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The Actomyosin ATPase: A Two-State System [and Discussion]

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The actomyosin ATPase: a two-state system

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SUMMARY

Studies of the interaction between actin and myosin subfragment 1 (S1) in solution have shown that the association reaction takes place in at least two steps. Initially the association is relatively weak to form a complex called the A state which can then isomerize to the R state. The rate and equilibrium constants for the isomerization have been measured and are shown to depend upon the nucleotide bound to the S1 ATPase site; with ATP bound the A state is preferred but as ATP is hydrolysed and the products are sequentially released then the complex gradually shifts to the A state. An extensive series of experiments have characterized the A-to-R isomerization both in solution and in contracting muscle fibres and have shown it to be closely associated with the key events in the ATP-driven contraction cycle: the conformational change from the A to the R state can be monitored by fluorescent probes on either actin or the nucleotide; the isomerization can be perturbed by increases in hydrostatic pressure; the actin-induced acceleration of the rate of product release from myosin is coupled to the A-to-R isomerization; tropomyosin may control actin and myosin interaction by controlling the ismoerization step and finally pressure perturbations of contracting muscle fibres shows there to be a close coupling between the isomerization of acto.S1 and the force generating event of muscle contraction.

1. INTRODUCTION

A fundamental property of proteins is their ability to change conformation and this property is often linked to the expression of their biological activity. One of the themes of the discussion at this meeting is how the conformation of a group of proteins is coupled to the ability of these proteins to recognize and hydrolyse nucleotide triphosphates. Following on from this property is how the conformation, governed by the nature of the nucleotide bound, can influence the interaction of the triphosphatases with other proteins. In the case of myosin we are dealing with an ATPase where the energy of hydrolysis is used to drive a cyclical interaction between myosin and actin and which leads to the development of a mechanical force in muscle (and some non-muscle motile systems). The thesis I want to consider is that this behaviour can be analysed in terms of two principal conformations of the actomyosin complex and that the preferred state of the complex (determined by both equilibrium and kinetic factors) depends upon the nature of the nucleotide bound to myosin, on the state of the calcium binding site of the tropomyosin complex (Tm) attached to actin and on the mechanical state of the complex. This whole ensemble represents an intricate information transfer and energy transducing system.

First consider myosin alone: most solution studies use a soluble proteolytic fragment of the insoluble parent myosin known as subfragment 1 (S1). This retains all of the actin binding and ATPase activity of the parent myosin but is a monomer compared to the parent dimer. The ATPase mechanism was elucidated in the early 1970s by several people but is shown here in the form developed by Bagshaw & Trentham (1974).

$$M + T \rightleftharpoons M.T \rightleftharpoons M^*T \rightleftharpoons M^{**}D.P_i \rightleftharpoons M^*D.P_i \rightleftharpoons M^*D \rightleftharpoons M.D \text{—} M + D$$

Where M is myosin or S1 and T, D, P_i are ATP, ADP and phosphate respectively. The asterisks refer to different conformations of the protein as observed by intrinsic protein fluorescence. The mechanism has been refined in recent years but remains essentially correct. As shown, ATP binds in a two-step process; complex formation followed by a conformational change to the M* state. Hydrolysis of ATP to ADP & P_i results in a further increase in fluorescence to the M** state. Dissociation of P_i results in return to the M* level of fluorescence followed by return to M with dissociation of ADP (both dissociation occurring as two step processes). The evidence from the intrinsic protein fluorescence can be interpreted to suggest three states of the protein and clearly the fluorescence change must represent a perturbation of the local environment around one or more tryptophans. However, if we examine the affinity of nucleotides for S1 (table 1) we can see that although ADP and ATP produce a similar fluorescence state in myosin their affinities are very different. A difference in the property of myosin is also seen if the interaction of different myosin.nucleotide complexes with actin are compared. Table 1 shows that the affinity of M*.ADP for actin is 100 times that of M*.ATP even though both are in the M* state whereas the affinity of M**.ADP.P_i and M*.ATP for actin are very similar even though the protein fluorescence is different. We need therefore to distinguish between

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Table 1. *The association constants for the interaction between actin, S1 and nucleotide*

nucleotide	K_{ass} M^{-1} nucleotide to S1	K_{ass} M^{-1} actin to S1.N	K_{ass} M^{-1} nucleotide to acto.S1
ATP	10^{11}	$< 10^4$	0.5×10^3
ATP γ	$\geqslant 10^7$	$\leqslant 10^4$	1.0×10^3
AMP.PNP	10^7	$< 10^5$	0.5×10^3
ADP	10^6	10^6	5.0×10^3

