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PII: S0144-8617(96)00017-3

Carbohydrate Polymers 29 (1996) 337–345

0144-8617/96/$15.00 + .00

Role of acetylation on metal induced precipitation of alginates

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(Received 22 August 1995; revised version received 8 January 1996; accepted 10 January 1996)

Acetylation dramatically effects both the solution properties and the metal induced precipitation of alginates. The presence of acetyl groups on both bacterial and seaweed alginate polymers marginally increased the weight average molecular weight (M_w) of each polymer by 7% and 11%, respectively. Acetylated bacterial alginate showed a significant increase in solution viscosity compared to its deacetylated counterpart. However microbial acetylation of seaweed alginate did not change its solution viscosity. Acetylation altered the calcium induced precipitation of both alginates. The presence of acetyl groups decreased the ability of each polymer to bind with calcium but increased their ability to bind with ferric lon (Fe^{3+}). By controlling the degree of acetylation on the alginate chains, it was possible to modify solution viscosity and cation induced precipitation of these polymers. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Alginate is one of the few polysaccharides that can be obtained from both eukaryotes and prokaryotes. It is a structural polymer in numerous species of brown seaweed, particularly members of the genera *Ascophyllum, Ecklonia, Fusarium, Laminaria*, and *Macrocystis*, constituting between 14 and 40% of the dry solids of these marine plants. Seaweed alginates are [1-4] linked block copolymers of β-D-mannuronic acid and α-L-guluronic acid (Lin & Hassid, 1966; Penman & Sanderson, 1972). Many pseudomonads produce alginate-like exopolysaccharides, "bacterial alginates", but the genes involved in bacterial alginate biosynthesis are not normally expressed during growth. *Azotobacter vinlandii* (Larsen & Haug, 1971) and several species of *Pseudomonas* (Hacking *et al.*, 1983; Linker & Jones, 1966) including *Pseudomonas aeruginosa* (Banerjee *et al.*, 1983; Fyfe & Govan, 1980) and *Pseudomonas syringae* (Fett *et al.*, 1986) produce bacteria alginates. The most conspicuous difference between seaweed and bacterial alginates is that the bacterial polymer is randomly organized and the mannuronic acid residues are acetylated (Davidson *et al.*, 1977). Most mannuronic acid residues in these alginates are mono-*O*-acetylated at the C-2 or C-3 position. However, some residues may be 2,3 di-*O*-acetylated (Sherbrock-Cox *et al.*, 1984; Skjak-Braek *et al.*, 1985).

Alginates containing high proportions of mannuronate residues adopt flat ribbon-like 2-fold chain conformations in the solid state, similar to those found in β-1,4 diequatorially linked Polymers such as cellulose (Atkins & Nieduszynski, 1973a; Rees, 1972), while polyguluronate rich polymers adopt a buckled 2-fold chain conformation (Atkins & Nieduszynski, 1973b). The mannuronate/guluronate (M/G) ratios in alginates vary depending on the of the polymer (Haug & Larsen, 1962; Haug & Smidsrod, 1965). The M/G ratio, along with the sequence (block or random), dictates the polymer solution properties and the properties of any resultant gels. Alginates with low M/G ratios generally produce lower viscosity solutions and, under certain conditions, more brittle gels than alginates with high M/G ratios.

The affinities of alginates for metal ions vary. Alginate prepared from *Laminaria digitata* (rich in mannuronate residues) have a different affinity for divalent metal ions than alginates prepared from *Laminaria hyperborea* stipes (rich in guluronate residues) (Haug, 1961). Adsorption affinities of various alginates from different algae strains have been studied (Darnall *et al.*,

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1986; Holan & Volesky, 1994; Nakajima *et al.*, 1982; Torresday *et al.*, 1988; Volesky & Prasetyo, 1994). The primary mechanism for metal adsorption is ion-exchange (Kohn, 1975; Kuyucak & Volesky, 1989) although covalent bonding also plays a role (Watkins *et al.*, 1987). Carboxylic groups are involved in this binding (Torresday *et al.*, 1990).

Acetyl groups have a marked effect on the macromolecular properties of the alginates. Acetylation of the polymer modifies its ionization properties by reducing the net negative charge of the polymers (Dentini *et al.*, 1984). It does not however, significantly alter the molecular weight or polydispersity of alginate polymers (Skjak-Braek *et al.*, 1989a). The objective of this study was to characterize the effects of acetylation of bacterial and seaweed alginate on solution viscosity and metal induced precipitation.

