The actin-activated Mg\(^{2+}\) -ATPase activity of Acanthamoeba myosin II minifilaments is dependent both on Mg\(^{2+}\) concentration and on the state of phosphorylation of three serine sites at the C-terminal end of the heavy chains. Previous electric birefringence experiments on minifilaments showed a large dependence of signal amplitude on the phosphorylation state and Mg\(^{2+}\) concentration, consistent with large changes in filament flexibility. These observations suggested that minifilament stiffness was important for function. We now report that the binding of nucleotides to dephosphorylated minifilaments at Mg\(^{2+}\) concentrations needed for optimal activity increases the flexibility by about 10-fold, as inferred from the birefringence signal amplitude increase. An increase in flexibility with nucleotide binding is not observed for dephosphorylated minifilaments at lower Mg\(^{2+}\) concentrations or for phosphorylated minifilaments at any Mg\(^{2+}\) concentration examined. The relaxation times for minifilament rotations that are sensitive to the conformation myosin heads are also observed to depend on phosphorylation, Mg\(^{2+}\) concentration, and nucleotide binding. These latter experiments indicate that the actin-activated Mg\(^{2+}\) -ATPase activity of Acanthamoeba myosin II correlates with both changes in myosin head conformation and the ability of minifilaments to cycle between stiff and flexible conformations coupled to nucleotide binding and release.

The heavy chain component of the multiple members of the myosin superfamily share a highly homologous head (but with sufficient sequence differences to allow their classification into 10 or 11 families) connected to highly variable tails (1). The ATP- and actin-binding sites, actin-activated Mg\(^{2+}\) -ATPase activity, and in vitro motility activity all reside in the conserved head domain. The tail is generally thought to determine the supramolecular organization of the myosins and their associations with specific cell structures and organelles. For example, the two N-terminal heads of type II myosin heavy chains are attached to a long rodlike C-terminal tail that self-associates into an α-helical coiled-coil and subsequently assemble into a bipolar filament (the heavy chains of other myosin classes either remain monomeric or form dimers but do not form filaments).

The heavy chains of most class II myosins can be proteolytically cleaved to light meromyosin (LMM), the C-terminal end of the α-helical, coiled-coil rod, and heavy meromyosin (HMM), the N-terminal portion of the α-helical, coiled-coil rod with its two attached globular heads (2). LMM retains the filament-forming properties of native myosin II, and HMM retains all of the catalytic activity and in vitro motility activity. HMM can be further proteolyzed to two monomers of subfragment 1 (S1), a single globular head with an α-helical C-terminal tail, and subfragment 2 (S2), the α-helical, coiled-coil portion of HMM. The individual S1 fragments retain most of the properties of HMM.

Class II myosins contain two pairs of light chains, essential light chains (ELC) and regulatory light chains (RLC), with one of each pair attached to the helical tail of each S1 domain (3). Some (molluscan) native type IIMyosins are activated by direct binding of Ca\(^{2+}\) to the ELC and others (smooth muscle, vertebrate nonmuscle, and Dictyostelium) by phosphorylation of the RLC (4). The light chains stabilize the helical tail of S1 (3, 5), and removal of the light chains or the helical tail of S1 greatly reduces the ability of S1 to move actin filaments in an in vitro motility assay (6), but neither the light chains nor the helical tail of S1 are necessary for maximal actin-activated Mg\(^{2+}\) -ATPase activity of S1. These data are consistent with the most recent structural model of the contractile cycle derived from x-ray crystallography of S1; conformational changes in the globular portion of S1 resulting from the binding and hydrolysis of ATP are transmitted through the ELC and generate a rotational movement of the helical tail of S1 (7). This model was supported by recent experiments that demonstrate tilting of the light chain region of the myosin head during muscle contraction (8).

