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The effect of myosin motor protein activity on the filamentous actin (F-actin) rheological response is studied using diffusing wave spectroscopy. Under conditions of saturating motor activity, we find an enhancement of longitudinal filament fluctuations corresponding to a scaling of the viscoelastic shear modulus $G_d(\omega) \sim \omega^{7/8}$. As the adenosine tri-phosphate reservoir sustaining motor activity is depleted, we find an abrupt transient to a passive, "rigor state" and a return to dissipation dominated by transverse filament modes. Single-filament measurements of the apparent persistence length support the notion that motor activity leads to an increase in the effective temperature for tangential motion.

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The globular protein actin plays an important role in cellular mechanics, motility, and morphology by polymerizing into filaments of diameter $d \simeq 7$ nm to form networks and higher-order structures. Because of their high bending rigidity κ, long persistence length $L_p = \kappa/k_bT \simeq 10{-}15$ μm, and ease of visualization, actin filaments are also important models for semiflexible polymer physics. Significant advances have been made in the understanding of actin rheology and its consequences for polymer theory, cellular organization, intracellular transport, and cell signaling [1–5]. To date, however, rheological and dynamical studies of F-actin have relied on probing passive reconstituted systems through either mechanical rheometry, light scattering, or microrheological techniques [3,6,7].

In frequency regimes governed by single polymer relaxation, the linear viscoelastic storage $G'(\omega)$ and loss $G''(\omega)$ shear moduli scale as $G'(\omega) \sim G''(\omega) \sim \omega^{3/4}$, a direct consequence of the dominant stress relaxation by end-to-end response upon affine deformation of the polymer [8,9]. This is accounted for by summing up transverse modes after assuming the incompressibility of the filament; however, if one could perturb the filament locally, the effects of finite propagation velocity of tension should give rise to a longitudinal response that scales as $\omega^{7/8}$ [4]. Such would be the case when pulling one end of the filament, or following the motion of a reticulation point between cross-linked filaments.

The mechanochemical activity of myosin molecular motors offers a unique opportunity to perturb F-actin filaments from their equilibrium thermal fluctuations both locally and anisotropically to study the resulting rheological response and relaxation mechanisms. In a reaction that consumes one adenosine tri-phosphate (ATP) per cycle, myosin binds to actin, produces a force through a mechanical "power stroke" by rotating a lever-arm domain parallel to the filament axis, then unbinds [10]. The actomyosin cycle proceeds perpetually as long as ATP is present. In the absence of ATP, myosin remains attached to filaments with a low dissociation $K_d = 500$ nM in what is known as the rigor state [11]. The actin-myosin cycle forms the basis of many cellular motility mechanisms, including the contraction of skeletal and smooth muscle. Motor activity may have profound effects on actin filaments and networks. Recently, it was shown that double-headed myosin fluidizes actin solutions by an enhancement of the slow relaxation of stress [12]. In addition, theoretical work suggests that motor activity introduces an active temperature T_{act} for tangential motion of filaments, which may enhance longitudinal modes [13]. Similar effects of activity have been reported for the dynamics of biological membranes [14], the two-dimensional analog of semiflexible filaments [15].

In this Letter, we report the rheology of F-actin using tracer particle microrheology with diffusing wave spectroscopy (DWS) [16] in the presence of *single-headed* myosin motor protein. We measure the linear viscoelasticity of "active" F-actin solutions on time scales that probe the relaxation of individual filaments from motor-driven modes. In addition, our experiments address a number of other important issues of actomyosin. For example, it is suggested that filament mechanics are altered by motor activity [17]. Such changes should modify the spectrum of fluctuations of the filaments, and could be identified through changes in the measured viscoelasticity. In addition, we discuss the implications of our work for the myosin power-stroke time scale and mechanism.

We will first summarize our experimental methods. Next, we present the dependence of the actomyosin viscoelastic response on ATP consumption, and examine the scaling behavior during motor activity. We conclude by deriving an expression for the active temperature and discussing the time scale and mechanism of the myosin power stroke.