MATERIALS AND METHODS

Materials

Alginate from the brown seaweed *Macrocystis pyrifera* was purchased from Sigma Chemical Company (St. Louis, MO).

Production and purification of bacterial alginate

Pseudomonas syringae subsp. *phaseolicola* ATCC 19304 was obtained from the American Type Culture Collection, Rockville, MD. Cultures were maintained at 4°C on a modified Dworkin and Foster (DF) agar (Dworkin & Foster, 1958). This medium contained (in grams per liter of deionized water): KH_2PO_4, 4.0; Na_2HPO_4, 6.0; $MgSO_4 \cdot 7H_2O$, 0.2; $(NH_4)_2SO_4$, 0.5; NaCl, 0.4; KNO_3, 9.1; gluconic acid, 20.0; and agar, 15.0. Gluconic acid was sterilized separately by autoclaving and then added to the basal medium under aseptic conditions. The pH of the medium prior to sterilization was between 6.7 and 7.0. Broth cultures were prepared by inoculating *P. syringae* ATCC 19304 from agar slants into 50 ml of DF liquid medium in 250 ml Erlenmeyer flasks. Cultures were incubated for 30–35 hours at 30°C and 180 rpm on a NBS Model G25-KC rotary shaker, after which the absorbance at 660 nanometers was standardized to between 1.9 and 2.0. Bacterial alginate was isolated either from the 'slime' removed from agar plates containing 20 ml of either DF or nutrient agar (Difco Labs, Detroit, MI), which were inoculated with 0.1 ml of the standardized starter culture and incubated at 30°C for 48 hours, or from 5 day shake flask cultures. Alginate production and the degree of acetylation were determined by calorimetric assay described below.

Purification of the bacterial alginate depended upon the production procedure. Alginate produced on agar plates was separated from the bacterial growth after it was scraped off the agar surface with a bent glass rod, and resuspended in 150 ml of deionized water. The bacterial alginate was separated by centrifugation at 27,000 × g for 30 minutes. Three volumes of isopropanol were added per volume of clarified supernatant to precipitate the polysaccharide. The precipitate was then washed in isopropanol and then in acetone and air dried. Bacterial alginate produced in shake flask culture was separated from the cells by centrifugation at 7,000 × g for 30 minutes. Three volumes of isopropanol were added per volume of clarified culture broth to precipitate the polysaccharide. This solution was gently mixed for 1 hour and then centrifuged at 8,000 × g for 1 hour. The precipitate was washed with isopropanol and acetone and then redissolved in deionized water and finally concentrated in a Büchi Model R110 rotary evaporator (Büchi Lab., Flawil, Switzerland). Purity of bacterial alginate was determined to be greater than 95% (w/w).

Alginate and acetyl quantitation

Alginate concentrations were determined as uronic acid according to the method of Blumenkrantz and Asboe-Hansen (Blumenkrantz & Asboe-Hansen, 1973). The degree of acetylation was measured according to the method described by McComb and McCready (McComb & McCready, 1957). Prior to assay, all samples were desalted either by dialysis against deionized water for 48 hours at room temperature or by passing through a Sephadex G-25 column. A standard curve for alginate was prepared using sodium alginate isolated from *Macrocystis pyrifera* (Sigma Chemical Co., St. Louis MO). A standard curve for acetyl was prepared using glucose pentaacetate (Sigma Chemical Co., St. Louis, MO).

Deacetylation of bacterial alginate

P. syringae ATCC 19304 bacterial alginate was deacetylated for comparison with other alginates, unacetylated seaweed alginate, and acetylated seaweed alginate. Bacterial alginate was dissolved in deionized water at a concentration of approximately 1 mg/ml. Three volumes of this solution were mixed with one volume of 1 M sodium hydroxide solution. After incubation for 20 minutes at room temperature with gentle agitation, one volume of 1 M hydrochloric acid was added to neutralize the solution (final pH was about 7.0) and stop the reaction. The deacetylated bacterial alginate was dialyzed extensively against deionized water to remove residual salts.