It seems likely, however, that the head and tail domains of class II myosins are not as functionally independent as the foregoing brief summary might imply. For example, the myosin superfamily can be grouped into essentially the same classes based on the overall structure of their tails (1), suggesting co-evolution of the head and tail domains that would presumably result from their functional interactions. Moreover, the actin-activated Mg\(^{2+}\) -ATPase activities of S1 from both ELC-regulated and RLC-regulated muscle myosins are unregulated (whereas HMM is regulated), suggesting that at least a portion of the tails is necessary for the appropriate coupling of S1 head and light chain conformations (4).

Acanthamoeba myosin II provides a striking example of the functional interaction of the head and tail regions of a myosin II; both the actin-activated Mg\(^{2+}\) -ATPase activity and the in vitro motility activity of minifilaments of Acanthamoeba myosin II are inactivated by phosphorylation of 3 serine residues in a short (29 amino acids), nonhelical tail piece at the C-terminal tip of the α-helical, coiled-coil rod (9, 10). Extensive experimental light chain; HMM, heavy meromyosin; S1, subfragment 1; S2, subfragment 2; AMPPNP, 5'-adenylyl-imidodiphosphate; DTT, dithiothreitol; RLC, regulatory light chain.

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The abbreviations used are: LMM, light meromyosin; ELC, essential light chain; HMM, heavy meromyosin; S1, subfragment 1; S2, subfragment 2; AMPPNP, 5'-adenylyl-imidodiphosphate; DTT, dithiothreitol; RLC, regulatory light chain.

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Nucleotide-dependent Flexibility of Myosin II Filaments

tal data (11–13) show that the activity of each molecule in the filament depends not on its own phosphorylation state but on the level of phosphorylation of the filament as a whole. This led to the hypothesis that the state of phosphorylation at the tip of the tail affects the conformation of the hinge region at the HMM-LMM junction in the tails of adjacent molecules in the minifilament and, thereby, the interactions with F-actin of the S1 heads at the ends of the HMM arms. Electric birefringence studies on chymotrypsin-treated Acanthamoeba myosin II parallel dimers (14) indeed indicated that the tip of one monomer was in close proximity to the hinge region of the other.

Further electric birefringence experiments showed that the signals from minifilaments are fundamentally different from other signals from minifilaments are fundamentally different from other.

Recent experiments (16) showing that binding of ATP to Acanthamoeba myosin II enhances the rate of papain cleavage of the heavy chain of monomers in the region corresponding to that which interacts with the ELC in chicken myosin II (3) are consistent with the proposal (7) that ATP induces conformational changes in S1. However, ATP also promotes papain cleavage within the C-terminal tail of phosphorylated minifilaments (16); this was not anticipated. We now investigate the effects of nucleotides on the flexibility of phosphorylated and dephosphorylated minifilaments. The electric birefringence signal amplitude of dephosphorylated minifilaments indicates that an order of magnitude increase in flexibility accompanies nucleotide binding at 4–5 mM Mg$^{2+}$. The optimal concentration for both catalytic and in vitro motility activities for dephosphorylated minifilaments occurs at about 4–5 mM Mg$^{2+}$. In contrast, filaments of phosphorylated myosin, which are inactive at all Mg$^{2+}$ concentrations, remain flexible even at 4 mM Mg$^{2+}$.

