Effect of Metal Cations on the Viscosity of a Pectin-Like Capsular Polysaccharide from the Cyanobacterium Microcystis flos-aquae C3-40

DOROTHY L. PARKER,1,* BRIAN R. SCHRAM,1† JOHN L. PLUDE,2 AND ROBERT E. MOORE2

Department of Biology and Microbiology1 and Department of Chemistry,2 University of Wisconsin Oshkosh, Oshkosh, Wisconsin 54901

Received 6 November 1995/Accepted 19 January 1996

The properties of purified capsular polysaccharide from the cyanobacterium Microcystis flos-aquae C3-40 were examined by capillary viscometry. Capsule suspensions exhibited similar viscosities between pH 6 and 10 but were more viscous at pH ≤ 4 than at pH 6 to 11. At pH 7, a biphasic effect of metal ion concentration on capsule viscosity was observed: (i) capsule viscosity increased with increasing metal ion concentration until a maximal viscosity occurred at a specific concentration that was a reproducible characteristic of each metal ion, and (ii) the viscosity decreased with further addition of that ion. Because the latter part of the biphasic curve was complicated by additional factors (especially the precipitation or gelation of capsule by divalent metal ions), the effects of various metal chlorides were compared for the former phase in which capsule viscosity increased in the presence of metal ions. Equivalent increases in capsule viscosity were observed with micromolar concentrations of divalent metal ions but only with 10 to 20 times greater concentrations of NaCl. The relative abilities of various metal salts to increase capsule viscosity were as follows: CdCl2 > Pb(NO3)2 > FeCl2 > MnCl2 > CuCl2, CaCl2 > NaCl. This pattern of metal efficacy resembles known cation influences on the structural integrity of capsule in naturally occurring and cultured M. flos-aquae colonies. The data are the first direct demonstration of an interaction between metal ions and purified M. flos-aquae capsule, which has previously been proposed to play a role in the environmental cycling of certain multivalent metals, especially manganese. The M. flos-aquae capsule and the plant polysaccharide pectin have similar sugar compositions but differ in their relative responses to various metals, suggesting that capsular polysaccharide could be a preferable alternative to pectin for certain biotechnological applications.

The cyanobacterium Microcystis flos-aquae C3-40 produces a polysaccharide capsule composed of 83% (wt/wt) galacturonic, 1.5% galactose, 2% glucose, 3% xylose, 5% mannose, 5.5% rhamnose, and no detectable protein (30), a composition which resembles that of the plant polysaccharide pectin (9, 39). Microcystis flos-aquae is the only bacterium known to produce a pectin-like capsular polysaccharide (20). Pectin, a commonly used gelling agent (39), requires chemical modification for certain applications in which a bacterial polysaccharide could be more suitable. For example, native pectin is highly methylated and must be deesterified to make low-methoxyl pectin, the only inexpensive alternative to pectin for certain applications. Since the M. flos-aquae capsule is an extracellular material that is readily washed from the surface of cells (30), it could be an inexpensive alternative to pectin for certain applications.

Large amounts of capsule are produced during the frequent and extensive Microcystis blooms in many eutrophic lakes worldwide (18). Both these blooms and Microcystis cultures have been shown to accumulate metal ions (4, 25, 30). The uptake of metals by Microcystis spp. can therefore be expected to influence the solubilization, precipitation, bioavailability, and cycling of metals in many aquatic environments. For example, the accumulation of manganese within the Microcystis capsule is involved in the precipitation of manganese nodules in freshwater lakes (1, 2, 26, 33).

An understanding of the metal specificities of the Microcystis capsule is required to model the capsule’s role in pertinent environmental phenomena and to evaluate the capsule’s biotechnological potential. To examine this question, we have investigated the differing effects of various metal ions on the viscosity and gelation of purified capsule from M. flos-aquae C3-40.