Acetylation by immobilized *P. syringae* cells:

A bioreactor containing carbon immobilized *P. syringae* ATCC 19304 cells was used for the continuous acetylation of seaweed alginate (Lee and Day, 1995). The feed

was 1.5 g/l *Macrocystis pyrifera* alginate and 1.0% (w/v) gluconic acid in 0.01 M phosphate buffer (pH 6.0). The feed rate was 0.02 h^{-1}. The temperature was maintained at 25°C and aeration was 0.4 standard liters per minute (SLPM). Air was filtered through a 6 inch glass wool-packed tube and a sterile Whatman Hepa-vent filter (Whatman Inc., Clifton, NJ). The reactor was a 700 ml Kontes Airlift Bioreactor (Kontes Life Science Products, Vineland, NJ) containing 25 g of carbon catalyst with a working volume of 500 ml. Acetylation continued for more than 15 days at a fixed flow rate. The average degree of acetylation of the seaweed alginate was about 30%. This means that the ratio of acetyl groups to monomeric residues in the seaweed alginate was 0.3. The $T_{1/2}$ of this system was 6.5 days at a dilution rate of 0.02 h^{-1}.

Purification of acetylated seaweed alginate

Acetylated seaweed alginate was mixed with an equivalent volume of isopropanol. This mixture was incubated for 12 hours at room temperature and then centrifuged at 8,000 × g for 40 minutes in a Sorval Superspeed Model RC-5B centrifuge (DuPont Co., Wilmington, DE). The precipitate was washed with isopropanol and redissolved in deionized water. It was dialyzed against 500 volumes of deionized water for 48 hours to remove low molecular weight compounds and then concentrated in a Büchi Model R110 rotary evaporator (Büchi Lab., Flawil, Switzerland). After concentration, it was freeze-dried and stored.

Nuclear Magnetic Resonance (NMR) spectra of alginates

Confirmation of acetylation of seaweed alginate and characterization of the position of acetylation was by NMR spectroscopy. All samples for NMR were deuterated three times by evaporation under reduced pressure with 0.5 ml of D_2O (Sigma Chemical Co., St. Louis, MO). ^{1}H-NMR spectra of each sample were obtained using a Bruker WM-400 NMR spectrometer (Bruker Instrument Co., Germany) operating in the Fourier transform mode. The sample volume for ^{1}H-NMR spectra was 0.5 ml. The alginate concentrations were 60 mg/ml except for bacterial alginate which was 40 mg/ml. The operation temperature was 70°C. Each ^{1}H-NMR spectra was scanned for 1 h.

Viscosities

The effects of acetylation on the solution viscosities of alginates were determined by the method of Allison and Matthews (Allison & Matthews, 1992) using a simple U-shaped capillary viscometer designed for small volume samples. The time taken for the adjusted concentration of each alginate sample to fall a fixed distance under gravity at a fixed temperature was expressed as a measure of comparative viscosity (N/N_o), where N is the time elapsed for the alginate solutions to fall a fixed distance and N_o the time for deionized water to fall that same distance. The concentration of all samples tested was 400 μg/ml. The volume of each sample was 2.5 ml. Measurements were repeated 5 times at each temperature.

Molecular weights

Molecular weights were determined by gel permeation chromatography (GPC) of alginate [100 μg/ml (w/v)] in deionized water. Alginate sizes and polydispersity indices were determined by measurement of multi-angle light scattering intensities using a DAWN-Photometer (Wyatt Technology, Santa Barbara, CA). The DAWN GPC detector measures the scattering intensities of a sample at 15 different angles and transmits the data to a computer for digital conversion and subsequent processing under control of the ASTRA™ (or ASTRA 202) software (Wyatt Technology, Santa Barbara, CA). Acetone and cyclohexane were used for instrument calibration. Concentrations were obtained from a calibrated Waters Model 410 Differential Refractometer (Millipore Corp., Milford, MA). Alginate sizes were determined using: T10, T40, and T500 dextrans (Pharmacia Co., Uppsala, Sweden) as external standards. Sample injection volumes were 100 μl and the GPC column was an Ultrahydrogel Linear column (Waters, Millipore Corp., Milford, MA). The running buffer was 0.1 M $NaNO_3$ and the temperature was 45°C.

Precipitation of alginates by metal ions

The relative precipitation by cations of the various alginates were compared. Each alginate sample was tested at a concentration of 400 μg/ml (w/v). Dependent on their solubilities, metal salts were dissolved in deionized water at concentrations of 0 to 20 or 0 to 100 mm with at least 7 intermediate concentrations. The metal ions tested were: Ca^{2+}, CS^{1+}, CO^{2+}, Fe^{3+}, Pb^{2+}, Mg^{2+}, Rb^{1+}, Sr^{2+}, and U^{6+}. All metal salts were obtained from Sigma Chemical Co., St. Louis, MO, except for uranyl acetate (Eastman Kodak Co., Rochester, NY). Four volumes of each alginate solution were mixed with one volume of each metal solution in triplicate. The solutions were incubated for 12 hours at room temperature to allow for maximum complex formation. They were then centrifuged (18,000 × g for 60 minutes) in a Sorval Superspeed Model RC-5B centrifuge. The supernatants were separated and the concentration of residual alginate in each supernatant was measured as previously described. The concentrations of alginate precipitated were calculated by difference and these values were used to determine the relative precipitation of each polymer.

