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Rheologically Interesting Polysaccharides from Yeasts

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ABSTRACT

We have examined the relationships between primary, secondary, and tertiary structures of polysaccharides exhibiting the rheological property of friction (drag) reduction in turbulent flows. We found an example of an exopolysaccharide from the yeast *Cryptococcus laurentii* that possessed high molecular weight but exhibited lower than expected drag reducing activity. Earlier correlations by Hoyt (*8,10*) showing that $\beta 1\rightarrow 3$, $\beta 2\rightarrow 4$, and $\alpha 1\rightarrow 3$ linkages in polysaccharides favored drag reduction were expanded to include correlations to secondary structure. The effect of sidechains in a series of gellan gums was shown to be related to sidechain length and position. Disruption of secondary structure in drag reducing polysaccharides reduced drag reducing activity for some but not all exopolysaccharides. The polymer from *C. laurentii* was shown to be more stable than xanthan gum and other exopolysaccharides under the most vigorous of denaturing conditions. We also showed a direct relationship between extensional viscosity measurements and the drag reducing coefficient for four exopolysaccharides.

Index Entries: Yeast, polysaccharide; structure/function; exopolysaccharides; drag reduction.

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INTRODUCTION

Microbial polysaccharides with important mechanical properties have significant impact in commercial applications. For example, the bacterium, *Xanthomonas campestris*, produces a polysaccharide, xanthan gum, whose properties are useful as an emulsifier in food additivies, as a thickener in enhanced oil recovery, and as a reducer of friction in turbulent flow (*1*). Xanthan gum's solution structure under various conditions is relatively well characterized, and correlations between its structure and function have been explored (*2-4*).

We had screened yeast strains growing on alcohols that produced exopolysaccharides that were active drag reducers (*5*). This search was made for two practical reasons: The exopolysaccharides from yeasts cells are usually more easily separated than in bacterial systems and, thus, are attractive for large scale production; and ethanol and methanol are abundant and inexpensive feedstocks. We identified one methylotroph (*Candida boidinii*) and several other heterotrophs that produced drag reducing polysaccharides (Table 1) and examined the effects of cultural and genetic modifications on the production and quality of drag reducing exopolysaccharides (*5*).

There were definite differences in polysaccharide performance as a function of cultural conditions. In addition, not all of the large polysaccharides (measured by gel permeation chromatography) from different strains were drag reducers. These observations prompted us to examine the relationship between structure and function in this class of polymers to determine which features promote good drag reduction. Using the set of polysaccharides produced from our screening effort, we could examine both good and poor drag reducers and compare their physical/chemical properties in order to gain insight into the molecular basis of this phenomenon.

The study of the drag reduction phenomenon the Toms effect (*6*) has spanned four decades, yet no completely satisfactory model or models have been produced to account for the phenomenon in terms of molecular or viscoelastic parameters. Two major barriers to this understanding are the accurate description of turbulence itself and the behavior of polymers in static solution. Research into understanding these independent features should lead to a better understanding of the overall phenomenon. Synthetic polymers were the primary source of materials to study these questions but are available in only a limited number of configurations. The polysaccharides from microbial sources represent a structurally diverse class of materials whose structural properties are only moderately well understood. By expanding the set of structures investigated, it is hoped that salient molecular features will be identified.

J. Hoyt has suggested that the key to the mechanism of drag reduction is the ability of a polymer to interfere with the time averaged velocities of fluid flow in the radial directions of pipe flow (*7*). The damping

Table 1

Drag Reducing Coefficients of Selected Yeast Strains and Xanthan Gum

Strain	Carbon source	Fraction	Concentration, wppm 10	25	40–60	100	200	500	1000
Candida boidinii, Y 2332	Glucose	Total			1.1	5.5	5.5[a]		
	Methanol	Total			3.4	4.0			
Cryptococcus laurentii, Y 1401	Glucose	Total					5.7[a]		
		Spoolable			35	41[a] 50			
	Ethanol	Spoolable			23[a] 20	31			
		Nonspoolable			11[a]				
Hansenula capsulata, YB 1842	Glucose	Total			0.5	1.5[a]		3.4[a]	5.6[a]
Lipomyces starkeyii, YB 1380	Ethanol	Total			3.9[a]	6.6[a]			
Rhinocladellia elatior, YB 4163	Glucose	Spoolable	18	36	57	66	70[a]		
Rhodotorula glutinis, Y 2502	Glucose	Total			25[a]				
	Ethanol	Total			28[a] 30	41			
Xanthan gum Sigma Chemical Co.	?	Total	6.0	14	20	20[a] 29			

[a] Glucose equivalent, all others are based on major sugar(s).

effect of flow additives on fluctuations in fluid flow often results in drag reduction. Hoyt has provided several examples of data from his own and other laboratories to corroborate this view (*7–10*). He has proposed that there exist four properties of materials that would then promote drag reduction

1. Sufficient length to attenuate crosswise fluctuations;
2. Sufficient mass to dampen both axial and crosswise fluctuations;
3. Rigidity to absorb fluctuations; and
4. Elasticity to provide a delayed response to fluctuations.

From these proposed contraints/requirements, he reasons that polysaccharides would be likely "optimum drag reducers" if very long and completely linear. A particular advantage of polysaccharides is the larger mass of their monomer units (for example, glucose compared to ethylene). The drag reduction effect is usually attributed to the mechanical properties of flow additives, with the chemical properties being relegated to secondary importance (*7*). A very good drag reducing synthetic polymer, poly(ethyleneoxide) can exhibit aspect ratios (length to "apparent width" ratios) of tens to hundreds of thousands, whereas most polysaccharides have aspect ratios on the order of hundreds. Thus, factors other than chain length and chain "width" must control the mechanical properties necessary to reduce drag. The common polysaccharides derived from microbial species are of relatively low molecular weight, hence their ability to reduce friction appears to result from their mass, rigidity, or elastic properties.

Since we had in hand a set of diverse drag reducing polysaccharides, we desired to evaluate them using some simple chemical/physical characterizations and to correlate aspects of structure and performance. Although this approach has been used before for certain available materials, there has been no systematic evaluation of these parameters. We felt that such an evaluation would permit us to determine if polysaccharides could serve as models for the study of the mechanism of drag reduction. Another factor that encouraged this approach was that published structural information existed for almost all of the polysaccharides that we had in hand. The data included backbone linkage, repeating unit composition, and primary and secondary structure (*11,12*).

In order to determine which molecular properties were important, we used the following approach

1. Examine drag reduction as a function of molecular weight and distribution;
2. Examine the effects of backbone linkage and monomer composition;
3. Examine secondary structures and their perturbation by chaotropic agents and correlate these effects on activity; and

4. Examine the importance of intermolecular interactions by testing drag reduction in controlled mixtures.

We had already noted in earlier work (*5*) that the commonly accepted correlations may have been too simplistic (*7,8*). In one case we found a counterexample to the prevailing notion that great length and linearity in a polymer is sufficient to ensure drag reducing activity. The linear and large polysaccharides from the yeasts *Hansenula capsulata* and *Candida boidinii* are poor drag reducers (*5*). Also, early observations by Hoyt indicated no correlation between the primary linkage of polysaccharides and drag reduction. However, when linkage type is correlated to secondary structure and drag reduction in polysaccharides, the relationships become more clear.

