MI1IQ is a complex of calmodulin and an epitope-tagged 85-kDa fragment representing the amino-terminal catalytic motor domain and the first of 6 calmodulin-binding IQ domains of the mammalian myosin I gene, rat myr-1 (130-kDa myosin I or MI130). We have determined the transient kinetic parameters that dictate the ATP hydrolysis cycle of mammalian myosin I by examining the properties of MI1IQ. Transient kinetics reveal that the affinity of MI1IQ for actin is 12 nM. The ATP-induced dissociation of actin-MI1IQ is biphasic. The fast phase is dependent upon [ATP], whereas the slow phase is not; both phases show a Ca2+ sensitivity. The fast phase is eliminated by the addition of ADP, 10 µM being required for half-saturation of the effect in the presence of Ca2+ and 3 µM ADP in the absence of Ca2+. The slow phase shares the same rate constant as ADP release (8 and 3 s⁻¹ in the presence and absence of Ca2+, respectively), but cannot be eliminated by decreasing [ADP]. We interpret these results to suggest that actin-myosin I exists in two forms in equilibrium, one of which is unable to bind nucleotide. These results also indicate that the absence of the COOH-terminal 5 calmodulin binding domains of myr-1 do not influence the kinetic properties of MI130 and that the Ca2+ sensitivity of the kinetics are in all likelihood due to Ca2+ binding to the first IQ domain.

MI1IQ was expressed in baculovirus and purified from insect lysates as described in the accompanying article (8). MI130 was prepared from rat liver as described previously (3). Rabbit skeletal muscle actin was prepared according to (11) and, in some cases, labeled with pyrene at Cys-374 according to Ref. 12. Muscle actin was prepared according to (11) and, in some cases, labeled with pyrene at Cys-374 according to Ref. 12.

EXPERIMENTAL PROCEDURES

Proteins—MI1IQ was expressed in baculovirus and purified from insect lysates as described in the accompanying article (8). MI130 was prepared from rat liver as described previously (3). Rabbit skeletal muscle actin was prepared according to (11) and, in some cases, labeled with pyrene at Cys-374 according to Ref. 12.

Transient Enzyme Kinetics—All kinetic experiments were performed at 19.8 °C in 20 mM MOPS, 100 mM KCl, 5 mM MgCl2, 1 mM dithiothreitol, and 1 mM CaCl2.

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‡ The abbreviations used are: MI1IQ, epitope-tagged motor domain and first IQ domain of 130-kDa myosin I; MI130, 130-kDa myosin I; A, actin; M, myosin; MI, myosin I; MOPS, 4-morpholinepropanesulfonic acid; D, ADP; T, ATP; S1, subfragment 1; sm, smooth muscle myosin.
Expressed Truncated Mammalian Myosin I: Kinetics

The transient kinetics of the interaction of MI1IQ with actin and ATP were examined and compared with our previous measurements on the native protein, MI130 (7). In all cases the results were very similar. Fig. 1A shows the fluorescent transient observed upon addition of 100 μM ATP to a 25 nm complex of MI1IQ and pyrene-labeled actin in the presence of Ca2+. The transient was well described by a single exponential with a kobs of 2.4 s⁻¹ and corresponds to the dissociation of actin from the complex. At low ATP concentrations (25–200 μM), the reaction was monophasic and kobs was linearly dependent upon [ATP] with an apparent second order rate constant, K[M]k₄, of 5.3 × 10⁴ M⁻¹ s⁻¹. Above 200 μM ATP, the reaction became biphasic and could be described by a two exponential term (Fig. 1B). The slow phase was independent of [ATP] with a kobs of 8 s⁻¹ and an amplitude of 25–30% of the total transient. The fast phase showed a hyperbolic dependence upon [ATP], and the best fit gave a maximum observed rate constant of 79 s⁻¹ with 1.5 mM ATP.

