Microrheometry underestimates the values of the viscoelastic moduli in measurements on *F*-actin solutions compared to macrorheometry

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(Received 31 August 1999; revised manuscript received 28 January 2000)

We present a systematic comparison of microrheological and macrorheological measurements of the viscoelastic storage and loss moduli, $G'(f)$ and $G''(f)$, respectively, of solutions of the semiflexible biopolymer *F*-actin. Using magnetic tweezers microrheometry and rotating disk macrorheometry, we show that microscopic values for $G'(f)$ and $G''(f)$ are significantly smaller than macroscopic results over the frequency range $f=0.004$–4 Hz, whereas the qualitative shape of the spectra is similar. These findings confirm recent theoretical predictions [A. C. Maggs, Phys. Rev. E **57**, 2091 (1998)]. The discrepancy affects not only absolute values of $G'(f)$ and $G''(f)$: although microscopic and macroscopic plateau regime are found in the same frequency range, the two methods yield different values for the entanglement time which determines the high-frequency end of the plateau. By investigating *F*-actin solutions of different mean filament lengths, we show that microscopic and macroscopic $G'(f)$ and $G''(f)$ converge, if the probe particle used in microrheometry becomes large compared to the length of actin filaments.

PACS number(s): 87.16.Ka, 83.85.Cg, 07.10.Pz

I. INTRODUCTION

Microrheometric techniques have become a major tool for investigating mechanical properties of biological material both *in vitro* and *in vivo*, e.g., solutions of filamentous actin (so-called *F*-actin; see below) and living cells [2,3], respectively. All techniques which are used presently are based on embedding colloidal spherical particles (the typical diameter is in the range of a few microns) as probes within the material. Viscoelastic storage and loss moduli, $G'(f)$ and $G''(f)$, respectively, can either be determined from the Brownian motion of the particles [4] or from the motion of the particles in response to an oscillatory external force [5].

Microrheometric techniques have recently been applied especially for investigating solutions of *F*-actin [6], which is considered to be a paradigm of the class of semiflexible filaments (cf. Refs. [7,8] for reviews). Networks of *F*-actin can be characterized by distinct length scales: the filament diameter and mean contour length, d and l, respectively; the persistence length, l_p; and the mesh size of the network, ξ. The diameter is 7 nm [9], ξ is of the order of 0.5 μm (see below), l_p is about 17 μm [10], and $l \approx 20$ μm [11], so that $d \ll \xi < l_p \sim l$. These unique features, and the fact that significant progress has recently been made in the development of theoretical models for the description of the viscoelastic behavior of solutions of semiflexible filaments [12–14], are reasons for the still growing interest in *F*-actin among physicists. Detailed knowledge of the viscoelastic properties of *F*-actin *in vitro* is also a prerequisite for understanding more complex natural actin networks.

Although significant discrepancies between results from the microscopic and standard macrorheometric techniques, like oscillating disk rheometry (e.g., Refs. [15,16]), have been predicted on the basis of theoretical considerations, especially for the case of *F*-actin [1], to our knowledge no attempts have yet been made to compare results from microrheometry and macrorheometry directly. This is an essential problem in view of the fact that some of the theoretical concepts mentioned before are adapted to calculations of absolute values of $G'(f)$ and $G''(f)$ in homogeneous shear fields as applied in macroscopic torsional experiments. An extension of theoretical concepts to experimental data obtained by microrheometry, which is associated with a different kind of (possibly inhomogeneous) shear fields, is only possible if potential differences between the two techniques are understood. This is of great practical importance, since microrheological techniques, like magnetic tweezers, become a standard tool in cell micro-mechanics.

In the present work, we compare viscoelastic impedance spectra of identical solutions of *F*-actin measured by microrheometry and macrorheometry. To test the influence of mesh size and filament length, we varied the actin concentration as well as the mean filament length.

II. MATERIALS AND METHODS

A. Microscopic magnetic tweezers rheometry

The physical principle and instrumental details of the magnetic tweezers rheometer (MTR) used in the present experiments have been described in a preceding publication [17]. In brief, spherical superparamagnetic beads with a diameter of $D=4.5$ μm (Dynabeads M-450, Deutsche Dynal, Hamburg) are embedded within the network. Beads can be manipulated by an external magnetic field (hence the name magnetic tweezers). The movement of a bead in response to the resulting force is monitored by videomicroscopy and image processing.

The magnetic force is controlled by the voltage signal of a function generator. For this purpose, the signal from the function generator is transformed into a defined voltage-proportional current which drives the magnet coils of the

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instrument. The details of the image-processing technique have been described elsewhere [2]. Furthermore, the voltage signal is simultaneously fed to a light-emitting diode (LED); the image of the LED is recorded on videotape together with the image of a probe particle embedded in the *F*-actin solution. Since the light intensity of the LED is proportional to the original voltage signal from the function generator, the force acting on the probe can always be determined from the brightness of the LED. In consequence, the phase shift between the force and movement of the bead is continuously monitored. By applying a sinusoidal force of frequency *f*, the viscoelastic storage and loss moduli, $G'(f)$ and $G''(f)$, respectively, can be determined from the phase shift between the oscillatory force and the response of the probe (cf. Ref. [5]) as a function of the force acting on the probe. The force was determined in a separate calibration experiment [18].

