Micro-bead mechanics with actin filaments

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Many experiments have been performed using microscopic beads to probe the small-scale mechanics of actin solutions. We calculate the minimum bead size needed to measure a valid macroscopic response function. We find that the quasi-static response is characterized by an anomalous scaling as a function of the size of the probing particles. [S1063-651X(97)12712-X]

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Actin filaments are a beautiful model system for the study of the dynamics and rheology of semidilute polymers [1,2]. They are characterized by length scales that are easily accessible with optical techniques allowing a detailed study of phenomena such as tube dynamics. However, the macroscopic rheology of these systems has been hard to master experimentally. Difficulties in purification and sample preparation lead to orders-of-magnitude variation in fundamental properties such as the value of the plateau modulus [3–7], the standard measure of the response of an entangled polymer solution to external perturbations.

To get around the problems of macroscopic sample preparation and also to probe the local viscoelastic behavior of these materials, a number of experimental groups have started using small, colloidal beads to study the mechanics of these materials [8–14]. One either pulls on the particles using a magnetic field or simply observes the fluctuations of the particles undergoing Brownian motion. In this paper, I shall try to attack the problem of what exactly one measures in these experiments. In particular, how large do these particles have to be in order to measure a macroscopic elastic modulus and when do we expect to be sensitive to the individual filament properties?

In contrast to flexible polymer solutions, there are two principal length scales present in a semidilute solution of actin: the mesh size and the persistence length. Naive application of scaling ideas thus becomes a highly ambiguous exercise because an arbitrarily large number of intermediate lengths can be created by considering $\xi^{1-\alpha} l_p^{\alpha}$ with ξ the mesh size and l_p the persistence length. This ambiguity in lengths also translates into an ambiguity in the plateau modulus, which can be expressed as $k_B T$ per characteristic volume. As an example of this difficulty we might quote two recent attempts to calculate the modulus in actin solutions with scaling approaches [15,16] where completely different results were found. Indeed, this proliferation of lengths is already known for the tube geometry where one finds both $\alpha = +\frac{1}{5}$ and $\alpha = -\frac{1}{5}$ [17–19]. We shall show in this article that an intermediate scale with $\alpha = \frac{3}{5}$ becomes crucial in the understanding of the elasticity of actin solutions at length scales probed with micrometer-sized beads. At these scales we show that the elasticity is characterized by an anomalous penetration of the response into the sample and unusual scaling with the size of the probing particle.

Most experiments using microbead rheology have been analyzed with the implicit assumption that if one examines the system on a scale only slightly larger than the mesh size one should at once approach a continuum limit in which standard elastic theory must apply. Thus, even the smallest particles should give a passable estimate of the macroscopic modulus. A more subtle argument implies, however, that much larger particles are needed: Macroscopic shear of a sample produces an affine deformation of the local geometry of the sample (in which straight segments are rotated but never bent). Pulling on a small bead produces above all a *bending* of nearby filaments with a characteristic radius comparable to the bead size. To eliminate this nonaffine deformation, one would argue that beads should be much larger than l_p, and thus should have a diameter of at least *several tens* of micrometers. This argument is rather negative. It would imply that none of the present generation of experiments with beads up to 5μ would be able to measure a valid macroscopic response. We shall conclude that the truth is somewhere between these two extremes. A continuum theory is valid at small length scales; however, there is an important contribution to the energy from the nonaffine component of the deformation. This component becomes small for beads larger than the new *intermediate* length scale; the present generation of experiments should be able to measure a macroscopic modulus.

Note that in this paper I am mostly interested in the low-frequency mechanics and thus I exclude from the discussion high-frequency fluctuation measurements (up to 20 kHz) which have been recently performed [12]; I will discuss the quasistatic regime between 10^{-3} Hz and 10^{-1} Hz. I conclude this paper, however, with a few remarks on the frequency range 10^{-1}–10^{+1} Hz, where a crossover is expected to a stiffer macroscopic modulus [26]. This intermediate-frequency regime requires the use of much larger beads in order to correctly study the macroscopic limit.