**Fig. 1.** ATP-induced dissociation of pyrene-labeled actin-MI130. A and B, 25 nm MI130 was incubated with 25 nm phallolidin-stabilized pyrene-labeled actin and pyrene fluorescence was measured immediately following addition of ATP in the presence of Ca²⁺. A, dissociation of actin-MI130 by 100 μM ATP resulted in an increase in fluorescence and the best fit to a single exponential is superimposed with kobs = 2.4 s⁻¹ and an amplitude of 28.4%. B, increasing ATP to 2.5 mM gave a biphasic reaction with kobs = 42.3 and 6.4 s⁻¹ with amplitudes of 21.8 and 8.3%, respectively. C, the dependence of kobs on [ATP] and Ca²⁺. The measurements were repeated in either 1 mM EGTA (○), 0.1 mM free Ca²⁺ (+Ca²⁺; squares), and kobs was plotted as a function of [ATP]. The best fits to kobs = K[M]k₄[ATP](1+K[A]) for the fast phase (closed symbols) are superimposed. The data were well described by hyperbolas with maximal observed rates kobs of 79 s⁻¹ and 36 s⁻¹ and 1.5 mM and 1.2 mM ATP required for half-maximal saturation (1/K[A]) with and without Ca²⁺, respectively. The values for kobs for the slow phase (open symbols) are not fitted.

**RESULTS**

The transient kinetics of the interaction of MI1IQ with actin and ATP were examined and compared with our previous measurements on the native protein, MI130 (7). In all cases the results were very similar. Fig. 1A shows the fluorescent transient observed upon addition of 100 μM ATP to a 25 nm complex of MI1IQ and pyrene-labeled actin in the presence of Ca²⁺. The transient was well described by a single exponential with a kobs of 2.4 s⁻¹ and corresponds to the dissociation of actin from the complex. At low ATP concentrations (25–200 μM), the reaction was monophasic and kobs was linearly dependent upon [ATP] with an apparent second order rate constant, K[M]k₄, of 5.3 × 10⁴ M⁻¹ s⁻¹. Above 200 μM ATP, the reaction became biphasic and could be described by a two exponential term (Fig. 1B). The slow phase was independent of [ATP] with a kobs of 8 s⁻¹ and an amplitude of 25–30% of the total transient. The fast phase showed a hyperbolic dependence upon [ATP], and the best fit gave a maximum observed rate constant of 79 s⁻¹ with 1.5 mM ATP.

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ATP required for half saturation of $k_{obs}$ (see Fig. 1C). By analogy with other actomyosin systems, the maximal rate and the ATP concentration at half the maximal rate were assigned to $k_{+2}$ and $1/K_1$, respectively, the rate constant for an isomerization of the actin-actomyosin complex, which limits the dissociation of actin and the affinity of ATP for the actin-actomyosin complex (Table I).

Repeating the measurement in the absence of Ca$^{2+}$ gave similar results, except that the slow component of the transient comprised 50% of the total amplitude with $k_{obs}$ of 3.4 s$^{-1}$ and the best fit to the ATP dependence of the $k_{obs}$ of the fast component gave $k_{+2} = 36$ s$^{-1}$ and $1/K_1 = 1.2$ mM for an apparent second order rate constant ($-Ca^{2+}$) of $3 \times 10^7$ M$^{-1}$ s$^{-1}$ (Fig. 1C).