Nucleotide-dependent Flexibility of Myosin II Filaments

EXPERIMENTAL PROCEDURES

Myosin Preparations—Acanthamoeba myosin II was isolated (17) and dephosphorylated by potato acid phosphatase as described (18). One portion of the dephosphorylated myosin II was then phosphorylated with partially purified myosin II heavy chain kinase (19). Dephosphorylated and phosphorylated myosin II were separated from phospha- tase and kinase, respectively, by Sepharose CL-4B gel filtration (13), dialyzed against 10 mM imidazole, pH 7.0, 0.1 mM KCl, 1 mM DTT, 10% sucrose, concentrated against solid sucrose, and stored at 4 °C. The extent of heavy chain phosphorylation was quantified as described by Garcia-Lamothe (13). Routinely, the incorporation was 3–5 pmol of myosin II. Myosin preparations were also characterized by their actin-activated Mg$^{2+}$-ATPase activities; only dephosphorylated myosin II was maximally active at 4–5 mM Mg$^{2+}$ at pH 7.4 than at 7.0. The previously reported signal amplitudes at 4 mM Mg$^{2+}$ at pH 7.0 require 4.5–5 mM at pH 7.4, depending on the specific myosin preparation. To avoid aggregation but also to achieve maximal signal amplitude and signal to noise ratio: standard conditions for the experiments reported here were a protein concentration of about 40 μM, a field strength E = 1.2 kV/cm, and a voltage pulse length of 140 μs. Optical signals from a single sample were averaged over 32 pulses. Occasionally, much longer pulse lengths (~800 μs) were used to ensure that more slowly relaxing birefringence components were not present. Signal amplitudes were also measured as a function of field strength E, up to about 2 kV/cm to verify that it scales with $E^2$. Signal amplitudes were also observed to vary linearly with protein concentration up to about 100 μg/ml.

Overview of the Electric Birefringence Signal from Minifilaments—Fig. 1 illustrates the general features of the electric birefringence signal of Acanthamoeba myosin II minifilaments reported previously (15). The overall signal is composed of at least two major components that are evident in the kinetics of both the signal rise when the electric field is applied (+E) and the decay after the field is removed (−E); (i) a com-

Fig. 1 illustrates the general features of the electric birefringence signal of Acanthamoeba myosin II minifilaments reported previously (15). The overall signal is composed of at least two major components that are evident in the kinetics of both the signal rise when the electric field is applied (+E) and the decay after the field is removed (−E); (i) a com-
The electric birefringence signal from minifilaments is characterized by a fast, positive birefringence component due to a component of the optical anisotropy that is perpendicular to the long axis (parallel to the dipole moment), and a more slowly relaxing, negative birefringence component due to an end-over-end tumbling rotation of the minifilament, illustrated in the lower right cartoon. Each component is characterized by an amplitude, A, and a relaxation time, τ. The overall signal amplitude, A_{total}, and the ratio of the birefringence change at the maximum, A_{max}, to the birefringence change at the minimum in the decay, ΔA_{decay}, are predicted to depend on the flexibility of the minifilaments.

Compared with this complicated set of signals from minifilaments, the electric birefringence signals from Acanthamoeba myosin II monomers and parallel dimers (14) are straightforward and provide a basis for understanding the minifilament signals. A large permanent dipole moment parallel to the axis of a filament comprising four LMM segments. The more slowly relaxing, negative birefringence component is due to an end-over-end tumbling rotation of the minifilament, illustrated in the lower right. Each component is characterized by an amplitude, A, and a relaxation time, τ. The overall signal amplitude, A_{total}, and the ratio of the birefringence change at the maximum, A_{max}, to the birefringence change at the minimum in the decay, ΔA_{decay}, are predicted to depend on the flexibility of the minifilaments.

Fig. 2. The effect of added ATP on the electric birefringence signal amplitude for dephosphorylated minifilaments in 5 mM Mg^{2+} at 20°C. The optical signal is shown normalized for field strength and protein concentration, δCE^2, as in Fig. 1. The start and end of the 140-μs long electric field pulse are indicated by the arrows. The increase in signal amplitude was about 10-fold with ATP binding. In addition to the Mg^{2+}, the samples also contained 1 mM KCl, 2 mM imidazole (pH 7.4), 1 mM DTT, 5% sucrose, and 40–50 μg/ml protein. The added ATP concentration was 250 μM.

Effect of Nucleotides on Signal Amplitude—Fig. 2 shows the effect of ATP on signal amplitudes in 5 mM Mg^{2+}. As found previously (15), the amplitudes of both the positive and negative birefringence signal components of dephosphorylated myosin II minifilaments in the absence of nucleotide are much smaller in 5 mM Mg^{2+} than in 2 mM Mg^{2+} (compare the upper curve in Fig. 2 to Fig. 1). The addition of ATP to dephosphorylated minifilaments in 5 mM Mg^{2+} increased the signal amplitude to a magnitude similar to that observed at the lower concentration of Mg^{2+} in the absence of nucleotide (compare the lower curve in Fig. 2 with Fig. 1).