MATERIALS AND METHODS

M. flos-aquae C3-40, culture conditions, and capsule extraction procedures have been described before (30). Acid-leached glassware or low-metal plastics were used for all procedures. Extracted capsule in 3,500-molecular-weight (MW)-cutoff dialysis tubing (Spectrum) was dialyzed for 6 days at 4°C against 100-fold volumes of distilled water that were changed at 8-h intervals. During the first 2 days of dialysis, the vessel also contained a dialysis bag filled with 10% (wt/wt) Chelex-100 cation exchange resin (Bio-Rad), which acted as a sink for divalent cations that otherwise were not completely removed from the capsule. Residual metals in Chelex-treated dialyzed capsule, assayed (11) with a Varian Spectra AA-200 HT atomic absorption spectrophotometer, were the following (in micrograms per gram of capsule): Mn, <1; Zn, 3; Cu, 3; Mg, 9; Fe, 29; K, 43; Ca, 131; Na, 270. Various capsule suspensions that had been adjusted to pH 7 with NaOH contained 3 to 4 mg of Na per g of capsule. The capsular polysaccharide was of heterogeneous molecular weight: >99% was retained by 10,000-MW-cutoff dialysis tubing (Spectrum). Freshly prepared dextran blue 2000 (MW, 2 x 106; Pharmacia) and alginate (MW, 12,000 to 80,000 [Sigma]; 95 to 100 mg of Na per g of polysaccharide) were included in initial experiments as comparison compounds.

Lyophilized capsule was suspended to a concentration of 0.3 or 0.35% (wt/wt) in deionized water, gently shaken, stored at 5°C overnight until dissolved, passed through a 0.45-µm-pore-size filter (Millipore), and assayed for carbohydrate by the anthrone method (16). Reagent-grade NaBr, NaI, KCl, CdCl2, FeCl3, MnCl2,
and CuCl$_2$ (all from J. T. Baker Co.); NaCl and Pb(NO$_3$)$_2$ (Mallinckrodt); LiCl (Sigma); CsCl (U.S. Biochemical Corp.); and CaCl$_2$ (Fisher) were each dissolved in ≥16 MΩ deionized water. Appropriate volumes of maximally diluted capsule and metal solutions were mixed to yield the desired final concentrations. Each capsule suspension or the corresponding solvent control was added to a Cannon-Fenske 100-bore, 10-ml capillary viscometer that was suspended in precise ver- capsular suspension or the corresponding solvent control was added to a Cannon-Fenske 100-bore, 10-ml capillary viscometer that was suspended in precise vertical orientation in a water bath equipped with a proportional temperature controller (Fisher) that maintained temperature at 25 ± 0.01°C. After 30 min for temperature equilibration, a portion of the suspension was drawn into the upper chamber of the viscometer. The time required for the meniscus to move from the upper to the lower fiduciary mark on the viscometer was measured with a MicroKonta stopwatch. Flow times were longer than 100 s. Viscometric data were analyzed according to the Huggins (19) and Schulz-Blaschke (35) equations:

$$\eta = \eta_0 + k' \eta_0^2$$

(Huggins equation) and

$$\eta = \eta_0 + k' \eta_0^2$$

(Schulz-Blaschke equation), where $c$ is the polymer concentration; $\eta_0$ is the specific viscosity, which can be measured in capillary viscometers as $(t - t_0)/t_0$, with $t$ being the flow time of the polymer solution and $t_0$ being the flow time of the solvent; $\eta_0$, the reduced viscosity, is $\eta_0$ per unit polymer concentration ($\eta_0/c$); $[\eta]$, the intrinsic viscosity, is $\eta_0$ at infinite polymer dilution; and $k'$ and $k''$ are proportionality factors. In studies of cation-promoted capsule precipitation, precipitates were detected visually or by a decrease in anthrone-reacting material after filtration through 0.45-μm-pore-size filters (Gelman).

**RESULTS**

**Effect of pH.** Capsule suspensions in 0.1 M NaCl were more viscous at pH 4 than at pH 6 to 11 but exhibited similar viscosities between pH 6 and 10 (Fig. 1). Capsule suspensions at pH ≥7 became exceedingly viscous or gelled so that flow times could not be measured. Since the pK$_a$ of the COO$^-$ groups in polygalacturonate is 3.6 (10), the increased capsule viscosity at pH ≥4 may be related to increased protonation of galacturonate residues.

**Effect of capsule concentration.** The effect of capsule concentration on viscosity at pH 7 was examined at various NaCl concentrations between 10$^{-5}$ and 1.5 M (Table 1). The data were analyzed by both the Schulz-Blaschke (35) and the Huggins (19) methods. Schulz-Blaschke plots of reduced viscosity versus specific viscosity yielded straight lines (Fig. 2 and 3); intrinsic viscosity, $[\eta]$, was estimated from the y axis intercept, and the Schulz-Blaschke coefficient ($k''$) was estimated from the slope of each curve for a particular NaCl concentration (Fig. 2 and 3; Table 1). Estimates of $[\eta]$ from the extrapolation of Huggins plots to zero capsule concentration were nearly identical to those determined by the Schulz-Blaschke method (Fig. 2). Schulz-Blaschke analyses were judged to be more trustworthy than Huggins analyses because the Huggins plots were not linear, especially at high capsule concentrations and low salt concentrations (Fig. 2).

