Strain-Stiffening Response in Transient Networks Formed by Reverse Wormlike Micelles

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Strain-stiffening, that is, an increase in material stiffness at large deformations, is a property of many biological materials. Currently, model systems for the study of this phenomenon are elastic networks (gels) of semiflexible filamentous biopolymers such as actin, keratin, or fibrin. Here, we demonstrate strain-stiffening in a class of *viscoelastic* solutions, comprising *reverse wormlike micelles*. These structures are formed by the coassembly of the physiological surfactants, lecithin and bile salt, in an organic solvent, cyclohexane. In contrast to the biopolymer gels, the networks here are transient and are formed by the physical entanglement of relatively flexible worms. Our results suggest that neither a permanent network nor a high filament rigidity is required for strain-stiffening. We suggest a different origin, based on a temporary strain-induced increase in the volume fraction of entangled worms. Our system can also serve as a convenient synthetic model for future studies into this phenomenon.

1. Introduction

Strain-stiffening is a nonlinear rheological response closely associated with biological materials such as blood clots, the cornea, and cytoskeletal networks.[1] The phenomenon refers to an increase in the modulus (stiffness) of a material when it is strained beyond its linear regime of deformations. Such behavior is unusual because most classes of soft matter (polymeric or colloidal) tend to soften monotonically when deformed in shear under nonlinear conditions.[2] A few examples of strain-stiffening in single-phase nonbiological fluids such as associating polymer solutions do exist,[3,4] but the effects in these cases have tended to be quite weak. Currently, model studies on strain-stiffening are almost always conducted with biopolymer gels, such as those of actin, keratin, or fibrin, which are networks of semiflexible filaments.[1,5,6] Indeed, the ubiquity of strain-stiffening in biology has led researchers to speculate if this property may have physiological relevance, since it could prevent biological materials from experiencing high deformations.[1]

Two different theories have recently been offered for strain-stiffening in filamentous networks.[1,7] The first is based on the nonlinear stretching of semiflexible filaments.[1] A filament is considered semiflexible when its persistence length l_p and contour length L_c are comparable.[1] For such filaments, the force required to stretch out the thermal bending fluctuations diverges dramatically at large deformations, which is believed to cause the stiffening behavior.[1] An alternate explanation has also been suggested,[7] which attributes strain-stiffening to a transition from a bending-dominated response to a stretch-dominated response of the filamentous network as strain is increased. Both these theories, however, apply only to *permanent* networks of chains or filaments, with the bending or stretching being associated with chain segments between adjacent cross-link points in such networks.

In this paper, we report the occurrence of strain-stiffening for *viscoelastic solutions* containing reverse wormlike micelles. Reverse worms (also called polymer-like reverse micelles) are long, cylindrical filaments[8] whose physical entanglement results in a *transient* network.[9,10] The term "reverse" refers to the fact that the micelles are self-assembled in nonpolar solvents, unlike "normal" micelles that form in water. Our observation of strain-stiffening in transient self-assembled networks of reverse worms is interesting because these are very different from the "permanent" biopolymer networks in which strain-stiffening is generally studied. Moreover, we will show that the reverse worms studied here have a low persistence length; that is, they are quite *flexible* (at least in bending modes) compared to the rigid or semiflexible biopolymer filaments. In other words, neither a permanent network nor the presence of rigid filaments seems to be a necessary condition for strain-stiffening to occur in complex fluids. An alternate explanation for this phenomenon will be suggested later in this paper.

The reverse worm samples in which we find strain-stiffening are mixtures of the phospholipid, lecithin with a small amount of the bile salt, and sodium deoxycholate (SDC) in a nonpolar organic solvent such as cyclohexane or *n*-decane.[10,11] The lecithin/SDC system represents a new class of reverse worms, which we have recently characterized using rheology and small-angle neutron scattering (SANS).[10,11] For comparison, we also study reverse worms based on the original and widely studied recipe, which is to combine lecithin with a small amount of water in organic solvents.[9] Both water and SDC are believed to induce growth of reverse spherical micelles of lecithin into reverse worms by an identical mechanism, which involves the formation of hydrogen bonds with the headgroups of lecithin.[9,10] However, a significant finding from the present study is that *lecithin/water*