The direction and magnitude of the effect of ATP on signal...
ADP was more consistent with a titration of binding sites; the total birefringence signal amplitude varied with the Mg$^{2+}$ concentration. In the absence of ATP but comparatively constant amplitude in its presence. In contrast, the birefringence signal amplitude of phosphorylated minifilaments was not affected either by the Mg$^{2+}$ concentration, as observed previously (15), or by the presence of ATP (Fig. 3, open symbols). The nucleotide concentration dependence of the amplitude of the birefringence signal for dephosphorylated myosin II minifilaments at 4.5 mM Mg$^{2+}$ is shown in Fig. 4 for ATP, ADP, and the nonhydrolyzable ATP analogue AMPPNP. The protein concentration in these experiments was 0.1 µM in myosin II monomers or 0.2 µM in S1 heads. The lowest concentration of AMPPNP used, 0.4 µM, was sufficient to achieve essentially the maximal increase in signal amplitude (Fig. 4, squares). Even when the protein concentration was increased to 0.35 µM S1, an AMPPNP concentration of 0.4 µM was still sufficient to obtain the maximal effect, i.e., even when the concentration of heads and AMPPNP were approximately equal (data not shown). Similarly, the addition of an approximately equimolar concentration of AMPPNP was sufficient to obtain the maximum decrease in the amplitude of the birefringence signal at 2 mM Mg$^{2+}$ (data not shown). These data indicate that the $K_d$ for AMPPNP binding is smaller than 0.2–0.4 µM, which is consistent with the $K_d$ of 0.09 µM that was estimated by differential scanning calorimetry.2

In contrast to the stoichiometric effect of AMPPNP, the increase in birefringence signal amplitude with added ATP or ADP was more consistent with a titration of binding sites between 0 and 4 µM nucleotide (Fig. 4). No further increase in signal amplitude was observed between 5 and 50 µM ADP or ATP (Fig. 4, inset). Consistent with this behavior, increasing the myosin concentration from 0.2 to 0.35 µM S1 significantly decreased the observed signal amplitude at 0.4 µM ADP and to a somewhat greater extent also at 0.4 µM ATP, but it had a much smaller effect at ADP and ATP concentrations higher than 1 µM (data not shown).

From the birefringence data, the $K_d$ for ADP is 0.1–2 µM, a not unreasonable value because ADP would be expected to bind to myosin more weakly than AMPPNP. In contrast to expectations, however, ATP also appears to bind to the myosin II minifilaments more weakly than AMPPNP. But, because the actin-independent Mg$^{2+}$-ATPase activity of dephosphorylated myosin II under the experimental conditions used for the electric birefringence measurements is 5–10 × 10$^{-3}$ s$^{-1}$, most of the ATP (for initial concentrations up to 4 µM) was likely hydrolyzed to ADP during the course of a typical 30–60-min experiment. Thus, the high $K_d$ estimated for ATP binding might actually have been for ADP binding instead.

**Relaxation Kinetics**—The relaxation time of the negative birefringence component of dephosphorylated minifilaments was insensitive to the total signal amplitude both in the presence and the absence of nucleotide. Fig. 5 shows a comparison of the signal decay for minifilaments in 3 and 4.5 mM Mg$^{2+}$, without added nucleotide and in 4.5 mM Mg$^{2+}$ with 5 µM AMPPNP. The average relaxation time, $t_{relax}$ was 235 ± 15 µs, corrected to water viscosity at 20 °C. The relaxation time of the slow component was also unchanged in 50 µM AMPPNP, 250 µM ATP, and 5 and 50 µM ADP (data not shown). Relaxation times of the negative birefringence component with and without added nucleotides at two Mg$^{2+}$ concentrations are summarized in Table I for both dephosphorylated and phosphorylated myosin II minifilaments. In general, the slow relaxation time is insensitive to myosin phosphorylation, Mg$^{2+}$ concentration, and nucleotide binding.