Protein preparation and characterization.—Actin is purified from chicken skeletal muscle acetone powder using the method of Pardee and Spudich [18] (actin purity greater than 99% based on SDS-PAGE). Monomeric actin (G-actin) is stored at $-70\,^{\circ}$C in G-buffer [5 mM Tris-HCl pH 7.8, 5 mM ATP, 100 μM $CaCl_2$, 1 mM

dithiothreitol (DTT), 0.01% NaN_3]. Prior to experiments, we thaw the actin rapidly until completely melted and dialyze against 100 volumes of fresh G-buffer overnight at 4 °C. Sample-to-sample variation in the microrheological response of the actin when subjected to one freeze-thaw cycle is minimal provided a sufficient amount of DTT is present [19]. Myosin subfragment 1 (S1) is a single-headed motor consisting of the motor, converter, and lever-arm domains. To characterize the kinetics and rate of mechanochemical events in our system, we measure ATP hydrolysis by the same F-actin (13 μM) and myosin S1 (6.75 μM) in high-ATP (14.5 mM) F-buffer by absorbance at 650 nm of molybdenum blue.

Microrheology.—The mean-squared displacement $\langle \Delta r^2(t) \rangle$ of particles embedded in an F-actin sample is measured by DWS using a transmission geometry and plane-wave Ar^+ laser source [20]. From $\langle \Delta r^2(t) \rangle$, we calculate the shear modulus amplitude $G_d(\omega)$ and phase angle $\delta(\omega)$ of the F-actin [16]. Note that $G_d(\omega)$ represents the total resistance to deformation, while $\delta(\omega)$ characterizes whether the resistance is in-phase (viscous) or out-of-phase (elastic) with the rate of strain [21]. They are related to the storage and loss moduli by $G'(\omega) + iG''(\omega) = G_d(\omega)e^{i\delta(\omega)}$. Within a small correction $G_d(\omega) \sim \langle \Delta r^2(t) \rangle^{-1}_{t=2\pi/\omega}$ and $\delta(\omega) \sim [d \ln G_d / d \ln t]_{t=2\pi/\omega}$, which essentially gives the scaling of $G_d(\omega)$ with respect to ω [22].

Particles (0.98 μm carboxylated polystyrene, Bangs Laboratories) are washed to exchange surfactant and impurities. We incubate tracer particles for 2 hours in the G-actin solution to protein coat the bead surfaces by nonspecific adsorption. Immediately prior to polymerizing the actin, myosin S1 is added to a final concentration between 0.01 and 10 μM and Mg_2ATP to 14.3 mM. Each sample is polymerized in glass cuvettes ($L = 0.5$ cm) by raising the KCl concentration to 50 mM and $MgCl_2$ to 1 mM.

Single-filament microscopy.—A 5 μM solution of F-actin fluorescently labeled and stabilized with rhodamine-phalloidin in F-buffer (25 mM imidazole, 2 mM $MgCl_2$, 0.5 mM ATP, 50 mM KCl, 0.5 mM DTT, 0.01% NaN_3) is diluted 500-fold with F-buffer and 0.05 mg/ml glucose oxidase, 0.01 mg/ml catalase, 2.3 mg/ml glucose, and 2% β-mercaptoethanol added to reduce photobleaching. After adding myosin S1 to 6.6 μM and Mg_2ATP to 10 mM, a drop of solution is placed between two BSA-coated coverslips using 1 μM latex particles as spacers to limit filament motion in the focal plane [23]. We calculate the tangent correlation $\langle \mathbf{t}(s)\mathbf{t}(s + x) \rangle$ as a function of contour length x from digitized images of filaments using a tracing routine that iteratively fits intensity profiles of successive intersecting arcs with a Gaussian, providing subpixel resolution.