A coherent picture of the large-scale mechanics of non-cross-linked actin solutions is now available. The actin system is usually polymerized [11] in conditions such that the mean distance between filaments ξ is between 0.3μ and 1μ. ξ can be linked with the concentration of monomers c by noting that $\xi \sim 1/\sqrt{cd}$ with d the size of actin monomers. A useful geometric quantity is the length of filament per unit volume $\rho \sim 1/\xi^2$. The filament is characterized by its persistence length l_p, which is close to 15μ [20]. For a single weakly bent filament the energy of a configuration is given by [21]

$$E = k_B T l_p / 2 \int [\partial_s^2 \mathbf{r}_\perp(s)]^2 ds, \tag{1}$$

where $\mathbf{r}_\perp(s)$ is the transverse fluctuation of the filament about its equilibrium configuration.

In a manner which is familiar from flexible polymers, the individual filaments are confined to a tube whose diameter scales as $\xi^{6/5}/l_p^{1/5}$, the filament is confined to the tube by collisions between the filament and its neighbors every $l_e \sim \xi^{4/5} l_p^{1/5}$ [17,18]. l_e is in some ways equivalent to the entanglement length in the Doi-Edwards tube model [22–24]. The long-time dynamics and mechanics are dominated by the reptation of filaments along their tubes [5,24,26]. This process has a characteristic time, the reptation time, which defines the time scale beyond which the sample behaves like a viscous fluid (rather than an elastic solid) and can be as long as several hours [5]. Under macroscopic shear, the longitudinal stresses in a filament relax relatively rapidly [26] leaving a residual contribution to the free energy that comes from the modification of the confinement of the filament in its tube. A simple argument for this free-energy contribution is to count $k_B T$ per collision of the tube with the filament. Thus the macroscopic modulus varies as

$$G \sim \rho k_B T / l_e \sim c^{7/5} / l_p^{1/5}, \tag{2}$$

as was recently confirmed by an explicit calculation [24,25].

This picture of filaments confined to a tube is only true on time scales that are long enough for the filament to dynamically sample fluctuations on the scale of l_e. This time, which is determined by the bending elasticity of the filaments, varies as $\tau_e \sim \eta l_e^4 / l_p k_B T \sim 10$ Hz [25]. This is our reason for restricting our treatment to lower frequencies; at higher frequencies one is presumably sensitive to individual filament dynamics (coupled by hydrodynamics), rather than the collective, entangled, modes that interest us in this paper. For frequencies lower than the inverse reptation time (i.e., frequencies comparable to 10^{-3} Hz) the sample behaves as a fluid and the bead moves freely as filaments slide out of the way of the particles.

Before moving on to the problem of the behavior of actin solutions, we shall revise a Peierls-like argument from which we can deduce the basic scaling behavior of a normal elastic solid. We shall then adapt this argument to the case of semiflexible filaments. Consider a bead of radius R embedded in an elastic medium in d dimensions. If we pull on the particle with a force f we can make the following variational ansatz in order to find the minimum energy configuration. Let us assume that the material is disturbed over a distance l from the bead; then the elastic energy will scale in the following manner:

$$E_{var} \sim G \int (\nabla a)^2 dV, \tag{3}$$

where a is an amplitude of displacement, G an elastic constant, and the integral is over the variational volume $V \sim l^d$. This scales as

$$E_{var} \sim G (a/l)^2 l^d. \tag{4}$$

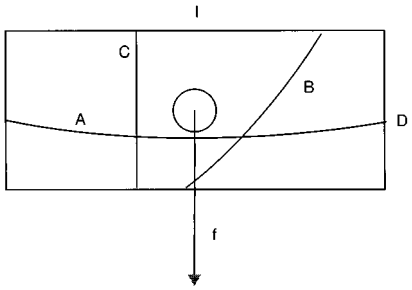

FIG. 1. Schematic representation of the bending of filaments in a region of dimensions $l \times l \times D$. Filaments crossing the volume horizontally are excited with wavelength l (A). Filaments passing vertically through the region are relatively undisturbed (C). In the geometry shown here (with $D < l$), filaments passing in diagonal are excited with wavelength comparable to D.