We had previously observed for MI$^{130}$ that the fast phase of the transient was eliminated by incubating the proteins with 20 μM ADP before initiating the reaction by mixing with ATP. A similar result was observed here for the expressed myosin I fragment (Fig. 2A). In this case we were able to titrate the fast phase of the transient, and a plot of the amplitude against [ADP] in the presence and absence of Ca$^{2+}$ is shown in Fig. 2B. A fit of the amplitude to a hyperbola gave a best fit of 10 and 3 μM, respectively, in the presence and absence of Ca$^{2+}$, and this is assigned to the affinity of ATP for the A.MI$^{114}$ complex ($K_{AD}$). The $k_{obs}$ of the slow phase remained almost constant over the range of [ADP] used (8.5 s$^{-1}$ in Ca$^{2+}$; 2.45 s$^{-1}$, -Ca$^{2+}$) and was assigned to $k_{-AD}$, the rate of ADP dissociation from A.MI$^{114}$.ADP. Since $K_{AD} = k_{-AD}/k_{+AD}$, it is possible to calculate $k_{+AD}$, the apparent second order rate constant of ADP binding to A.MI$^{114}$. This gave values of $0.8 \times 10^7$ M$^{-1}$ s$^{-1}$ (+Ca$^{2+}$) and $1.1 \times 10^6$ M$^{-1}$ s$^{-1}$ (-Ca$^{2+}$), i.e. the apparent second order rate constant of ADP binding is Ca$^{2+}$ independent and 15–40 fold faster than the apparent second order rate constant of ATP binding to A.MI$^{114}$.

To verify that the rate of ADP binding is faster than ATP binding, we examined the rates of competitive binding of ADP and ATP to A.MI$^{114}$. A.MI$^{114}$ at 30 nM was mixed in the stopped flow apparatus with 1 mM ATP and 0–40 μM ADP. The amplitude of the fast phase decreased, whereas $k_{obs}$ increased with increasing [ADP] (data not shown). This demonstrates that ADP effectively competes with ATP even at less than one-tenth of the concentration. If the rate of ADP dissociation from the A.M.D complex is much less than the rate at which ATP and ADP bind to A.M (i.e. $[A.M.D] \cdot k_{-AD} \ll [ATP] \cdot K_{AD} + [ADP] \cdot k_{+AD}$, then $k_{obs} = [ATP] \cdot K_{AD} + [ADP] \cdot k_{+AD}$. At a fixed ATP concentration, $k_{obs}$ increased from 23 to 42 s$^{-1}$ at 20 μM ADP and further increased to 73 s$^{-1}$ at 40 μM. The data over this limited range are therefore compatible with a value of $k_{+AD}$ of $0.95 \pm 0.25 \times 10^6$ M$^{-1}$ s$^{-1}$ in agreement with the estimate above.

The slow phase of the ATP-induced dissociation of A.MI$^{114}$ has a $k_{obs}$ that is very similar to $k_{-AD}$, the rate constant of ADP dissociation from the A.MI$^{114}$-D complex even in the absence of added ADP. It is possible, therefore, that the slow phase rep-

### Table I

**Transient kinetic analysis of native rat MI$^{130}$ and expressed fragment MI$^{114}$**

|                          | Rate/eq constant | Units                  | MI$^{130}$ (7) | MI$^{114}$ | Rb S1 (222) |
|--------------------------|------------------|------------------------|----------------|------------|-------------|
|                          |                  |                        | +Ca$^{2+}$ | -Ca$^{2+}$ | +Ca$^{2+}$ | -Ca$^{2+}$ |
| Nucleotide binding to acto-M | $K_1 \cdot k_{+2}$ | μM$^{-1}$ s$^{-1}$ | 0.023 | 0.017 | 0.053 | 0.03 | 2.1 |
|                          | $1/K_1$          | min                    | 3.2 | 1.9 | 1.5 | 1.2 | 5.7 |
|                          | $k_{+2}$         | s$^{-1}$               | 74 | 32.5 | 79 | 36 | 12,000 |
|                          | $K_{AD}$         | μM                    | ≤10 | ≤10 | 10 | 3 | 200 |
|                          | $k_{-AD}$        | s$^{-1}$               | 6 | 2 | 8.0 | 3.5 | >500 |
| Actin binding            |                  |                        |              |            |            |            |
|                          | $K_{AD}$         | nm                     | 13 | 13 | 12 | 30 | 1000 |
|                          | $k_{AD}$         | nm                     | 60–110 | 60–110 | 1200 |
| ATP binding              |                  |                        |              |            |            |            |
|                          | $K_1 \cdot k_{+2}$ | μM$^{-1}$ s$^{-1}$ | 0.1 | 0.1 | 0.1 | 0.1 | 2.3 |
|                          | $k_{-AD}$        | s$^{-1}$               | 0.8 | 1.1 | 0.023 | 0.017 | 0.053 | 0.03 | 2.1 |