(Conditions: ionic strength 0.12 M, pH 7.0, 20°C. References: ATP, Trentham *et al.* (1976); Millar & Geeves (1983); ATP & S, Geeves *et al.* (1986), Bagshaw *et al.* (1974); AMP.PNP, Konrad & Goody (1982); ADP, Geeves (1989).)

information which tells us about local perturbation of protein structure, which may reflect nucleotide recognition or docking process, and those structural perturbations which are transmitted to other parts of the protein and modify interactions with other molecules. Clearly the two events are not totally separate as pointed out by Goody & Holmes (1983) more than 10 years ago. There is an inverse relation between the affinity of a nucleotide for S1 and the affinity of S1.N for actin (table 1). The goal of understanding muscle contraction is closely associated with understanding the differential binding of nucleotides to myosin and the transmission of this information to the actin binding site.

2. THE TWO-STEP MODEL OF ACTO.S1 INTERACTION

The key to understanding the relationship between actin and nucleotide binding to S1 was suggested by Goody & Holmes (1983) and developed by Geeves *et al.* (1984) when they suggested that the relationship could be explained by a two-state model of actomyosin association

$$A + M.(N) \underset{K_I}{\rightleftharpoons} A{-}M.(N) \underset{K_{II}}{\rightleftharpoons} A.M(N)$$

In this model actin binds initially to form a complex (A-M.N), called the A state, in which actin is relatively weakly bound and nucleotide is tightly bound. This complex then isomerizes to the R state in which actin is more tightly bound and nucleotide is weakly bound (the nucleotide will therefore dissociate if the concentration of free nucleotide is less than K_d). The preferred state of the actomyosin complex is defined by K_{II}. As shown in table 2, K_{II} is less than 1 for ATP and ADP.P_i and the A state is preferred. For ADP and no nucleotide then K_{II} is greater than 1 and the R state is preferred. Thus a cycle of ATP hydrolysis results in the actomyosin complex alternating between the A state and the R state and the lifetime in each state is controlled by the rate of hydrolysis of ATP and the rates of product dissociation.

This is similar to the models for other nucleotide triphosphatase proteins; in this case the actomyosin and actomyosin.ADP are the ones which are 'active'

Table 2. *Equilibrium constants for the two step association between actin and S1.nucleotide complexes*

nucleotide	K_I (M^{-1})	K_{II}
none	5×10^4	200
ADP	5×10^4	10
ATP	$< 10^4$	$\leqslant 10^{-2}$

(Conditions as table 1. References: no nucleotide, Coates *et al.* (1985); ADP, Geeves (1989); ATP, Millar & Geeves (1983); Geeves *et al.* (1987).)

in that these are the states capable of binding actin tightly and holding onto actin against a mechanical load. The crossbridge cycle (figure 1) is then the result of the transition from an A state to an R state being coupled with a structural transition which can generate a mechanical force.

The work in Bristol has centred on characterizing the transitions between the A and R states for a range of nucleotides. The problem with presenting the evidence for these differing complexes is that the equilibrium and rate constants for the transitions vary over wide range. The characterization of each actomyosin.nucleotide complex therefore requires a different experimental approach. Only one or two representative experiments will be presented here before the implications of the results are discussed

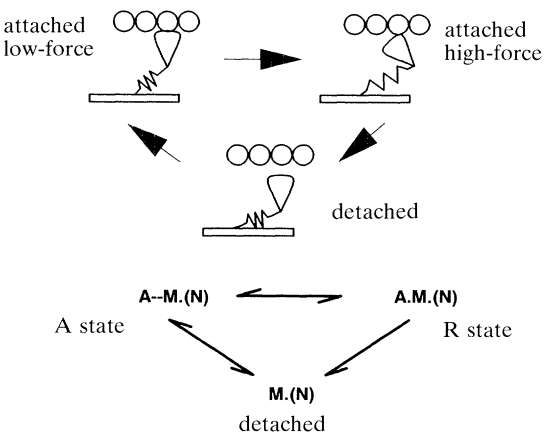

Figure 1. The relationship between the crossbridge cycle in muscle and the two-state model of acto.S1 interactions. (*a*) The simplest crossbridge model which consists of three mechanical states. The detached crossbridge attaches to actin rapidly and reversibly to form the 'low-force' crossbridge. Transition from the low-force to the high-force state is shown as a change in the angle at which the attached crossbridge binds to actin and which occurs with the stretching of an elastic element drawn here as a spring in the connection between the myosin head and the thick filament. (*b*) The two-step association of actin to S1(nucleotide) complexes drawn to emphasize the correspondence with the crossbridge model. Actin binds reversibly to myosin nucleotide complexes to form the A state. The A state is in equilibrium with the R state and the equilibrium position is determined by the nucleotide bound (see table 2). Binding of ATP to the R state results in irreversible dissociation of actin; hydrolysis of ATP and product dissociation leads to repopulation of the R state.