In order to relate any information derived in this study, it is necessary to relate drag reduction to typical viscoelastic measurements and determine any correlations. This has not previously been done and an initial analysis of these relationships was carried out.

An eventual goal of this work is to develop a set of simple rules that permits a simple and quick identification of potential drag reducing polysaccharides, and an estimate of the potential performance of the polysaccharides. We attempted to evaluate our data in relation to the currently accepted models for drag reduction.

MATERIALS AND METHODS

Media and Growth Conditions

YM Broth (YM) and Yeast Nitrogen Media (YNB) were from Difco (Difco, Ann Arbor, MI). For the culture of *R. elatior* the medium suggested by Burton et al. (*13*) was employed substituting yeast extract for yeast autolysate paste and called BNC medium in this report. This organism was cultured as described by Burton et al. (*13*) and Sanford et al. (*14*). Other media were based on the Yeast Carbon Base Medium from Difco and constituted into a standard formulation as described in a previous report and called Yeast Standard Synthetic Medium (YSS) or Yeast Standard Synthetic Medium with Phosphate (YSSP) (*5*). Some of the media contained kanamycin and/or streptomycin to prevent bacterial contamination during the extended times required in culturing.

Stock cultures were made by inoculating 25 mL of YM medium with single colonies of yeast cultures. Subsequent cultures were made by using the stock culture to inoculate test cultures (100–120 mL) that were grown at 25–28°C in 250 mL DeLong shake flasks for 6–8 d in gyratory shaker incubators and mixed at 250–300 rpm. Larger batch scale production of polysaccharide, was accomplished by growing cultures in 500 mL of medium in 1L Erlenmeyer flasks. Growth was monitored by measuring turbidity at 600 nm.

Isolation of Polysaccharides

Whole cell cultures were centrifuged by low speed centrifugation at 1700 xg for 20–30 min in a refrigerated centrifuge to separate cells from the supernatant. The exopolysaccharides in the culture supernatants were isolated by precipitation with alcohol. Three volumes of 95% ethanol or neat isopropanol were added to one volume of culture supernatant and stored at −20°C for 24 h. The resultant precipitates were isolated by centrifugation in 250 mL aliquots at 4080×g for 5 min in a Sorvall GSA rotor (less efficiently flocculating precipitates sometimes required centrifugation at 10,400×g). The pellets were vacuum dried at room temperature overnight (small amounts of precipitate often were dry within 2 h). The dried precipitates were either weighed analytically and taken up in a standard solution for use in rheological measurements, 0.01 *M* NaCl with 0.05% sodium azide, or dissolved directly in the standard solution to provide a 1–5% (w/v) solution. The concentration of polysaccharide was estimated with the phenol/sulfuric acid method for total carbohydrates For some exopolysaccharides, notably those from *R. elatior* and *C. laurentii,* ethanol precipitation gave a filamentous mass that could be mechanically separated by spooling on a glass rod. Sometimes a "nonspoolable" second fraction was isolated as a precipitate after overnight storage at −20°C and collected by low speed centrifugation. This was most evident in *C. laurentii* cultures.

Sugar Analysis

The determination of total hexose sugars employed the phenol/sulfuric acid procedure described by Hofreiter and Hodge. (*15*). Concentrations of homopolysaccharides were determined using a monomer sugar as a standard. The concentration of heteropolysaccharides was determined by analyzing a matrix of mixed sugar concentrations of the two most abundant monomers (for example, glucose and mannose) by the phenol/sulfuric acid method. These values were then analyzed by plotting them as part of a standard surface (SURFER, Golden Software, Inc., Golden, CO) where x coordinate was the total hexose, the y coordinate was the mole fraction of the most abundant sugar and the z coordinate was the absorbance at 490 nm. By knowing the mole fraction of one of the sugars it was possible to estimate the total sugar content in the samples. The values obtained by this approach were considered to be accurate to two significant figures.

Gel Permeation Chromotography

Gel permeation chromotography was carried out in a 1.5 cm by 90 cm glass Econocolumn obtained from BioRad (Richmond, CA) or a similar column from Spectrum with low volume fittings (Fisher, Los Angeles).

The solid matrix was Sephacryl 400 (Sigma) equilibrated in 0.01 *M* morpholinopropanesulfonic acid (MOPS) pH 6.8 and 0.02% sodium azide. The samples containing 1-5 mg of polysaccharide were layered on the column and eluted with the MOPS buffer. Twenty drop fractions (1 mL) were collected using a Gilson Model 201 fraction collector and a 90–100 cm pressure head of buffer. Fractions were assayed by removing a 0.5 mL aliquot from each fraction and measuring total carbohydrate content with the phenol/sulfuric acid method. A standard curve of molecular weights was obtained using pullulan standards (polyglucose with $\alpha 1 \rightarrow 4$ and $\times 1 \rightarrow 6$ linkages in a 2:1 ratio). This curve was used to evaluate the molecular weight distribution in samples of exopolysaccharides.

Rheology

Measurement of Drag Reduction

A turbulent flow rheometer was fabricated at JPL from a design provided by Jack Hoyt, Dept. of Mechanical Engineering, San Diego State University. This particular rheometer operates at a fixed Reynolds number of 16,500 (*8,9*). The principle of operation is the measurement of pressure at two fixed points 10 cm apart along a 1 mm diameter pipe in which the turbulence of the fluid flow is fully developed. Fluid is forced through the pipe at a constant velocity using a 100 mL syringe driven by a linear actuator and motor that is overrated so as to eliminate irregularities due to back pressure. The rheometer gives reproducible results (±1–2%) from run to run and from month to month. The calculation of the drag reduction coefficient was made as follows

$$\%\ \text{Drag Reduction} = \{[(\text{PD Solvent}) - (\text{PD Solution})]/(\text{PD Solvent})\} * 100$$

where PD Solvent and PD Solution are the pressure differences in the solvent and solution, respectively, recorded on a strip chart recorder. The maximum practical drag reduction coefficient that can be measured with this rheometer design is 67–70% (as measured in our laboratory and from information supplied by J. Hoyt). Between samples the rheometer was rinsed with tap water until the baseline returned to normal. Sample size was 100–120 mL into which were diluted aliquots of the stock solutions to give concentrations between 10–1000 weight parts per million (wppm). All measurements were made with water as the solvent. Samples to be analyzed were made up by diluting stock solutions into the rheological buffer. Solutions of standard drag reducing polymers, such as xanthan gum, polyethylene oxide, and polyacrylamide produced drag reduction coefficients that compared well with published results (*9*).