Saturation of acetylated seaweed alginate by ferric ions

The breakthrough values for continuous concentration of ferric ions were measured by passing a ferric chloride solution from the reservoir to a stirred cell (Amicon Inc., Beverly, MA) with a YM50 membrane, containing 150 ml of 2,000 μg/ml alginate solution, under a pressure of 20 Ibs/in^2. Ferric ions bound to the alginate and were concentrated in the stirred cell. The filtrate passed through the membrane. The concentration of ferric ions in the filtrate was measured using a Leeman Labs Model PS 3000 Inductively Coupled Plasma Emission Spectrophotometer (Leeman Lab., Lowell, MA).

RESULTS

Proof of acetylation

The presence of acetyl groups in both the bacterial and acetylated seaweed polymers was confirmed by ^{1}H-NMR spectroscopy. Acetylation of the seaweed alginate produced a new, distinct signal at approximately 2.1 ppm in the ^{1}H-NMR spectrum. This signal was absent in the ^{1}H-NMR spectrum of the native seaweed polymer (Fig. 1). The location of the proton shift (2.1 ppm) is characteristic of methyl groups in an acetylated region. The partly overlapping peaks of acetyl protons in this region suggests the presence of either di-acetylated units and/or two mono-acetylated units (Skjak-Braek *et al.*, 1989b). Bacterial alginate shows the same signal at 2.1 ppm on ^{1}H-NMR spectrum. This signal is absent in the ^{1}H-NMR spectrum of deacetylated bacterial alginate.

Alginate characterization

Chemical analysis of the bacterial and seaweed alginates showed M/G ratios of 82:18 and 60:40 respectively, and maximum ratios of acetyl groups to monomeric residues in bacterial and seaweed alginates were 1.2 and 0.3, respectively. The weight average molecular weight (M_w) and the number average molecular weight (M_n) were also determined for each polymer. The acetylated alginates were 7 to 11% larger than their deacetylated counterparts (Table 1). All the polymers had polydispersity indices of approximately 3.0, indicating a wide variation of molecular sizes in each sample.

Viscosity changes:

The viscosity of polymer solutions are dependent on molecular weight, rigidity of solute, and environmental factors, especially temperature. Acetylation increased the viscosity of aqueous solutions of bacterial alginate by 38% at 30°C but solutions of seaweed alginate were relatively unaffected. The comparative viscosity for each alginate solution at a concentration of 400 μg/ml (w/v) was independent of temperature up to 52°C. Above 52°C the viscosities of the solutions decreased linearly. Between 52°C and 85°C the comparative viscosities of the seaweed alginate sample and the acetylated seaweed alginate decreased at the same rate, ~3.4% per °C, while acetylated and deacetylated bacterial alginate viscosities decreased at different rates, ~9.2% and 6.5% per °C, respectively. At 85°C, the comparative viscosities of all the alginate solutions were identical (Fig. 2).

Cation precipitation by alginates

The relative abilities of 9 different cations to precipitate the various alginates were compared by $P_{1/2}$ values. The $P_{1/2}$ is defined as the concentration of cations, in mM, required to precipitate 50% of the polymer from a 400 μg/ml alginate solution. The typical plots for calculating the $P_{1/2}$ values for Ca^{2+} and Fe^{3+} are shown in Figs 3 and 4. This value can be used to prospect the relative binding ability or affinity of alginate for cations. The relative order of precipitations of seaweed, acetylated seaweed, bacterial, and deacetylated bacterial alginate by these ions follows:

Seaweed alginate: Pb^{2+}, U^{6+} > Fe^{3+}, Sr^{2+} > Ca^{2+} > Co^{2+} > Mg^{2+} Cs^{1+}, Rb^{1+}
Seaweed alginate (acetylated): Fe^{3+} > U^{6+}, Pb^{2+} > Sr^{2+} > Ca^{2+} > Co^{2+} > Mg^{2+}, Cs^{1+}, Rb^{1+}
Bacterial alginate (deacetylated): Pb^{2+}, Fe^{3+}, U^{6+} > Sr^{2+} > Ca^{2+} > Co^{2+} > Mg^{2+}, Cs^{1+}, Rb^{1+}
Bacterial alginate (acetylated): Pb^{2+}, Fe^{3+}, U^{6+} > Sr^{2+} > Ca^{2+} > Co^{2+}, Mg^{2+}, Cs^{1+}, Rb^{1+}

Acetylation of seaweed alginate reversed the binding preference for only two ions, Fe^{3+} and Pb^{2+}. Acetylation significantly increased the $P_{1/2}$ for Co^{2+}, Ca^{2+} and Sr^{2+} (Table 2).

