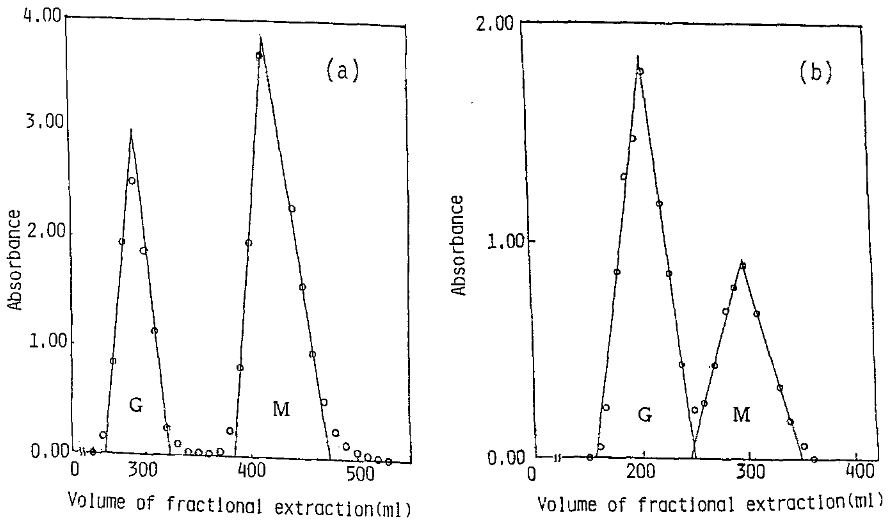

Figure 1. UV absorbance plotted against fractional volume for hydrolyzed products: (a) Alg-M_2G_1 and (b) Alg-M_1G_2

Table 1. M/G ratios and molecular weights of samples used

Sample	M/G ratio		Molecular Weight
	Chem.proced.	^{13}C-NMR	
Alg-M_2G_1	1.84	1.79	77,000
Alg-M_1G_2	0.55	0.55	59,500

