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## Viscoelastic Properties of an Exocellular Polysaccharide Produced by a *Lactococcus lactis*

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The viscoelastic properties of a well-characterized exocellular polysaccharide (EPS) produced by the lactic acid bacterium *Lactococcus lactis* subsp. *cremoris* strain B40 were investigated. Dynamic rheological measurements were made as a function of frequency and EPS concentration. The bead–spring model of Rouse could reasonably describe the dynamic properties. Concentrated EPS solutions have a significant elasticity ( $G' > G''$ ) at high frequencies. The relatively high  $G'$  values at high concentrations and high frequencies are indicative of significant normal stress differences, and we put forward a hypothesis that suggests that these normal stresses may explain the contribution of EPSs to the ropy behavior of yogurts.

### Introduction

In situ production of exocellular polysaccharides (EPSs) in yogurt gives rise to a better texture of the product,<sup>1</sup> and therefore the properties of these EPSs are of special interest. The production of EPSs can lead to a ropy consistency.<sup>2–4</sup> Ropiness or sliminess is characterized by a threadlike behavior on pouring the yogurt. Consequently, the bacterial strains that are used as starters for ropy yogurts are called ropy strains.<sup>4</sup> From the surface of a ropy yogurt one can easily pull out a thread of the yogurt and pouring the yogurt shows these threads as well. These phenomena imply that ropy yogurts are able to generate normal stresses upon elongation.

Previously,<sup>5,6</sup> we have analyzed various physical properties of an EPS produced by the lactic acid bacterium *Lactococcus lactis* subsp. *cremoris* NIZO B40. The structure of the repeating unit of this EPS was analyzed by van Casteren et al.<sup>7</sup> and is also reported in ref 5. The repeating unit contains three  $\beta(1\rightarrow4)$ -linked monosaccharides in the backbone; two D-glucose residues and one D-galactose unit. Additionally, the galactose carries two side groups. The C3 of galactose is linked to a phosphoric acid group of which one oxygen atom is bound to the C1 of an  $\alpha$ -D-galactose. Via a (1 $\rightarrow$ 2) bond, the galactose in the backbone also has a covalent bond with an  $\alpha$ -L-rhamnose residue.

It was shown that in a 0.10 M salt solution the EPS behaves as a flexible chain with excluded volume.<sup>5</sup> Both the concentration and the shear-rate dependence of the viscosity of solutions of this EPS have been described theoretically.<sup>6</sup>

As is well-known, concentrated polymer solutions also exhibit elastic properties that are related to normal stresses. To investigate the elastic properties and the relation between elastic and viscous properties, we report the viscoelastic properties of EPS solutions and we show how the simple model of Rouse,<sup>8</sup> based on molecular properties of polymers, gives a reasonable description of the measurements.

### Theory

**Oscillatory Flow.** The viscoelastic properties of a material can be determined by measuring the stress response in an oscillating shear field.<sup>9</sup> In such a dynamic experiment, a periodic sinusoidal strain is imposed on the system and the concomitant shear stress is determined as a function of time. A single experiment allows a determination of both the storage (elasticity) and the loss (viscosity) moduli,  $G'$  and  $G''$ , respectively, as a function of the frequency,  $\omega$ . The ratio  $G''/G'$  is called  $\tan \delta$ , with the loss angle  $\delta$ . From Lodge's constitutive equation,<sup>10</sup> it can be derived that, in the limit of low frequencies,  $G'$  and  $G''$  are related to stationary shear flow by<sup>9</sup>

$$\lim_{\omega \rightarrow 0} \frac{G'}{\omega^2} = \frac{\psi_{1,0}}{2} \quad \lim_{\omega \rightarrow 0} \frac{G''}{\omega} = \eta_0 \quad (1)$$

where  $\eta_0$  is the zero-shear viscosity and  $\psi_{1,0}$  is the first normal stress coefficient, which equals  $N_1/\dot{\gamma}^2$  at low shear rate, with  $N_1$  the first normal stress difference. These expressions underline the relationship of Coleman and Markovitz (see ref 9), which says that steady shear flow and sinusoidal shear deformations can be directly related in the limit of low frequencies. We will check whether the relationship of Coleman and Markovitz applies for our system.

