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Rheological studies of the interaction of mucins with alginate and polyacrylate

Asira Fuongfuchat [a], Alexander M. Jamieson [a,*], John Blackwell [a], Thomas A. Gerken [b]

[a] *Department of Macromolecular Science, Case Western Reserve University, Cleveland, OH 44106, USA*
[b] *W.A. Bernbaum Cystic Fibrosis Research Center and Department of Pediatrics and Biochemistry, Case Western Reserve University, Cleveland, OH 44106, USA*

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Abstract

The associative interaction of purified ovine and porcine submaxillary mucins (OSM and PSM) with sodium alginate was evaluated by comparing the rheological properties of mixtures against those of pure alginate and mucin in dilute, semi-dilute, and concentrated solutions. These systems were investigated as models for the interaction of mucin with the extracellular alginate produced by *Pseudomonas aeruginosa*. In dilute solution, evidence for such interaction cannot be obtained because aggregate species exist both in the OSM–alginate mixtures as well as in pure OSM. However, in the semi-dilute regime, mixtures containing a higher proportion of mucin show systematically higher viscosities than those predicted by simple additivity. In concentrated solutions containing higher proportion of mucin, an enhanced elastic response is observed. These results demonstrate a substantial binding interaction of mucins with alginate. This property is not observed in mixtures containing a high proportion of alginate, suggesting that mucins possess relatively low numbers of interacting sites. Introduction of 3 mM Ca^{2+} ions to all mucin–alginate mixtures enhances the elasticity due to gelation of alginate. Finally, comparison of the rheological properties of PSM–alginate mixtures with those of PSM–polyacrylate mixtures indicates that the binding strength of alginate to mucin is significantly weaker than that of polyacrylate.

Keywords: Mucins; Alginate; Polyacrylate; Rheological studies

* Corresponding author.

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1. Introduction

The surface of epithelia are covered by a layer of mucus, a continuous hydrated, viscoelastic gel. The macromolecular component principally responsible for the viscoelastic properties of mucus is a high molecular weight glycoprotein known as mucin. The macromolecular structure of mucin is composed of elementary glycoprotein subunits, which contain both heavily and sparingly glycosylated regions and are linked end-to-end via disulfide bridges [1] as shown in Fig. 1. Molecular weights of the intact mucin molecules have been reported [2–4] in the range $2–20 \times 10^6$. Physical [4] and electron microscopic [5,6] investigations have established that the mucin molecules adopt a semi-flexible linear random coil configuration in solution. As a result of this relatively expanded chain conformation, as well as the high density of oligosaccharide side chains, mucins tend to self-associate and also bind to foreign particles. In the pulmonary clearance mechanism, tracheobronchial mucus adheres to and traps bacteria, which are borne away by mucociliary transport. However, certain pathogens such as *Pseudomonas aeruginosa* are not easily removed by this process. *P. aeruginosa* generates an extracellular alginate that is reported to exhibit a strong adhesive interaction with tracheobronchial mucus [7], thereby affecting the rheological properties of mucus and inhibiting mucociliary transport. For pharmacological applications, a number of charged and neutral polymers are classified as mucoadhesive, since they are known to bind very strongly to mucus via non-covalent bonds.

Mucoadhesion is a complicated phenomenon [8,9], but is thought to involve two general kinetic processes [10]. First, the formation of non-covalent bonds (electrostatic, hydrophobic and hydrogen bonding) at the interface between the mucus layer and polymer; and second, the interdiffusion and/or interpenetration of polymer chains and mucin across the interface. Several authors [11,12] have suggested that rheological synergism of mucin–polymer or mucus–polymer mixtures can be related to the strength of mucoadhesion. Thus, associative interactions between mucin and polymer and the related rheological behavior are of interest with regard to potential mucoadhesive behavior. Effective mucoadhesives generally are linear or lightly cross-linked polymers with numerous hydrophilic functional groups that can form ionic bridges and hydrogen bonds, such as carboxyl, hydroxyl, amide, and sulfate groups [13].

In the present paper, we are interested in using rheological methods to investigate whether purified mucin glycoproteins have a strong associative interaction with sodium

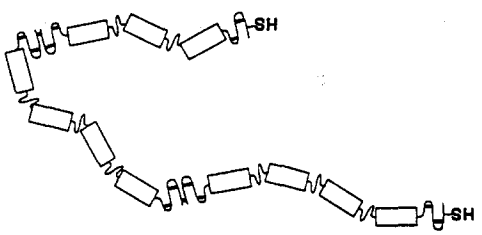
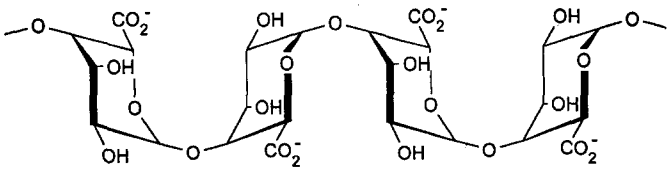
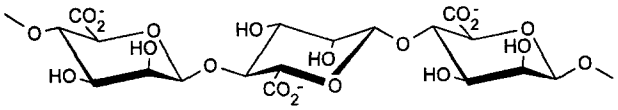