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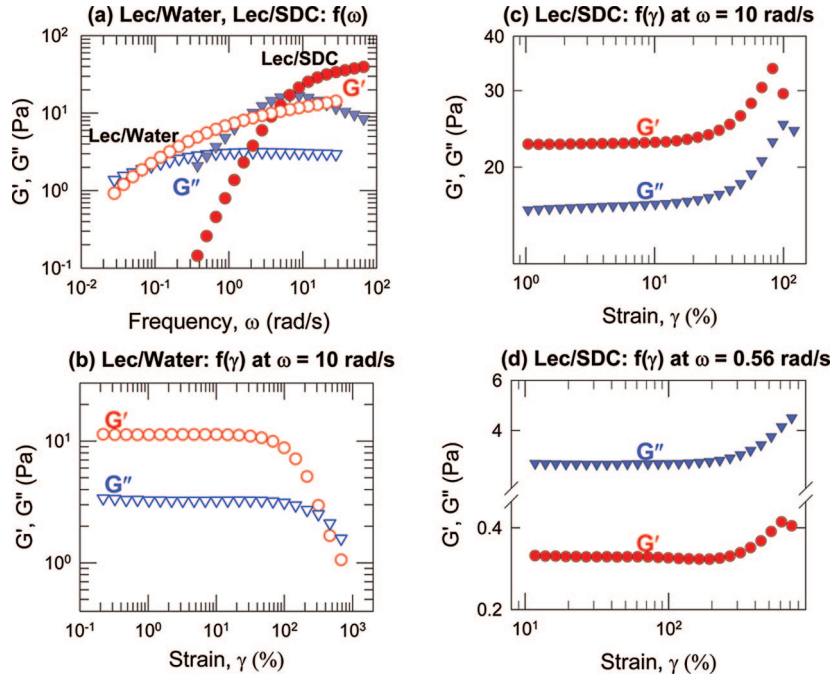

Figure 1. Linear and nonlinear rheology of typical lecithin/water and lecithin/SDC samples in cyclohexane at 25 °C. The former is a mixture of 35 mM lecithin and 315 mM water, while the latter contains 35 mM lecithin and 15 mM SDC. (a) Linear rheology as a function of frequency for both samples (filled symbols: SDC, unfilled symbols: water). (b) Strain sweep at 10 rad/s for the lecithin/water sample showing strain-softening. (c) Strain sweeps for the lecithin/SDC sample at 10 rad/s and (d) at 0.56 rad/s, with both showing strain-stiffening.

reverse worm samples do not exhibit strain-stiffening, while lecithin/SDC ones do. Possible reasons for these differences will be discussed later in the paper.

2. Experimental Section

Materials. The bile salt, SDC (97%), and the solvent, cylcohexane, were purchased from Sigma-Aldrich, while the zwitterionic lipid, soybean lecithin (95%), was purchased from Avanti Polar Lipids, Inc. Deuterated cyclohexane (99.5% D) for the SANS experiments was obtained from Cambridge Isotopes.

Sample Preparation. Lecithin/water reverse worms in cyclohexane were prepared by adding the organic solvent into dry lecithin (dried in a vacuum oven at room temperature for 48 h), followed by stirring until the lecithin was completely dissolved. Deionized water was then added to the lecithin solutions, followed by heating and stirring until the sample became homogeneous.

Lecithin/SDC reverse worms in cyclohexane were prepared by the procedure described in our earlier papers.[10,11] First, stock solutions of lecithin and SDC were made in methanol, and then samples of desired composition were prepared by mixing these stock solutions. Methanol was removed by drying the samples in a vacuum oven at room temperature for 48 h. The final samples with desired concentrations were obtained by adding cyclohexane, followed by stirring until the solutions became transparent and homogeneous. The above procedure ensured the removal of any residual water from the sample and thereby facilitated reproducibile sample preparation.

Rheology. Rheological experiments were conducted at 25 °C on a Rheometrics RDA-III strain-controlled rheometer using a couette geometry with a solvent trap to prevent evaporation of cyclohexane. The experiments were also reproduced on an AR2000 stress-controlled rheometer (TA Instruments) using either parallel-plate or couette geometries. The key experiments in this work are nonlinear rheological experiments, and these are analyzed in the conventional manner via the rheometer software. Alternate approaches in terms of Lissajous curves have been applied by some researchers.[12]

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Small-Angle Neutron Scattering (SANS). SANS measurements were made on the NG-7 (30 m) beamline at NIST in Gaithersburg, MD. Three sample−detector distances were used to probe a wide range of wave vectors from 0.004 to 0.4 Å^{-1}. Samples were studied in 2 mm quartz cells at 25 °C. The scattering spectra were corrected and placed on an absolute scale using calibration standards provided by NIST. The data are shown for the radially averaged intensity I versus the wave vector $q = (4\pi/\lambda)\sin(\theta/2)$, where λ is the wavelength of incident neutrons and θ is the scattering angle.