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**Relaxation Times.** To obtain theoretical expressions for the dynamic moduli one needs a molecular model which describes the relaxation of polymer coils upon deformation. We have found that the simple Rouse model, which is straightforwardly related to the molecular properties of polymer molecules, can describe our system reasonably.

In the model of Rouse,<sup>8</sup> a polymer molecule is described as a chain consisting of springs connected by beads. It is assumed that there is only hydrodynamic interaction between the beads and the solvent molecules. The number of springs is usually set equal to the number of Kuhn segments  $N_K$ . The motions of these bead-spring systems can be subdivided into independent relaxation modes. Every mode has a characteristic relaxation time  $\tau_p$ . The longest relaxation time  $\tau_R$ , which is dominant, is given by the first mode ( $p = 1$ )

$$\tau_R = \frac{6(\eta_0 - \eta_s)M}{\pi^2 RTc} \quad (2)$$

where  $c$  equals the polymer concentration,  $\eta_s$  the viscosity of the solvent,  $M$  the molar mass of the polymer molecule,  $R$  the gas constant,  $T$  the temperature, and  $\eta_0$  the zero-shear viscosity. For the EPS of *L. lactis* strain B40, we have shown<sup>6</sup> that the concentration dependence of the zero-shear viscosity can be described with

$$\frac{\eta_0}{\eta_s} = 1 + [\eta]_0 c + \frac{1}{25} ([\eta]_0 c)^{3.5} \quad (3)$$

where  $[\eta]_0$  is the zero-shear intrinsic viscosity.

Higher order relaxation times (for  $p < N_K/5$ ) can be calculated using  $\tau_p = \tau_R/p^2$ . For a good description of the relaxations of a polymer molecule under shear, Fixman<sup>11</sup> showed that it is sufficient to consider the 10 longest relaxation times. From the Rouse model, it then follows for the dynamic moduli

$$G'(\omega) = \frac{cRT}{M} \sum_{p=1}^{N_K} \frac{\omega^2 \tau_p^2}{1 + \omega^2 \tau_p^2} \quad (4)$$

$$G''(\omega) = \omega \eta_s + \frac{cRT}{M} \sum_{p=1}^{N_K} \frac{\omega \tau_p^2}{1 + \omega^2 \tau_p^2} \quad (5)$$

where the relaxation times can be calculated from eq 2 and  $\tau_p = \tau_R/p^2$ .

Zimm<sup>12</sup> extended the Rouse model by adding hydrodynamic interactions between the beads of the polymer. At low concentrations the theory of Zimm gives a good description for polymer solutions with a narrow size distribution.<sup>13</sup> In the entangled region the Rouse theory gives a better description of the experimental data, especially for high molar mass polymers. This is explained by a screening of the hydrodynamic interactions between the beads with increasing concentration and chain length.<sup>13</sup> All theories mentioned were developed for ideal chains but they can be applied to polymers in a good solvent since experimental results for  $G'(\omega)$  and  $G''(\omega)$  hardly depend on the solvent quality.<sup>13</sup>

At very high polymer concentrations and in polymer melts entanglements govern the relaxation behavior of the system. For a highly entangled system, the entanglements can be regarded as topological constraints which only enable snake-like motions (reptations) of a polymer chain in a "tube".<sup>14,15</sup> The model of Doi and Edwards<sup>15</sup> gives a poorer description of our measured data, both qualitatively and quantitatively. The Doi-Edwards theory predicts a different relaxation domain than is observed experimentally. This means that the investigated solution does not satisfy the reptation conditions; the entanglement density is probably not high enough to assume that the polymers only exhibit snakelike motions.