Extensional Flow Viscometry

Measurements were made using an extensional viscometer constructed at Stanford University (*16*). The unit is designed to study low viscosity fluids at high strain rates and can measure the enhancement of extensional viscosity caused by the addition of additives to a standard solvent. Polysaccharides were supplied in concentrated solution form in 0.01 *M* NaCl and 0.05% sodium azide with concentrations based on glucose equivalents of sugars. Measurements were made of the extensional viscosity (a measure of the degree of deformation and/or orientation of a molecule), the extensional velocity gradient, and the shear viscosity (the flow conditions obtained in capillary viscometers) of the solutions. The data were analyzed by comparing the extensional viscosity normalized by the zero shear viscosity of the solution against the extension rate or the normalized (wppm-glucose equivalents) drag coefficient. The former analysis permits the qualitative evaluation of polymer conformation by noting flow characteristics of the solution. Experiments have shown that with this viscometer, it is possible to qualitatively differentiate between the flow behavior of a flexible polymer such as polyacrylamide and a more rigid and rod like polymer such as xanthan gum. A flexible polymer tends to orient more slowly in the flow and, hence, produces a slowly rising, characteristic curve, whereas a rigid polymer quickly aligns with the flow and exhibits a different viscosity ratio curve.

Reagents

The pullulan standards for gel permeation chromotography were obtained from Polysciences (Warrington, PA). Samples of gellan, rhamsan, and welan gum were provided by G. Veeder of Kelco, a Division of Merck & Co., San Diego, CA. All other chemicals and biochemicals were of reagent grade quality and obtained from Fisher (Los Angeles, CA) and Sigma (St. Louis, MO) unless otherwise noted.

RESULTS

Performance Measurements in Relation to Standards

The species of yeast studied are shown in Table 1 along with measurements of the drag reduction coefficients for their partially purified exopolysaccharides at several concentrations. Some concentrations were based on a glucose equivalent concentration of polymer and were not corrected for composition. The use of glucose as a standard is satisfactory for comparative purposes only.

It can be seen that three strains produce a good drag reducing polysaccharide. The polymer from *Rhinocladellia* is an outstanding material that performs on a level approaching poly(ethyleneoxide). Even 10 ppm solutions of this material have good drag reducing properties. Figure 1 shows a comparison of drag reduction for poly(ethyleneoxide), and the *Rhinocladellia* polysaccharide and commercial xanthan gum.

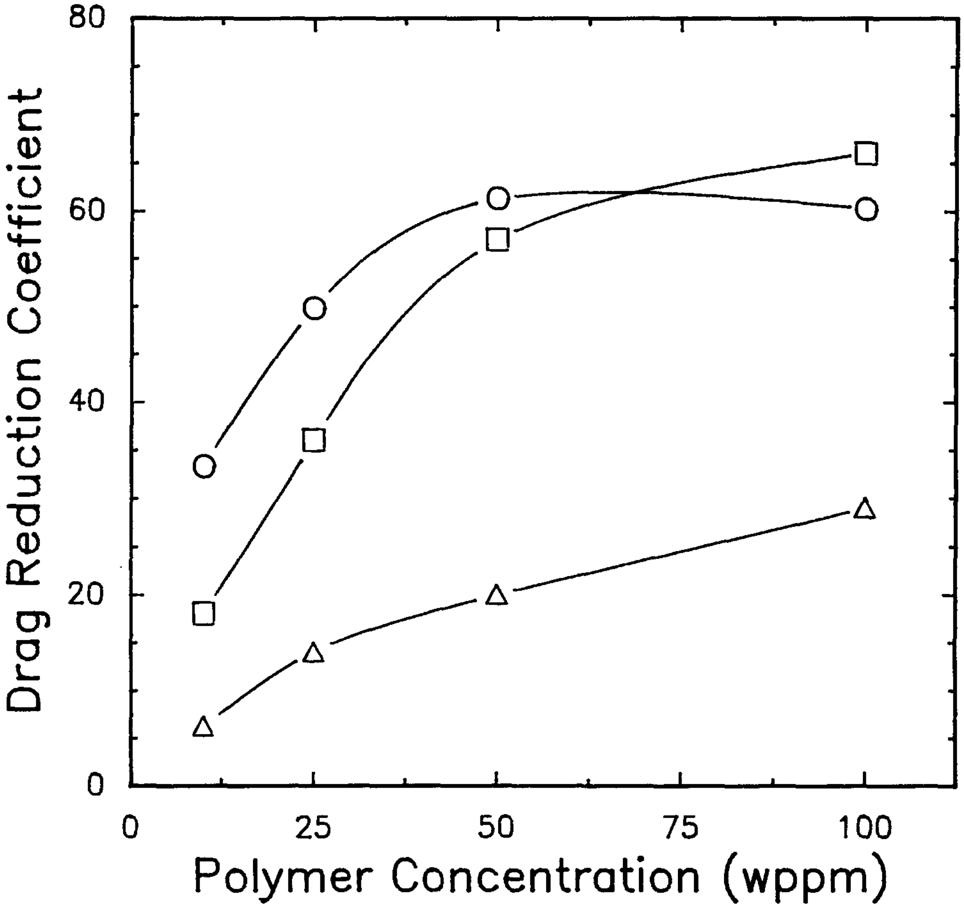

Fig. 1. Comparison of drag reduction activities. ○ Drag reduction coefficients measured for poly(ethyleneoxide); xanthan gum from Sigma △; and, polysaccharide from *R. elatior* □.

A comparison of structural features and drag reduction activities from the literature and our own data are compiled in Table 2. The table lists the various polysaccharides, their linkages, approximate monomer composition, and drag reduction coefficient obtained either directly by us or extracted from the literature. All drag reduction measurements were made on a Hoyt type rheometer. As Hoyt and others have observed, there is little correlation between monomer composition and drag reducing performance (*7,8,10*). However, those monomers that possess ionic character and thus form polyelectrolytes do tend to make good drag reducers, examples of which are hyaluronic acid, xanthan gum, carrageenans, and the exopolysaccharide from *R. elatior*.

Influence of Size on Performance

Results from our earlier work indicated that large size and linearity were not necessarily sufficient to induce drag reducing activity. To further

Table 2
Linkage Data in Microbial and Plant Polysaccharides

Species or source	Backbone linkage(s)	Component sugars	Drag reduction coefficients (wppm)	
C. laurentii	$\alpha 1 \rightarrow 3$	Man,xyl, D-GlcUA	57 (200)	JPL
H. capsulata	$\alpha 1 \rightarrow 6$, $1 \rightarrow 2$	Man	1.5 (200)	JPL
H. holstii	$\alpha 1 \rightarrow 3$, $1 \rightarrow 2$	Man	0 (200)	JPL
L. starkeyi	$\alpha 1 \rightarrow 2$	Gal,Man, D-GlcUA	6.6 (100)	JPL
R. glutinis	$\beta 1 \rightarrow 4$, $1 \rightarrow 3$	Man	41 (97)	JPL
X. campestris (Xanthan gum)	$\beta 1 \rightarrow 4$	Glc & Man	29 (100)	JPL
R. elatior	$\beta 1 \rightarrow 4$ or $\beta 1 \rightarrow 3$	2-acetamido-2-deoxy-GlcUA	66 (100)	JPL
lambda Carrageenan	$\alpha 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$	Gal,Gal-SO_4	19 (100)[a]	Hoyt
Na Carrageenan	$\alpha 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$	Gal,Gal-SO_4	27 (100)[a]	Hoyt
Okra gum	$\alpha 1 \rightarrow 4$	Gal	>25[b]	Hoyt
Rhodomenia palmata "Dulsan extract"	$\beta 1 \rightarrow 3$	Xyl	>25[b]	Hoyt
Hyaluronic acid	$\beta 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$	D-GlcUA and 2-acetamido-2-deoxy-GlcUA	16 (63)[a]	Hoyt
Guar gum	$\beta 1 \rightarrow 4$	Man	54 (100)[a]	Hoyt
Locust bean gum	$\beta 1 \rightarrow 4$	Man	37 (100)[a]	Hoyt
Sodium alginate	$\beta 1 \rightarrow 4$	D-ManUA L-GlcUA	32 (120)[a]	Hoyt