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along with experiments to test some of the predictions from this work.

The key to characterizing the A to R transition is the availability of an optical probe which monitors the transition and the ability to perturb the transition specifically. The probe is a pyrene molecule covalently attached through an acetamide group to Cys-374 of actin. The fluorescence of this pyrene group is high when the actin is free of S1 but quenched by 70% when the R state is formed (Kouyama & Mihashi 1981; Criddle *et al.* 1985). It is not the association of S1 with actin which causes the quenching of fluorescence but the formation of the R state specifically (Coates *et al.* 1985; Geeves *et al.* 1986). This signal can be used to show that in the presence of ATP less than 1% of the acto.S1 complex is in the R state (Geeves *et al.* 1986); 10% is in the R state in the presence of ADP (Geeves & Jeffries 1988) and more than 99% is in the R state in the absence of nucleotide (Coates *et al.* 1985). Kinetic experiments to measure the rate of interconversion of the A and R states then allows a more precise estimate of the equilibrium constants to give the values in table 2.

The transition between the A and the R state can be perturbed specifically by changes in hydrostatic pressure. Estimates suggest that the volume change between the two states is 100 $cm^3\ mol^{-1}$ (Coates *et al.* 1985). Such volume changes are normally caused by changes in hydration shells around the protein, not by intrinsic changes of the volume of the protein itself. This is usually the result of a change in the number charged or hydrophobic groups exposed to the bulk solvent which results in a change in the ordering (and therefore the volume) of water around the protein surface. As a result of this volume change application of 10 MPa (100 atmospheres) pressure to a solution of acto.S1 results in a twofold reduction in K_{II} (Coates *et al.* 1985). Rapid release (200 μs) of the elevated pressure can then be used to follow the re-equilibration of the system back to its equilibrium position at ambient pressure. Such an experiment is shown in figure 2. At high protein concentrations only a rapid fluorescence transient is seen which characterizes the re-equilibration of the A-to-R transition. At lower protein concentrations a significant fraction of the acto-S1 which is forced into the A state at high pressure will dissociate and so two relaxations are observed. The fast and slow relaxations correspond respectively to the uncoupled A-to-R transition and the A-to-R transition coupled to reassociation of acto.S1. If the reaction is monitored by light scattering or if association between actin and S1 is studied using a stopped-flow spectrophotometer, using either fluorescence or light scattering to monitor the reaction, only the slow process is observed. For a simple two-step scheme the fast process ($1/\tau_1$) defines $k_{+2}+k_{-2}$ and the slow process ($1/\tau_2$) defines $k_{+1}([A]+[M])+k_{-1}/(1+K_2)$. The ratio of the two amplitudes allows the two equilibrium constants to be defined (see legend to figure 2).

These experiments then define the equilibrium and rate constants of the two-step association of actin with

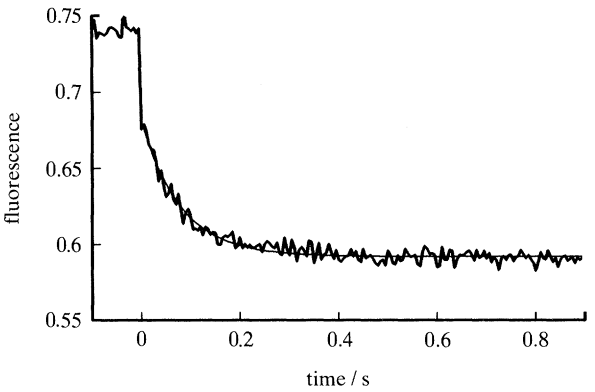

Figure 2. Pressure induced relaxation of a solution of pyrene-actin.S1. Observed fluorescence changes following rapid (200 μs) release of 10 MPa pressure applied to a solution of 2.6 μM pyrene labelled actin and 4.2 μM S1. Fitting the data to the two-step model of association gives $1/\tau_1 \geqslant 2000\ s^{-1} = k_{+II}+k_{-II}$ and $1/\tau_2 = 12.5\ s^{-1} = k_{+1}([A]+[M])+k_{-1}/(1+K_{II})$, and the ratio of the amplitudes $A_1/A_2 = K_1([A]+[M])+1/K_{II}$ (Coates *et al.* (1985).) Conditions: ionic strength 0.12 M, pH 7.0, 20°C. Fluorescence excitation at 365 nm and emission observed at 407 nm.