Fig. 1. Typical mucin structure: (block) heavily glycosylated regions, (wavy line) sparingly glycosylated region, and (=) intra- and inter-molecular disulfide bonds (ref. [3]).



poly(α-L-Guluronate)



poly(β-D-Mannuronate)

Fig. 2. The structure and conformation of alginates (ref. [18]).

alginate. In nature, alginate is predominantly found in brown algae (Phaephyceae) [14]. It is also produced by bacteria, such as *Azotobacter vinelandii* [15] and several species of *Pseudomonad* [16]. Alginate is an unbranched copolymer of two types of uronate residues, β-D-mannuronate and, its C-5 epimer, α-L-guluronate (Fig. 2). These uronate residues are linked via (1 → 4) glycosidic bonds in widely varying proportions and sequences [17,18]. Within a single polysaccharide chain, there are homopolymeric poly-β-D-mannuronate (poly-M) and poly-α-L-guluronate (poly-G) sequences, as well as heteropolymeric poly-MG sequences in which the two uronate species are linked randomly with terminal excesses of β-D-mannuronate.

Alginate in solution at neutral pH is a negatively charged polyelectrolyte. Concentrated alginate solutions are highly viscous and are capable of forming gels in the presence of multivalent cations [19,20] including Ca^{2+} [21,22]. The gelation of alginate is a diffusion-controlled process. All residues in alginate bind divalent cations, but the strength of the interaction increases with the proportion of poly-G blocks whose conformation facilitates efficient coordination of the cation with the oxygens of other electronegative groups in addition to carboxylate. Otherwise, the binding of cations occurs by ionic interaction alone.

It has been reported that alginate shows excellent adhesion on mucus layers in vivo [23], and increases the elasticity of mucus secretions [7,18]. Smedley et al. [7] found that, in the presence of bacterial alginate, there is an increase in the dynamic storage modulus and viscosity of mucus. Furthermore, the interaction of alginate with mucus is enhanced in the presence of Ca^{2+}. The concentration of Ca^{2+} in the extracellular fluid from a cystic fibrosis (CF) patient is approximately 3 mM, which is close to the precipitation point observed for poly-G-containing alginate solutions [24].

The mucoadhesive properties of polymers have been studied by various types of mechanical measurements including failure of polymer–mucus interfaces in tensile or shear deformation, and surface tension analysis [13,23,25–29] and the interaction of a

polymeric microparticle with a mucous layer [30]. In addition, associative interactions between mucin glycoproteins and mucoadhesive polymers have been demonstrated by comparing the viscoelastic properties of mucin–polymer mixtures with those of the mucin and polymer alone [11,12,31,32]. The latter approach is adopted in the work described below. We have compared the rheological properties of solutions containing mixtures of alginate with ovine and porcine submaxillary mucin (OSM and PSM) versus those of the solutions of the components. The experiments have been carried out in the dilute, semi-dilute, and concentrated regimes. We have also compared the rheological behavior of PSM–alginate mixtures against those of PSM–polyacrylate mixtures. Polyacrylate (or polyacrylic acid), in common with other polyanionic species having a high charge density, shows excellent mucoadhesive properties [23,25–33].

The OSM and PSM specimens used in our studies have well-defined chemical and physical characteristics [34–38]. The two mucin specimens differ in the lengths of the carbohydrate side chains and, from the available data, in their sequence of the protein cores [36,38]. It is of interest to ascertain whether the difference in oligosaccharide structure influences the interaction with alginate.

2. Experimental

Materials.—Sodium alginate (Fluka), derived from *Laminaria hyperborea*, has a reported molecular weight of 48|000–186|000; tris(hydroxymethyl)aminomethane hydrochloride (Tris · HCl), guanidine hydrochloride (GdnHCl), sodium azide, and calcium chloride were from Sigma Chemical Co. Polyacrylic acid of molecular weight 90|000 was obtained as a 25% (w/v) aqueous solution from Aldrich Chemical Co.

Purification and characterization of submaxillary mucins.—OSM and PSM were isolated from sheep and pig submaxillary glands, respectively, as described previously [34,39] using a modified procedure based on those of Hill et al. [38] and De Salegui and Plonska [40]. Mucin solutions in saline buffer were dialyzed exhaustively against water at 4 °C. Weight-average molecular weights ($\overline{M}_w$) of these OSM and PSM were determined by static light scattering from solutions in 6 M GdnHCl, 10 mM phosphate buffer, pH 7.01. Zimm plot analyses [39] yielded $\overline{M}_w = 2.81 \times 10^6$ g/mol for OSM and 5.93×10^6 g/mol for PSM.

Solution preparation.—Mucin and alginate solutions were prepared in 0.1 M Tris · HCl and 0.02% sodium azide, pH 7.4 (Tris · HCl buffer). Concentrated solutions of OSM were prepared by direct dissolution of lyophilized mucin in 0.1 M Tris · HCl buffer. The solutions were shaken slowly at 4 °C for a week to allow complete dissolution. However, because of the difficulty of dissolving PSM in the Tris · HCl buffer, 20 mg/mL PSM was first dissolved in 3 M GdnHCl and 0.1 M Tris · HCl buffer and then dialyzed first against 3 M GdnHCl and 0.1 M Tris · HCl buffer for 2 days, against deionized water for 3 days, and finally dialyzed against 0.1 M Tris · HCl buffer for 1 day. All procedures were performed at 4 °C, and the stock solutions were stored at 4 °C.