Breakthrough of ferric ions on acetylated and deacetylated seaweed alginate

In order to determine the significance of the reversal of binding order with ferric ion due to acetylation, saturation levels of Fe^{3+} for alginates were determined from the breakthrough point of a known amount of alginate in a flow cell (Fig. 5). The equation for the binding concentration of ferric ions for seaweed alginate, generated from the plot of ferric ion in against ferric ion out is $Y = 40.21 - 0.15X + (1.40 \cdot 10^{-4}) \cdot X^2 - (1.83 \cdot 10^{-8}) \cdot X^3$, ($X$ = the concentration of ferric ion in, Y = the concentration of ferric ion out, and $R^2 = 1.00$). The equation for acetylated seaweed alginate is $Y = 42.67 - 0.15X + (1.34 \cdot 10^{-4}) \cdot X^2 - (1.80 \cdot 100^{-8}) \cdot X^3$, ($R^2 = 1.00$). From these equations, the saturation binding concentrations for ferric ion for 300 mg of seaweed alginate or acetylated seaweed alginate were calculated to be 659

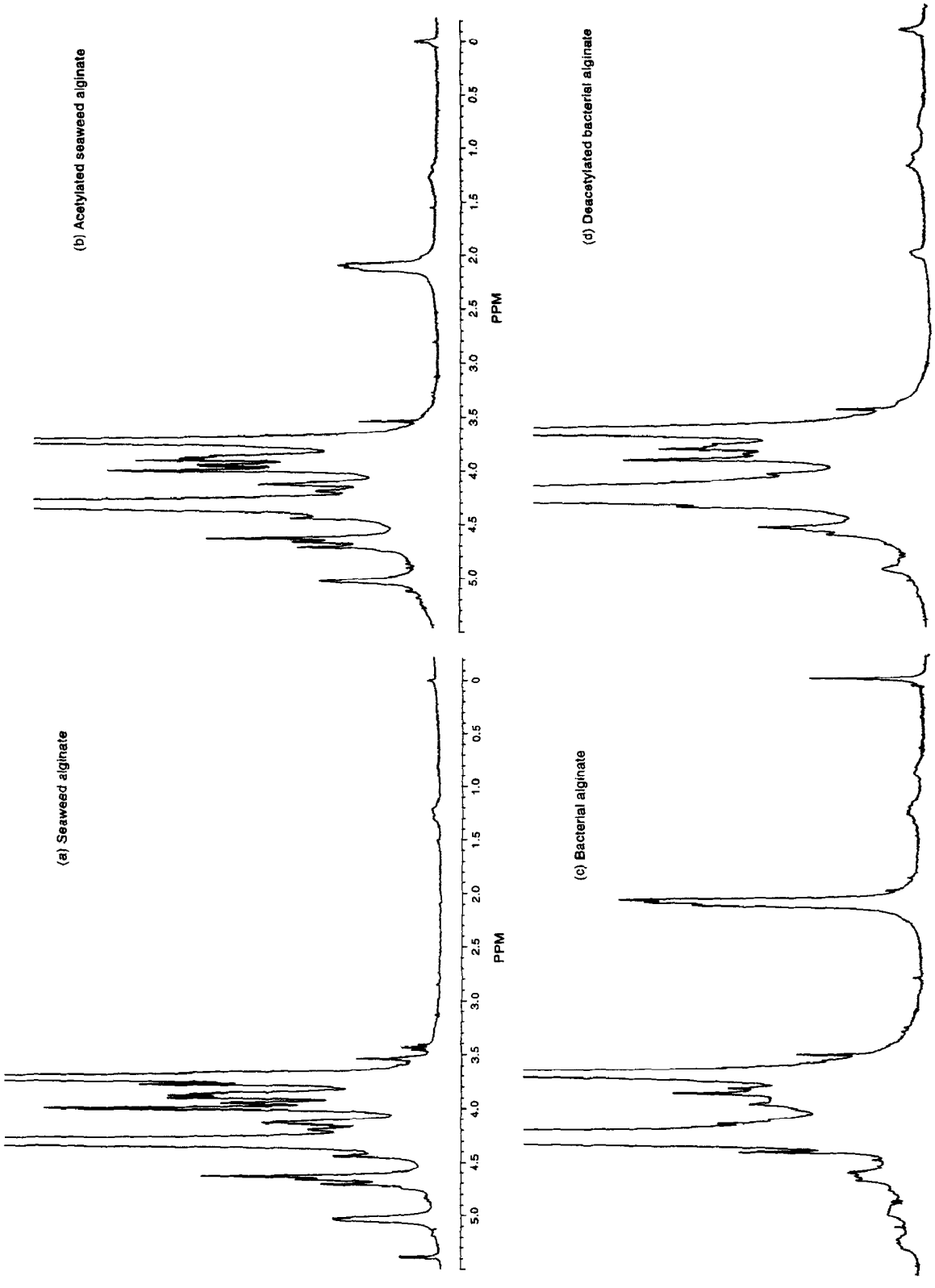

Fig. 1. The 400 MHz ^{1}H-NMR spectra of alginates: (a) seaweed alginate (*Macrocystis pyrifera*) (60 mg/ml), (b) seaweed alginate acetylated by carbon immobilized *P. syringae* ATCC 19304 (60 mg/ml), (c) bacterial alginate isolated from *P. syringae* ATCC 19304 (40 mg/ml), (d) deacetylated bacterial alginate (60 mg/ml) in D_2O (pD 7.0) at 70°C.

Table 1. Alginate characterization

Alginate sample	M_n[a] ($\times 10^4$)	M_w[b] ($\times 10^4$)	M_w/M_n[c]	M/G ratio	Acetylation[d]
Seaweed	1.4	4.7	3.36	60:40	0