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2 M. Zolkiewski, M. J. Redowicz, E. D. Korn, and A. Ginsburg, unpublished observation.
The nucleotide-dependent flexibility of myosin II filaments was markedly dependent on both Mg
+2 and AMPPNP (at two Mg
+2 concentrations) for dephosphorylated myosin II at 4–5 mM Mg
+2. The increase in the fast relaxation time for dephosphorylated minifilaments at 4.5–5 mM Mg
+2 was about a factor of 10 smaller (32). The electric birefringence signal of native Acanthamoeba W
+HMM-LMM bend is essential for the average fast relaxation time, depending on the Mg
+2 concentration. The absence of a significant change in the relaxation time of the slow component, \( t_{\text{slow}} \), upon the addition of nucleotides to dephosphorylated minifilaments further indicates that nucleotide binding does not cause a large change in the overall structure of the minifilament. The basic structure, the number of monomers, the repeat distance between monomers, and the length of the central bare zone, is not significantly different in the presence or absence of nucleotide. In particular, there is no evidence for aggregation, consistent with analytical ultracentrifugation data (16). Therefore, the order of magnitude of increase in the signal amplitude with nucleotide binding at 4–5 mM Mg
+2 most probably results from a substantial increase in the flexibility of minifilaments. The decrease of \( -\Delta A_{\text{rise}}/\Delta A_{\text{decay}} \) at 4–5 mM Mg
+2 from 0.4 to 0.2 (Table I) with added nucleotide is also consistent with an increase in flexibility (28). This ratio will depend on stiffness through the kinetics of the bending motion and a value of ~0.2 is characteristic of other conditions that give approximately the same signal amplitude.

Because nucleotide binding can either increase or decrease the signal amplitude, depending on the Mg
+2 concentration, the effects of nucleotides and Mg
+2 are neither independent nor simply additive. Rather, both likely regulate the flexibility of either the same or tightly coupled sites. We previously suggested that the bending site was at the HMM-LMM junction rather than the S1-S2 junction or an elastic flexing of the S2 rod itself based on the estimated bending kinetics extracted from \(-\Delta A_{\text{rise}}/\Delta A_{\text{decay}}\). This has been confirmed by more recent experiments on Acanthamoeba myosin II rod and rods in which the bend at the HMM-LMM junction has been removed or modified by amino acid substitution. The results demonstrate that the S1 heads are not necessary to obtain full signal amplitude but that the “native” HMM-LMM bend is essential (32). The electric birefringence signal of native Acanthamoeba myosin II minifilaments almost certainly arises from bending at the HMM-LMM junction. Preliminary data also show that

\[ A_{\text{fast}}/A_{\text{slow}} \] of dephosphorylated minifilaments as the Mg
+2 concentration was increased from 3 to 4.5 mM was not reversed by adding nucleotide. In fact, the binding of ADP slightly increased this ratio even further. As with the average fast relaxation time, \( A_{\text{fast}}/A_{\text{slow}} \) is not tightly linked to signal amplitude and flexibility.

**DISCUSSION**

Structural Implications of the Change in Total Signal Amplitude—We previously reported that the electric birefringence signal amplitude of dephosphorylated Acanthamoeba myosin II minifilaments decreases significantly as the Mg
+2 concentration increases from 1 to 4 mM, i.e., to the concentration range necessary for the optimal expression of actin-activated Mg
+2-ATPase activity. We now find that the total signal amplitude is also dependent on nucleotide binding at the higher Mg
+2 concentrations. Upon the addition of low concentrations of ADP, ATP, or AMPPNP, \( A_{\text{total}} \) increases about 10-fold at 4–5 mM Mg
+2. This increase in signal amplitude is not simply due to an increase in the dipole moment that might accompany binding of a charged ligand because nucleotides cause a decrease in the signal amplitude for dephosphorylated minifilaments at Mg
+2 concentrations between 1 and about 3 mM. Moreover, nucleotides have no effect on the signal amplitude of phosphorylated minifilaments at 1–5 mM Mg
+2 concentration even though nucleotides bind as well to phosphorylated minifilaments as to dephosphorylated minifilaments.