To ensure that linear response is probed in MTR measurements, experiments of the following type were carried out: Using one distinct probe particle, spectra of $G'(f)$ and $G''(f)$ of an *F*-actin solution were determined with force amplitudes of 2 and 8 pN, respectively (data not shown). No indication was found that the results depend on the amplitude of force. We assume that our results are not affected by nonlinear behavior of the *F*-actin solutions, since the results presented below hold for forces below 8 pN. Since observation of a single probe enables one to determine viscoelastic properties on a local scale of a few micrometers, the MTR can be regarded as a ''microrheometric'' method.

B. Macroscopic rotating disk rheometry

The instrumental details of the magnetically driven rotating disk rheometer have also been published prior to this work [15,19]. Care was taken to keep the angular strain below 1% to probe linear response. At the upper bound of the frequency range around 3 Hz (the precise value depends on actin concentration), the results from the instrument are no longer reliable for very soft *F*-actin solutions: At lower actin concentration the frequency range is limited by instrumental inertia effects, because the mechanical response of the system (consisting of polymer solution plus disk) is dominated by an instrumental contribution. Results obtained with the rotating disk rheometer are only shown for frequencies where these instrumental effects could be neglected.

The sample volume for these experiments is 0.4 ml which is large compared to the volume probed locally by the MTR. Therefore, measurements which were performed with the rotating disk rheometer will be referred to as ''macrorheometric.''

C. Microrheometry and macrorheometry of a Newtonian fluid

To demonstrate that microrheometry and macrorheometry yield the same results for the case of a simple fluid, a Newtonian fluid (mixture of 80 wt % glycerol and 20 wt % water) was investigated first as a control. The result is shown in Fig. 1. Although microrheometer and macrorheometer were calibrated independently of each other, the agreement of both methods is excellent over the whole frequency range. As expected for a purely viscous fluid, the loss modulus obeys a power law $G''(f) \propto f^1$. The agreement of both methods ensures that any possible differences between microscopic and macroscopic results on the more complex *F*-actin solutions do not arise from instrumental artifacts (e.g., inaccurate calibrations of both instruments). Furthermore, it can be assumed that a ''macroscopic limit'' of microrheometry is indicated by an equality of microscopic and macroscopic loss moduli. Therefore, besides comparing microscopic and macroscopic results for $G'(f)$, we will in particular focus on a comparison of results for $G''(f)$ in what follows.

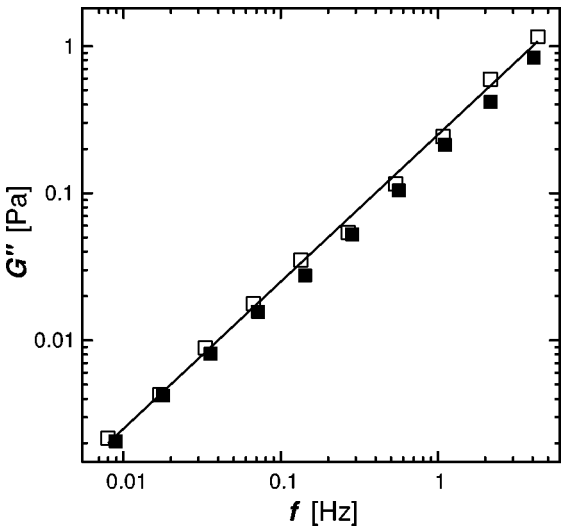

FIG. 1. Comparison of loss modulus $G''(f)$ as a function of frequency from microrheometry (open squares) and macrorheometry (filled squares) for the case of a Newtonian (i.e., purely viscous) liquid (mixture of 80 wt % glycerol and 20 wt % water). The results from both methods are in excellent agreement. The drawn line has slope 1, i.e., it represents a linear dependence of $G''(f)$ on frequency as it is expected for a viscous fluid.

D. Proteins

Actin. Monomeric (globular) actin (so-called *G*-actin, $M_W = 42$ kDa) was prepared from rabbit skeletal muscle following the method of Spudich and Watt [20]. In order to remove residual cross-linking and capping proteins, it was purified by an additional step using gel column chromatography (Sephacryl S-300) as described by MacLean-Fletcher and Pollard [21]. *G*-actin was kept in *G*-buffer (consisting of 2 mM Imidazol, 0.2 mM $CaCl_2$, 0.2 mM DTT, 0.5 mM ATP, and 0.005 vol % NaN_3, pH = 7.4) at 4 °C, and was used within 14 days after preparation. The concentration of *G*-actin was determined by absorption spectroscopy assuming an extinction coefficient of 0.63 $mg^{-1}\,ml^{-1}$ for absorption at 290 nm [22].