(continued)

Table 2 (Continued)

Species or source	Backbone linkage(s)	Component sugars	Drag reduction coefficients (wppm)	
Hydroxyethyl cellulose	$\beta 1 \rightarrow 4$	D-Glc	>25[b]	Hoyt
Hydroxy propylmethyl cellulose	$\beta 1 \rightarrow 4$	D-Glc	>25[b]	Hoyt
Sodium carboxy methyl cellulose	$\beta 1 \rightarrow 4$	D-Glc	>25[b]	Hoyt
Karaya gum	$\beta 1 \rightarrow 4$	D-GalUA	33 (245)[a]	Hoyt
Deoxyribo-nucleic acid	$3' \rightarrow 5'$	2-deoxy-ribose	66 (200)[a]	Hoyt
Dextran	$\alpha 1 \rightarrow 6$ main chain and $\alpha 1 \rightarrow 2$ side chains	D-Glc	low[a]	Hoyt
Amylose	$\alpha 1 \rightarrow 4$ highly branched	D-Glc	low[a]	Hoyt
Gellan gum	$\beta 1 \rightarrow 4$ & $\beta 1 \rightarrow 3$	Glc, GlcUA, Rha	3 (100)	JPL
Rhamsan gum	Same as Gellan[c]	Glc, GlcUA, Rha	59 (100)	JPL
Welan gum	Same as Gellan[d]	Glc, GlcUA, Rha or Man	0 (100)	JPL

[a] Estimated from figures in Refs. (*7–10*) by Hoyt.

[b] Value given by Hoyt (*8*) for materials considered to have good drag reducing coefficients.

[c] Has one side chain per unit comprised of two glucose monomers.

[d] Has one side chain per unit comprised of one monomer of mannose or rhamnose.

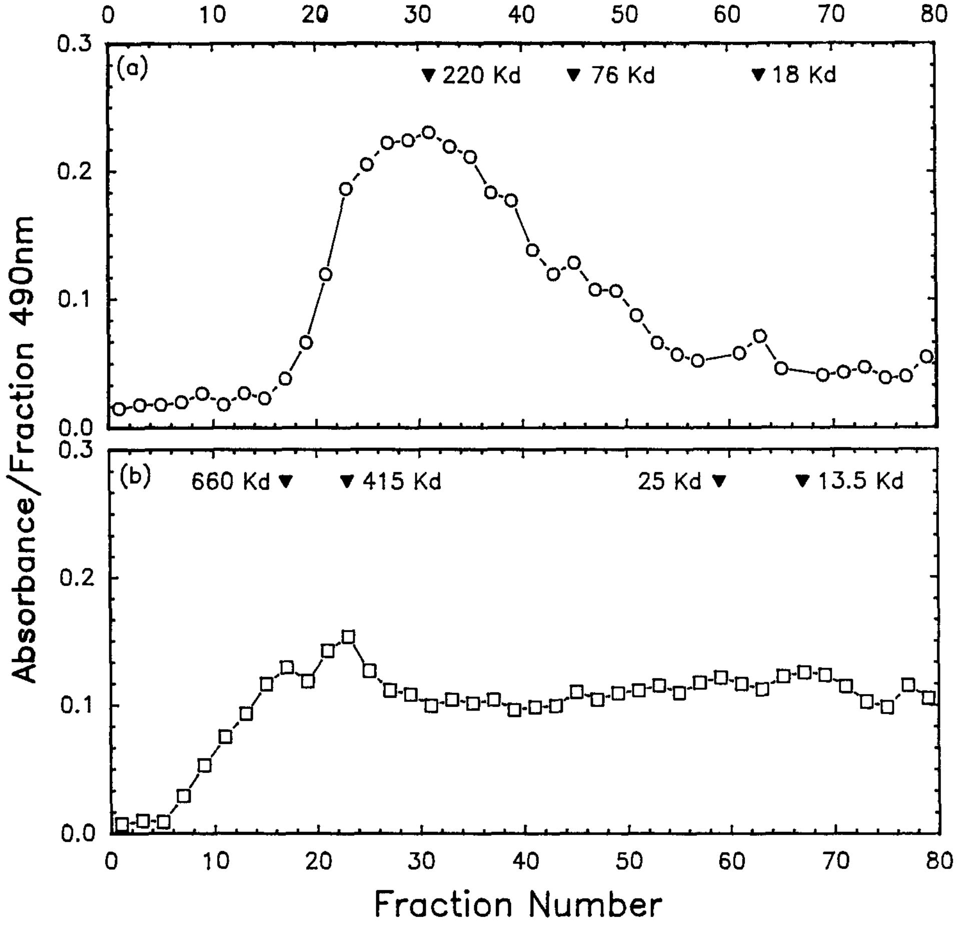

Fig. 2. Molecular weight distributions for xanthan gum and *L. starkeyi* exopolysaccharide. Xanthan gum (a) and *L. starkeyi* polysaccharide (b) were analyzed by gel permeation. The major component peaks are labeled with molecular weight values based on pullulan standards.

assess the role of size, the molecular weight distributions of the exopolysaccharides from *R. glutinis, C. laurentii,* and *L. starkeyi* as well as xanthan gum were obtained. The exopolysaccharides from *H. capsulata* and *C. boidinii* had previously been characterized (5). Figure 2 shows the molecular weight distributions of the polysaccharide from commercial xanthan gum and the ethanol grown yeast *L. starkeyi*. Note that *L. starkeyi* produces polymer(s) that are factors of two to three times larger than xanthan gum but its drag reducing coefficient is significantly lower (Table 1). The large mole fraction of lower molecular weight forms probably reduces the weight normalized drag reducing activity of the exopolysaccharide. The dilution effect of molecular weight components may also apply in the case of the exopolysaccharide from *R. glutinis*.