Acetylated seaweed	1.6	5.2	3.25	60:40	0.3
Bacterial	4.3	12.7	2.95	82:18	1.2
Deacetylated bacterial	3.8	11.9	3.13	82:18	0

[a]Number average molecular weight.
[b]Weight average molecular weight.
[c]Polydispersity.
[d]ratio of acetyl groups to monomeric sugarresidues.

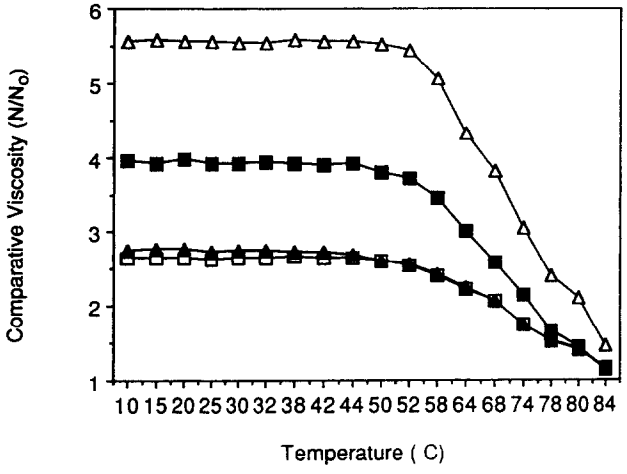

Fig. 2. Comparative viscosities, (N/N_o), of seaweed alginate (□), acetylated seaweed alginate (▲). Alginate solutions were tested at 400 μg/ml (w/v) concentrations.

and 770 μM, respectively. This confirmed the difference in affinities between the two polymers but also highlighted the small magnitude of the change acetylation made to the binding of this ion.

DISCUSSION

Acetylation altered the solution viscosities and the precipitation characteristics of alginate with some cations. A naturally acetylated alginate, its deacetylated equivalent, non-acetylated alginate from *Macrocystis pyrifera* and its acetylated equivalent were employed in this study. This acetylation was confirmed by ^{1}H-NMR. Acetylation of the seaweed alginate resulted in a 10.6% increase in the M_w of the polymer. This translated into a 4% increase in comparative viscosity for the acetylated polymer at 30°C. The bacterial alginate solutions showed a much greater viscosity difference between the acetylated and deacetylated polymers at equivalent concentrations. At 30°C, the comparative viscosity of the acetylated polymer was approximately 38% higher than the deacetylated polymer. This large difference also may