Active and rigor steady states.—We will first comment on the relationship between the kinetics of the actin-myosin reaction cycle and changes on the viscoelastic properties. During DWS measurements at high S1 concentration, we observe two dynamical regimes: an active steady state associated with ATP hydrolysis by actin-myosin and a final steady state due to myosin rigor (Fig. 1). At 5 μM S1, myosin activity during the active steady state dramatically alters the scaling behavior of the modulus, shown in Fig. 1 by the rotation downward in $G_d(\omega)$ and the corresponding shift upward in $\delta(\omega)$ relative to the actin-only sample. By contrast, the rigor state is marked by a nearly identical response for $\delta(\omega)$ when compared to actin alone, indicating a dominance of passive dissipation by the semiflexible filaments. Moreover, there is a uniform increase in $G_d(\omega)$ by a factor of 2 over actin only that can be accounted for from the increase of the filament hydrodynamic radius to 40 nm due to the attachment of rigor myosin, since there is no difference in L_p (Fig. 2).

The onset time of the transient from the active steady state to the rigor state is consistent with the depletion rate of the ATP reservoir by myosin activity. Our direct measurement of ATP hydrolysis gives a consumption rate of 6 μM/s for 6.7 μM S1, in agreement with

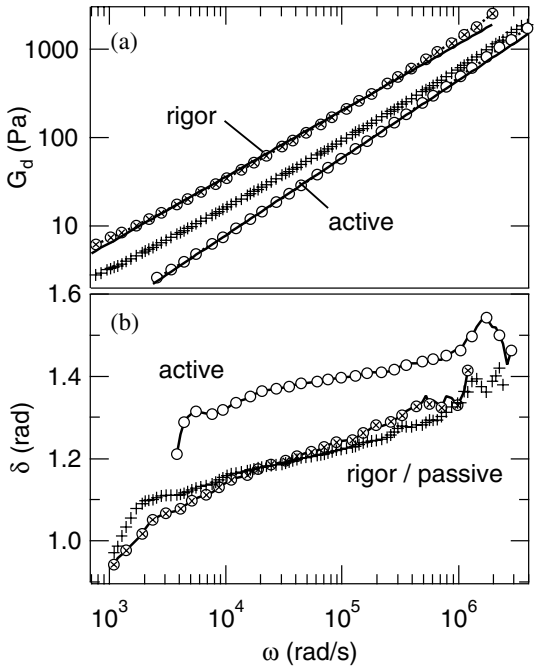

FIG. 1. The (a) viscoelastic shear modulus amplitude $G_d(\omega)$ and (b) phase angle $\delta(\omega)$ for 13.7 μM F-actin and 5 μM myosin S1 (open and crossed circles) compared to actin alone (crosses). During myosin activity (open circles), we observe a new scaling $G_d(\omega) \sim \omega^{7/8}$ (represented by the lower solid line) corresponding to $\delta(\omega) \simeq 1.37$. After depletion of the ATP reservoir (crossed circles), myosin binds to actin in the rigor state, with a factor of 2 increase in $G_d(\omega)$ and similar $\delta(\omega)$ compared to actin alone. The passive and rigor scaling agrees with previous reports, $G_d(\omega) \sim \omega^{3/4}$ (solid line compared to rigor data).

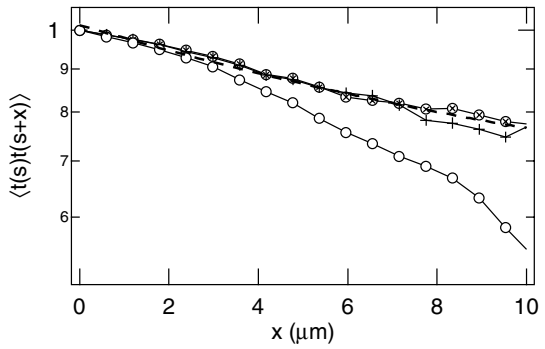

FIG. 2. Tangent correlation function $\langle \mathbf{t}(s)\mathbf{t}(s+x)\rangle = e^{-x/2L_p}$ for F-actin (crosses), F-actin with 6.6 μM S1 myosin with ATP (open circles), and F-actin and myosin without ATP (crossed circles). For passive and rigor conditions, we find $L_p \simeq 16.7 \pm 0.3$ μm (dashed line). Myosin activity decreases the apparent L_p by roughly a factor of 2.

the cyclic reaction model of actomyosin using rate laws determined from biochemical and motor assays [11]. Under the microrheology experimental conditions, the two highest S1 concentrations show transients from active to rigor at times consistent with ATP depletion predicted by the model. We observe abrupt transients at 25–36 min and 77–85 min for 10 and 5 μM, respectively, compared to 26–29 min and 52–58 min from the model. Below 5 μM we find only active steady state behavior for the duration of the experiments.