Solutions of filamentous actin (*F*-actin) were prepared by adding 1/10 of the sample volume of tenfold concentrated *F*-buffer (20 mM Imidazol, 1 M KCl, 2 mM $CaCl_2$, 20 mM $MgCl_2$, 2 mM DTT, and 5 mM ATP, pH = 7.4). Immediately after initializing polymerization, the required volumes were pipetted into the sample cells of the two rheometers. Polymerization took place within these sample cells overnight at 4 °C. For microrheometry an amount of 2 μl of bead solution was added to a volume of 400 μl of *F*-actin solution. The beads had been coated with BSA (bovine serum albumin; Sigma, Deisenhofen, Germany) to inhibit un-

specific binding of protein to the bead surface, and washed carefully before adding them to the samples. For macrorheometry no beads had been added.

Gelsolin. Gelsolin ($M_W = 90$ kDa) was prepared from bovine plasma serum according to Cooper *et al.* [23], dissolved in *G*-buffer, and stored at 4 °C for several weeks. The concentration of gelsolin was determined according to Bradford [24], with BSA as a standard.

Gelsolin was used in several of the experiments to adjust the mean filament length, l, of *F*-actin. In these cases, gelsolin was added to *G*-actin before initiating polymerization. According to Janmey *et al.* [25], l can be calculated using the relation

$$l = \frac{1}{370\ r_{G:A}}\ \mu\text{m}, \tag{1}$$

where $r_{G:A}$ denotes the molar ratio of gelsolin to actin.

The purity of the proteins was checked by SDS-PAGE (sodium dodecyl polyacrylamide gel electrophoresis). After staining with coomassie blue [26], only one single band was detected.

III. RESULTS

Microrheological and macrorheological measurements of $G'(f)$ and $G''(f)$ were carried out on identical solutions of *F*-actin in the concentration range $c_A = 0.5$–2 mg/ml. Frequencies from $f = 4$ mHz up to $f = 4$ Hz could be studied with both instruments. In a second series of experiments, the mean filament length was varied in the range $l = 1.5$–$10\ \mu$m at a fixed actin concentration of $c_A = 2$ mg/ml. The diameter of the magnetic beads used in microrheometry was $D = 4.5\ \mu$m for all experiments.

In general, the frequency spectrum of viscoelastic moduli of entangled *F*-actin networks in the frequency range mentioned above has the following shape: At low frequencies, a plateau regime is found where $G'(f) > G''(f)$ and $G'(f) \approx$ const, while $G''(f)$ passes through a minimum. The high-frequency end of the plateau is given by the condition $G'(f_e) = G''(f_e)$. The reciprocal of f_e defines a relaxation time of the network, the entanglement time, $t_e = 1/f_e$. At higher frequencies, $f > f_e$, one finds an ascending branch with $G'(f) < G''(f)$. Another characteristic time of the network, t_{min}, can be defined as the reciprocal of the frequency, f_{min}, at which a minimum in the course of the loss tangent, $G''(f)/G'(f)$, is found. Usually, f_{min} equals approximately the frequency at which the minimum of $G''(f)$ is found within the plateau regime. For the case of networks made up of very short filaments, one is also able to detect the low-frequency end of the plateau within the range of measurement. It is given by crossover to a regime where $G''(f)$ is the dominant quantity again. This low-frequency regime can only be seen in few of the measurements presented below, and will therefore not be discussed in detail here.

Figure 2 summarizes measurements of the viscoelastic impedance for *F*-actin solutions with concentrations $c_A = 0.5$–2 mg/ml corresponding to mesh sizes $\xi = 0.5$–$0.2\ \mu$m [27]. The filament length has not been controlled in these experiments. It is easily seen that microscopic and macroscopic results for $G'(f)$ and $G''(f)$ differ by about a factor of 3 over the whole frequency range with microrheometry underestimating the macroscopic result. Another remarkable discrepancy is the following: Although the plateau is found with both methods to extend roughly from about 0.1 Hz to low frequencies, a significant difference exists at the high-frequency end of the plateau regime: The frequencies corresponding to $f_e = 1/t_e$ have been marked in Fig. 2 by arrows. A markedly smaller value for t_e is found at all concentrations by MTR. Usually, a minimum or at least an inflection point in the course of $G''(f)$ is detected at $f = f_{min}$ too. In the graphs of Fig. 2, we have marked $f_{min} = 1/t_{min}$ as derived from an analysis of $G''(f)/G'(f)$ by arrows. A minimum of $G''(f)/G'(f)$ is found within the range of measurement only for the microscopic experiments at $c_A = 1$ and 2 mg/ml.

The effect of mean filament length on the discrepancies between microrheometry and macrorheometry was studied in a second set of measurements. Gelsolin was added to *F*-actin solutions with a concentration of $c_A = 2$ mg/ml at molar ratios of gelsolin to actin in a range $r_{G:A} = 1{:}3700$–$1{:}555$. According to Eq. (1) this corresponds to a decrease of the mean filament lengths from $l = 10$ to $1.5\ \mu$m. The results are summarized in Fig. 3. An agreement of absolute values from microrheometry and macrorheometry is only seen at higher frequencies above 0.3 Hz for the case of the shortest filaments [Fig. 3(D)]. A more detailled analysis of the dependence of the disagreement of absolute values of $G'(f)$ and $G''(f)$ on l as well as on c_A will be given below.