The sample solutions were prepared by mixing mucin and alginate stock solutions to the concentrations required. After mixing, the solutions were slowly shaken at 4 °C for 2

days to allow for equilibration. To explore the effect of Ca^{2+} ions, aliquots of 15 mM calcium chloride were added during the second day of shaking to give a final Ca^{2+} concentration of 3 mM.

Sample solutions of PSM and polyacrylate were prepared by mixing stock solutions of 20 mg/mL PSM and 125 mg/mL [12.5% (w/v)] polyacrylic acid in Tris · HCl buffer in the desired proportion. These solutions were thoroughly equilibrated by gentle shaking overnight prior to the measurements.

Dynamic light scattering and viscometric analyses.—Dilute solutions were filtered through Millipore SCWP (pore size: 5 μm for OSM and 8 μm for PSM), sealed in sample cells, and centrifuged at 5000 rpm for 30 min prior to measurements.

Dynamic light scattering experiments were performed at 25 °C using a He–Ne laser ($\lambda = 632.8$ nm) and a BI 240 photogoniometer with BI 2020 autocorrelator (Brookhaven Instruments Corp., Ronkonkoma, NY). The intensity autocorrelation function of the scattered light was analyzed by using the cumulant method in order to determine the z-average translational diffusion coefficient as a function of wave vector, $q = (4\pi/\lambda)\sin(\theta/2)$ where θ is the angle between the incident and scattered light and ranged 25°–90°. The diffusion coefficient was independent of concentration over the range 0.1–1.0 mg/mL. In our studies, the mucin concentration used was 0.2 mg/mL. The translational diffusion coefficient $D^0_{t,z}$ was obtained by extrapolation to zero wave vector [41]. The hydrodynamic radius of the solute is calculated from the Stokes–Einstein equation:

$$D^0_{t,z} = \frac{kT}{6\pi\eta_0}\left\langle R_h^{-1}\right\rangle_z \tag{1}$$

where k is the Boltzmann constant, T is the absolute temperature, and η_0 is solvent viscosity and $\langle R_h^{-1}\rangle_z$ indicates that we measure the z-average of the inverse hydrodynamic radius, R_h.

A Cannon–Ubbelohde No. 25 viscometer in a water-bath controlled temperature at 25 ± 0.1 °C was used to determine the viscosities for dilute solutions. Intrinsic viscosities were calculated using the Kraemer and Huggins equations.

Rheological measurements.—Dynamic oscillatory measurements on solutions of mucin, alginate, and mucin:alginate mixtures in 0.1 M Tris · HCl buffer were performed using the Rheometrics Fluids Spectrometer (Rheometrics, Inc., Piscataway, NJ) with cone-plate geometry (cone radius 25 mm and cone angle 0.02 rad) at 25 ± 0.20 °C. The storage modulus (G'), loss modulus (G''), loss tangent (tan δ), and dynamic complex viscosity (η^*) were measured as a function of angular frequency (ω) ranging from 10^{-1} to 10^2 rad/s, at a constant strain of 5%. This falls in the linear viscoelastic, i.e., strain-independent, region. The steady shear viscosity was also measured in the shear rate γ range of 10^{-1} to 10^2 s^{-1}.

3. Results and discussion

Dilute solutions.—Table 1 contrasts the intrinsic viscosities of polymer–mucin mixtures with the values predicted from the intrinsic viscosities of the individual

Table 1
Intrinsic viscosity of OSM, sodium alginate, and their mixtures in the Tris·HCl buffer

Solution	$[\eta]$ [a] (mL/g)
OSM	674 ± 7
Alginate (sodium salt)	553 ± 7
1:1 [b] OSM–alginate	472 ± 4
Excess intrinsic viscosity ($[\eta]^E$)	−141 ± 4

[a] Mean ± SD, where $n = 3$.
[b] $c[\eta]$ ratio. For example, 1:1 is a mixture of OSM and alginate with equal hydrodynamic volume fractions, $c[\eta]$.

components using an additivity relation. The latter is based on the Einstein–Simha equation for the solution viscosity, $\eta = \eta_s(1 + \nu\phi)$, where η_s is the solvent viscosity. The total hydrodynamic volume fraction of the solutes, $\phi = c[\eta]\nu^{-1}$, is assumed to be the sum of the volume fractions of all individual species, $\phi_i = c_i[\eta]_i\nu_i^{-1}$, where the shape factor, ν, of all solutes is assumed to be identical, i.e., $\nu = \nu_i$. Here, c is the total mass concentration (g/mL) of all polymer species, and $c = \sum_{i=1}^{N} c_i$ where c_i is the concentration of each component. The calculated intrinsic viscosity of the mixture, $[\eta]_{M,cal}$, is related to those of the individual species, $[\eta]_i$, as shown below:

$$[\eta]_{M,cal} = \sum_{i=1}^{N} c_i[\eta]_i \Big/ \sum_{i=1}^{N} c_i \tag{2}$$

If there are associative interactions among the constituent species present in solution, the measured intrinsic viscosity should differ from that calculated via eq (2) by an amount referred to as the excess intrinsic viscosity:

$$[\eta]^E = [\eta]_{M,meas} - [\eta]_{M,cal} \tag{3}$$

Experimentally, we find a negative value of $[\eta]^E$ as shown in Table 1. This indicates that the hydrodynamic volume fraction of the mixture, ϕ_M, is smaller than that computed as the sum of each component.