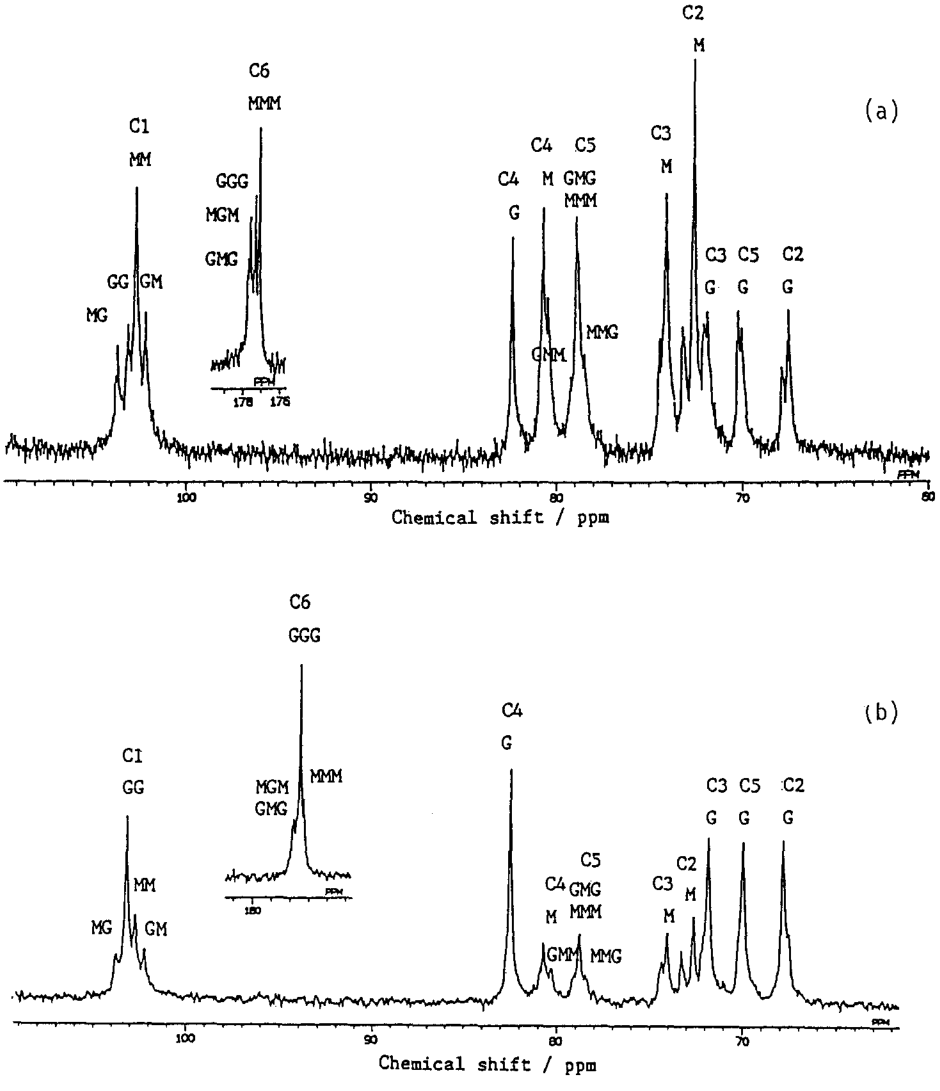

Figure 2. ^{13}C-NMR spectra (100 MHz) of sodium alginate(50mg/ml) in D_2O at pD=5.7-6.0 and 70°C : (a) Alg-M_2G_1 and (b) Alg-M_1G_2.

100 MHz ^{13}C-NMR spectra of Alg-M_2G_1 and Alg-M_1G_2 are shown in Figure 2 (a) and 2 (b), in which C6 MMM is assumed to be 177.1 ppm, and all the assignments of spectra were made after Grasdalen (18).

To obtain M/G ratio, it is required to carry out measurements under the condition that relative intensities of the spectra are proportional to the carbon numbers. Grasdalen (11, 18) pointed out that T_1 values of all the ring carbons are essentially similar (0.25 $\pm$ 0.05 s) and nuclear Overhauser effect is always 2.3, and accordingly, the relative intensity obtained by the complete decoupling method is in accord with that obtained by the gated decoupling method within less than 10% error. To confirm this result, we measured T_1 of Alg-M_2G_1 by the complete decoupling method with 100 MHz ^{13}C-NMR at 50°C and 2s recycle time. As indicated in Table 2, the spin-lattice relaxation times T_1 of carbonyl carbon are 3.5-5.1s and those of ring carbons are 0.3-0.65s.

Table 2. ^{13}C spin-lattice relaxation times(s) of carbons in Alg-M_2G_2 chain measured at 100 MHz and 50°C.

C1				C2				C3			
MG	GG	MM	GM	M		G		M		G	
0.62	0.51	0.59	0.65	0.64	0.52	0.31	0.41	0.65	0.59	0.41	0.38

C4			C5			C6			
M		G	M	G		GMG	MGM	GGG	MMM
0.54	0.58	0.49	0.62	0.41	0.38	5.1	4.6	3.5	5.1

As obvious from the table, T_1 values for G-residues are somewhat lower than those for M-residues. This result indicates that mobility of guluronate is lower than that of mannuronate.

The M/G ratios were obtained from respective area ratios of C1, C4, and C5, and these three values were averaged. Diad and triad frequencies were calculated from C1 and C5 spectra by the equations: $F_{MMM} + F_{GMM} = F_{MM}$, and $F_{GMG} + F_{MMG} = F_{MG}$. Results of calculations were summarized in Table 3.

Table 3. M/G ratio, and diad and triad frequencies determined from ^{13}C-NMR spectra.