![Fig. 2](http://www.jbc.org/Downloaded from http://www.jbc.org/)

**Fig. 2. Influence of ADP on the ATP-induced dissociation of acto-MI$^{130}$**. A, addition of 25 mM ATP to 25 mM pyrene-actin-MI$^{130}$ in the absence of ADP resulted in a rapid increase in fluorescence best described by a double exponential ($k_{obs} = 43.8$ and 8.5 s$^{-1}$ and amplitudes of 18.8 and 9.4%, respectively). In the presence of 20 μM ADP, the change in fluorescence can be described by a single exponential with a $k_{obs} = 10.3$ s$^{-1}$ and an amplitude of 19.8%; however, the data can be equally described by a double exponential with $k_{obs} = 37.7$ and 8.5 s$^{-1}$ and amplitudes of 3.8% and 17.3%, respectively. B, titration of the amplitude of the fast phase against [ADP] added to the protein before mixing with ATP in the presence and absence of Ca$^{2+}$. The data were fitted to a hyperbola in each case, and the apparent affinity ($K_{AD}$) for ADP is 10 μM in the presence of Ca$^{2+}$ and 3 μM in its absence.
expressed a fraction of A-MI<sub>11Q</sub> that is isolated with ADP bound. Extensive treatment of the protein with apyrase did not eliminate the slow phase of the reaction. In contrast, if the protein was treated with 20 mM ADP such that the fast phase was eliminated, then treatment with apyrase restored the fast phase but only to the same extent as in the original measurement. Thus, apyrase treatment does eliminate the protein bound ADP effectively. We therefore conclude that the slow phase is not caused by the presence of ADP bound to the protein.

Another possibility is that ATP could be the source of contaminating ADP. ATP normally contains about 1% ADP. If ADP binds to the protein faster than ATP (as shown above), then the contaminant ADP could bind a fraction of the protein to produce the slow phase. The true substrate for myosin is MgATP, and the product is MgADP. Since ATP binds Mg<sup>2+</sup> more tightly than ADP, reducing the free Mg<sup>2+</sup> concentration to a minimum should reduce the contaminant MgADP concentration; however, under limiting Mg<sup>2+</sup> concentrations, the slow phase remained constant (data not shown). Furthermore, addition of up to 5% ADP into the ATP had no effect on the amplitude of the slow phase (see above). These results indicate that the slow phase is not due to ADP contamination in the ATP.

The effect of Ca<sup>2+</sup> on the rate of ADP release from MI<sub>13O</sub> was measured by determining in buffers containing fixed amounts of Ca<sup>2+</sup>, the rate of the ATP-induced dissociation of pyrene-labeled actin-MI in the presence of saturating amounts of ADP (Fig. 3). The <i>k<sub>obs</sub></i> was plotted as a function of [Ca<sup>2+</sup>], and the line represents the best fit of the MI<sub>11Q</sub> data to the Hill equation with a Ca<sup>2+</sup> affinity of 6.9 ± 0.8 μM with <i>k<sub>obs</sub></i> in the presence and absence of Ca<sup>2+</sup> of 8.3 ± 0.4 and 1.5 ± 0.2 s<sup>-1</sup>, respectively. The Hill coefficient was not well defined by the data, and an acceptable fit could be achieved with any value from 2 to 5.