To gain further insight, the hydrodynamic radii of the solutes were determined directly by dynamic light scattering as shown in Table 2. Note that the radii of mucin

Table 2
Translational diffusion coefficients, $D^0_{t,z}$, and hydrodynamic (Stokes) radii, $1/\langle R_h^{-1}\rangle_z$, of OSM, alginate, and OSM–alginate mixtures in the Tris·HCl buffer

Solution	$D^0_{t,z} \times 10^9$ (cm^2/s)	$1/\langle R_h^{-1}\rangle_z$ (Å)
OSM	6.61	3296
Alginate (sodium salt)	7.18	3034
99:1 [a] OSM–alginate	7.54	2891
9:1 [a] OSM–alginate	9.12	2391
1:1 [a] OSM–alginate	9.20	2370

[a] $c[\eta]$ ratio

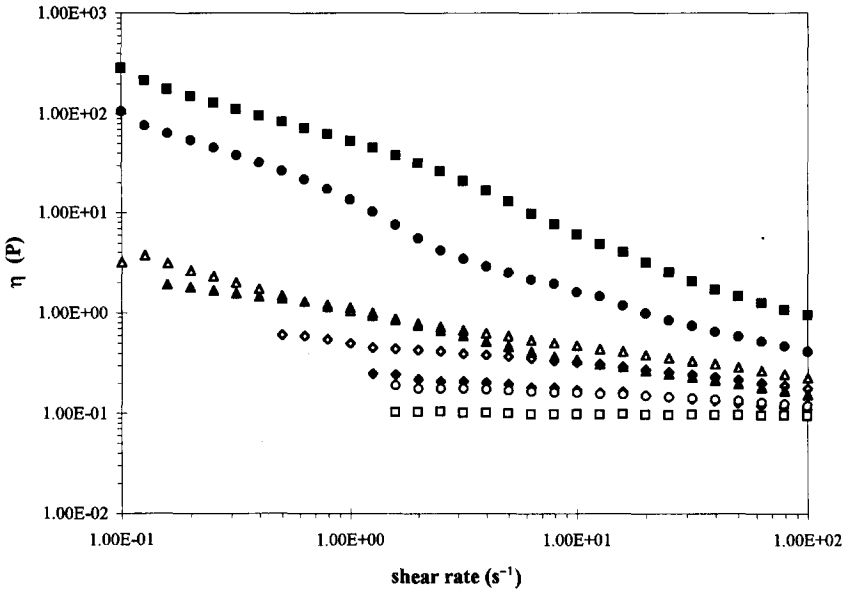


Fig. 3. Plots of the steady shear viscosity against shear rate for OSM (◇), alginate (□), and their mixtures (○, 1:1 and △, 3:1) at $c[\eta] = 2.7$ in the presence (filled symbols) and absence (open symbols) of 3 mM Ca^{2+} in the Tris·HCl buffer.

and alginate are larger than expected for single chains based on experimental relations generated earlier [39]. Consequently, we deduce that each species is present as an aggregate. Table 2 shows the hydrodynamic radii observed for the mixtures are significantly smaller than those of pure mucin and alginate. Thus, dilute-solution viscometry and dynamic light scattering both indicate that the hydrodynamic sizes of the mixed solutes are smaller than those of the pure polymers, i.e., mucin–alginate interactions lead to the formation of smaller aggregates. Any interaction of alginate with mucin is, therefore, obscured by the self-associative behavior of the component polymers and/or by shape changes on complex formation.

Semi-dilute solutions.—Rheological studies were performed on solutions of OSM (4 mg/mL), alginate (5 mg/mL) and mixtures of these solutions with different hydrodynamic volume fraction, $c[\eta]$, ratio, specifically OSM:alginate = 1:1, 2:1, and 3:1, respectively. All solutions had an identical total solute volume fraction, i.e., $c[\eta] = 2.7$, at which c is above the overlap concentration, $c_0 \sim 1.08/[\eta] = 1.6$ mg/mL. The dynamic response of these solutions was below the lower limit of the transducer sensitivity. Therefore, only the steady shear viscosities were measured.