Sample	M/G ratio	F_{MM}	F_{MG}	F_{GM}	F_{GG}	F_{MMM}	F_{GMM}	F_{MMG}	F_{GMG}	F_{GGG}	F_{MGG}	F_{GGM}	F_{MGM}
Alg-M_2G_1	1.79	0.47	0.17	0.17	0.19	0.39	0.08	0.09	0.08	0.12	0.07	0.07	0.10
Alg-M_1G_2	0.55	0.23	0.13	0.14	0.50	0.17	0.06	0.07	0.06	0.45	0.05	0.05	0.09

As indicated in Table 1, the M/G ratios obtained from ^{13}C-NMR spectra are in accord with the results obtained by the chemical procedure. Table 3 demonstrates that Alg-M_2G_1 sample is rich in MM and MMM, and Alg-M_1G_2 sample is rich in GG and GGG.

Now we refer to the conformational behaviors of five alkali-metal alginates (Li,'Na, K, Rb, and Cs). As obvious from Table 4, ion radius of alkali-metals increases from 0.60 to 1.69A in the order of $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$, and electronegativity of the elements decreases with increasing ion radius. Though not shown here, hydration number of alkali-metal decreases with decreasing electronegativity.

Table 4. Ion radius and electronegativity of alkali-metals

Element	Li	Na	K	Rb	Cs
Ion radius (Å)	0.60	0.95	1.33	1.48	1.69
Electronegativity	0.98	0.93	0.82	0.82	0.79

Relative chain dimension in solution was estimated from the viscosity number at low polymer concentration. In Table 5, the viscosity numbers of alkali-metal alginates in water at c = 1.0g/1000ml were shown. We point out that the chain dimension is largly affected by the kind of alkali-metal ion and decreases with increasing ion radius, i. e., electrostatic repulsion along the chain decreases with increasing ion radius of the counter ion.

Table 5. Viscosity numbers of five alkali-metal alginates in water at c = 1.0g/1000ml

Sample	Viscosity number				
	Li	Na	K	Rb	Cs
Alg-M_2G_1	1.47	1.31	1.09	1.01	0.92
Alg-M_1G_2	1.94	1.75	1.45	1.32	1.07

The chain dimension of lithium alginate is much higher than that of caesium alginate, in other words, the former is the chain behavior in good solvent, and the latter is that in poorer solvent. When polymer membrane is prepared by casting method from solution, the void size in the membrane formed is larger with poorer solvent. The aformentioned information that the permeation velocity of alkali-metal alginate membranes increases with increasing ion radius of the alkali-metal, is well interpreted by polymer chain dimension in solution before casting.

The exchange ratio (%) of alkali-metal ion by calcium ion, and the selectivity coefficient K for calcium-alkali metal ion-exchange reaction were determined for Alg-M_2G_1 and Alg-M_1G_2, both carrying five kinds of alkali-metals. The results at polymer concentration c=1.0g/1000ml are shown in Table 6. Obviously, the affinity of the carboxy group of alginate for calcium ions is higher with G-rich sample than M-rich sample. Such result supports the egg-box formation (6-8) between G-blocks via Ca^{++} ion. Our experimental data indicate that G-blocks are primarily responsible for egg-box formation, but M-blocks are also substituted by Ca^{++} ions. With increasing ion radius, and decreasing electronegativity of alkali-metal, the exchange ratio by Ca^{++} ions increases. The electronegativity is a measure of electron attractive force of carbonyl residue, so the exchange ratio is increased with increasing difference between the electronegativity of Ca (1.00) and that of alkali-metal. Thus, higher exchange ratio is obtained by alkali-metal with lower electronegativity.

Table 6. Ion exchange ratio(wt %) and selectivity coefficient K of alkali-metal alginates by Ca^{++} at c=1.0g/1000ml

		Alkali-metal alginate				
		Li	Na	K	Rb	Cs
Exchange ratio(%)	Alg-M_2G_1	58.0	62.2	67.4	72.1	77.8
	Alg-M_1G_2	75.3	76.6	90.9	88.4	91.5
Log K	Alg-M_2G_1	0.419	0.650	0.947	1.277	1.629
	Alg-M_1G_2	1.415	1.565	3.009	2.642	3.083

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