Subdiffusion and Anomalous Local Viscoelasticity in Actin Networks

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We use magnetic tweezers to study local viscoelastic response in filamentous actin networks. The choice of magnetic, colloidal particles of varying size allows us to explore properties on the relevant micron and submicron scales. At these scales the mechanical response is determined by the bending properties of individual filaments and described by an anomalous power-law behavior. In the absence of external forces the particles exhibit a subdiffusive motion. [S0031-9007(96)01627-4]

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Complex molecular systems, such as polymer solutions, polymer melts, gels, (micro)emulsions, and foams, often display a combination of the elastic properties of solids and the viscous properties of fluids. Using classical rheological methods [1], the viscoelastic properties of such materials have been described at scales much larger than the molecular dimensions, and the systems under study have mostly been treated as homogeneous media. In many situations, however, local mechanical properties are of critical importance. For instance, the shape and motility of living cells, as well as cytoplasmic transport, are strongly influenced by the mechanical properties of cytoskeleton networks [2] at submicron and micron scales. Actin filaments (*f*-actin), formed upon polymerization of globular actin proteins, are major components of the cytoskeleton and are involved in both transport and motility [3]. Easily purified and polymerized *in vitro*, actin is a model system for the study of the mechanics and assembly of biopolymers [4,5]. In this paper we show, using the example of actin filaments, how micromechanical measurements can provide information about *local* viscoelastic properties of the medium.

f-actin is a rigid polymer with a persistence length L_p of the order of 15 μm [6]. At high enough concentrations, in the so-called semidilute regime, the polymers form a three-dimensional network with a mesh size L. L is typically of the order of a micron and thus much smaller than L_p. Viscoelastic properties of this inhomogeneous medium can be locally studied by inserting colloidal magnetic beads and perturbing them with external magnetic forces. In fact, such simple methods have been used for many years to explore the cytoplasm [7]. For beads with diameter, d, much larger than L, the mechanical perturbation is macroscopic. On the other hand, if d is much smaller than the mesh size, the bead is expected to probe only the solvent viscosity and geometrical constraints introduced by polymers. Therefore, the regime that is relevant for exploring the local network mechanics is one for which d is comparable to L. In this case, the bead is moving inside a "cage" of typical linear size L. To move further, it has to perturb the polymers of the cage, either through the influence of an external force or via thermal fluctuations. In both cases, one can study the viscoelastic properties on micron scales by observing the motion of individual beads. We show below that this approach can be made quantitative, and that local mechanical properties of the network can be related to the bending elasticity of individual filaments.

We built magnetic tweezers [8] which allowed us to apply constant or time-dependent forces and simultaneously follow the movement of the magnetic bead under a microscope (see Fig. 1). At the center of the device, formed by four pole pieces, the resulting magnetic forces are parallel to the focal plane and directed towards one of the poles, the intensity being quite uniform throughout the microscope field [9]. An electronic feedback circuit, based on four Hall probes located on the pole pieces, is used to accurately control the magnetic field, with a response time of the order of the video frequency. Although static magnetic fields cannot establish a true three-dimensional trap, we can still use our system as a tweezer to manipulate beads in a dense viscoelastic medium [8]. Under our experimental conditions, actin filaments had a mean length comparable with, or slightly exceeding, the persistence length. The actin concentration, of the order

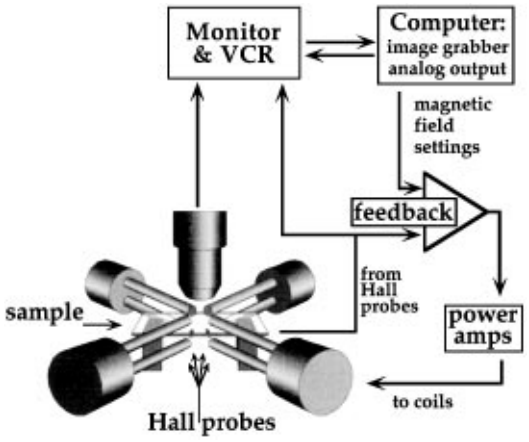

FIG. 1. Composite diagram of the microscope-based apparatus built to impart controlled uniform forces on magnetic beads and to record their position together with the imposed magnetic field [8].

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of 0.1 mg/ml, was such that the mesh size of the filament network is of the order of 1 μm [10]. We thus used beads of comparable size, ranging from 0.3 to 2.8 μm. Only beads located in the horizontal midplane of the capillary were analyzed, where their distance to the wall (200 μm) is much larger than their radius. An adequate analysis of video images of the beads (obtained typically through a 40× long working distance objective with a low resolution CCD; in contrast, diffusion of 0.3 μm beads was imaged by fluorescence microscopy using oil immersion optics) provided us with 30 Hz temporal and 20 nm spatial resolution of the position of their center of mass.