We found that only mixtures containing excess OSM have a higher steady shear viscosity compared to those of pure OSM and alginate solutions. As shown in Fig. 3, this is particularly evident for the 3:1 OSM–alginate solution and clearly indicates the interactions of alginate with mucin. However, the 1:1 mixture has a viscosity intermediate between that of pure PSM and pure alginate. On addition of 3 mM Ca^{2+}, the 1:1 mixture shows a substantial increase in steady shear viscosity. However, addition of

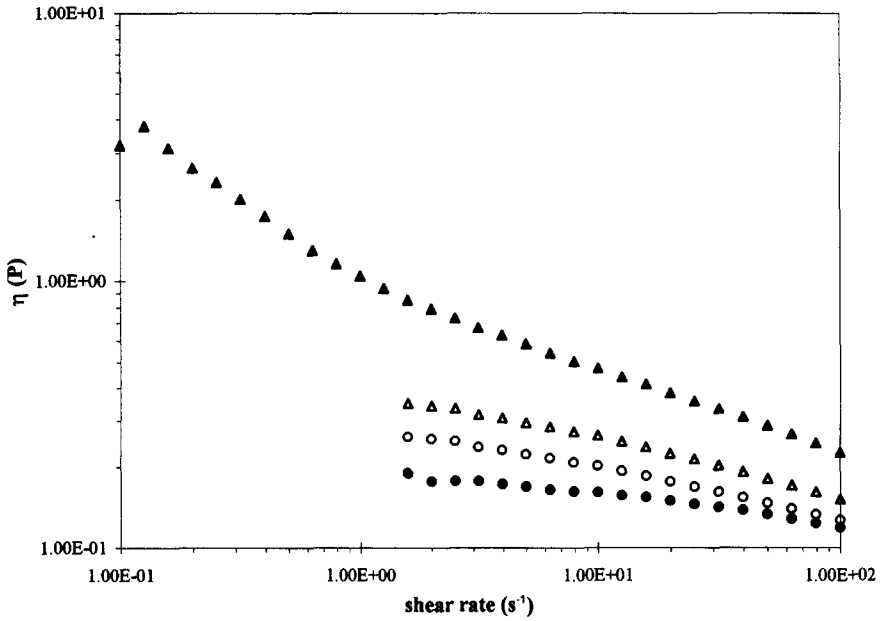


Fig. 4. Plots of the steady shear viscosity (filled symbols) and the calculated shear viscosity (open symbols) [eq (4)] against shear rate for OSM–alginate mixtures (○, 1:1 and △, 3:1) with $c[\eta] = 2.7$ in Tris·HCl buffer.

Ca^{2+} does not significantly change the viscosity of the 3:1 mixture, indicating that mucin–alginate interactions are dominant. The viscosities of all mixtures containing Ca^{2+} are lower than that of the pure alginate solution, which has a gel structure as a result of Ca^{2+}-bridging, but are higher than that of the pure OSM solution which has no Ca^{2+} cross-linking.

To further illustrate the interaction between mucin and alginate, we compared the experimental viscosity of the mixtures versus that calculated from the sum of the component viscosities, as proposed by Hassan and Gallo [31] and Rossi et al. [12]:

$$\eta_{M,cal} = \eta_m + \eta_p \tag{4}$$

where $\eta_{M,cal}$ is the calculated viscosity of the mixture; and η_m and η_p are the viscosities of mucin and the polymer, respectively.

Fig. 4 shows plots of the measured ($\eta_{M,meas}$) and calculated ($\eta_{M,cal}$) steady shear viscosities for different OSM–alginate mixtures. In these semi-dilute solutions, a signifant interaction between alginate and OSM is observed, for the 3:1 mixture, since the measured viscosity is substantially larger than the calculated. However, the measured viscosity of the 1:1 mixture is actually slightly smaller than the calculated value. We note further that the viscosities of the mixtures containing 3 mM Ca^{2+} (not shown in Fig. 4) are always smaller than calculated values because the predominant contribution comes from the highly elastic alginate gel via the formation of Ca^{2+}-bridges [42] that are disrupted by the addition of mucin.

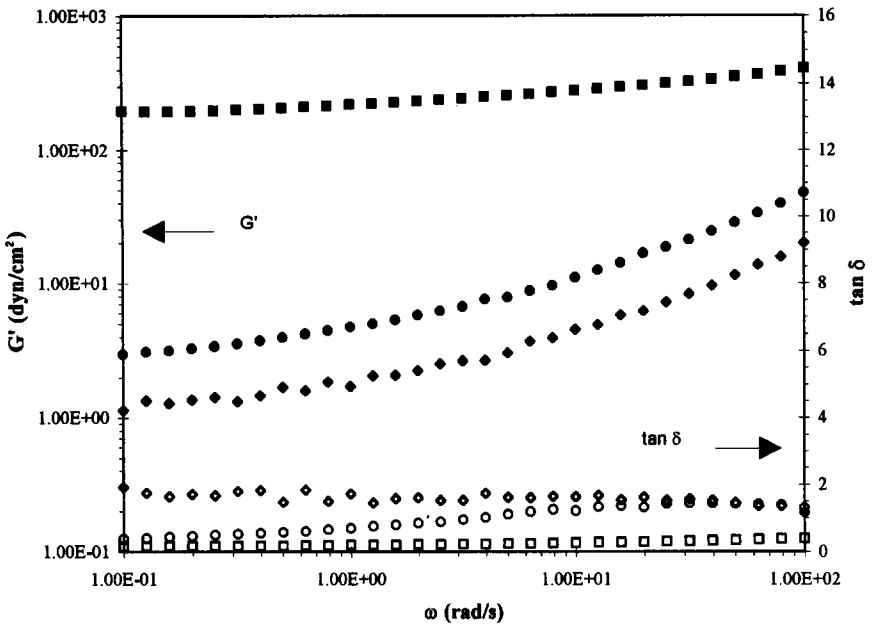


Fig. 5. The effect of sodium alginate concentrations on G and tan δ of 20 mg/mL OSM solution in 0.1 M Tris·HCl buffer versus ω. (◇) OSM, (○) 20:3 and (□) 20:5.