**DISCUSSION**

Our results demonstrate that MI<sub>11Q</sub> is essentially indistinguishable from MI<sub>13O</sub> in terms of its interaction with actin and nucleotide, indicating that the presence of the 5 additional IQ domains and associated calmodulins in the parent molecule has no effect on these properties (Table I). The truncated myosin I shows the same actin-activated Mg<sup>2+</sup>-ATPase activity as the parent molecule (8) and the ATP, ADP, and actin binding to A-MI<sub>11Q</sub> are identical to the parent. Since the actin and nucleotide binding to MI<sub>11Q</sub> are unchanged, the coupling between actin and nucleotide binding is also expected to remain unaffected by the missing IQ domains and their associated calmodulins. In addition, the
Ca\(^{2+}\) sensitivity of all of the above properties is identical to that of the parent molecule, MI\(^{130}\). These results demonstrate that the Ca\(^{2+}\) sensitivity of these properties is not a function of the missing calmodulins and must result from Ca\(^{2+}\) binding to the first calmodulin or from a novel independent Ca\(^{2+}\)-binding site in the motor domain.

All of the events in the acto-MI ATPase cycle that we have measured are significantly faster than the turnover rate and are therefore not rate-limiting in the ATPase reaction. It is therefore likely that P\(_i\) release is rate-limiting, as has been observed for other myosins, and in this case the P\(_i\) release must be regulated by Ca\(^{2+}\), as previously observed for scallop muscle myosin II (19). A biphasic fluorescent transient was observed upon introduction of ATP to complexes of pyrene-labeled actin with both MI\(^{130}\) and MI\(^{130}\). The amplitudes and rates of the two phases were similar for the two proteins, and in both cases the rate constant of the slow phase was very similar to the rate constant limiting ADP release from A-MI. To address the possibility that the slow component was due to ADP present either in association with the expressed myosin I or as a trace contaminant in the ATP, several experiments designed to reduce ADP levels were performed. In all cases, the slow phase remained unaltered.

For the native protein we considered the alternative possibility that the slow phase in the ATP dissociation reaction was due to the presence of a fraction of damaged myosin I, e.g. missing one or more of the calmodulins, or that a contaminant myosin I could be present and therefore responsible for the biphasic nature of the ATP-induced dissociation. It seems unlikely, however, that the same contaminant would be present in both myosin I preparations (i.e. the native protein isolated from liver and the expressed fragment from insect cells) since they derive from different cell types and involve different purification schemes. We are therefore forced to conclude that this represents an intrinsic property of both the expressed protein and the parent MI\(^{130}\).

The simplest explanation of the biphasic nature of the ATP-induced dissociation is that the protein exists in two forms: one to which ATP can bind readily (at an apparent second order rate constant of 5.3 \(\times 10^{4} \text{ M}^{-1} \text{ s}^{-1}\) in the presence of Ca\(^{2+}\)) and the other, which cannot bind ATP without first isomerizing. One possible model is shown in Fig. 5, where the nucleotide pocket must open before nucleotide can bind. (In this model, we have assumed a direct link between the opening of the nucleotide pocket and an ATP-induced structural change, i.e. “wagging” of the myosin neck; see below.) The rate constant for the opening of the pocket is very similar to the net rate constant of ADP dissociation suggesting a similar process limits ADP re-