In contrast, the relaxation time of the fast, positive birefringence component was markedly dependent on both Mg
+2 and nucleotide. Fig. 6 illustrates the -3.5-fold difference in the average fast component relative times, \( t_{\text{fast}} \), for dephosphorylated minifilaments in 4.5 mM Mg
+2 with 5 mM AMPPNP and in 3 mM Mg
+2 without added nucleotide (12 \( \mu \)M). This difference in relaxation times was observed even though the total signal amplitudes for these two experimental conditions were comparable. Average fast component relaxation times for other conditions of nucleotide and Mg
+2 concentration are summarized in Table I for dephosphorylated and phosphorylated minifilaments. The increase in the fast relaxation time for dephosphorylated minifilaments depended on the nucleotide species bound, ranging from 40–45 \( \mu \)s with bound ADP to 25–30 \( \mu \)s with AMPPNP and 20–25 \( \mu \)s with ATP. In contrast, the average fast relaxation time for phosphorylated minifilaments actually decreased somewhat with added ATP from 12–13 to 8–9 \( \mu \)s.

**Effect of Nucleotide on Other Birefringence Parameters—** Table I summarizes the effect of added nucleotides (ATP, ADP, and AMPPNP) at two Mg
+2 concentrations on several parameters extracted from the birefringence experiment for both dephosphorylated and phosphorylated minifilaments. In addition to the total signal amplitude, \( A_{\text{total}} \), and the relaxation times of the positive and negative birefringence components, \( t_{\text{fast}} \) and \( t_{\text{slow}} \), already discussed, two other parameters are also given in Table I. As noted previously (15), the difference in total signal amplitude between dephosphorylated and phosphorylated minifilaments at 4.5–5 mM Mg
+2 was accompanied by a difference in \( \Delta A_{\text{rise}}/\Delta A_{\text{decay}} \) (cf. Fig. 1) and a small difference in \( A_{\text{total}}/A_{\text{slow}} \). The nucleotide-dependent increase in \( A_{\text{total}} \) for dephosphorylated myosin II at 4–5 mM Mg
+2 was also coupled to a decrease in the ratio \( \Delta A_{\text{rise}}/\Delta A_{\text{decay}} \) from about 0.4 to 0.2. In contrast, the small increase in the ratio of the positive to negative birefringence amplitudes, \( A_{\text{fast}}/A_{\text{slow}} \), of dephosphorylated minifilaments as the Mg
+2 concentration was increased from 3 to 4.5 mM was not reversed by adding nucleotide. In fact, the binding of ADP slightly increased this ratio even further. As with the average fast relaxation time, \( A_{\text{fast}}/A_{\text{slow}} \) is not tightly linked to signal amplitude and flexibility.
nucleotides have no effect on the electric birefringence of the rods, as expected if nucleotides interact only with S1 heads.

The bending force spring constant of monomers in the minifilament can be estimated (28) from the magnitude of the electric birefringence signal, assuming that the bending is Gaussian and planar and occurs at the HMM-LMM junction and that the S1 heads make little contribution to the birefringence compared with the coiled-coil $\alpha$-helices of the rest of the molecule. For a bending spring constant $\alpha$ in units of energy/ radian$^2$ and thermal energy kT, reduced spring constants, $\alpha^2 = a^2/(2kT)$, for dephosphorylated minifilaments at 4–5 mM Mg$^{2+}$ with and without bound nucleotide are estimated as $-15$ and $-300$, respectively. These values correspond to root mean square angular fluctuations, $\langle \phi^2 \rangle$, of about 10 and 2°, respectively.