**Relation between the Storage Modulus and Normal Stresses.** The relation between  $G'/\omega^2$  and  $N_1/\dot{\gamma}^2$  as satisfied by eq 1 makes a connection between the elastic component as measured in shear flow,  $G'$ , and the first normal stress difference,  $N_1$ , in static shear flow. Significant first normal stress differences lead to the Weissenberg effect, i.e., rod-climbing behavior of solutions. Later, we will use eq 1 to make a connection between the measured  $G'$  and the elongational properties of the EPS solutions in order to speculate on the relation between sliminess or ropiness and normal stresses, as measured via the storage modulus. By combining eqs 1, 2, and 4 the normal stress difference can be expressed as

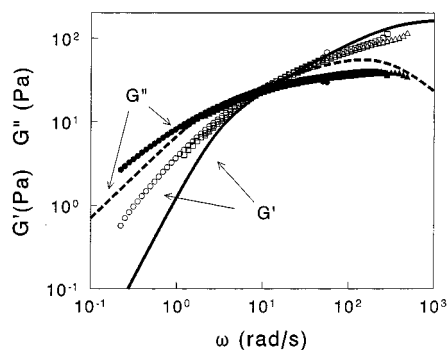
$$N_1 = \dot{\gamma}^2 \frac{36(\eta_0 - \eta_s)^2 M}{\pi^4 RTc} \quad (6)$$

From eq 3 we can see that  $\eta_0$  only depends on the polymer concentration and  $[\eta]_0$ , which in turn depends on the molar mass and the hydrodynamic radius of the polymer.<sup>14</sup> Assuming that the Rouse model holds, the normal stress difference thus only depends on polymer concentration, shear rate and molecular properties of the polymer.

## Materials and Methods

EPS was produced on a pilot-plant scale. A pre-inoculum of 1% of the strain *L. lactis* subsp. *cremoris* NIZO B40 was made and used to inoculate a whey permeate medium. This medium consisted of 350 L of whey permeate, 7 kg of lactose, 7 L (25%) of yeast extract (Bio Springer), and 3 L of a 40% sodium phosphate buffer solution. The EPS production was described previously.<sup>5</sup> After fermentation the culture broth was centrifuged, microfiltered and ultrafiltered. Subsequently, the retentate was diafiltered with deionized water, after which the EPS product was freeze-dried. All EPS solutions were investigated in aqueous solutions containing 0.10 M NaCl.

Dynamic rheological measurements were carried out with a Carri-med rotational rheometer (TA-instruments, type CSL2500 A/G H/R). Temperature control was established by connection with a Julabo F25 cooling/heating bath. We used a double concentric cylinder geometry in order to achieve a maximum torque domain. The frequency domain was 0.1–250 rad/s. The signal-to-noise ratio was too small below 1 Pa for a reliable measurement. The most concentrated EPS solution (10 g/L) was used to detect the linear



**Figure 1.** Dynamic shear loss ( $G''$ , closed symbols) and storage ( $G'$ , open symbols) moduli as a function of the frequency of a 10 g/L EPS solution in 0.10 M NaCl as obtained from time–temperature superposition to 20.0 °C. The different symbols refer to measurements at different temperatures (5 (triangles), 20 (squares), and 50 °C (circles)). The solid and dashed curves are model predictions from the Rouse theory.

region. We found that  $G'$  became nonlinear above  $\gamma = 0.3$ , where  $\gamma$  is the deformation, and  $G''$  lost its linearity with  $\gamma$  above 0.8. Therefore, we chose  $\gamma = 0.1$  during the experiments.

To achieve a broad frequency spectrum we changed the temperature in the range from 5.0 to 50.0 °C and made use of the time–temperature superposition principle proposed by Williams et al.<sup>16</sup> This principle states that one is allowed to shift experimental data horizontally over the log-frequency scale in such a way that they match data for the same system at another temperature. It is assumed that there are no conformational transitions or supramolecular structure formations under the shifting operations.<sup>17</sup> The temperature  $T_0$  to which the data were shifted is called the reference temperature.

## Results and Discussion

**Description of Dynamic Moduli.** For several EPS concentrations we measured  $G'$  and  $G''$  as a function of the frequency  $\omega$ . In Figure 1 we report the results for a concentration of 10.0 g/L (ionic strength 0.10 M) at various temperatures: 5.0, 20.0, and 50.0 °C (they covered the measurements at 10 and 30 °C). By making use of time–temperature superposition, we shifted all curves to that of 20.0 °C. The superposition procedure applies to our system; the data points from the various temperatures follow a single curve. The shapes of the curves are characteristic for concentrated polysaccharide solutions.<sup>17</sup> With increasing concentration both moduli increase. The elastic contribution increases more strongly than the viscous one at higher concentrations. This means that upon making the solution more viscous by adding EPS, one also makes a system more elastic. We now compare the measured curves with theory as presented in the theoretical section.