3. Results and Discussion

Figure 1 compares the linear and nonlinear rheological behavior of lecithin/water and lecithin/SDC reverse worms under oscillatory shear. The samples being compared have the same concentration of lecithin (35 mM) and are combined with either 315 mM water or 15 mM SDC. Figure 1a shows their linear response, that is, their elastic (G') and viscous (G'') moduli as functions of the frequency ω at a low strain amplitude (ca. 10%) within their respective linear viscoelastic regimes. Both samples show the expected viscoelastic response,[9,10] with a predominance of G' at high frequencies and G'' at low frequencies. Note that the plateau modulus (value of G' at high frequencies) is higher for the lecithin/SDC sample (ca. 40 Pa) over the lecithin/water sample (ca. 15 Pa). Mesh sizes for the transient networks estimated from these values are 47 and 65 nm, respectively.[8] We will return to this point later in the paper.

Next, we turn to the key data in Figure 1, which are the strain-sweeps, that is, plots of G' and G'' as functions of the strain-amplitude γ (in %) at a constant frequency. Lecithin/water samples exhibit a *strain-softening* response at high strains, as typified by Figure 1b: here, both G' and G'' decrease at high γ (above 30%) relative to their values in the linear regime at low γ. Similar strain-softening is observed for all lecithin/water reverse worms irrespective of the imposed frequency. In contrast, lecithin/SDC reverse worms exhibit *strain-stiffening*; that is, their moduli increase over a range of strains. The strain sweep at 10 rad/s is shown in Figure 1c and at 0.56 rad/s in Figure 1d. Note from

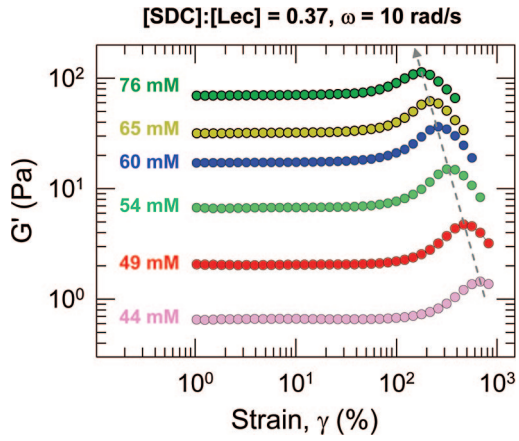

Figure 2. Strain sweeps at 25 °C for lecithin/SDC/cyclohexane samples over a range of lecithin concentrations (shown on the plot), with the molar ratio of [SDC]/[lecithin] fixed at 0.37. All samples show strain-stiffening.

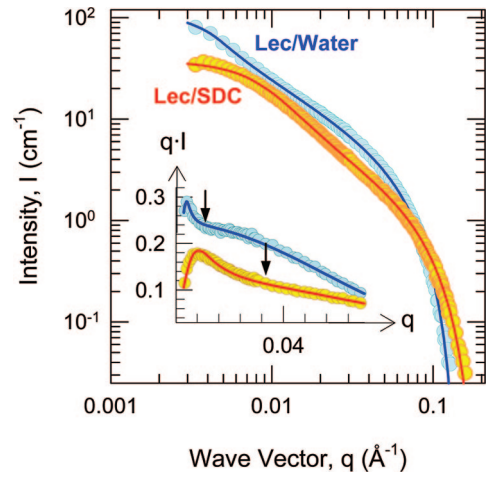

Figure 3. SANS data at 25 °C for lecithin/water and lecithin/SDC reverse worms in deuterated cyclohexane. The solid lines are fits to a flexible cylinder model. The inset shows the same data in a plot of (Iq) vs q.

the linear response of this sample in Figure 1a that there is a crossover of G' and G'' around 7 rad/s. Thus, at 10 rad/s, the behavior is elastic ($G' \gg G''$), reflecting the unrelaxed reverse worm network, whereas at 0.56 rad/s the reverse worms have relaxed and the behavior is viscous ($G'' \gg G'$). Interestingly, however, strain-stiffening is seen at both the above frequencies. The increase in moduli is more pronounced at 10 rad/s: in this case, G' and G'' are constant until $\gamma \approx 20\%$, whereupon they show a sharp increase up to a maximum. The highest value reached by G', that is, G_{max}, is about 50% higher than its linear value G_0. At the lower frequency of 0.56 rad/s, the onset of strain-stiffening occurs at a higher γ (~300%).