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**Fig. 5. Proposed model depicting the isomerization of the nucleotide-binding pocket that must occur before ATP or ADP can bind or ADP can be released.** The conformational change is represented as a swing of the converter domain of the myosin head with respect to the actin binding domain, which is coupled to the accessibility of the nucleotide binding pocket. The data in Figs. 1 and 2 allow assignment of all of the rate and equilibrium constants. Analysis of the amplitudes of the ATP-induced dissociation reaction in the presence of Ca\(^{2+}\) shows a 60:30 ratio of the two forms. This ratio defines the equilibrium constant between the two forms of A-M, \(K_{eq}\), with a value of \(-2.5\). Since \(k_{\text{on}} = 8 \text{ s}^{-1}\) (the slow phase of ATP binding) and \(K_{eq} = k_{\text{on}}/k_{\text{off}}\), then \(k_{\text{off}} = 3.2 \text{ s}^{-1}\). In the absence of Ca\(^{2+}\), the amplitudes of the two phases are similar, consistent with the equilibrium lying closer to the closed form of A-M with \(K_{eq} = 1-2\) and since \(k_{\text{on}} = 3.4 \text{ s}^{-1}\), \(k_{\text{off}}\) is unchanged by Ca\(^{2+}\) at 3-4 s\(^{-1}\). Since (i) the rate constant of ADP dissociation from A-M-D (\(k_{\text{ADP}}\)) and the rate constant of the isomerization of A-M (\(k_{\text{iso}}\)) are similar and (ii) they are both reduced 2-3-fold on removal of Ca\(^{2+}\), it suggests that the two events are closely related and may represent the same isomerization of the A-M complex as shown. The displacement of ADP from A-M occurs in a single phase and suggests little occupancy of A-M-D; therefore, in both the presence and absence of Ca\(^{2+}\), \(K_{ADP}/K_{M} < 0.1\). The affinity of ADP for the complex, \(K_{ADP}\), is defined by \(K_{ADP} = K_{M}/K_{ADP}\) and has a value of 10 \(\mu\text{M}\) in the presence of Ca\(^{2+}\) and 3 \(\mu\text{M}\) in the absence of Ca\(^{2+}\). Although \(K_{ADP}\) and \(K_{M}\) are not defined individually, \(K_{ADP}\) is \(k_{\text{on}}/k_{\text{off}}\) and therefore \(K_{ADP} = K_{M}/k_{\text{off}}\) or, after rearranging, \(k_{\text{on}}/k_{\text{off}} = k_{\text{on}}/k_{\text{off}}\). Since \(k_{\text{off}}\) has a value of 8 \(\text{ s}^{-1}\) (Ca\(^{2+}\)) and 3.4 \(\text{ s}^{-1}\) (Ca\(^{2+}\)), the apparent ADP on rate, \(k_{\text{on}}/k_{\text{off}}\), has a value of 0.8 \(\times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}\) in the presence of Ca\(^{2+}\) or 1.1 \(\times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}\) in the absence of Ca\(^{2+}\). This is in good agreement with the directly measured values and is independent of calcium. In this interpretation the rate of the conformational change giving access to the site is Ca\(^{2+}\)-dependent but independent of ADP bound to the pocket, whereas reversal of the conformational change is ADP-dependent and Ca\(^{2+}\)-independent.
lease ($k_{1a} = k_{1a+} = 8 \text{ s}^{-1}$, Fig. 5). From the data presented in Figs. 1 and 2, we can define the rate and equilibrium constants of each of the transitions shown in Fig. 5. The assignment of the constants is described in the legend to Fig. 5, and the values are listed in Table II.

The evidence points toward a significant proportion (~30%) of the conformation with a closed nucleotide pocket being present in the absence of nucleotide. By increasing the rate constant of pocket closing, ADP causes almost all of the myosin I to be in the closed pocket form. In contrast, the presence of Ca2+ increases the proportion of the open pocket form by increasing the rate of the opening of the pocket in both the presence and absence of ADP. In terms of nucleotide binding, Ca2+ lowers the affinity of A-MI for ADP by increasing the net rate constant of ADP release ($k_{1a+}$). Ca2+ also increases the rate of ATP-induced dissociation of actin ($k_{2+}$). Scheme I and Table I from A-MI, but does not affect the affinity of ATP for A-MI ($K_{z}$). Thus, Ca2+ can stimulate the detachment of actin from A-MI-D by increasing the rate of ADP release and the rate of the subsequent dissociation by ATP. Note, however, that the effects of Ca2+ are small (in all cases, values do not differ by more than a factor of 3) and therefore probably do not represent an on/off switch but rather a modulator of activity.

At physiological nucleotide concentrations of 1 mM ATP and 10–50 mM ADP, the net rates of nucleotide binding to A-MI will be 18 s−1 for ATP and 10–50 s−1 for ADP in the absence of Ca2+. Thus, the A-MI that has released ADP is as likely to rebind ADP as to bind ATP. The low efficiency for binding ATP and detaching from actin supports our proposal that myosin I is designed for tension maintenance not motility [7]. The presence of Ca2+ increases the net rate of ATP binding ($K_{a} k_{2+}$) but does not alter the rate of ADP rebinding ($k_{1a+} K_{ADP}$).