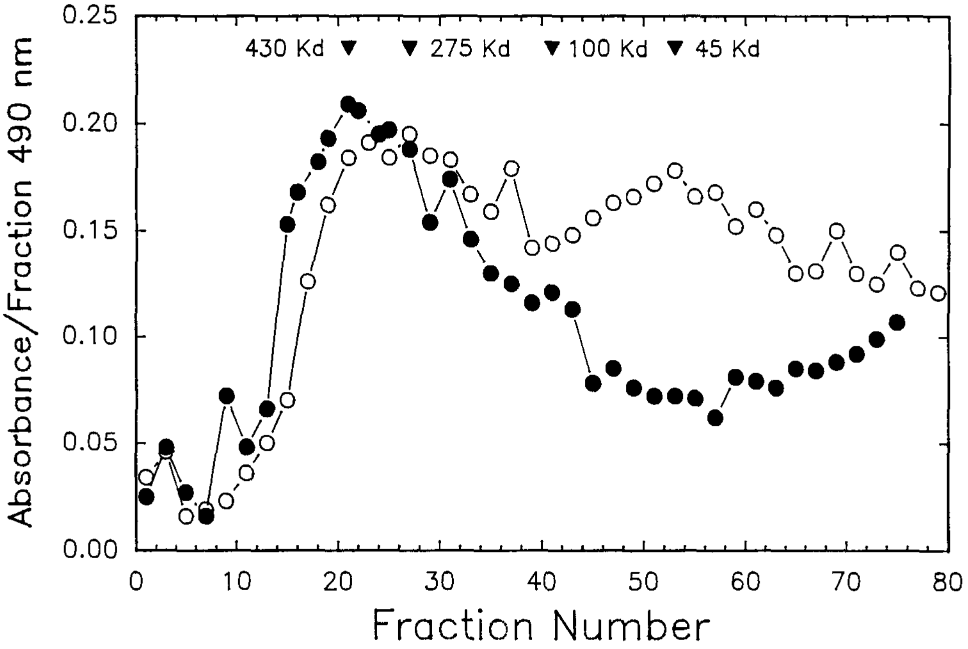

Fig. 3. Molecular weight distribution for *R. glutinis*. The exopolysaccharide from *R. glutinis* cultured on glucose ○ or ethanol ● was analyzed by gel permeation. The major component peaks are labeled with molecular weight values based on pullulan standards.

Figure 3 compares the molecular weight distributions for exopolysaccharide(s) from *R. glutinis* grown in glucose and ethanol. The largest band of material is in the molecular weight range of about 275–430 Kd, which is similar to xanthan gum (but smaller than the polysaccharides from *H. capsulata* and *C. boidinii*). Note that glucose derived material is composed of a larger fraction of low molecular weight components than is the ethanol derived product. It also performs slightly worse than ethanol grown material as a drag reducer (Table 1). Additional insights into the relationships between molecular weight and drag reduction are seen when the exopolysaccharides from *C. laurentii* were examined by gel chromatography.

Figure 4 shows the molecular weight distribution of *C. laurentii* polysaccharides. By using the simple mechanical separation of the alcohol precipitated product, two fractions of the exopolysaccharide produced by this organism could be isolated—the spoolable and nonspoolable fractions. This crude separation provided a convenient opportunity to observe drag reduction as a function of exopolysaccharide fraction. For the spoolable fraction of both glucose and ethanol grown cultures, there exists a similar mole fraction of components at the 400–600 Kd range, with the ethanol grown material producing a larger mole fraction of low mole-

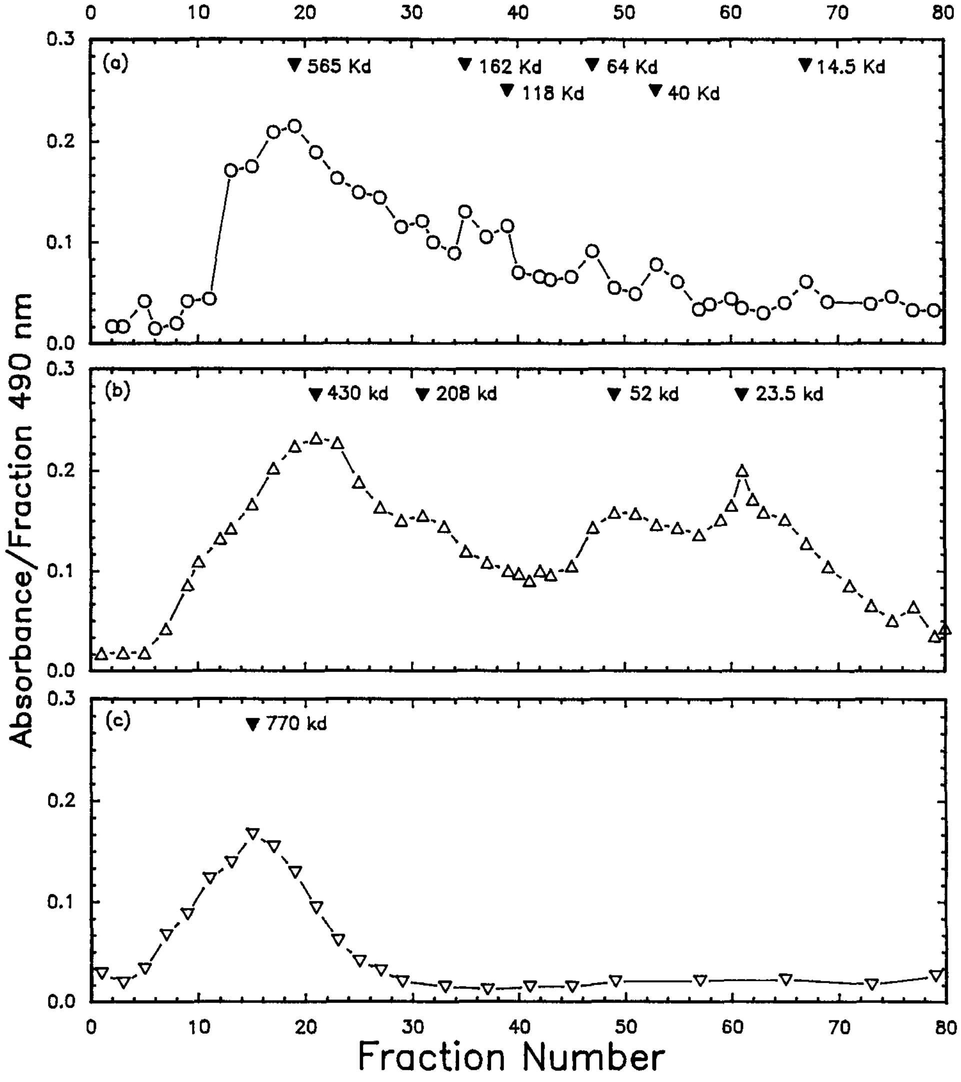

Fig. 4. Molecular weight distributions for the different components of *C. laurentii* exopolysaccharide. The spoolable and nonspoolable fractions of *C. laurentii* exopolysaccharide were analyzed by gel permeation. Glucose grown spoolable component (a); ethanol grown spoolable component (b); and, ethanol grown nonspoolable component (c). The major component peaks are labeled with molecular weight values based on pullulan standards.

cular weight components. For the nonspoolable fraction of the ethanol derived material, a homogeneous fraction of components is centered near 770 Kd. The molecular weight distributions of these individual materials take on more meaning when examined along with the drag reduction coefficient values shown in Table 1.