First let us consider macroscopic and microscopic relaxation times which can be derived from the results of Fig. 3. In accordance with the results of Fig. 2, the plateau regime [if defined simply by the condition $G'(f) \approx$ const] is found roughly in the same frequency regime by microrheometry and macrorheometry, i.e., at frequencies of 0.1 Hz and below. The shorter the filaments, the better the agreement between the microscopic and macroscopic results for t_e (corresponding frequencies marked by arrows). From microrheometry there is no evidence of a dependence of t_e on mean filament length. On the other hand, the value of t_e obtained by macrorheometry gradually approaches the microscopic result, and eventually agrees with the latter for $l = 2.5\ \mu$m. Furthermore, we are now able to derive t_{min} from microrheometry as well as from macrorheometry. Similar to t_e, microrheometry yields constantly smaller values of t_{min} than macrorheometry, while the values of t_{min} converge with decreasing l. To clarify this, t_{min} has again been calculated as the reciprocal of the frequency at which a minimum of $G''(f)/G'(f)$ (not shown) is found. The corresponding frequencies have been marked by arrows in Fig. 3.

To provide a concise analysis of the c_A and l dependences of the discrepancies between the absolute values measured by microrheometry and macrorheometry, we now consider the ratio of macroscopic to microscopic loss modulus, $G''_{macro}(f)/G''_{micro}(f)$. It is expected that a ''macroscopic limit'' of microrheometry is indicated by the condition $G''_{macro}(f)/G''_{micro}(f) = 1$ (cf. Fig. 1). In Fig. 4, $G''_{macro}(f)/G''_{micro}(f)$ is plotted against frequency for the two sets of measurements of Figs. 2 and 3. The result from the measurement on a Newtonian fluid (Fig. 1) has been added in both cases as a reference to represent the ''ideal'' ratio of