The possibility that myosin I exists in two conformational states, as shown by the kinetic evidence presented here, is of great interest since an isomerization of A-MI-D can be responsible for the second displacement seen in laser trap assays for MI[10], brush border myosin I [6], a close relative to MI[10], and smooth muscle myosin S1 (smS1) [20]. It is also required for the structural changes or “tail wagging” seen in three-dimensional reconstructions from electron micrographs for some actomyosins including brush border myosin I and smS1 [9, 21]. If our assignment is correct, then the identified isomerization provides a direct link between the accessibility of nucleotide to its binding pocket and the mechanical transient and lends strong support for the role of nucleotide release in providing a strain-sensing mechanism [22]. We previously argued that a MI cross-bridge bearing the typical isometric tension could have ADP release reduced up to 100-fold. In the current model, the strain would act directly on the isomerization shown in Fig. 5. Any load or strain on the cross-bridge would inhibit the swing of the tail against the load. The effect would be to reduce $k_{1a+}$ and hence $K_{1a}$.

The Ca2+ sensitivity of the A-MI isomerization also establishes a link between the binding of Ca2+ (presumably to the remaining calmodulin), and the biochemical, structural, and mechanical events discussed above. If the protein isomerization is a mechanical sensor that slows down the rates of both ADP release and ATP binding in the presence of strain, then the effect of Ca2+ may be far more dramatic for a head bearing strain. Ca2+ could act on the strained head by binding to calmodulin to alter the elasticity of the calmodulin-IQ complex and so reduce the strain leading to acceleration of the isomerization, ADP release, and cross-bridge detachment. The other 5 calmodulin-IQ domains could also contribute to the Ca2+-assisted release of strain if they are elastically distorted in a strained cross-bridge. All that is required is for the Ca2+-induced change in calmodulin conformation to alter the length of each calmodulin-IQ domain such that the strain on a A-MI-D head is modulated. It has previously been noted that Ca2+ binding reduces the affinity of calmodulin for the IQ domain but probably not enough to cause calmodulin dissociation at cellular concentrations of calmodulin.

A remaining question about the A-MI conformation is its relation to conformational changes in MI alone and to similar structural changes in other myosins. To date we have no evidence for two conformations of MI in the absence of actin and the rate of ATP binding is reasonably fast (0.1 × 108 M−1 s−1); however, the fluorescent signal changes that monitor ATP binding are very small and the presence of a second component cannot be eliminated.

smS1 shows a similar mechanical and structural change on binding ATP to A-MI, yet a close examination of earlier data [22] shows no evidence for a second phase in the ATP-induced dissociation of A-smS1. There are two possible explanations for the lack of a second component. Either $K_{a}$ is much larger (>10), such that in the absence of ADP the closed conformation is not significantly occupied, or the rate of pocket opening is very fast ($K_{1a+} > 200$ s−1), such that the opening can only be observed at very high ATP concentrations. The first possibility is compatible with the smS1 data, where electron micrographs indicate an ADP-induced swing of the myosin neck. The second possibility is formally equivalent to a strain-dependent transition state for nucleotide binding and release proposed for skeletal muscle myosin [23]. The proposed model may therefore reflect a property of myosins in general. In this respect it is noteworthy that the neck of the related molecular motor, kinesin, has recently been proposed to adopt various nucleotide-dependent conformations when attached to its microtubule track [24]. The interpretation of our data and of the recent kinesin data allows for a strain-sensitive ADP to ATP exchange mechanism. In the kinesin mechanism, this could provide gating of the interaction between the two heads of kinesin. In the case of myosin I, it provides a mechanism for a single-headed molecule to maintain tension with low ATP turnover. If this myosin I clusters on a membrane or vesicle as has been proposed for Acanthamoeba myosin I [25], then a similar gating mechanism could also apply.