Concentrated solutions.—The above studies of the rheological behavior of alginate in semi-dilute solution indicated that the interaction of alginate with mucin is more effectively analyzed using mixtures containing excess mucin. This conclusion was reinforced by similar studies performed in the concentrated solution regime, $c[\eta] \gg 1$. We compared the rheological properties of mixtures of OSM (20 mg/mL) containing small amounts of alginate (3 and 5 mg/mL) with those of mixtures of alginate (20 mg/mL) containing small amounts of mucin (3 and 5 mg/mL). Similar studies were also done for PSM–alginate mixtures (10:3 and 10:5).

Mixtures containing alginate (20 mg/mL) and low concentrations of mucin (3 and 5 mg/mL) did not show any increase in viscosity and dynamic moduli relative to those for pure alginate, either in the presence or absence of 3 mM Ca^{2+} (data not shown). The experimental results are dominated by the alginate rheology [43]. On the other hand, as shown in Figs. 5 and 6, mixtures concentrated in mucin (20 mg/mL OSM and 10 mg/mL PSM) and dilute in alginate (3 and 5 mg/mL) show viscosities and dynamic moduli that are much higher than those of the pure mucins, both in the presence and absence of 3 mM Ca^{2+}. Furthermore, the addition of alginate converts the rheological behavior from a viscous fluid (tan $\delta > 1$) to an elastic gel (tan $\delta < 1$). Concentrated solutions of pure OSM and pure PSM either in the presence or absence of 3 mM Ca^{2+} show primarily viscous behavior, i.e., $G'' \geq G'$ and tan $\delta \geq 1$. Note that on addition of Ca^{2+} to OSM, we observed [43] a significant decrease in the viscosity, which may reflect previous observations in dilute solution that Ca^{2+} induces a contraction or

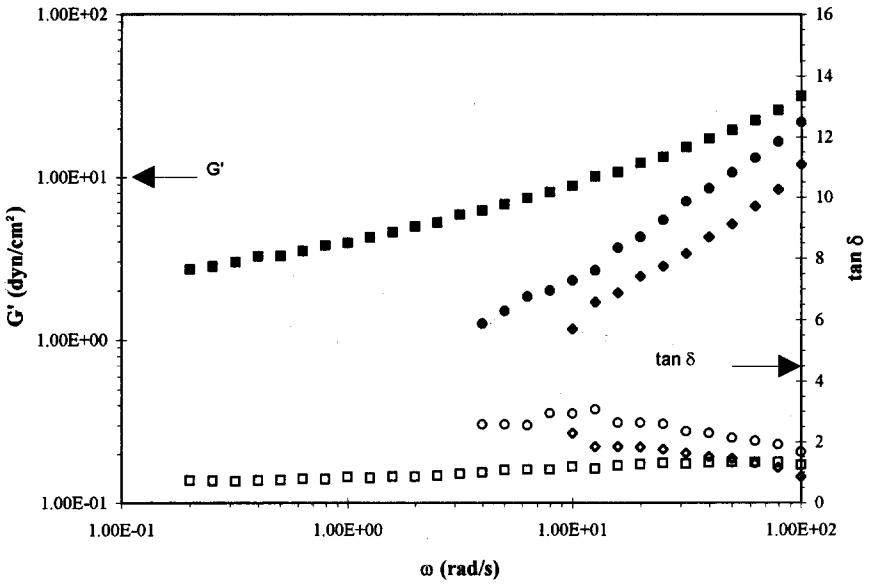


Fig. 6. The effect of sodium alginate concentrations on G and tan δ of 10 mg/mL PSM solution in 0.1 M Tris·HCl buffer versus ω. (◇) PSM, (○) 10:3, and (□) 10:5.

folding of OSM [42,44,45]. Addition of Ca^{2+} has a negligible effect on the viscosity of PSM.

Fig. 5 shows further that the increase in elasticity of a concentrated OSM solution (20 mg/mL) is a strong function of the concentration of addition alginate, and that the moduli are much larger than those for pure OSM. The 20:3 OSM–alginate mixture is a gel, since tan $\delta < 1$ ($G' > G''$) at low frequencies, and the 20:5 mixture is a strong gel, for which tan $\delta < 1$ over the entire frequency range. As evident in Fig. 6, the PSM–alginate mixtures have smaller moduli than those of OSM–alginate mixtures. However, Fig. 6 clearly indicates that there is also a strong interaction between PSM and alginate, and, based on tan δ values, the 10:5 PSM–alginate mixture is also beyond the gel point. Comparing mucin–alginate mixtures with the same mixtures containing Ca^{2+}, we observed [43] that 3 mM Ca^{2+} facilitates the gelation of concentrated mucin solutions containing small amounts of alginate, as evidenced by further increases in moduli (data not shown).