We see that in less than two dimensions an arbitrarily small force is able to displace the bead large distances because E_{var} can be made small by increasing the variational parameter l. In three dimensions, however, the energy diverges with l and has a lower bound for small l due to the short-wavelength cutoff coming from the finite size of the bead. Thus the minimum energy is found for $l \sim R$ and we deduce that $E_{var} \approx G a^2 R$. At constant force the displacement scales inversely with the bead size,

$$a \approx f / GR. \tag{5}$$

A full calculation of the response of an isotropic viscoelastic material has recently been performed and confirms this simple scaling argument [12].

This argument is perhaps a little too simple. We see that there is an asymmetry in the problem coming from the direction in which we apply the force f, and we should worry that the volume excited is not spherical as has been assumed in the argument. Let us perform a slightly more elaborate variational treatment where we assume that the volume V is characterized by a disk of dimensions $l \times l \times D$ where the particle excites modes of wavelength l that penetrate a distance D into the sample in the direction of f. In this case our estimate for E_{var} is

$$E_{var} \sim (l^2 D) G [(a/l)^2 + (a/D)^2], \tag{6}$$

where a/l and a/D are estimates of the components of the strain tensor in the material. Taking D as a variational parameter, one sees that $D \sim l$ and the problem reduces to that considered above.

How must this argument be modified in the actin system? Experiments are performed with beads which vary in size from 0.2μ to 5μ. The smallest beads pass between the filaments and diffuse almost freely [11]; they will not concern us any further. Are we able to use continuum elastic arguments (like that above) to deduce the experimental stress-strain relationships? We now argue that in actin solutions there are now two contributions to the variational energy E_{var}. For large beads the normal affine elasticity (summarized above) dominates; for smaller beads, however, a different elastic response is found. Consider a volume V distorted by a force on a particle of size R (Fig. 1). Again we take this volume as anisotropic with dimensions $l \times l \times D$. In this vol-

ume the filaments that traverse the volume perpendicular to f bend with a wavelength l. Those filaments that are parallel to f do not contribute to the bending energy, since they are free to slide along their tubes; however, filaments somewhat inclined to f will be bent with a wavelength D.

The total nonaffine contribution to the energy from (1) is thus

$$E_1 \sim (l^2 D)(a^2 k_B T l_p / l^4 + a^2 k_B T l_p / D^4)\rho. \tag{7}$$

The three multiplicative factors are, respectively, the volume excited, the bending energy per unit length of filament, and the filament density within the volume. a is again the typical amplitude of the excitation in the volume. To this bending contribution one must add the equivalent of E_{var} in Eq. (6): When we impose the bending on the volume V there is also a variation in the geometry of the confining tubes. For instance, in the direction of f the confining tubes are compressed by a factor a/D, which is coupled to the macroscopic modulus. There is thus a contribution to the energy of the form

$$E_2 \sim (l^2 D)[(a/l)^2 + (a/D)^2](\rho k_B T / l_e), \tag{8}$$

where we have again, respectively, the volume, the square elastic strain, and the macroscopic elastic modulus from Eq. (2). We can now optimize $E_1 + E_2$ by minimizing over D, finding $D \sim l$. Substituting for D in $E_1 + E_2$ one finds

$$E_{eff} \sim a^2 \rho k_B T (l_p / l + l / l_e). \tag{9}$$

We conclude that there is an important new length scale in the problem. For excitations with wavelengths greater than

$$l_c = \sqrt{l_e l_p} \sim \xi^{2/5} l_p^{3/5} \tag{10}$$

the contributions in E_2 are going to dominate the elasticity and we are back to the case of normal affine elastic theory. However, the structure of the energy (9) is very different from the corresponding equation for normal elastic solids (4). The short-wavelength cutoff is no longer determined by the bead size but rather by the intrinsic properties of the solution itself; even if we excite the medium with a smaller particle the minimum-energy configuration is one in which the energy cost is shared by the bending of the filaments and compression of the confining tubes. The optimum size of excitation is given by $l \sim \sqrt{l_e l_p}$. Substituting Eq. (10) in Eq. (9) gives

$$E_{eff} \sim \rho a^2 k_B T \sqrt{\frac{l_p}{l_e}}. \tag{11}$$

This is one of the important results of this paper and allows us to calculate the response due to small beads. From Eq. (11) we find that under the influence of thermal fluctuations the typical excursion of a small bead should scale as

$$a \sim \xi / (l_p / l_e)^{1/4} \tag{12}$$

independent of R. What happens if we interpret the fluctuations as a macroscopic modulus and examine its behavior as a function of concentration? In this case we would find that

$$G_{eff} \sim c^{6/5} l_p^{2/5}, \tag{13}$$

thus the effective modulus scales with a smaller power of the concentration than the true modulus Eq. (2).