The intrinsic viscosity of capsule in 0.1 M NaCl was 10.9 d/l/g (Table 1), similar to the intrinsic viscosities of pectin components from various plants (15). Intrinsic viscosities for dextran (2 × 10$^8$ MW) and alginate (12,000 to 80,000 MW), which were characterized in parallel with capsule as controls, were 3.4 and 17.6 d/l/g, respectively.

Viscosities at pH 7 in 0.1 M solutions of LiCl, KCl, CsCl, NaBr, and NaI were each determined at eight capsule concentrations between 0.07 and 0.16% (data not shown). The results were indistinguishable from those for 0.1 M NaCl (Fig. 3).

**Effect of salt concentration.** NaCl, CaCl$_2$, CdCl$_2$, CuCl$_2$, MnCl$_2$, FeCl$_2$, and Pb(NO$_3$)$_2$; at numerous concentrations between 10$^{-6}$ and 1 M were each added to aliquots of two capsule suspensions containing 0.06 and 0.02% (wt/wt) cap-

![FIG. 1. Effect of pH on the viscosity of a capsule suspension in 0.1 M NaCl. Standard deviations of triplicate measurements are smaller than symbol diameters.](http://aem.asm.org/)

![FIG. 2. Viscosities of capsule suspensions in 10$^{-5}$ M NaCl.](http://aem.asm.org/)

<table>
<thead>
<tr>
<th>NaCl concn (M)</th>
<th>$[\eta]^{b}$</th>
<th>$k''^{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>7.8</td>
<td>0.26</td>
</tr>
<tr>
<td>1.0</td>
<td>8.0</td>
<td>0.27</td>
</tr>
<tr>
<td>0.75</td>
<td>8.5</td>
<td>0.26</td>
</tr>
<tr>
<td>0.50</td>
<td>8.9</td>
<td>0.26</td>
</tr>
<tr>
<td>0.40</td>
<td>8.9</td>
<td>0.27</td>
</tr>
<tr>
<td>0.30</td>
<td>8.9</td>
<td>0.28</td>
</tr>
<tr>
<td>0.20</td>
<td>10.2</td>
<td>0.23</td>
</tr>
<tr>
<td>0.10</td>
<td>10.9</td>
<td>0.21</td>
</tr>
<tr>
<td>0.005</td>
<td>25.1</td>
<td>0.31</td>
</tr>
<tr>
<td>10$^{-3}$</td>
<td>10.6</td>
<td>0.55</td>
</tr>
</tbody>
</table>

$^{a}$ Based on triplicate measurements for each of more than six capsule concentrations in each NaCl solution except 0.005 M NaCl, for which only two capsule concentrations were examined.

$^{b}$ From the y axis intercept of Schulz-Blaschke plots.

$^{c}$ From the slope ($[\eta]\kappa''$) of Schulz-Blaschke plots.

Table 1. Effect of NaCl concentration on the intrinsic viscosity ($[\eta]$) and Schulz-Blaschke coefficient ($k''$) of *M. flos-aquae* C3-40 capsule suspensions.
solute. In all cases a biphasic pattern was observed: $\eta_{red}$ increased with increasing salt concentration until a maximal viscosity was observed at a specific salt concentration between 0.05 and 5 mM, but the viscosity decreased with further addition of that salt (Fig. 4). The salt concentration at maximal $\eta_{red}$ was reproducible for each salt and differed considerably among the various compounds that were compared (Table 2). Table 2 also shows the concentration of each salt that was required to increase the suspension’s reduced viscosity to 40 dl/g. In terms of ability to cause equivalent increases in capsule viscosity, the salts made up four groups: (i) CdCl$_2$, Pb(NO$_3$)$_2$, and FeCl$_2$, which acted at the lowest concentrations; (ii) MnCl$_2$, which acted at slightly higher concentrations; (iii) CuCl$_2$ and CaCl$_2$, which acted at intermediate concentrations; and (iv) NaCl, which acted only at much higher concentrations.