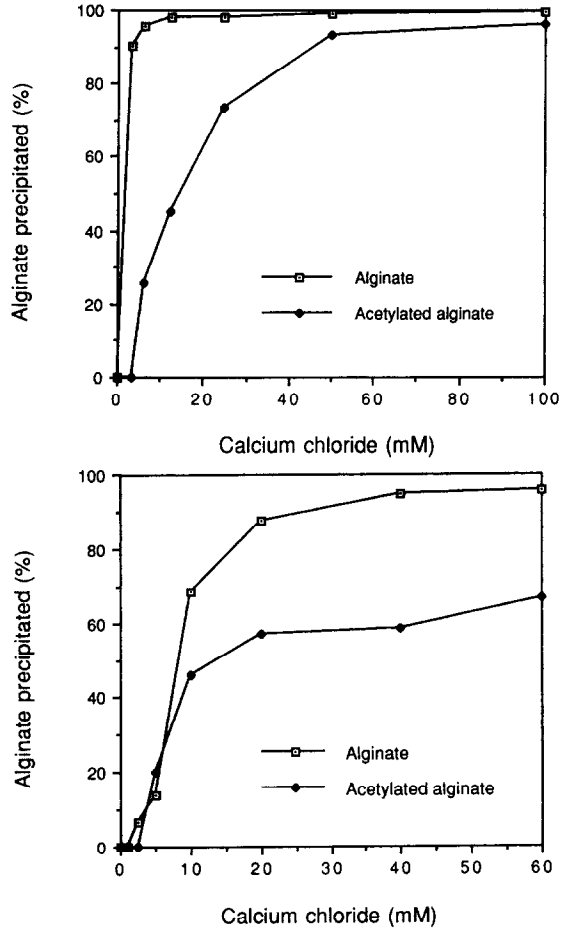

Fig. 3. The effects of calcium ions on alginate precipitation: (a: top) seaweed alginate and (b: bottom) bacterial alginate.

be due in part to the increase in average molecular weight, but it is more likely due to the higher degree of acetylation, and the more extended ribbon-like tertiary structure of the bacterial polymer.

The acetylated alginates showed major differences in their abilities to precipitate with some cations compared to their deacetylated counterparts. The only increase in extent of precipitation, albeit small, was with ferric ions. It was apparent that acetylation generally decreased the polymer's ability to precipitate with those divalent

Table 2. Precipitation of alginates by cations

Ions	Periodic Group	$P_{1/2}$[a]			
		Seaweed Alginate	Acetylated Seaweed Alginate	Acetylated Bacterial Alginate	Deacetylated Bacterial Alginate
Cs^{1+}, Rb^{1+}	IA	No affinity[b]	No affinity	No affinity	No affinity
Mg^{2+}	IIA	No affinity	No affinity	No affinity	No affinity
Ca^{2+}		2.6±0.5	11.7±1.9	13.5±1.7	8.4±1.2
Sr^{2+}		1.8±0.5	5.2±1.0	8.5±0.9	3.8±0.6
Fe^{3+}	VIIIA	1.8±0.6	0.6±0.1	0.6±0.1	0.8±0.2
Co^{2+}		9.6±0.4	47.0±5.6	No affinity	45.0±7.2
Pb^{2+}	IVB	0.7±0.1	1.7±0.6	0.5±0.1	0.6±0.1
U^{6+}	Actinide metal	0.9±0.2	1.4±0.5	0.7±0.1	0.5±0.1

[a] $P_{1/2}$ is the concentration of metal ions (mM) required to precipitate 50% (w/v) of the alginate from 400 μg/ml (w/v) alginate solutions.
[b] 'No affinity' signifies that the ion did not precipitate 50% of the alginate sample up to 100 mM ion concentration.