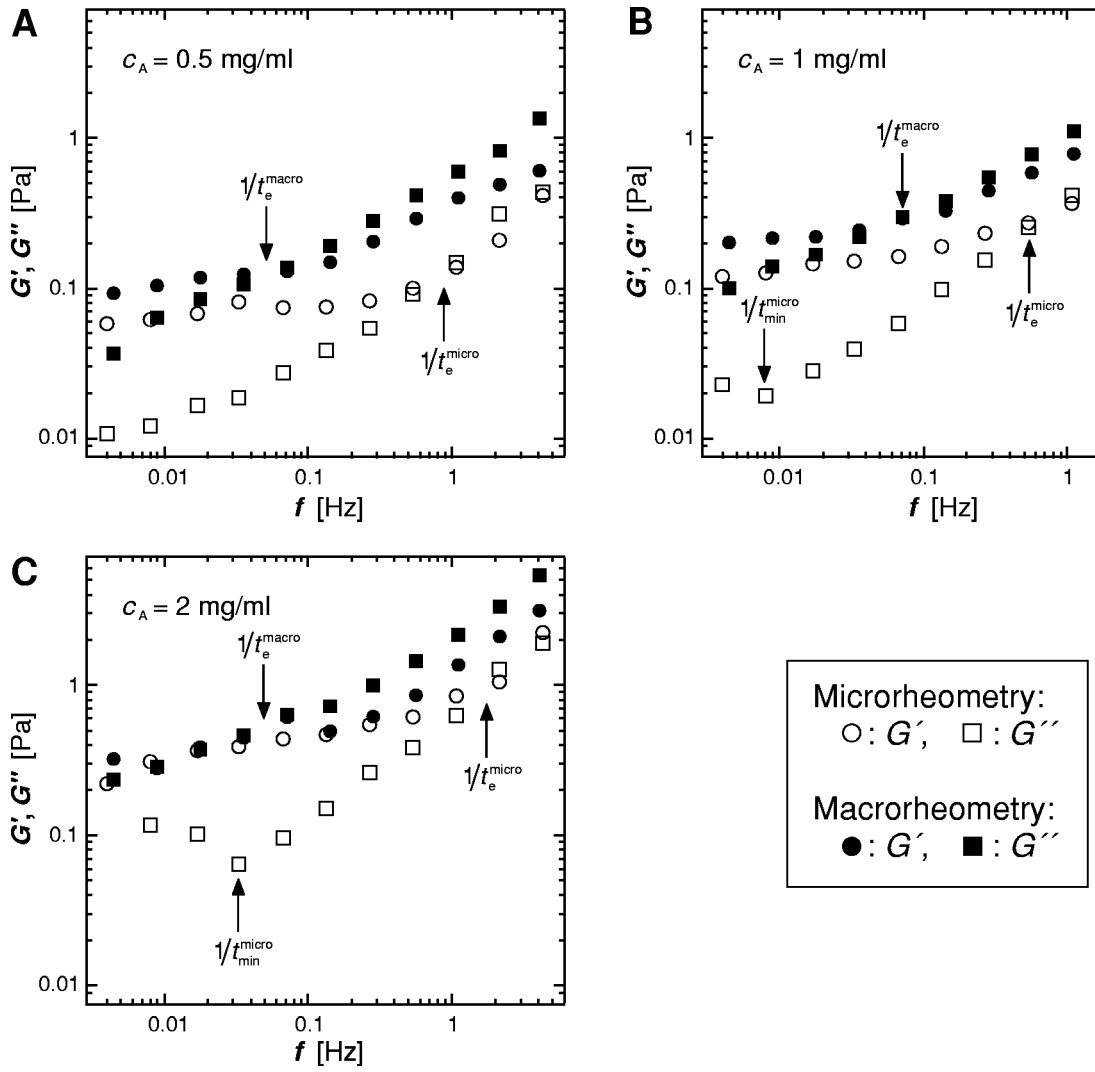

FIG. 2. Comparison of storage and loss moduli, $G'(f)$ (circles) and $G''(f)$ (squares), respectively, from microrheometry (open symbols) and macrorheometry (filled symbols) for the case of *F*-actin solutions with concentrations $c_A = 0.5$ mg/ml (A), $c_A = 1$ mg/ml (B), and $c_A = 2$ mg/ml (C). The values of $G''(f)$ obtained by microrheometry and macrorheometry differ by about a factor of 3 over the whole frequency range. Note that the difference is most significant in the plateau regime. The reciprocal entanglement time, $1/t_e$, is determined by the intersection of $G'(f)$ and $G''(f)$ at the high-frequency end of the plateau (arrowheads). $1/t_e^{micro}$ and $1/t_e^{macro}$ refer to results from microrheometry and macrorheometry, respectively. Results for t_e from both methods differ by about an order of magnitude. We also indicate the characteristic time $1/t_{min}$ at which a minimum of the loss tangent, $G''(f)/G'(f)$, is found.

1. In Fig. 4(A), $G''_{macro}(f)/G''_{micro}(f)$ is shown for the experiments at different actin concentrations (no control of filament length). The ratio $G''_{macro}(f)/G''_{micro}(f)$ exhibits a maximum in the plateau region; the results indicate no clear dependence of $G''_{macro}(f)/G''_{micro}(f)$ on concentration. Figure 4(B) shows the ratio for the measurements on solutions with different mean filament lengths. In this case, the ratio clearly becomes smaller with decreasing l, i.e., the values of $G''(f)$ from both methods converge with decreasing filament length. Eventually, $G''_{macro}(f)/G''_{micro}(f)$ reaches a value of 1 for $l = 1.5$ μm around $f = 0.3$ Hz.

IV. DISCUSSION

The results of Figs. 2 and 3 show that microrheometry yields substantially smaller values for both $G'(f)$ and $G''(f)$ of solutions of entangled *F*-actin than macroscopic torsional rheometry. Only if the mean length of actin filaments becomes distinctly smaller than the size of the probe particle used in microrheometry, the agreement of both methods is observed. First let us discuss these differences in absolute values for $G'(f)$ and $G''(f)$ from microrheometry and macrorheometry in more detail. At the end of this section, we will address the question concerning the origin of different relaxation times from both methods.

Naively, one would expect that viscoelastic moduli measured by microrheometry and macrorheometry converge, i.e., $G''_{macro}(f)/G''_{micro}(f) = 1$, if the bead diameter D is large compared to the mesh size of the *F*-actin network, ξ. Our measurements show that this is not the case. For the measurements presented in Fig. 2, the condition $9 \leqslant D/\xi \leqslant 19$ holds. Nonetheless, microrheometry yields values of the viscoelastic impedance which are smaller than macroscopic results by a factor of about 3. The near agreement of microscopic and macroscopic $G'(f)$ in the plateau regime at $c_A = 2$ mg/ml in Fig. 2 could be attributed to the formation of