Substituting typical values for material constants, $\xi \sim 0.5\mu$, $l_p \sim 15\mu$ we find that $l_e \sim 1\mu$. The crossover length scale $l_c \sim 4\mu$. These values are clearly only estimates, since simple scaling arguments are incapable of giving precise numerical values. However, we feel that they should be at least a reasonable guide to the experimental situation. We thus expect a series of crossovers as a function of probing wavelength. (a) The smallest beads diffuse freely in the solution. (b) For $\xi < l < l_c$ nonaffine excitations of the solution are important with anomalous penetration of the excitation into the sample. (c) For $l > l_c$ the elasticity becomes affine.

We conclude that to measure a valid macroscopic response function, particle sizes should be at least l_c; the second argument of the introduction concerning the bead size for which affine elasticity becomes valid is too pessimistic. Recent experiments seem be consistent with some of the results given here [12]. At high frequencies the experimental amplitudes scale as $1/R$. However, at low frequencies the authors explicitly remark that the amplitudes are only weakly dependent on the bead size in the range 0.5μ to 5μ.

Until now we have considered the low-frequency response of a sample, that is, times long enough for all longitudinal stresses to have relaxed along the tube. It has been shown [26] that one expects two plateau moduli as a function of frequency. The low-frequency plateau used in the above discussion comes from variation in tube geometry under sample deformation. The second, much larger, contribution, which dominates at higher frequencies, comes from coupling of the shear to the longitudinal density fluctuations of the filament in its tube. Can we see the crossover between the low-frequency and high-frequency behavior with micro-bead techniques? This question is difficult to answer; the static approach used above is not adapted to answering this dynamic question; however, we can certainly expect that the frequency of crossover between the two regimes will vary with the bead size.

The regime of the high plateau in macroscopic rheology is delimited by the two times $\tau_e \sim 0.1$ s and $\tau_e (l_p / l_e)^2 \sim 10$ s. This second time is the time needed for excitations to diffuse a distance l_p along the tube. It is important because macroscopic shear produces density fluctuations along the tube that are coherent over a distance l_p. When we excite a sample with a wavelength l, which is smaller than l_p, we expect that the window of times for the observation of this high plateau is reduced to the interval between τ_e and $\tau_e (l / l_e)^2$. For the smallest beads, this high second plateau should almost completely disappear. Even with larger beads, the elastic modulus should be substantially underestimated over certain frequency ranges. More detailed discussion of this regime seems to be difficult without a detailed *dynamic* theory of the coupling of the bend and longitudinal degrees of freedom. Thus, even though the smaller, long-time modulus should be accessible to micro-bead techniques it may prove much harder to study the larger short-time modulus. For this case, the beads should really be large compared with l_p and not in comparison with the smaller length l_c.

To conclude, actin mechanics shows a quite rich series of crossover in the response function $G(q,\omega)$. We have simple arguments for the wave-vector dependence of this function at frequencies between 10^{-3} and 10 Hz. Further work requires a full dynamic theory of the coupling between bending and density fluctuations. We have shown that the present generation of microbead experiments should be capable of measuring a valid macroscopic modulus at low frequencies, contrary to some simple arguments. In the time range $\tau_e - \tau_e(l_p/l_e)^2$ the micro-bead technique probably substantially underestimates the modulus.

Smaller beads should display an anomalous scaling of response as a function of bead size. Recent experiments already seem to show this effect [12]. It should also be noted that the results will be rather sensitive to the length of filaments in the sample and that the above results are only valid in the limit that the mean length is greater than l_c. When the filaments are shorter than this, the schematic representation of Fig. 1, where filaments cross the excitation volume, is no longer valid. For such short filaments, it has also been shown [24] that there is an important orientational contribution to the elastic response which should also be added to the present discussion. Since the length of filaments can be modulated with capping agents, one expects an interesting evolution of the response as a function of the capping concentration.

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