From our studies of numerous lecithin/SDC reverse worm samples, we have found that strain-stiffening is ubiquitous for these samples, regardless of the experimental conditions. To verify that the stiffening is real and not an artifact, we have reproduced the same behavior on different rheometer geometries as well as on a stress-controlled rheometer. A few systematic trends in the strain-stiffening response are evident from our data. As suggested by Figure 1c and d, the critical strain at the onset of stiffening γ_{crit} is a monotonically decreasing function of frequency. Also, the extent of stiffening, that is, the ratio G_{max}/G_0, weakly increases with increasing frequency.

We have also studied variations in the stiffening response as a function of the total amphiphile (lecithin + SDC) concentration. Results for G' versus γ (at $\omega = 10$ rad/s) are shown in Figure 2 for samples at different lecithin concentrations and a constant molar ratio of SDC/lecithin of 0.37. All samples show strain-stiffening, and the data clearly exhibit three regimes: first, G' is constant at low γ; next, it increases to a maximum over a range of γ; and finally it decreases. The onset strain γ_{crit} and the strain γ_{max} at which G' shows a maximum both shift to lower values with increasing concentration. Also, the stiffening ratio G_{max}/G_0 slightly decreases with increasing concentration. These trends are consistent with those reported previously for biopolymer gels.[1,5,6] The extent of strain-stiffening in the present system is comparable to that reported by Storm et al.[1] for F-actin but less than those for vimentin or collagen.

Why does strain-stiffening occur for lecithin/SDC reverse worms and not for lecithin/water reverse worms? If this phenomenon is indeed associated with stiff filaments as suggested by theory, one might expect the SDC-based worms to be stiffer (i.e., have higher persistence lengths l_p) than the water-based ones. To test this hypothesis, we have used SANS to extract l_p for the two types of reverse worms. Figure 3 shows typical SANS data (I vs q) for reverse worms based on 10 mM lecithin in deuterated cyclohexane with 90 mM water or 4 mM SDC (we used a low lecithin concentration to minimize structure factor effects). To analyze the SANS data, we used a flexible cylinder model, detailed by Magid et al.[13] The corresponding fits are shown as solid lines through the data. From the fits, we obtain a shorter l_p of 91 Å for SDC-induced worms compared with 378 Å for water-induced worms. This suggests that the SDC-based worms are the more flexible structures. To corroborate this result, we also replot the $I(q)$ data in a plot of $qI(q)$ versus q (called a Holtzer or bending rod plot) that is shown as the inset of Figure 3. Here, each curve goes through a maximum at low q and then exhibits a plateau (indicating the $I \sim q^{-1}$ scaling that is typical of cylinders[13]). The onset of the plateau region is expected to occur at $ql_p \sim 1.9$ for semiflexible chains.[13,14] Clearly, the transition to the plateau is broader and shifted to higher q for the SDC-based worms, indicating that they have a lower value of l_p.

The above results and analysis imply that neither a permanent network nor rigid filaments are necessary for strain-stiffening. How then can we account for this unusual phenomenon? One suggestive point that we had observed earlier in Figure 1a is that the plateau modulus G_p was much higher for the lecithin/SDC sample over the lecithin/water one at the same lecithin concentration. In fact, this difference is seen over the entire range of lecithin concentrations. Also, the power-law exponent for the variation of G_p with volume fraction is higher for lecithin/SDC worms over lecithin/water worms (3.9 compared to 2.3; data not shown).[10] Generally, a higher G_p implies that the filaments in the network are more rigid: for example, actin solutions have much higher G_p values than polystyrene solutions at the same concentration.[15] However, a higher rigidity of lecithin/SDC filaments would be inconsistent with our SANS data. While this aspect needs resolution, the G_p data show that lecithin/SDC *networks* are stiffer than those of lecithin/water. It is therefore plausible that the previous theories for strain-stiffening might still apply to the transient, but stiff, networks of lecithin/SDC worms.