Dynamic shear modulus and scaling behavior.—Under passive and rigor conditions (Fig. 1), the scaling behavior of G_d is identical to previously reported theoretical and experimental values for reconstituted actin networks [2,7–9], where $G_d(\omega)$ scales as $\sim\omega^{3/4}$ giving rise to a phase angle $\delta(\omega) = (\pi/2)(3/4) \approx 1.17$. The amplitude $G_d(\omega) \sim 10$ Pa at 10^4 rad/s for actin only is in agreement with single-particle tracking studies using protein-coated beads [24].

In Fig. 3 we compare $G_d(\omega)$ and $\delta(\omega)$ for actin-only samples to the active steady states for several S1 concentrations. Increasing the myosin concentration enhances the measured response from passivelike behavior to an essentially saturated active behavior at 1, 3, and 5 μM S1. The phase angle with respect to myosin concentration, shown in Fig. 4, is bounded between $1.17 \leq \delta(\omega) \leq 1.39$. The upper value of $\delta(\omega)$ is in excellent quantitative agreement with the expected phase angle for longitudinal fluctuations generated by local pulling, where $G_d(\omega) \sim \omega^{7/8}$ and thus, $\delta(\omega) = (\pi/2)\ (7/8) \approx 1.37$ [4]. Liverpool and coworkers have suggested that the high-frequency modulus for active filaments should scale as $\omega^{1/8}$ [13]; however, we note that their results strictly apply only to systems where the myosin has aggregated into motile centers in the network, allowing filaments to slide relative to one another. Instead, the observed limits and intermediate responses suggest that the local tensions generated

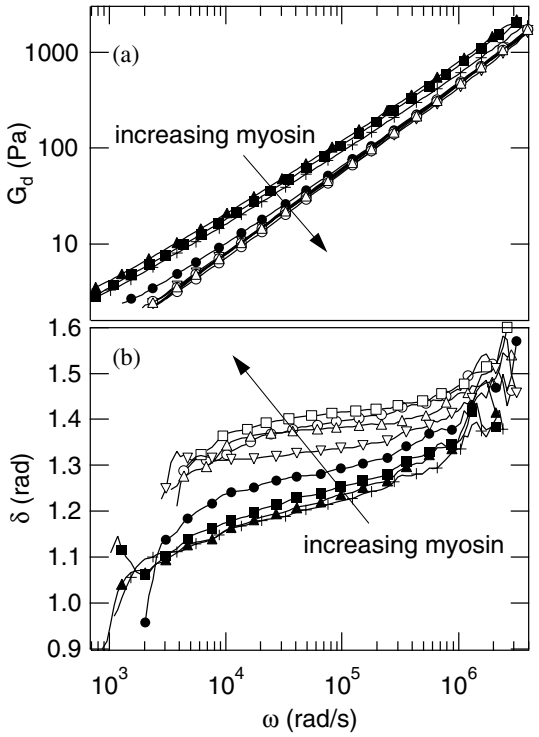

FIG. 3. (a) $G_d(\omega)$ and (b) $\delta(\omega)$ during the active steady state for 13.7 μM F-actin containing myosin S1 at 5 μM (open circle), 3 μM (open square), 1 μM (upwards open triangle), 0.5 μM (downwards open triangle), 0.1 μM (closed circle), 0.05 μM (closed square), and 0.01 μM (closed upwards triangle) compared to actin alone (crosses). The measured response rises monotonically with increasing S1, and saturates at $G_d(\omega) \sim \omega^{7/8}$ (see Fig. 4).

on actin by myosin contribute together with the global affine deformation due to the probe particles. However, the motor-driven excitation and dissipation of longitudinal modes dominates the response as the myosin concentration increases.