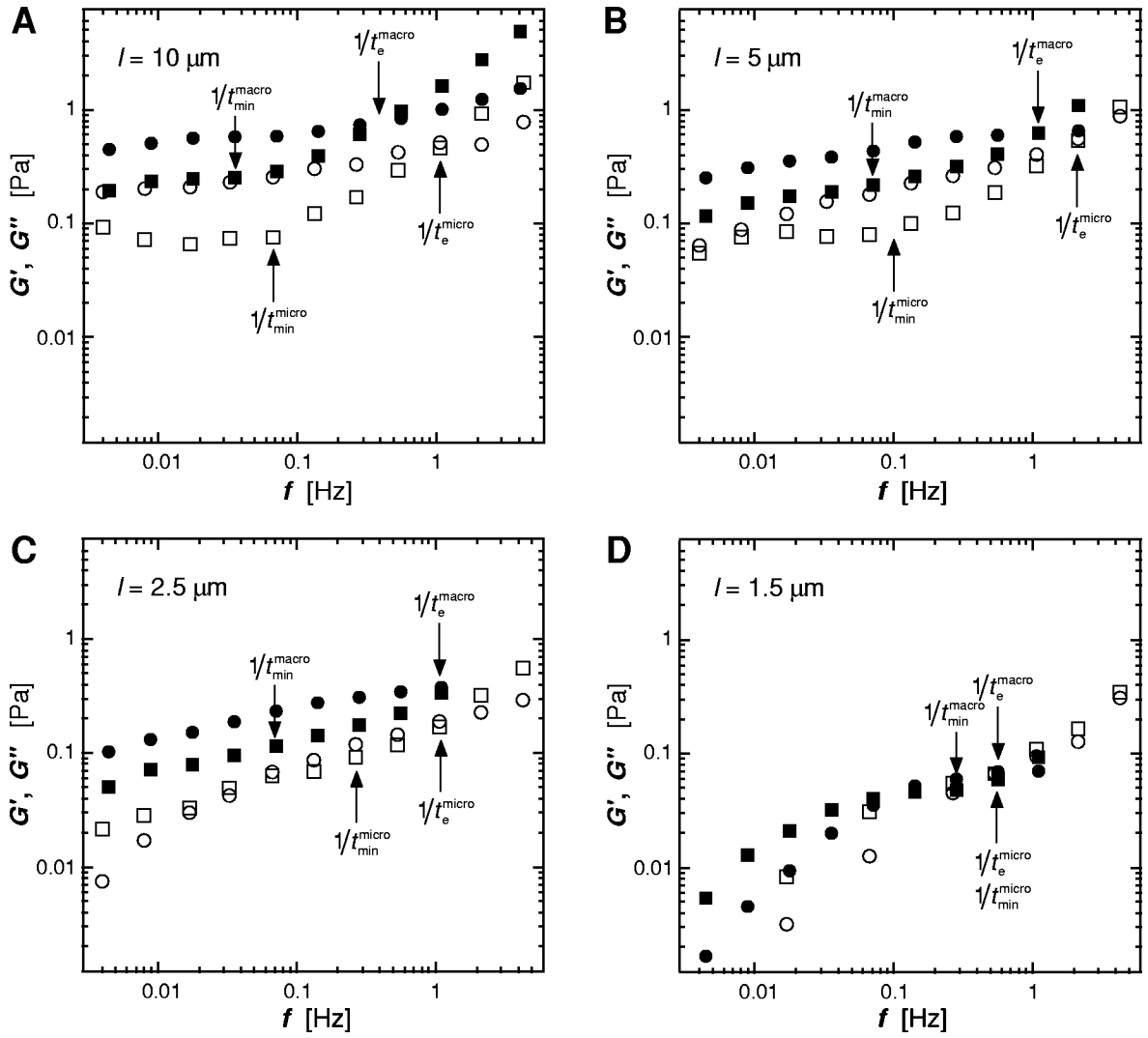

FIG. 3. Comparison of $G'(f)$ (circles) and $G''(f)$ (squares) determined by microrheometry (open symbols) and macrorheometry (filled symbols) for the case of *F*-actin solutions with a concentration of $c_A = 2$ mg/ml in the presence of different molar ratios, $r_{G:A}$, of capping protein gelsolin to actin. (A) $r_{G:A} = 1{:}3700$, corresponding to a mean filament length of $l = 10\ \mu$m. (B) $r_{G:A} = 1{:}1850$ (i.e., $l = 5\ \mu$m). (C) $r_{G:A} = 1{:}925$ (i.e., $l = 2.5\ \mu$m). (D) $r_{G:A} = 1{:}555$ (i.e., $l = 1.5\ \mu$m). Convergence of the viscoelastic impedance spectra is observed in the plateau regime of the sample with the shortest filaments (D).

nematic phases. As shown by several groups (cf., e.g., Ref. [7]), entangled *F*-actin solutions of uncontrolled average filament length tend to form nematic phases above a certain threshold concentration. In addition it is possible that macroscopic strain induces a nematic phase at frequencies within the plateau regime. This would provide a further reason for the agreement of $G'(f)$. Finally, for actin solutions of the same concentration but shorter filaments ($l = 10\ \mu$m in Fig. 3), the values of $G'_{macro}(f)$ and $G'_{micro}(f)$ no longer agree. The latter observation is quite plausible, since for shorter filaments the concentration, above which nematic ordering is observed, is shifted to higher values [28].

In addition to the condition $D \gg \xi$, the results of Fig. 3 show that $D \gg l$ has to be met to warrant agreement of the absolute values of $G'(f)$ and $G''(f)$ from microscopic and macroscopic measurements. As shown in Fig. 3(D), the onset of an agreement of microrheometry and macrorheometry is found for filaments with a mean length of $l = 1.5\ \mu$m, i.e., for $D/l = 3$. In a separate series of experiments, we studied solutions of the even shorter ($l = 0.9\ \mu$m) and monodisperse filamentous bacteriophage fd [29]. This virus forms semiflexible filaments similar to *F*-actin, but with an about tenfold smaller persistence length of $l_p = 2.2\ \mu$m. In this case the ratio D/l is $D/l = 5$, and excellent agreement of microrheometry and macrorheometry was found over the whole frequency range.