The complex and steady shear viscosities of all OSM–alginate mixtures show strongly non-Newtonian behavior, whereas PSM–alginate mixtures are only weakly non-Newtonian [43]. In each case, the viscosities of the mixtures show synergistic increments with increasing alginate concentration and are also higher than those of the corresponding pure mucin solutions. Addition of 3 mM Ca^{2+} to the mixtures further enhances the viscosities (data not shown). We note that, at low deformation rates, the magnitude of the dynamic complex viscosity is higher than the steady shear viscosity probably because steady shear destroys the intermolecular cross-links that contribute to the magnitude of the dynamic complex viscosity at small strains. However, we observed

that the dynamic viscosities of solutions measured 30 min after steady shear experiments are not significantly different from those recorded in earlier dynamic measurements. Thus, the intermolecular cross-links are reversible [43].

These rheological results indicate that there are substantial associative interactions of alginate with both OSM and PSM. At equivalent hydrodynamic volume fraction of mucin, these interactions appear stronger for OSM, but this may reflect the higher viscosity of the original OSM solution. Since the increase in moduli and viscosities appears only in mixtures concentrated in mucin and dilute in alginate, it appears that alginate possesses a relatively high number of mucin interacting sites, whereas mucin contains relatively few sites for alginate interaction. Furthermore, these interacting sites apparently form cross-links between the mucin chains at high concentration of mucin, leading to gel formation. We note further that the magnitudes of the viscoelastic functions of the concentrated mixtures are dramatically enhanced on addition of Ca^{2+}, presumably due to formation of alginate cross-links. Finally, the interaction of alginate with mucin was further investigated [43] at physiological temperature (37 °C). The results showed no significant difference in the rheological properties of mucin–alginate mixtures at 37 °C from those at 25 °C (data not shown). Physiologically, the rheology of mucin–alginate mixtures may be relevant to the pathological rheology of mucus of CF patients, in which interaction of mucin with the extracellular alginate polysaccharide of pneumococcal bacteria may occur [7]. One source of the abnormally thick and viscous mucus of CF patients could be the cross-linking effect of alginate on the secreted mucin [7]. In addition, the higher level of calcium ions detected in the sputum of CF patients could facilitate the gelation of mucin–alginate mixture by cross-linking of alginate, leading to a further increase of the viscosities and dynamic moduli of CF mucus. However, here it should be noted that bacterial alginate may contain relatively low levels of poly-G sequences which generate the strongest Ca^{2+} binding sites [18].

Comparison with polyacrylate.—Rheological analyses were performed on mixtures of 10 mg/mL PSM with 1.0, 3.0, and 6.3 mg/mL polyacrylate, and compared with their pure component solutions. As noted above 10 mg/mL PSM is a viscous solution with dynamic and steady shear viscosities that show slightly non-Newtonian characteristics. In addition, solutions of polyacrylate at concentration of 1.0, 3.0, and 6.3 mg/mL show Newtonian behavior with viscosities in the range $0.98–1.06 \times 10^{-2}$ P, which are much lower than the viscosities observed of PSM (10 mg/mL).

As shown in Fig. 7, mixtures of PSM with polyacrylate show primarily elastic behavior: $G' > G''$ and $\tan \delta < 1$ over the range of dynamic frequencies, i.e., the mixtures form strong gels. The dynamic and steady shear viscosities of these mixtures (not shown) show strongly non-Newtonian behaviors [43]. We found that the steady shear viscosities are higher than the dynamic shear viscosities and show an abnormal increase at low shear rates reflecting some degree of stress overshoot. However, we note that in small-strain dynamic experiments, PSM–polyacrylate mixtures show linear viscoelastic behavior over the entire frequency range, and the dynamic moduli measured at 30 min after a steady shear experiment are insignificantly different from the original values [43].

The results indicate that the addition of very small amounts of low viscosity polyacrylate solutions to PSM solutions causes a large increase in the shear moduli and

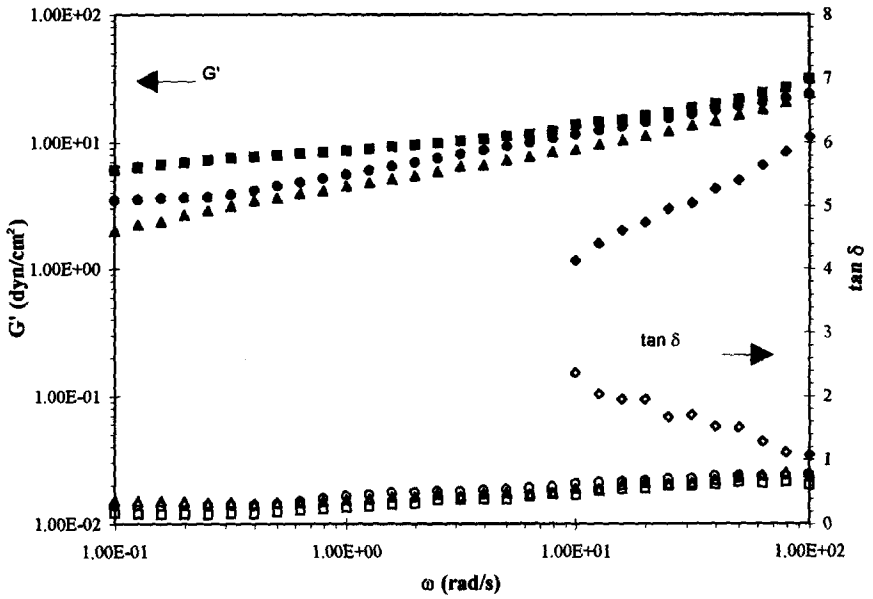


Fig. 7. The effect of polyacrylate concentration on G and tan δ of 10 mg/mL PSM solution in 0.1 M Tris·HCl buffer versus ω. (◇) PSM, (△) 10:1, (○) 10:3, and (□) 10:6.3.