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REFERENCES

1. Coluccio, L. M. (1997) Am. J. Physiol. 273, C347–C359
2. Ruppert, C., Kroschewski, R., and Bahler, M. (1993) J. Cell Biol. 120, 1393–1403
3. Coluccio, L. M., and Conaty, C. (1993) Cell Motil. Cytoskel. 24, 189–199
4. Coluccio, L. M. (1994) J. Cell Sci. 107, 2279–2284
5. Williams, R., and Coluccio, L. M. (1994) Cell Motil Cytoskel. 27, 41–48
6. Veigel, C., Coluccio, L. M., Jones, J. D., Sparrow, J. C., Milligan, R. A., and Molloy, J. E. (1999) Nature 398, 530–533
7. Coluccio, L. M., and Geeves, M. A. (1999) J. Biol. Chem. 274, 21575–21580
8. Perreault-Micale, C., Shushan, A. D., and Coluccio, L. M. (2000) J. Biol. Chem. 275, 21618–21623
9. Jones, J. D., Wilson-Kubalek, E. M., and Milligan, R. A. (1995) Nature 378, 751–753
10. Jones, J. D., and Milligan, R. A. (1997) J. Cell Biol. 139, 683–689
11. Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871
12. Criddle, A. H., Geeves, M. A., and Jeffries, T. (1985) Biochem. J. 232, 343–349
13. Moisescu, D. G., and Thieleczek, R. (1978) J. Physiol. 232, 246
14. Ashley, C. C., and Moisescu, D. G. (1977) J. Physiol. 270, 627–652

TABLE II

| $K_a$ | $k_{1a}$ | $k_{-a}$ | $K_{ADP}$ | $K_{1a}$ | $k_{1a+}$ |
|-------|---------|---------|---------|---------|---------|
|       | s−1     | s−1     | μM−1 s−1| s−1     | μM      |
| +Ca2+| 2–3     | 8       | 3–4     | ≤0.1    | 0.8     | ≥100    |
| −Ca2+| 1–2     | 3.4     | 3–4     | ≤0.1    | 3.4     | 1.1     | ≥30    |
21630

Expressed Truncated Mammalian Myosin I: Kinetics

15. Kurzawa, S. E., and Geeves, M. A. (1996) J. Muscle Res. Cell Motil. 17, 669–676
16. Bagshaw, C. R., Eccleston, J. F., Eckstein, F., Goody, R. S., Gut Freund, H., and Trentham, D. R. (1974) Biochem. J. 141, 351–364
17. Millar, N. C., and Geeves, M. A. (1988) Biochem. J. 249, 735–743
18. Siemankowski, R. P., and White, H. D. (1984) J. Biol. Chem. 259, 5045–5053
19. Wells, C., and Bagshaw, C. R. (1985) Nature 313, 696–697
20. Veigel, C., Kendrick-Jones, J., Sellers, J. R., Sparrow, J. C., and Molloy, J. E. (1999) Biophys. J. 76, A145
21. Whittaker, M., Wilson-Kubalek, E. M., Smith, J. E., Faust, L., Milligan, R. A., and Sweeney, H. L. (1995) Nature 378, 748–751
22. Creno, C. R., and Geeves, M. A. (1998) Biochemistry 37, 1969–1978
23. Smith, D. A., and Geeves, M. A. (1995) Biophys. J. 69, 524–537
24. Rice, S., Lin, A. W., Safer, D., Hart, C. L., Naber, N., Carragher, B. O., Cain, S. M., Pechatnikova, E., Wilson-Kubalek, E. M., Whittaker, M., Pate, E., Cooke, R., Taylor, E. W., Milligan, R. A., and Vale, R. D. (1999) Nature 402, 778–784
25. Ostap, E. M., and Pollard, T. D. (1996) J. Cell Biol. 132, 1053–1060
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