The main results of the measurements may be summarized as follows. When a constant force is exerted at time $t = 0$ on a bead with diameter d larger than L ($d/L \sim 3$, see Fig. 2), there exists a well-defined regime in which the movement of the bead's center of mass, $x(t)$, is described by an anomalous power law:

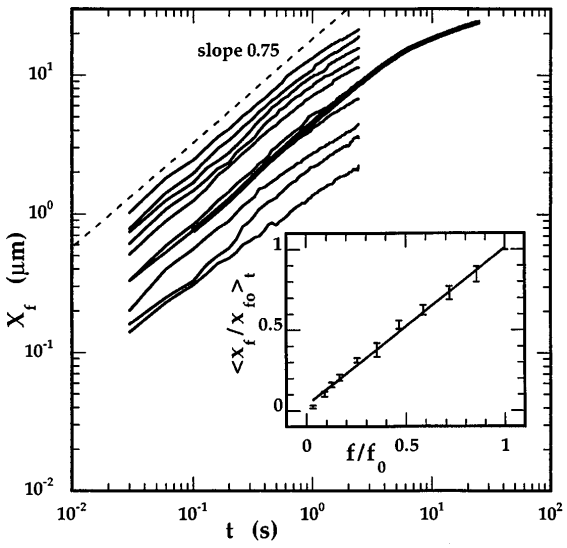

FIG. 2. Time-displacement responses, $X_f(t) - X_f(0)$, of 2.8 μm diameter beads (M280, Dynal) dispersed in a 0.1 mg/ml solution of actin filaments and subjected to a square wave force oscillating between $\pm f$ with a 5 s period. All half period responses were analyzed and superposed on the same origin to compute the average response. Each thin line corresponds to the mean response for a given value of f. The heavy line is the response of a bead subjected to a constant force for 25 s ($f/f_0 = 0.354$). The dashed line shows the theoretically predicted power law $X_f(t) - X_f(0) \propto t^{0.75}$. The *inset* shows $\langle X_f(t)/X_{f_0}(t)\rangle_t$ as a function of the ratio f/f_0 and confirms the linearity of the response. While the f/f_0 ratio is controlled by a computer generated gain parameter with an accuracy better than 1% [8], f_0 is only known approximately, and varies a lot between different beads [11]. In the present magnetic field pattern, calibration experiments indicate that the average value of f_0 is 1.9 pN. This maximal force is obtained with a 0.061 T field and a 14.6T m^{-1} gradient at the sample position. The ratio $X_f(t)/X_{f_0}(t)$ of the response at a given force f to the response at the maximum value of the applied force f_0 is nearly constant as a function of time. The errors bars correspond to 2σ.

$$X_f(t) - X_f(0) \propto t^p \quad \text{with } p = 0.76 \pm 0.03\,. \tag{1}$$

In the absence of external magnetic forces (see Fig. 3), $X_f(t)$ follows the usual Brownian diffusion $\langle x(t)^2\rangle \propto t$ for beads with $d/L < 1$. The movement of the bead's center of mass for larger beads with $d/L > 1$, on the other hand, is characterized by a subdiffusive rather than a diffusive power law:

$$\langle x(t)^2\rangle \propto t^q \quad \text{with } q = 0.73 \pm 0.01\,. \tag{2}$$

These observed anomalous power laws (1) and (2) can be readily explained if we consider the bead deforming the filaments of the cage surrounding it. Indeed, let us consider such a filament with bending constant κ, in a solvent of viscosity η. If s is the internal curvilinear coordinate along the polymer and $r(t, s)$ the transverse deformation of the filament, then the equation of movement for $r(t, s)$ is

$$\eta \frac{\partial r}{\partial t} = \kappa \frac{\partial^4 r}{\partial s^4} + f(t, s)\,, \tag{3}$$

where $f(t, s)$ is the force acting on the filament. It is straightforward to show that the Green function, $F(t, s)$,