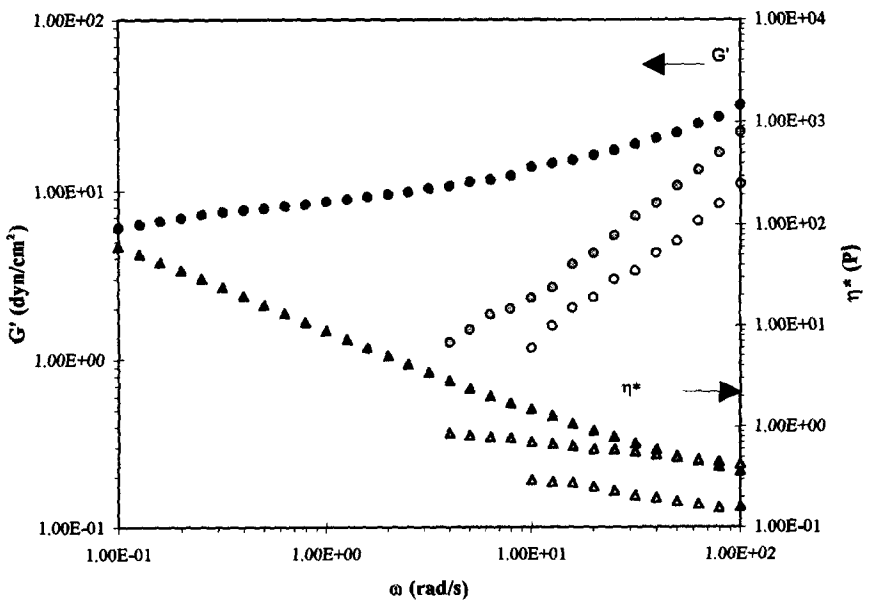


Fig. 8. G (○) and η (△) of 10 mg/mL PSM (open symbol), 10:3 PSM–alginate mixture (grey symbol) and 10:3 PSM–polyacrylate mixture (filled symbol) as a function of ω.

viscosities of solutions and, indeed, results in gel formation. The steady shear viscosities of the mixtures were very much higher than those predicted as the sum of the steady shear viscosities of the components [cf. eq (4)] [43]. Note that the viscosity of a dilute solution of polyacrylate is much smaller than that for PSM solution; therefore, the calculated viscosity is approximately the same as that for the pure PSM solution. Our results are consistent with the recent rheological analyses of Mortazavi et al. [32] that show a strong interaction between pig gastric mucin and Carbopol, a cross-linked polyacrylate.

Fig. 8 compares the storage moduli and dynamic complex viscosities of a PSM:alginate mixture and a PSM–polyacrylate mixture, each containing 10 mg/mL PSM and 3.0 mg/mL of alginate and polyacrylate, respectively. Polyacrylate strongly enhances the dynamic moduli and complex viscosities of PSM solutions, whereas alginate has a much smaller effect. Indeed, the 10:3 PSM:alginate mixture is a viscous solution ($\tan \delta > 1$), whereas the 10:3 PSM–polyacrylate mixture is a gel ($\tan \delta < 1$). This suggests that the binding interaction of polyacrylate to PSM is much stronger than that of alginate. In this context, it is interesting to compare our present data with earlier studies of the mucoadhesive property of alginate and polyacrylate. Each of these polymers has excellent mucoadhesive behavior as evidenced by in vivo evaluation [23] and in vitro tensile testing [27]. In the latter study the binding of alginate to gastric mucus was significantly weaker than that of polyacrylate.

4. Conclusion

The associative interaction between water-soluble polymers and mucin glycoproteins can be evaluated by comparing the viscoelastic properties of mucin–polymer mixtures against those of the pure components. Alginate has a significant interaction with both OSM and PSM, as evidenced by an increase in dynamic moduli and viscosity, and a decrease in loss tangent in semi-dilute and concentrated mixtures rich in mucin. We deduce that the alginate molecules possess higher numbers of interacting sites than do OSM and PSM, and can form cross-links between the mucin chains leading to gelation at sufficiently high mucin concentration. The nature of the binding sites remains unclear. However, in the present case where the mucoadhesive polymers including mucin are polyanionic, it appears that non-covalent interactions other than electrostatic must play an important role. Addition of calcium ions can cause a further increase in the elasticity of the mucin–alginate gel probably due to the formation of Ca^{2+} bridges between the alginate chains. Finally, rheological analysis indicates that the interaction of polyacrylate with mucin is stronger than that of sodium alginate. This is consistent with previous conclusions based on tensile adhesive strength of polymer specimens to gastric mucus [27].

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