We also wish to discuss an alternate mechanism for strain-stiffening, and this is one in which filament stiffness does not

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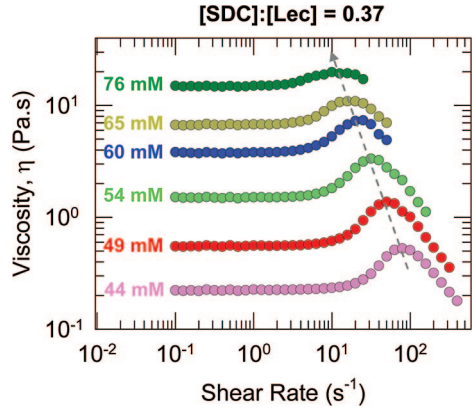

Figure 4. Steady-shear data (viscosity vs shear rate) at 25 °C for lecithin/SDC samples in cyclohexane over a range of lecithin concentrations, with a fixed [SDC]/[lecithin] molar ratio of 0.37. The samples all show shear-thickening.

play a central role. Strain-stiffening, under this mechanism, would be attributed to a strain-induced clustering of worms, or equivalently an increase in the volume fraction of entangled worms. This idea is not new; similar ideas have been proposed before to explain shear-thickening (i.e., an increase in viscosity η at high shear rates) in solutions of associating polymers as well as in dilute solutions of aqueous worms.[16–19] It is interesting that, for associating polymer solutions, strain-stiffening in dynamic rheology and shear-thickening in steady-shear rheology generally occur hand in hand. In this context, it is significant that the lecithin/SDC reverse worm samples that display strain-stiffening are also found to exhibit shear-thickening in steady shear, as shown by Figure 4. Note from this figure that, for each sample, the viscosity increases over a range of shear rates (in some cases, by as much as a factor of 3). The onset of the viscosity increase and the location of the viscosity maximum both shift to lower shear rates as the lecithin concentration increases, similar to the trend in Figure 2. We must emphasize that these lecithin/SDC samples are semidilute solutions, with the worms entanlged into networks (as can be judged from the linear rheology in Figure 1a). Shear-thickening is very unusual for such cases: typically, this phenomenon occurs for worms only in the dilute, unentangled regime.[20] Also, importantly, entangled reverse worms of lecithin/water do not show shear-thickening (data not shown); instead, their viscosities steadily decrease at high shear rates beyond their Newtonian plateau regions.

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The occurrence of both strain-stiffening and shear-thickening for lecithin/SDC networks suggests a common mechanism for the two phenomena, a likely candidate for which would be the shear/strain-induced clustering of micelles. It is known that a population of worms is always highly polydisperse, with an exponential distribution of filament lengths. Thus, some worms would be too short to fully entangle with the rest of the chains in the transient network. We speculate that, at high strains, these "free" worms become incorporated into the network, leading to the increase in moduli (i.e., strain-stiffening). The free worms may connect either with other free worms and thus increase their overall length substantially, or they might connect with worms that are already part of the entangled network.[16–19] In either case, the density of physical entanglements in the network will be enhanced at high strains.

How can this second mechanism explain the differences between lecithin/SDC and lecithin/water samples? Note that high strains not only can connect free worms but also can act to disentangle them from the network. If the dominant effect is to break up network connections, we speculate that strain-softening would occur, which is presumably the case for lecithin/water worms. On the other hand, if the balance is tilted in favor of induced clustering of free worms, the result would be strain-stiffening (and likewise, shear-thickening). The latter may predominate in lecithin/SDC samples, perhaps because a higher fraction of free worms exist in these samples. Alternately, the higher stiffness of the lecithin/SDC network may help to ensure that existing connections are maintained and thereby help tilt the balance toward strain-stiffening.

4. Conclusions

In conclusion, we have described the unusual nonlinear rheology exhibited by viscoelastic networks of lecithin/SDC reverse worms. The samples show strain-stiffening (increase in G' and G'' with strain) in dynamic rheology as well as shear-thickening (increase in η with shear rate) in steady-shear rheology. We propose that these phenomena are caused by increases in connectivity of the micelles at high strain amplitudes or shear rates. Our studies also suggest that strain-stiffening is not unique to biopolymer networks but may be exhibited by a variety of self-assembled molecular networks. Since lecithin/SDC samples can be easily prepared from widely available and inexpensive precursors, they may serve as a convenient model system for future studies into this unusual phenomenon.

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