Basically it is not surprising that $D \gg \xi$ is not a sufficient condition for a conformity of both methods: In contrast to networks of flexible filaments, which can be characterized by one parameter alone (the mesh size, ξ), an additional parameter (the persistence length, l_p) is needed for a complete characterization of a semiflexible network. Therefore it is reasonable to assume that the size D of the microscopic probe has to be compared to a second length scale besides ξ. From our experimental results it appears that this second parameter is l. l_p does not seem to be of relevance in this context, which could be understood in the following way: If filaments become shorter than l_p ($l_p \approx 17\ \mu$m for *F*-actin), the parameter l_p becomes eventually irrelevant as one approaches the limit of rigid rods. This is the case for the

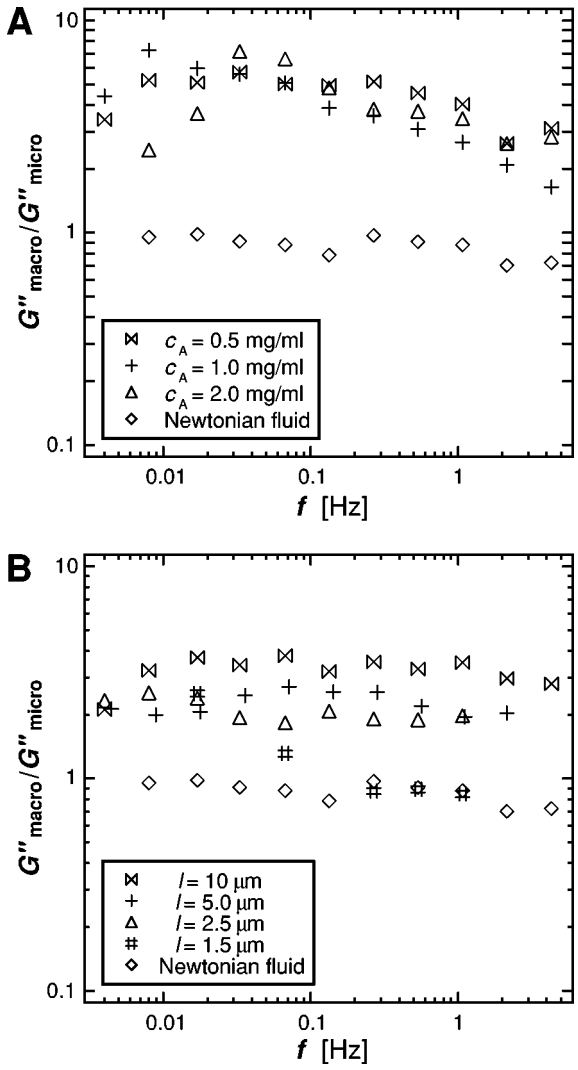

FIG. 4. Comparison of the ratio of macroscopic to microscopic loss modulus, $G''_{macro}(f)/G''_{micro}(f)$, as a function of frequency. (A) $G''_{macro}(f)/G''_{micro}(f)$ for different actin concentrations calculated from the results of Fig. 2. No systematic dependence of the result on concentration is found. (B) $G''_{macro}(f)/G''_{micro}(f)$ for different mean filament lengths calculated from the results of Fig. 3. The discrepancy between microrheometry and macrorheometry decreases with decreasing filament length, i.e., $G''_{macro}(f)/G''_{micro}(f)$ gradually approaches a value of 1. In both graphs, the value of the ratio for the case of a Newtonian fluid (cf. Fig. 1) is also plotted as a reference.

experiments summarized in Fig. 3. As a consequence, l becomes the important second parameter.

On the basis of theoretical considerations [1] the second relevant parameter besides ξ is expected to be

$$l_c = \sqrt{l_e l_p}$$

rather than l. Here l_e denotes the entanglement length, which is a measure of the average contour length between two collisions of the filament with the wall of its tube. l_e obeys the scaling law [30]

$$l_e = \xi^{4/5} l_p^{1/5}.$$

Maggs [1] argued that in the case $D > l_c$ microrheometric and macrorheometric plateau moduli should converge as long as the condition $l > l_c$ is met. For solutions of F-actin, one finds $l_c \approx 4$ μm.

We have $D = 4.5$ μm in all our experiments, so that $D > l_c$ and $l > l_c$ are fulfilled for the experiments presented in Fig. 2 and also for those of Figs. 3(A) and 3(B) where $l = 10$ and 5 μm. Nonetheless we still find a significant difference between microscopic and macroscopic. This discrepancy between theory and experiment is not surprising, since l_c is only a rough estimate based on scaling assumptions.

The condition $l > l_c$ no longer holds for the measurements on the samples with $l = 2.5$ and 1.5 μm presented in Figs. 3(C) and 3(D), respectively. In this regime no theoretical prediction is available yet. Here we observe that the microscopic result approaches the macroscopic result with decreasing mean filament length.