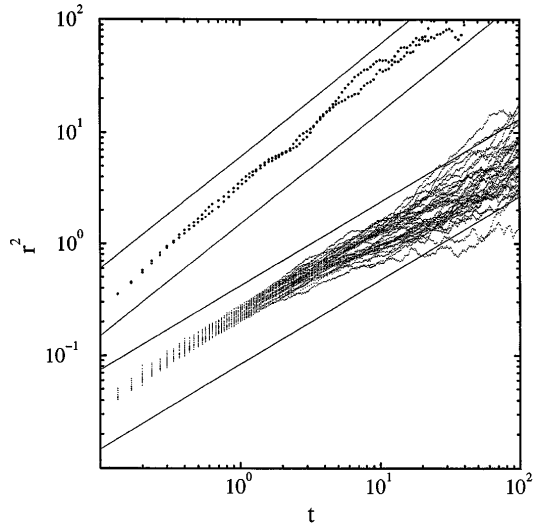

FIG. 3. Diffusion and subdiffusion of 0.3 μm (upper data set, circles) and 2.8 μm (lower data set, dots) diameter beads dispersed in a 0.1 mg ml^{-1} solution of filamentous actin. The median-square displacement of one rectangular coordinate (μm^2) is shown as a function of time (s). The video noise was removed by using the median instead of the mean-square displacement. The former was computed from the distribution of the squared displacement over successive overlapping time intervals, which cover the whole recorded time series of positions. The median-square displacement of one coordinate does not depend on the direction along which it is measured, provided anisotropy of the video noise is taken into account [8]. For the 2.8 μm beads, the data from 28 trajectories are shown, whereas two trajectories are analyzed for the 0.3 μm beads. The lines bracketing the upper set of data have a slope of 1, corresponding to usual diffusion, while the lines bracketing the lower set of data have a slope of 3/4.

associated with this equation can be written in the following scaling form:

$$F(t,s) = \frac{1}{\eta s} F\left(\frac{\eta s^4}{\kappa t}\right) \equiv \frac{1}{\eta^{3/4}\kappa^{1/4}t^{1/4}} \tilde{F}\left(\frac{\eta s^4}{\kappa t}\right). \quad (4)$$

If, for simplicity's sake, we assume that the magnetic bead applies a constant point force, f at $s = 0$, then the displacement of the center of mass of the bead is given by

$$x \approx \langle r(t,0)\rangle = f \int_0^t F(t - t', 0)\, dt' \approx f \frac{\tilde{F}(0) t^{3/4}}{\eta^{3/4}\kappa^{1/4}}. \quad (5)$$

In the absence of the external force, the bead movement is dominated by the thermal motions of the surrounding filaments to which it is coupled. In this case, the mean-square displacement of the bead is the mean-square displacement of the filament,

$$\langle x^2\rangle = \langle r^2(t,s)\rangle = k_B T \eta \int ds' \int_0^t F^2(t - t', s - s')\, dt' \propto \frac{k_B T t^{3/4}}{\eta^{3/4}\kappa^{1/4}}. \quad (6)$$

It is interesting to note that, when one evaluates the numerical prefactors in (5)and (6), one obtains a semiquantitative agreement with known values of the f-actin rigidity [12]. The power laws of the time dependence described by (5) and (6), $t^{3/4}$, are not altered if one considers the bead interacting simultaneously with several filaments of the cage. The details of local geometry should modify numerical prefactors in these theoretical formulas; undoubtedly the bead has to push against several filaments simultaneously while the confined geometry must change the effective friction constants involved. We note that the time scales involved in our experiments are very short compared with the reptation time of the filaments; we thus neglect all longitudinal dynamics of the filaments in their tube.

The power law (1) can be interpreted in terms of the usual storage and loss moduli, $G'(\omega)$ and $G''(\omega)$ [1]; $G'(\omega) \sim G''(\omega) \sim \omega^{3/4}$. One should contrast this viscoelastic behavior found locally with that determined in rheological macroscopic measurements of the bulk medium [13]. The power law (2) is in agreement with quasielastic light scattering results on actin networks [14]. The observed behavior (1) and (2) is a manifestation of the bending elasticity of individual filaments rather than coming from the collective behavior and interactions of molecular components.

We now discuss some experimental aspects of the observed behavior (1) and (2). In our setup, magnetic beads were attracted towards a pole by generating a stronger magnetic field RB_0 at the pole, which then spread out towards the three other poles where the field is B_0. The ratio R was precisely adjusted to an optimal value close to 3 [8]. Practically, in the range over which B_0 was varied (10 to 600 G), both the field at the center B_c and its gradient were proportional to B_0. The magnetization M of superparamagnetic beads was measured and the forces calibrated against a homogeneous viscous fluid [8,9]. In the regime described by Eq. (1), the response is proportional to the force, which ranged between 0.17 and 1.9 pN (see inset of Fig. 2). This suggests that such forces do not induce irreversible alteration of the local network. In addition, the power law holds well for displacements that do not exceed a critical distance of the order of 10 to 20 μm. When the duration and the intensity of the force are such that the displacement exceeds this limit, the velocity is reduced (Fig. 2). For larger displacements, one observes a strong influence of the pre-existing disorder of the semidilute solution [13], and also history dependence (data not shown).