In Figure 1 the result for the Rouse theory (eqs 2–5) for a polymer concentration of 10 g/L is plotted. The solid curve corresponds to  $G'$  and the dashed curve to  $G''$ . From separate measurements on dilute solutions, we know the intrinsic viscosity  $[\eta]_0$  (3.2 m<sup>3</sup>/kg<sup>6</sup>) and the molar mass ( $1.47 \times 10^3$  kg/mol<sup>5</sup>). We used only the lowest 10 relaxation modes as suggested by Fixman.<sup>11</sup> Above  $\omega = 5$  rad/s the predicted  $G'$

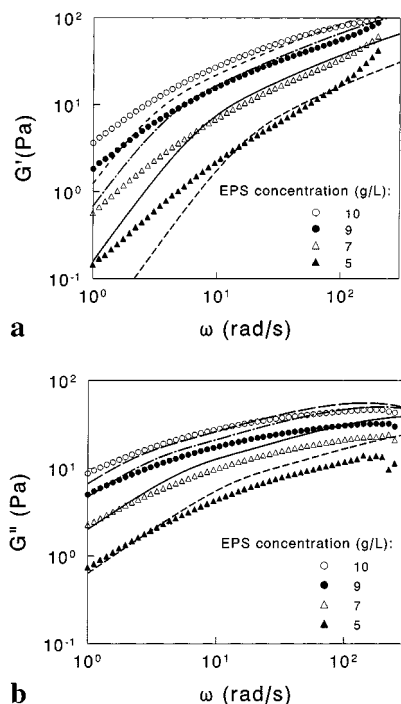
values (solid curve) are in reasonable correspondence with the experimental data; note that there are no adjustable parameters. At lower frequencies the theoretical prediction is poorer and the limiting behavior  $G' \sim \omega^2$  is not yet achieved. This may be due to polydispersity effects. The Rouse theory assumes that the polymers are monodisperse. Kokini et al.<sup>18</sup> showed that polydispersity of polysaccharides shifts the  $G'$  of polysaccharide solutions to higher values at low frequencies. Furthermore, entanglements are not taken into account in the Rouse model. The disentanglement time is longer than the “Rouse time” which would lead to a deviation from the Rouse behavior at low frequencies.

The calculated loss moduli from the Rouse theory (dashed curve) agree reasonably well with the experimental data points; the deviation at low frequencies is only small. At high frequencies ( $\omega > 10^2$ ) the theoretical curve passes through a maximum, which is also found experimentally, albeit at a slightly higher frequency. The position of the maximum in the theoretical value of  $G''$  strongly depends on the number of relaxation modes taken into account; the restrictions to 10 modes may be responsible for the discrepancy between theory and experiment at high frequencies. Furthermore, polydispersity tends to broaden the relaxation spectrum and therefore smooth out the maximum of  $G''$  in the experimental curve.<sup>18</sup> We conclude that our system can be described reasonably by the Rouse model using 10 relaxation modes, without invoking adjustable parameters.

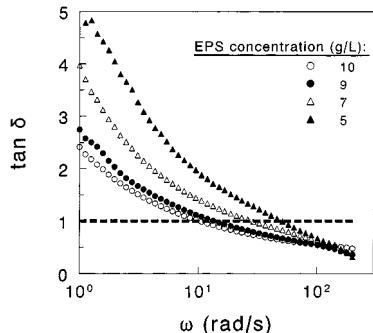
**Concentration Dependence of the Moduli.** We measured the dynamic shear moduli at various concentrations, and some representative measurements at 25 °C are plotted in Figure 2a for  $G'(\omega)$  and in Figure 2b for  $G''(\omega)$ . The curves are again calculated from the theory of Rouse. Except for the low-frequency  $G'$  data, we observe that the theoretical curves also describe the measured data points for the various concentrations in a reasonable way. In particular, the  $G''$  predictions match the experimental data rather accurately.