The better performance of glucose derived exopolysaccharide versus ethanol derived material appears to be a result of the dilution effect of a low molecular weight fraction of poor activity. This is consistent with the data shown in Figs. 2 and 3. However, an interesting result occurs with the performance of the nonspoolable fraction. In this case, a large homogeneous fraction is present, but its drag reducing performance is lower than that expected for a material of such large, homogeneous composition.

The molecular weight distribution for *R. elatior* was not determined. It is a polyelectrolyte and interacts strongly with the gel matrix as it could not be desorbed from the Sephacryl, even with 1.0 *M* NaCl.

The Influence of Backbone Linkage on Performance and Secondary Structure

By examining the information in Table 2, important correlations between linkage type and performance were found. Of the 25 species, those exhibiting strong drag reduction possessed β1→4 linkages (14/25), β1→3 linkages (5/25), and α1→3 linkages (3/25). The other two drag reducers have either α1→2 or 3′→5′ linkages. Other data (not shown) indicate that some α1→4 linkages (the common linkage for material such as glycogen and amylose) exhibit variable drag reducing properties, but most of these materials are highly branched and globular. Hoyt noted similar correlations, but did not relate them to the secondary structures resulting from such linkages. The β1→4 and the α1→3 linkages usually produce a polymer with an extended ribbon conformation (*2,17*), and, as shown here, they also possess high drag reduction activity. Similarly, good drag reduction activity usually occurs when β1→3 and α1→4 linkage types are present and these linkages promote a hollow helix conformation, as do 3′→5′ (DNA) linkages (*17*). However, certain linkages are associatedh with poor drag reduction. Polysaccharides with or β1→6 and α1→2 linkages are of poor quality.

These observations are consistent with the requirements that Hoyt placed on good drag reducers. One obvious property of the 1→6 linkage between sugar monomers is the three degrees of rotation around this bond. This would be expected to lead to a floppy or random coil type conformation that is not rigid. On the other hand, the presence of β1→3 and β1→4 and α1→3 and α1→4 linkages permits only two degrees of freedom around the glycosidic bond and brings neighboring hydroxyl and pyranose oxygen groups within easy hydrogen bonding distances. Hydrogen bonding is known to occur between monomers with such linkages. The steric constraints present in these configurations would promote the rigidity and extended form apparently required for good drag reduction performance.

Using these "rules" as a basis for selecting new drag reducers, a search of the literature was made for an exopolysaccharide known to possess favorable linkages with a predisposition toward hydrogen bonding. The exopolysaccharide from the yeast-like fungus, *Rhinocladellia elatior* Mangenot, possessed either β1→3 or β1→4 linkages of the monomer 2-acetamido-2-deoxy-β-D-glucuronic acid (*18*). Either beta or alpha conformations provide hydrogen bonding opportunities between the adjacent sugars at the pyranose oxygen and the acetamido or the hydroxyl groups. Our predictions were confirmed, since the polysaccharide is an excellent drag reducer (Table 1). Many polyelectrolytes are good drag reducers, indicating possible contributions of ionic character to polymer rigidity.

Influence of Side Chains on Secondary Structure and Performance

One very useful opportunity to examine a single structural feature was provided when the drag reducing activity was measured in the gellan gum series (Table 2). Gellan, welan, and rhamsan gum possess identical backbones with the favored linkages but with different side chain components. Gellan contains no side chains, and rhamsan and wellan possess a monomeric or dimeric side chain per repeating unit, respectively. The drag reduction values are markedly different, indicating that side chains strongly influence performance. The largest side chain in rhamsan gum (di-glucose) dramatically enhanced performance, whereas a monosaccharide side chain did little to enhance performance. It has been reported that an increase in the size of the side chain in this series of polymers increases the "stiffness" (or rigidity) of the polymer (*19*). This data confirms general observations discussed by Hoyt (*7*) that extended side chains can be correlated with drag reduction in more turbulent flow. It may be speculated that the larger side chain extension confers additional rigidity or extended conformation to the structure, or that the side chains permit or prevent some sorts of inter- and/or intra-molecular interactions that promote drag reduction.

Perturbation of Secondary Structure and its Influence on Performance

Chaotropic agents that disrupt hydrogen bonding and other intramolecular interactions were employed to evaluate the possible contribution of these factors to the drag reducing performance of polysaccharides. Samples of ethanol-precipitated polysaccharides were subjected to four conditions: variation in pH (pH 5 and 3), high salt (0.5 and 1.0 *M* NaCl), 6 *M* urea, and heat denaturation at 90°C. These results are shown in Figs. 5 and 6. These values are plotted as the percent drag reduction under the designated conditions vs percent drag reduction under standard conditions. Those values appearing below the diagonal represent a loss of drag reduction performance and those above the diagonal represent an improvement in drag reduction. Because of the limits of the instrument, only values deviating >2–3% should be considered significant. The wppm concentrations for all samples differ and were chosen to provide reasonable drag reduction coefficients with concentrations $\leq$200 wppm.