**Structural Implications of the Change in Relaxation Time**—In contrast to the relative insensitivity of $\tau_{\text{slow}}$ to Mg$^{2+}$ concentration and nucleotide binding, the relaxation time of the fast, positive birefringence component shows a substantial dependence on conditions (Table I). Even under conditions that give similar signal amplitudes, the average fast component relaxation times depend significantly on myosin phosphorylation, Mg$^{2+}$ concentration, and nucleotide species.

The decay of the positive birefringence component is through the spinning rotation of the long axis (cf. Fig. 1) and therefore is sensitive to the distribution of mass perpendicular to the filament axis, in particular to the angle between S2 rods and the minifilament axis, the angle between S1 and S2, the angle between S1 heads, and S1 conformation. These structural parameters are not as yet well enough characterized to justify hydrodynamic calculations. Given the comparative insensitivity of $\tau_{\text{fast}}$ to experimental conditions (Table I), however, the changes in $\langle \tau_{\text{fast}} \rangle$ likely reflect changes either in S1 conformation or in the orientation of S1 relative to the minifilament axis, rather than in S2. A change in isolated S1 head conformation with nucleotide binding has been observed previously (33–35).

**Correlation Between Structural Data and Actin-activated Mg$^{2+}$-ATPase Activity**—We had previously (15) proposed that minifilament stiffness is directly related to enzymatic activity for three reasons: the actin-activated Mg$^{2+}$-ATPase activity of dephosphorylated myosin II minifilaments is maximally expressed at 4–5 mM Mg$^{2+}$ when the filaments show substantial stiffness; phosphorylation greatly inhibits the actin-activated Mg$^{2+}$-ATPase activity and simultaneously inhibits the stiffening of minifilaments at 4–5 mM Mg$^{2+}$; and the nonlinear dependence of enzymatic activity of minifilament copolymers on the ratio of phosphorylated and dephosphorylated monomers parallels their change in stiffness. The present data require modification of this proposal. In the presence of 5 mM Mg$^{2+}$ and 250 $\mu$M ATP, minifilaments of catalytically active, dephosphorylated myosin II are a factor of 10 more flexible than without bound ATP and only a factor of 2 stiffer than inactive, phosphorylated minifilaments. Thus, the present data suggest that enzymatic activity may correlate more closely with the ability of minifilaments to cycle between stiff and flexible conformations coupled to ATP binding, hydrolysis, and subsequent release. This transition between flexible and stiff conformations...
is consistent with the large difference in axial compressibility of muscle fibers under rigor and relaxed conditions (36, 37). It is also consistent with recent proposals based on spectroscopic experiments (38, 39) that the myosin structural transition from the weakly actin bound, prefence state (or states) to the strongly bound rigor state is coupled to a dynamically disordered to ordered configurational change.

The substantial differences in <$\tau_{\text{fast}}$> seen with nucleotide binding and myosin phosphorylation are perhaps even more closely linked to enzymatic activity, given the dependence on nucleotide species, i.e., actin-activated Mg$^{2+}$-ATPase activity may also correlate with nucleotide-dependent conformational changes in S1 or at the S1-S2 junction. Because there is no direct correlation between <$\tau_{\text{fast}}$> and $A_{\text{total}}$, the postulated nucleotide-dependent changes in S1 structure (and enzymatic activity) are not simply or directly coupled to changes in filament flexibility. The nucleotide-dependent conformational changes in S1 inferred from the electric birefringence are entirely consistent with the current model for the cross-bridge activation. Changes in proteolytic susceptibility have also shown that the conformation of the S1 head is coupled to C-terminal phosphorylation (40) and that the conformation of the C-terminal tail is coupled with nucleotide binding to the S1 head (16). Other than that both demonstrate that the opposite ends of the myosin II molecule are linked; however, there is no firm basis for comparing the conformational changes detected by limited proteolysis and those detected by electric birefringence.

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