We prepared a highly purified and concentrated monomeric actin from chicken breast muscles, following standard methods described in [15]. Actin acetone powder was first prepared from chicken breast muscles, from which actin was then extracted through two cycles of polymerization-depolymerization. High salt washes were performed with 0.65 M KCl during 30 min, and filaments were depolymerized by rapid overnight dialysis in G buffer (Tris-HCl 2 mM, pH = 8.0, Na-ATP 0.5 mM, β-mercapto-ethanol 0.5 mM, $CaCl_2$ 0.2 mM and NaN_3 0.01%) followed by ultracentrifugation. Highly purified G-actin solution was stored at $-80\,^{\circ}$C. Frozen aliquots were quickly thawed at 37 °C, ultracentrifuged at 500 kg for 5 min. The supernatant was assayed for actin concentration and kept on ice for same-day sample preparations. Samples were prepared by adding a solution of 1M KCl, 40 mM $MgCl_2$ and 20 mM Na-ATP to obtain final concentrations of 2.5 μM actin, 50 mM KCl, 2 mM $MgCl_2$ and 1 mM Na-ATP. 2.8 μm diameter beads (M280, streptavidin-coated, Dynal, Oslo) were typically dispersed to a final concentration of 10^5 ml^{-1} giving a 10^{-6} volume fraction. The samples were then immediately loaded into a flat capillary. The main experiment was performed at room temperature (20 °C ± 2 °C). Using rhodamine-phalloidin labeling [5], fluorescent filaments could be imaged, and their mean length was estimated to be 20 μm, which was an indication that the preparation was not contaminated by actin binding proteins hindering filament polymerization. The total concentration of contaminant proteins (larger than 5 kD) was less than 1% of the actin concentration, as checked by SDS-PAGE electrophoresis. Importantly, beads were mixed with monomeric actin at the onset of polymerization so that the network was not sheared by the introduction of the beads and was isotropic around them. Capillaries were attached to a slowly rotating wheel to avoid sedimentation during the polymerization, which was allowed to proceed for 30 min before measurements were done. Plain polystyrene as well as streptavidin-coated magnetic beads were simply diluted

from the surfactant containing stock solution to the final concentration through one or two intermediate dilutions in water. Additional treatments consisting of carefully removing the surfactant, and/or adsorbing a carrier protein (bovine serum albumin), to prevent possible sticking of the beads to the actin filaments, gave identical results. In addition, diffusion of 3.0 μm polystyrene beads gave similar results to those shown here with 2.8 μm diameter streptavidin-coated beads. This is a strong indication that the present observations are *not* resulting from the interaction between the filaments and the surface chemistry of the beads. This conclusion is also reinforced by the observation of free diffusive behavior of beads with $d/L < 1$ (Fig. 3). Typically, 30 min after the onset of polymerization, beads were allowed to diffuse for 5 to 60 min. The results also did not depend on whether the measurements were performed 30 min or 24 h after the onset of the polymerization, which indicates that the anomalous diffusion is not due to a modification of the network during the course of the measurement. We also verified that the subdiffusive behavior (2) was isotropic.

The exact form of observed anomalous power laws (1) and (2) is limited to semidilute solutions of rigid (wormlike) polymers such as f-actin. However, the approach presented here may be generalized for many other complex, inhomogeneous molecular systems. In particular, measurements of local diffusion and viscoelasticity provide information complementary to traditional macroscopic experiments [16]. One can deduce from them the local properties of molecular constituents. We have also demonstrated that the introduction of active feedback in a magnetic micromanipulator allows one to gather accurate data similar to those obtained, for instance, with optical traps [17]. Although still more difficult to use, the magnetic tweezers provide the possibility of exploring molecular media which are sensitive to heating or strongly diffusive, in which case the use of optical traps or light scattering techniques is very difficult. By scaling down the separation of the magnetic poles, the range of generated forces can also be extended by orders of magnitude. All these points are of particular interest for the study of biological systems, e.g., micromechanics and transport in the cytoplasm or the nature of cell motility.

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[12] For example, in the case of force free diffusion, one can estimate as in [14] that $\langle x^2 \rangle \cong \frac{2k_BT}{\pi\kappa^{1/4}}(\frac{5.5t}{4\pi\eta})^{3/4}$. Evaluating this expression for $t = 1$ s, and using a persistence length of 15 μm to evaluate κ, one finds $\langle x^2 \rangle = 0.27\ \mu\text{m}^2$. Experimentally, we find $\langle x^2 \rangle = 0.2 \pm 0.05\ \mu\text{m}^2$.

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