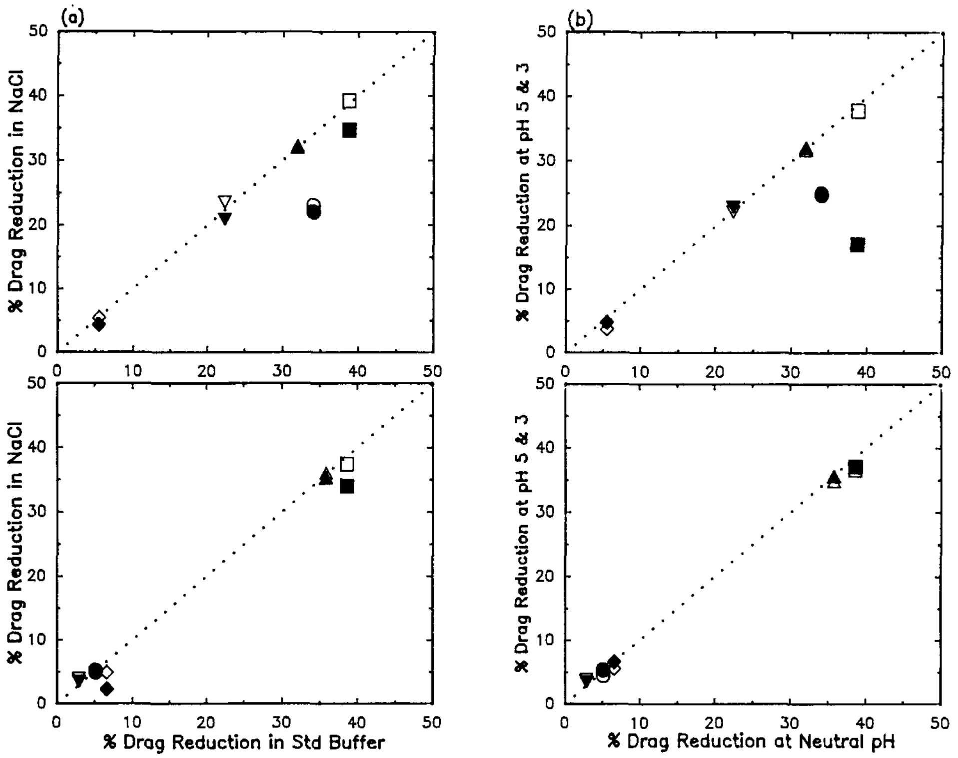

Fig. 5. Effects of high ionic strength and pH on drag reduction activity for exopolysaccharides. The effect on the drag reduction coefficient of polysaccharides analyzed in the rheometer in 0.5 *M* NaCl (open symbols) and 1.0 *M* NaCl (closed symbols) is shown in panel (a). The effect on the drag reduction coefficient of polysaccharides analyzed in the rheometer at pH 5 (open symbols) and 3 (closed symbols) is shown in panel (b). Upper panels of (a) and (b): xanthan gum ○; *C. laurentii* total exopolysaccharide (spoolable and nonspoolable components were not separated) from glucose grown cultures △ and ethanol grown cultures ▽; *R. elatior* exopolysaccharide □; and, *H. capsulata* exopolysaccharide □. Lower panels of (a) and (b): Exopolysaccharides from *C. boidinii* ○; *R. glutinis* glucose grown △ and ethanol grown □; *L. starkeyi* glucose grown ▽ and ethanol grown □.

High ionic strength shown in Fig. 5a does not strongly influence performance for any of the polysaccharides except xanthan gum and the material from *R. elatior.* Only the latter material exhibits any incremental response to increasing ionic strength. The effect of pH is similar to that of salt, with most strains producing material insensitive to pH changes (Fig. 5b). Xanthan gum and *R. elatior* polysaccharide were the most strongly disrupted, with the latter being most sensitive.

The effect of 6 *M* is unusual in that almost all the materials are better drag reducers in this milieu (Fig. 6a). Urea is known to shield hydrophobic

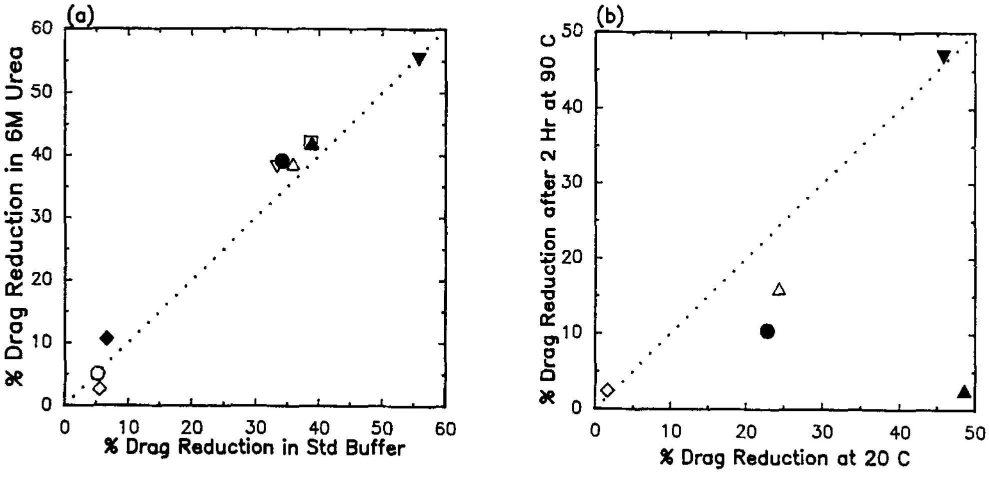

Fig. 6. Effects of 6 *M* urea and heat denaturation on drag reduction activity of exopolysaccharides. The effect on the drag reduction coefficient of polysaccharides analyzed in the rheometer in 6 *M* urea is shown in (a). The effect of heating solutions of selected polysaccharides to 90°C for two h and then rapidly cooling to 4°C is shown in (b). Xanthan gum ●; exopolysaccharides from *C. boidinii* ○; *R. glutinis* glucose △ and ethanol □ grown cultures; *L. starkeyi* ethanol grown culture ■; *C. laurentii* spoolable components of glucose ▼ and ethanol ▽ grown cultures; *R. elatior* ▲; and, *H. capsulata* □.

bonds from the solvent and stabilize structure in proteins. Such behavior has been proposed to occur in solutions of xanthan (*20*). We speculate that urea may act to stabilize a molecular conformation, resulting in improved drag reduction performance. We cannot ignore the possibility that the structure of the turbulence itself in the solvent may be altered, since we know that 6 *M* urea relative to water increases drag.

The effect of heat denaturation is marked for three of the five materials tested (Fig. 6b). The polymers from *C. laurentii* and *H. capsulata* were very stable (the latter material exhibited such a low drag reduction value that this increase may not be significant). The most dramatic effect is found with the material from *R. elatior* where the loss of performance is nearly complete. The heat stability of the polysaccharide from *C. laurentii* is remarkable and is consistent with its performance under other denaturing conditions.

The Effect of Intermolecular Interactions on Drag Reduction Performance

The interactions of different polymers were evaluated by mixing different materials and measuring their drag reduction coefficients. Three different results would be expected (although the latter two are subsets of a result that implies intermolecular interactions). If there were no inter-

Table 3
Interactions of Mixtures of Conformationally Different Polysaccharides[a]

	Xanthan gum	*H. Capsulata*	*C. boidinii*	*C. laurentii* glucose	*C. laurentii* ethanol	*R. elatior*
Xanthan gum	1	1.00	1.06	0.76	0.80	0.80
H capsulata	1.00	1	—	0.97	—	—
C. boidinii	1.06	—	1	1.00	—	—
C. laurentii glucose	0.76	0.97	1.00	1	0.76	0.98
C. laurentii ethanol	0.80	—	—	0.76	1	—
R. elatior	0.80	—	—	0.98	—	1

[a] Observed/Expected value of drag reduction in mixtures of equal wppm. Values below the diagonal are mirror image values for those above the diagonal.

actions and the polysaccharides operate independently of one another, the drag reduction observed ought to be additive. Alternatively, there could be interactions of the polymers. If a positive synergism occurs, it will be manifested in improved drag reduction (greater than the additive value). If negative interactions occur, drag reduction coefficients will lie between the observed values for single polysaccharides and the additive value.

Table 3 shows the data for the measurements of drag reduction resulting from the mixing of selected polysaccharides. The ratio of the observed to expected drag reduction coefficient were calculated for the mixtures examined. Only combinations with xanthan gum and exopolysaccharide from *C. laurentii* (glucose derived) were measured. From this table it can be seen that two of the possible outcomes occur. In five of the nine cases, the drag reduction coefficients were additive, indicating no interaction of the polymers. In each of the other four mixtures, the drag reduction coefficient experimental value was lower than the additive value. For both xanthan gum and *C. laurentii*, the additive values were found with the same two exopolysaccharides (from *H. capsulata* and *C. boidinii*), whereas, for *C. laurentii* another additive result occurred with *R. elatior*. We conclude that independent mechanisms occur and that some sort of negative interactions occur. No positive or synergistic interactions were discovered, but this was a relatively small set of examples that we examined, and additional combinations would need to be analyzed to make any conclusive evaluation of the effects of polymer mixing. The data do show that some effect does occur.