To compare the elastic and loss moduli as a function of frequency and concentration, we have plotted  $\tan \delta = G''/G'$  as a function of frequency for the four EPS concentrations in Figure 3. The curves clearly show that  $\tan \delta$  strongly decreases with increasing concentration and increasing frequency. Increasing the EPS concentration or decreasing the time scale thus makes the solution more elastic. The point where  $\tan \delta$  becomes smaller than unity marks the onset of the elasticity-dominated regime.

**Relationship between Static and Oscillatory Shear Flow.** The relationship of Coleman and Markovitz states that the  $\eta'(\omega)$  curve from a dynamic shear experiment should be similar to the  $\eta(\dot{\gamma})$  curve from a static shear experiment.<sup>19</sup> In Figure 4,  $\eta'(\omega)$  for  $c = 6.5$  g/L is shown together with the  $\eta(\dot{\gamma})$  flow curve determined earlier.<sup>6</sup> In this figure, we have included the Rouse prediction for  $\eta'(\omega)$  calculated from eqs 2, 3, and 5 with the intrinsic viscosity of 3.2 m<sup>3</sup>/kg as measured previously.<sup>6</sup> The curves are comparable, showing that the behavior of  $\eta'(\omega)$  and  $\eta(\dot{\gamma})$  match quite well. As before, the Rouse model describes the measurements in a reasonable way. The results given in Figure 4 show that the relationship of Coleman and Markovitz applies to the investigated EPS solution.

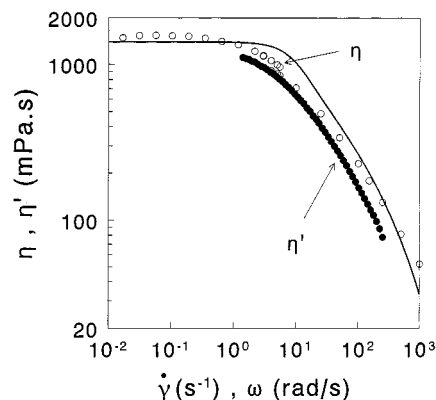


**Figure 2.** (a) Experimentally determined dynamic storage modulus (symbols) of EPS solutions in 0.10 M NaCl at various concentrations as indicated as a function of the frequency. The curves are calculated from the Rouse theory. The measurements were made at  $25.0 \pm 0.5$  °C. (b) Dynamic loss modulus (symbols) of EPS solutions in 0.10 M NaCl at various concentrations as indicated as a function of the frequency. The curves are calculated from the Rouse theory. The measurements were made at  $25.0 \pm 0.5$  °C.

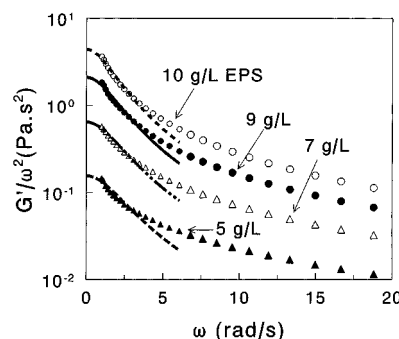


**Figure 3.** Loss tangent ( $\tan \delta$ ) as a function of the frequency of EPS solutions in 0.10 M NaCl at 5, 7, 9, and 10 g/L as indicated. The measurements were made at  $25.0 \pm 0.5$  °C. The dashed horizontal line identifies the point where  $G' = G''$ .