Viscosities could be determined only at low concentrations of divalent metal ions because at high concentrations the capsule precipitated or formed flocs. The absence of datum points at high salt concentrations in Fig. 4 indicates the range at which precipitation or flocculation was observed with each salt. Capsule precipitation was most pronounced with Pb(NO$_3$)$_2$ and FeCl$_2$, which also produced maximal capsule viscosity at the lowest salt concentrations (Fig. 4; Table 2). No precipitation or flocculation was detected with NaCl at any salt or capsule concentration examined. In another experiment, CaCl$_2$ effects were examined under conditions suitable for bulk precipitation during capsule purification, a procedure which included overnight storage of CaCl$_2$-treated material at 5°C. The CaCl$_2$ concentration that gave maximal precipitation of capsule after overnight storage was 0.7 mM (Table 3), in surprising agreement with the maximal $[\eta]$ at 0.8 mM CaCl$_2$ after 30 min at 20°C (Table 2). The above observations apply to dilute suspensions of $\leq 0.06%$ capsule. With more concentrated capsule, gels were observed at the divalent cation concentrations that yielded maximal viscosity with $\leq 0.06%$ capsule. The similarities among the conditions required for increased viscosity, gelation, and precipitation of capsule by divalent cations suggest that at least some of the mechanisms involved in these phenomena are similar.

**DISCUSSION**

Mono- and divalent metal cations differed in their effects on the viscosity and gelation of purified capsular polysaccharide from *M. flos-aquae* C3-40. Whereas divalent cations caused concentrated capsule suspensions to gel and dilute suspensions to precipitate, monovalent cations did not. Furthermore, equivalent increases in capsule viscosity were achieved at low concentrations of divalent cation chlorides (i.e., 0.05 mM CdCl$_2$ or FeCl$_2$) but only with much higher concentrations of monovalent chlorides (1 mM NaCl [Table 2]). In terms of ability to increase capsule viscosity, the various metal salts showed the following relative efficacies: CdCl$_2$, Pb(NO$_3$)$_2$, FeCl$_2$, MnCl$_2$, CuCl$_2$, CaCl$_2 > NaCl$. Since most of these salts are chlorides (except Pb, because its dichloride is too insoluble), it can be assumed that the major difference among the salts resides in the metal cations.

Preferential interaction with divalent versus monovalent metal ions has been reported for other acidic polysaccharides, including low-methoxyl pectin (23, 36, 39), which resembles *M. flos-aquae* C3-40 capsule in sugar composition (30). The thickening of pectin suspensions is thought to involve the cross-linking of polysaccharide chains by bonds between metal ions and the carboxylates, ring oxygens, and bridging oxygens of polygalacturonate “junction zones” on adjacent chains (6, 12, 14, 17, 21, 22, 32, 38). The increased viscosity of low-methoxyl pectin in the presence of Ca$^{2+}$ has been interpreted by Thibault and Rinaudo (37) as a reflection of polymer chain-chain association in early stages of cation-induced gelation. Our data support a similar conclusion because the conditions favoring increased capsule viscosity resemble those required for capsule gelation or precipitation. Thus, concentrated capsule suspensions gelled at divalent cation concentrations similar to those that yielded maximal viscosity with dilute capsule suspensions. Precipitation or flocculation of dilute capsule was observed at divalent cation concentrations in excess of the ones that yielded maximal viscosity. These precipitates were most pronounced with Fe$^{2+}$ and Pb$^{2+}$, which also acted to increase capsule viscosity at exceptionally low cation concentrations (Fig. 4; Table 2). Furthermore, the optimum CaCl$_2$ concentration for the precipitation of dilute capsule suspensions after overnight storage at 4°C (Table 3) was close to that for maximal viscosity after 30 min at 20°C (Table 2).

Metal ions at low concentrations caused an increase in capsule viscosity, whereas the same ions at higher concentrations prompted a decrease in viscosity (Fig. 4; Table 1). For divalent (but not monovalent) cations the decline in viscosity correlated with the formation of visible metal-capsule precipitates or flocs. The probable situation for divalent cations therefore is as follows: (i) the increased viscosities at low cation concentrations reflect various effects, including the initial stages of polymer chain association, with resulting changes in radii of polymer giration or the formation of loose flocs with large amounts of trapped solvent; and (ii) the decreased viscosities seen at high divalent cation concentrations are influenced by the removal of capsule from solution during the formation of condensed precipitates, as well as the other factors discussed below for NaCl. Analysis of intrinsic viscosities and Schulz-Blaschke coefficients ($k^*$) at various NaCl concentrations (Table 1) indicates that decreased capsule viscosities at high NaCl concentrations result from changes in solvation, polymer configuration, and chain-chain interactions not leading to precipitation. Solvent effects are indicated by the Schulz-Blaschke coefficient $k''$, which has been reported to decrease as solvent-polymer interactions increase (25). This coefficient was 1.3 times larger for capsule suspensions in 0.3 M than in 0.1 M NaCl (Table 1). The relative influences of polymer configuration and chain-chain interactions can be assessed by comparing the reduced viscosity, $\eta_{red}$, with the intrinsic viscosity, $[\eta]$; both reflect polymer configuration, but $\eta_{red}$ is influenced by chain concentrations.
FIG. 4. Effect of various concentrations of different metal ions on the reduced viscosity of solutions containing 0.06% (wt/wt) capsule in 10 μM NaCl, pH 7. Standard deviations of triplicate measurements are smaller than symbol diameters. □, NaCl; △, CaCl₂; ○, CdCl₂; ◦, CuCl₂; △, MnCl₂; ■, FeCl₂; •, Pb(NO₃)₂.
The accumulation of iron and manganese by capsule may also play a nutritional role, since both metals are relatively insoluble in aerobic alkaline waters (26, 27) and are essential nutrients for cyanobacterial growth (10, 31). Other metals, especially cadmium and lead, also interact with capsule at low metal concentrations, a phenomenon which could influence the bioavailability, solubilization, or precipitation of these toxic metals in aquatic environments.