In addition to his predictions about the relation between microrheometry and macrorheometry in the plateau regime, Maggs [1] also argued that microrheometry should underestimate the macroscopic result in the frequency range $f \sim 0.1$–10 Hz as long as $D < l_p$ (which is the case for all our experiments). This is confirmed by the results of Fig. 2.

Before we come back to a possible explanation for the discrepancies of absolute values of $G'(f)$ and $G''(f)$, we discuss the differences of the characteristic times t_e and t_{min} obtained by microrheometry and macrorheometry. A close inspection of Figs. 2 and 3 shows that the two methods yield identical results for t_e only for F-actin solutions with $l \leqslant 2.5$ μm (cf. Fig. 3). For longer filaments, we find higher values of t_e by macrorheometry, and the difference becomes as large as one order of magnitude for the case of F-actin solutions without gelsolin (cf. Fig. 2). A possible explanation for this finding is that the mechanism of shear differs in microrheometry and macrorheometry on a scale of the filament length. In macrorheometry the strain is homogeneous throughout the whole sample, so that all filaments (or, to be more precise, the tubes of the respective filaments) are affected by an external strain in the same way. In contrast, it is expected that microrheometry probes, in particular, shorter filaments for the following reason: An oscillation of a probe particle (diameter $D = 4.5$ μm) with an amplitude below 1 μm will cause a deformation of the actin network which can be characterized by a penetration depth with a maximum value around 10 μm or so [17,18]. For this reason, filaments with a contour length of 15 μm and more are not deformed in the same way as in a macroscopic experiment. Therefore, the viscoelastic response on a microscopic level might be dominated by the shorter filaments within the polydisperse actin network.

The latter hypothesis is supported by the following observations: No dependence of t_e on l is found in our microscopic measurements (cf. Fig. 3). Furthermore, microscopic and macroscopic values for t_e differ by more than one order of magnitude for the natural contour length $l \approx 20$ μm (Fig. 2), but are already quite similar for $l = 10$ μm [Fig. 3(A)] and almost agree for $l = 5$ μm [Fig. 3(B)]. On the basis of theoretical concepts (for the macroscopic case), no l dependence is expected for $G'(f)$ in the plateau regime for entangled networks of semiflexible filaments [13,31]. On the

other hand, $G''(f)$ should exhibit a strong dependence on l in the same frequency range, because the zero-shear viscosity, η_0, should depend on l like $\eta_0 \propto l^3$ for flexible filaments as well as for rigid rods [32]. The clearly different l dependence of $G'(f)$ and $G''(f)$ explains the shift of t_e to smaller values with decreasing l which has been observed with macrorheometry. The microscopic finding that t_e is independent of l fits into this picture, if a sensitivity of microrheometry especially on short filaments is assumed. Considering the estimates for relevant lengths given above, the observed agreement for filaments with $l = 5$ μm appears quite plausible.

The observed underestimation of absolute values for $G'(f)$ and $G''(f)$ by microrheometry can also be explained by the fact that MTR probes mainly short filaments in a polydisperse *F*-actin solution: This would result in a reduction of the effective actin concentration, i.e., the concentration which is actually deformed by the probe particle. Since the viscoelastic impedance of isotropic *F*-actin solutions decreases with decreasing concentration, the discrepancy between microrheometry and macrorheometry in $G'(f)$ and $G''(f)$ is no longer astonishing in this picture. An alternative explanation would be the formation of a depletion zone in the immediate vicinity of a probe particle [33]. Long actin filaments could be repelled from the surface of the beads for the following reason: To maintain the random structure of the network in the presence of probe particles, especially long filaments would have to bend more strongly in the vicinity of a bead surface than short ones. The resulting depletion of long filaments in the neighborhood of beads would again lead to a reduction of the effective actin concentration which is probed by the particle.

In summary, our comparative study shows that microrheometry and macrorheometry are complementary techniques. However, it also shows systematic discrepancies between the viscoelastic impedance spectra obtained by microscopic and macroscopic methods. Thus our findings provide a basis for future comparison of results obtained by the two techniques. An important result is the following: The discrepancies are the smaller, the larger the ratio of bead size to mean filament length becomes. For the case of semiflexible filaments, agreement within experimental reproducability of the two techniques is achieved, if the bead diameter is considerably larger than mesh size and mean filament length. These findings provide a basis for the interpretation of microrheological experiments. The present results are of interest from a physical point of view, since they might explain discrepancies between theoretical predictions and microscopic experiments in recent studies [12]. Microrheometry is an essential tool for studies of biological materials, in particular living cells which are microscopic per se. They are highly heterogeneous, so that viscoelastic parameters are local quantities.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) under Contract No. Sa.246/22-3 and by the Fonds der Chemischen Industrie. We are very grateful to K. Kroy, F. C. MacKintosh, and especially J. Wilhelm for helpful discussions and ideas. Futhermore, we thank H. Hirsch, H. Kirpal, and K. Vogt for preparation of proteins. F.G.S. wishes to thank F. Ziemann for fruitful and pleasant cooperation over many years.

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