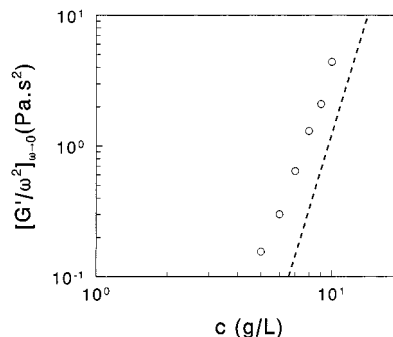
This gives a strong argument in support of the use of eq 6. Experimentally,  $N_1/\dot{\gamma}^2$  can be calculated from  $G'/\omega^2$  by extrapolation to  $\omega \rightarrow 0$ . In Figure 5 we plot  $G'/\omega^2$  as a function of  $\omega$ . Equation 4 shows that at low frequencies  $G'/\omega^2$  should equal  $cRT[\tau_R^2/(1 + \omega^2\tau_R^2)]/M$ ; a fit using this relation was made for the lowest measured frequencies in order to estimate  $[G'/\omega^2]_{\omega=0}$ . The calculated  $[G'/\omega^2]_{\omega=0}$  values are plotted as a function of the concentration in Figure 6. The dashed line represents the relation  $N_1/\dot{\gamma}^2 = cRT\tau_R(c)^2/M$ , which follows from eqs 1 and 4 for  $\omega \rightarrow 0$ . The experimental data seem to agree with the theoretical prediction, although the experimental  $N_1/\dot{\gamma}^2$  values are systematically higher. This is because the measured  $G'$  values in the low-frequency range are smaller than the predicted ones due to polydispersity of the EPS and/or effects of sticky parts on the EPSs.



**Figure 4.** Comparison of the shear-rate dependence of the viscosity<sup>6</sup> with the dynamic viscosity as a function of the frequency of a 6.5 g/L EPS in 0.10 M NaCl solution. Open symbols are data points obtained from static shear measurements and closed symbols were obtained from dynamic rheological measurements. The solid curve represents the Rouse prediction for  $\eta'(\omega)$ .



**Figure 5.** Dynamic storage modulus normalized over the squared frequency as a function of the frequency of EPS solutions at various concentrations as indicated. The curves are best fits to the relation  $G'/\omega^2 = cRT[\tau_R^2/(1 + \omega^2\tau_R^2)]/M$  as explained in the text.



**Figure 6.** Dynamic storage modulus normalized over the squared frequency as a function of the EPS concentration. Open symbols refer to the experimentally determined  $G'/\omega^2$  values from extrapolation as shown in Figure 5. The dashed line is the theoretical prediction from the theory of Rouse.

On the basis of the results presented, we now put forward a hypothesis on a possible explanation for the ropy behavior of EPS-containing yogurts. Ropy behavior is usually tested by (poorly defined) elongation of the yogurt. When the normal stress is large enough to compensate for the weight of the yogurt film, one calls the yogurt ropy. The ropy behavior therefore seems to be related to the magnitude of the normal stresses in the yogurt. We can estimate the normal stresses resulting from the shear flow from our dynamic experiments. For a typical thread length of 1 cm, the stress required to prevent the film from falling equals approximately



100 Pa. For 10 g/L EPS and a typical shear rate of  $10 \text{ s}^{-1}$  the normal stress difference equals  $\sim 100 \text{ Pa}$ , which is thus enough to prevent the yogurt thread from falling. Typical average EPS concentrations in yogurt are on the order of 100 mg/L. However, in yogurt a large fraction of the volume is occupied by casein micelles, thus leaving only a small volume of continuous phase for EPS. As a consequence, EPS is concentrated in the continuous phase of yogurt. It may be questionable whether this effect is sufficient to explain the ropiness. Interactions between EPSs and the casein gel might also result in excess normal stresses, explaining the ropy behavior.

### Conclusions

We characterized the viscoelastic behavior of the EPS produced by the lactic acid bacterium *L. lactis* subsp. *cremoris* NIZO B40. With the help of the Rouse model, which does not contain adjustable parameters, we can reasonably describe the loss and storage moduli. Further the relationship of Coleman and Markovitz applies to our system since the viscous part of the complex viscosity as a function of the frequency compares well with the shear-rate dependence of the viscosity.

With increasing polymer concentration the elastic component strongly increases and dominates the viscous one at high frequencies. It is shown that this agrees with significant normal stress differences. In yogurt, the average concentration of EPS is rather small. One might conclude that normal stresses are thus negligible. However, it should be realized that yogurt contains a large effective volume fraction of casein micelles, which concentrates the EPS. This might be an explanation for the rheological characteristics of "ropy" yogurt.

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