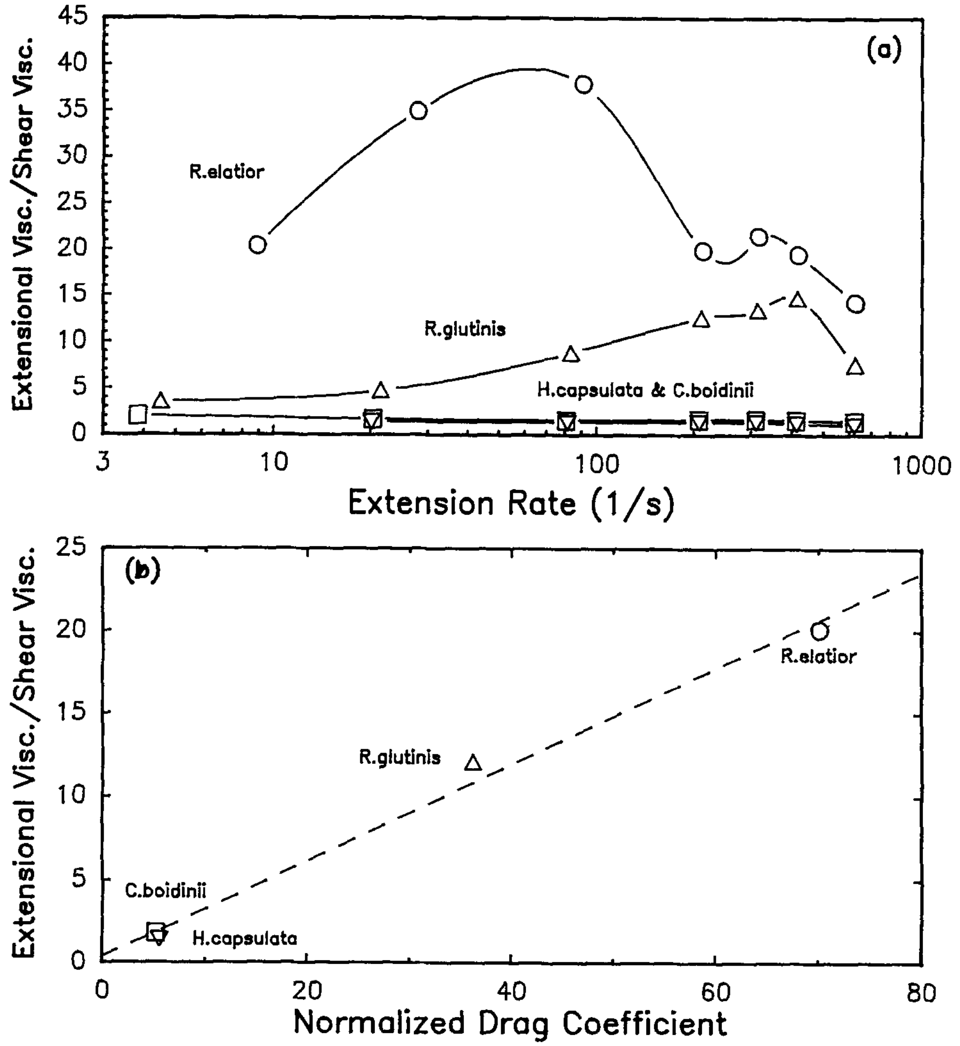

Fig. 7. Extensional viscosity vs extension rate and drag reduction coefficient. Measurements in the extensional flow viscometer of solutions of polysaccharides from *R. elatior* ○, *R. glutinis* △, *C. boidinii* □, and *H. capsulata* ∇ were carried out in a solvent comprised of 90/10 glycerin and water. Panel (a) shows values relative to the extension rate that have been fitted to a cubic spline curve. Panel (b) shows values at an extension rate of about 210 s^{-1} relative to the drag reduction coeffficient measured at 200 ppm (glucose equivalents). A regression curve was drawn through these points which showed a correlation coefficient of 0.95.

Relationships to Viscoelastic Parameters

The analysis of four samples by the extensional viscometer revealed additional information that correlates quite well with the analysis obtained above. Figure 7a shows the extensional viscosity (normalized to the zero shear viscosity of the same solution) vs extension rate (in inverse seconds) for the polysaccharides from *R. elatior, H. capsulata, R. glutinis,* and *C.*

boidinii. The polyelectrolyte polymer from *R. elatior* behaves as a rigid rod in much the same manner as semidilute collagen or xanthan gum (*16*). The neutral polymer from *R. glutinis* shows a response typical of a deformable or more flexible polymer chain. The polysaccharides from *H. capsulata* and *C. boidinii* showed little response, indicating that they were either of low molecular weight, had a globular nature, or the chains were very short and rigid. It was shown that the *Hansenula* and *Candida* polymers were of substantial molecular weight (0.8–1.3 Md, Ref. *5*). It is known, for the *Hansenula* strain, that the polymer does not possess a short, very rigid structural conformation (*12*). The data indicate, therefore, that these two polymers are probably random coils that in solution are difficult to deform (at $R_e = 16{,}500$) and may be collapsed rather than extended structures.

When the ratio of extensional to shear viscosity is compared to the normalized drag reduction values, a linear correlation is observed, as shown in Fig. 7b. This is an important finding, since it relates major viscoelastic parameters to drag reduction activity. Such relationships are particularly important since they have predictive value for identifying drag reducers and since extensional viscosity can be directly related to other molecular, polymeric features.

DISCUSSION

The results of this work confirm and extend the structure/function correlations observed by others who have examined structure/function in drag reduction. Requirements for size, linearity, and rigidity are generally similar. We found, however, that the polysaccharides provide diverse structures suitable for examining the fine molecular structure of drag reducing polymers. For this reason, they appear to be useful tools for examining the rheology of dilute polymer solutions. In addition, correlations between viscoelastic parameters and drag reduction have been made that set constraints for fluid dynamic analyses.

This work represents an initial examination of the mechanical properties of microbial polysaccharides. This proof of application justifies a more intensive examination of the relationships between drag reduction and

1. Discrete molecular weight/size fractions;
2. Pendant group length, composition, and location on the repeating unit;
3. Backbone linkage; and
4. Intramolecular interactions.

The polysaccharides discussed in this work provide a good core sample upon which to build a structure/function model. For such studies we find that these materials are enormously versatile and possess a great deal of "tailorability." These capabilities provide significant advantages over synthetic polymers for mechanistic studies.

We also suggest that the polysaccharide from *C. laurentii* be further evaluated to understand why it produces a material that is so thermally and ionically stable as well as being very resistant to shear degradation. It will be necessary to examine the linkage differences, if any, between the "spoolable" and "nonspoolable" exopolysaccharide, since such differences could explain the differences in drag reducing activity. This polysaccharide appears to be a very valuable commodity material surpassing xanthan gum in utility.

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