ACKNOWLEDGMENTS

This research was supported by a University of Wisconsin System Applied Research Grant and by University of Wisconsin Oshkosh grants FDR 350 and FDS 117.

REFERENCES

14. Filipp, M. P., M. S. Komissarenko, and R. Kohn. 1988. Investigation of ion interactions whereas [η] (being η_red at infinite polymer dilution) is not. Both η_red and [η] decreased with increasing NaCl concentration, but η_red changed more than [η] did (Table 1).

The M. flos-aquae C3-40 capsule differs from pectin in its relative responses to calcium, copper, and cadmium ions. That is, Cd2+ affected capsule viscosity at lower cation concentrations than did either Ca2+ or Cu2+ (Table 2), whereas the reverse has been reported for pectin gelation (37) or other metal effects on pectin (21, 22, 34). Capsule resembles pectin in sugar composition, but not necessarily sugar linkages, degree of methyl esterification of galacturonate residues, or three-dimensional structure (30). Future studies that contrast metal specificities and structural differences between pectin and capsule may help to elucidate the nature of the constraints on cation-mediated phenomena such as thickening and gelation. The relatively greater interaction of cadmium with capsule compared with pectin suggests that capsule could be utilized as a Cd-specific material.

Capsule as it surrounds Microcystis cells exists in a gel-like form that can be dissociated by the removal of cations (13, 29). The following minimum concentrations of various salts are needed to prevent dissociation of the capsule gels that enclose M. flos-aquae C3 cells: FeCl₂, 10 μM; CaCl₂, 80 μM; MgCl₂, 100 μM; and KCl, NaCl, NaNO₃, or NaH₂PO₄, 700 to 1,000 μM (13, 29). In these natural gels, multivalent cations influence capsule structure at lower concentrations than do monovalent cations, in parallel with the viscosity data (Fig. 4). Furthermore, the ratio of various ion concentrations needed to maintain the aggregation of these natural gels is very similar to the ratios at maximal capsule viscosity; in both cases, these molar ratios were as follows: NaFe > 25; NaCa = 8 to 13. In both instances interactions with multivalent cations were detected at micromolar salt concentrations. Viscometric properties therefore seem to be related to phenomena that occur in native Microcystis colonies and thus may reflect at least certain

### Table 2. Salt concentrations that yielded equivalent increases in the viscosity of M. flos-aquae C3-40 capsule suspensions

<table>
<thead>
<tr>
<th>Metal salt</th>
<th>Concentration at equal increase in η_red of 0.06%/ capsule (mM)</th>
<th>Concentration at maximal η_red of 0.06%/ capsule (mM)</th>
<th>Concentration at maximal η_red of 0.02%/ capsule (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdCl₂</td>
<td>0.05</td>
<td>0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>Pb(NO₃)₂</td>
<td>0.05</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>FeCl₂</td>
<td>0.05</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>0.055</td>
<td>0.2</td>
<td>0.25</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>0.15</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.1</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>NaCl</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

* η_red increased from 12 (no added salt) to 40 dℓ/ɡ after the specified concentration of each salt was added.

* Shown in Fig. 4.

### Table 3. Anthrone-reacting carbohydrate in the supernatant after CaCl₂ treatment of a capsule suspension, with overnight storage at 5°C

<table>
<thead>
<tr>
<th>CaCl₂ concn (mM)</th>
<th>Carbohydrate in supernatant (µg/ml)</th>
<th>% Carbohydrate precipitated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>0.17</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>0.34</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>0.68</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>1.02</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>1.36</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>