As mentioned previously, it has been suggested that motor activity in an actin solution can be modeled as an active temperature $T_{\rm act}$ for tangential motion of filaments, which could lead to the observed enhancement of the longitudinal modes [13]. To estimate $T_{\rm act}$, we assume the activity of the single-headed myosin generates a mechanical force on a filament by viscous drag on a length scale of the rotating lever-arm domain $a \simeq 10$ nm and time scale τ of magnitude $f_m \sim \eta a^2/\tau$, taking $\eta \simeq 10^{-3}$ Pa $\cdot$ s as the solvent viscosity. We estimate τ from the relaxation time scale of the power stroke initiated by the unlatching of the strained lever arm [10], $\tau \simeq \eta a/\kappa_m$, where $\kappa_m \simeq 0.1$ pN/nm is the measured myosin stiffness [25,26]. $T_{\rm act}$ is defined by the correlation function of the linear force dissipated by the filament ζ, $\langle \zeta_i(t,s)\zeta_j(t',s')\rangle = \eta k_b T_{\rm act}\delta_{ij}\delta(t - t')\delta(s - s')$. Setting the magnitudes of the forces equal, $T_{\rm act}/T \sim \eta a^3/k_b T\tau \simeq 10$. An active temperature due to myosin activity also implies that the apparent persistence

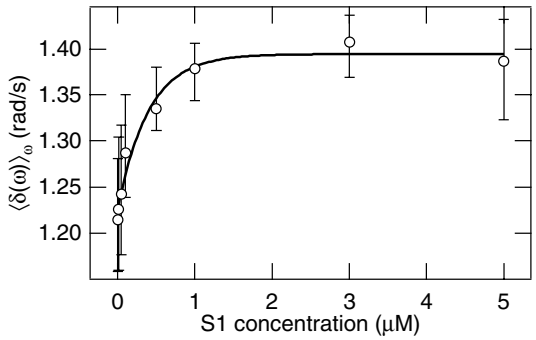

FIG. 4. Averages of $\delta(\omega)$ over $10^4 < \omega < 10^6$ for each S1 concentration demonstrates that the microrheological response increases exponentially with saturation at $\delta(\omega) = 1.39 \pm 0.04$ and characteristic concentration 0.4 ± 0.1 μM. $\delta(\omega) \simeq 1.17$ corresponds to $G_d(\omega) \sim \omega^{3/4}$ and $\delta(\omega) \simeq 1.37$ to $G_d(\omega) \sim \omega^{7/8}$. The error bars represent minimum and maximum values over the averaged frequency range.

length will be lower [27], which is confirmed by single-filament measurements, shown in Fig. 2.

It is important to note that the time scales over which we measure changes in the response are short compared to myosin power-stroke durations inferred from biochemical assays ($\tau_{\rm ps} \sim 1$ ms) [28], but are consistent with a "latched-spring" model of the rotating lever arm [10,11]. According to this model, the power stroke is initiated upon release of the ATP γ-phosphate which functions as a latch for the strained prestroke state [10]. In contrast to our dynamical measurements, the temporal resolution of biochemical assays are limited to the entire duration of myosin attachment, which includes the rate-limiting pre- and poststroke states. Finally, although cooperative structural modifications of actin upon myosin binding have been reported [17], the measured active response is consistent with hydrodynamic forces generated by the rotation of the myosin lever arm. Thus, it is unlikely that local mechanical changes of filament are behind the active response. In addition, we do not find changes in the filament persistence length due to rigor binding.

In this Letter we reported the viscoelastic response of semidilute F-actin solutions in the presence of active myosin. We observe two steady states, the first corresponding to active ATP hydrolysis and a second rigor state reached after the depletion of free ATP. The transient between active and rigor is in good agreement with the known actomyosin enzyme kinetics. In the rigor state, samples exhibit filament mechanics and rheological properties characteristic of passive semiflexible dynamics. The response in the active state is in excellent agreement with the scaling of longitudinal modes, $G_d(\omega) \sim \omega^{7/8}$, consistent with an active temperature generated by random mechanochemical force events along the filament axes, and confirms the notion that local tension propagates along semiflexible filaments at a finite velocity.

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