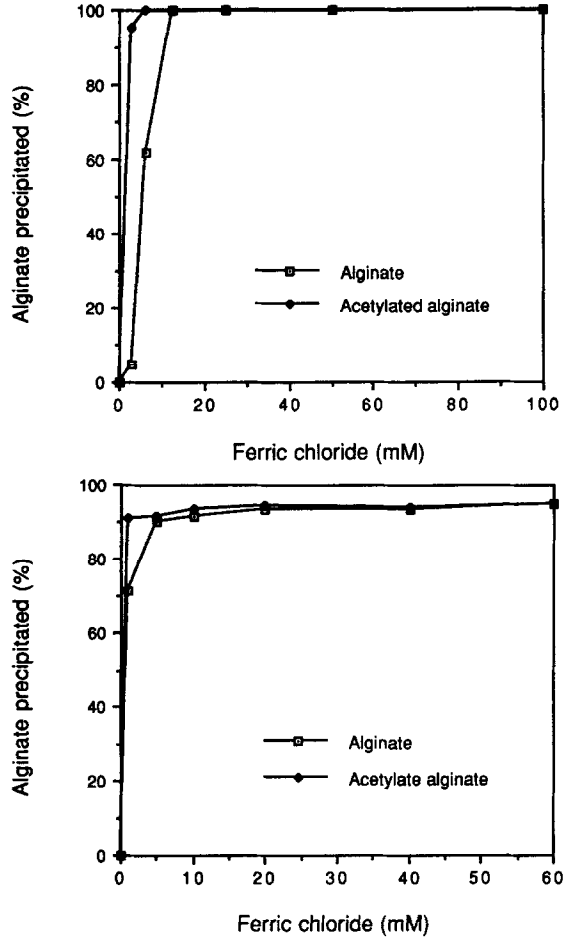

Fig. 4. The effects of ferric ions on alginate precipitation: (a: top) seaweed atginate and (b: bottom) bacterial alginate.

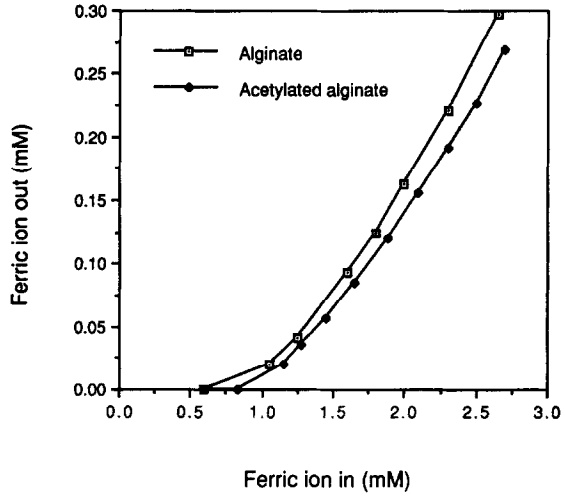

Fig. 5. The concentration of ferric ions bound by seaweed alginate and acetylated seaweed alginate.

cations which showed a normally high affinity for alginates. The high affinity of seaweed alginate for divalent ions, especially calcium ions, is due to the structural characteristics of the polymer; polyguluronate residues in seaweed alginate, hydroxyl groups and carboxyl groups of polyguluronate residues, and the net negative charge, as well as the molecular size and net charge of the calcium ion are all implicated in this interaction (Atkins & Nieduszynski, 1973b; Nilsson, 1992; Rees, 1972). Although the ionic effect of the carboxyl group is unlikely to be dramatically influenced, acetylation of seaweed alginate probably disturbs the basic relationships between the polymer and calcium or divalent ions by modifying the ionization properties of the polymer and sterically hindering the binding of these ions to the polymer (Morris *et al.*, 1978; Morris & Rees, 1980).

Because of a higher viscosity and a lower affinity for calcium ions, acetylated alginates can be favorably substituted for nonacetylated alginates when used as emulsifiers, stabilizers, and gelling agents in many industrial applications. The lower affinity of acetylated alginate for calcium ions confers a more soluble state on the polymer in aqueous solution. Acetylated alginate thus becomes a more desirable emulsifier and stabilizer. More viscous solutions can be made with lower concentrations of acetylated alginate than nonacetylated alginate, reducing the amount of polymer required in a given application.

Acetylated alginates have the potential to concentrate some toxic, heavy, and/or valuable metals. They were found to bind Fe^{3+}, U^{6+}, and Pb^{2+} ions more readily than Ca^{2+} ions. Given the small changes in binding capability brought about by acetylation, unless selective ion exchange is to be conducted in high calcium environments, there seems to be no advantage in using acetylated instead of non-acetylated alginates. Calcium induced gel formation of seaweed alginate is due to the physical binding of calcium ions to guluronate residues based on a charge to charge interaction between the positive charge from calcium ions and the negative charge from the guluronic acid carboxyl groups. The size of the calcium ion allows it to fit into the space formed by guluronate residues between antra and inter molecules of seaweed alginates (Morris *et al.*, 1978; Morris and Rees, 1980). This is the basis of the Rees "egg-box" model. Acetylated seaweed alginate does not appear to comply with the basic tenets of this model, because the affinity of acetylated seaweed alginate for calcium ions is decreased, but the concentrations of guluronate are unaffected. In light of this, and the calcium induced gelation of acetylated bacterial alginates that have few or no polyguluronate blocks (Gacesa, 1988; Larsen and Haug, 1971; Skjak-Braek *et al.*, 1989b), it might be wise to reinvestigate the theory of gelation of this polymer.

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