S1 and S1 nucleotide complexes. One word of caution, this two-step model is a simplification because in most cases the formation of the A state has been shown to be too slow for it to be diffusion limited reaction and there probably exists a preceding collision complex (Geeves & Gutfreund 1982; Coates *et al.* 1985). Some recent data from Taylor's laboratory can also be interpreted as demonstrating the existence of a step preceding the formation of the A state (Taylor 1991). The A state itself is therefore not a collision complex but an identifiable complex between the two proteins.

The one complex we have failed to characterize in its interaction with actin is the M.ADP.P_i complex. This complex (the predominate complex in the myosin ATPase steady state) is difficult to define in the presence of actin because in the forward direction the complex is a short-lived transient and in the reverse direction the binding of P_i is estimated to be greater than 1 M. Therefore, the A and R states in the presence of ADP and P_i cannot be analysed. The actomyosin.ADP.P_i complex is thought to be significantly occupied during the steady-state turnover in a muscle fibre and will be referred to later.

3. IMPLICATIONS AND TESTS OF THE TWO-STATE MODEL

One implication of this analysis of the interaction between actin and myosin is that the A state is an attachment between actin and S1 but the formation of this state is not capable of generating force between the two filaments. Thus the formation of the A state should not accelerate the ATPase rate as futile ATP hydrolysis would result. This can be tested in the case of the complexes with ADP by measuring the rate of ADP displacement from the complex. This is done by using mant.ADP a fluorescent analogue of ADP (Hiratsuka 1983) which increases its fluorescence

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more than 2-fold on binding to S1 (Cremo *et al.* 1990). Displacement of mant.ADP from S1 by addition of an excess of ATP is observed as an exponential process which defines the mant.ADP dissociation rate constant as 0.2 s^{-1} (Woodward *et al.* 1991). Displacement of mant.ADP from acto.S1 by ATP, under conditions where there is little free S1, shows different results when the reaction is monitored by mant.ADP fluorescence or by light scattering, (which monitors the ATP induced dissociation of acto.S1 limited by the dissociation of mant.ADP from the ternary complex). As reported by Woodward *et al.* (1991) and shown in figure 3 the light scattering signal shows a biphasic process with observed rate constants of 500 s^{-1} and 0.5 s^{-1} with amplitudes in the ratio 10:1 as expected for the proportions of the A and R state in the presence of ADP. Thus the rate of ADP release from the A state is similar to that from free S1 and 10^{-3} times that from the R state and compatible with the proposed model. The fluorescence signal only shows the slower process suggesting that the fluorescence of the majority A.M.mant.ADP is similar to that of free mant. ADP while that of A-M.mant.ADP is similar to that of S1.mant.ADP.

The experiments with mant.ADP confirm another requirement of the model. We have already seen that the structural transition changes the affinity of actin for S1 but now it also shows that the transition simultaneously changes the affinity of the nucleotide for the S1 nucleotide site as suggested by Goody & Holmes (1983). The fluorophore on mant.ADP senses this transition with a 2-fold change in fluorescence as does the pyrene fluorophore on Cys-374 of actin. Thus two fluorophores are simultaneously sensing this structural change which indicates that the transition involves perturbation of both nucleotide and actin binding sites on myosin, sites which are generally believed to be at least 3 nm apart. The volume change on the A-to-R transition referred to previously also suggests a substantial structural transition in the complex as this compares with the volume changes seen in denaturation of ribonuclease or chymotrypsin (Davis & Gutfreund 1976). Thus these properties are compatible with this transition being of sufficient magnitude to produce the 4–10 nm transition of the two muscle filaments required from mechanical measurements (Ford *et al.* 1977).

The suggestion that the A-to-R transition is involved in force generation can be tested since, if the transition is involved directly in force generation, then anything which inhibits the transition will inhibit force generation. There is some evidence that changes in ionic strength or addition of organic solvents to the bathing medium of contracting muscle fibres inhibits the tension developed in the way predicted by the effects on K_{II} (for review see Geeves (1991)). More clear cut is the effect of pressure on tension generation. An increase in pressure, on acto.S1 in solution, to 10 MPa reduces K_{II} by a factor of 2. If the tension a muscle can generate or hold is directly proportional to the number of cross bridges in the R state then application of pressure to a contracting muscle fibre

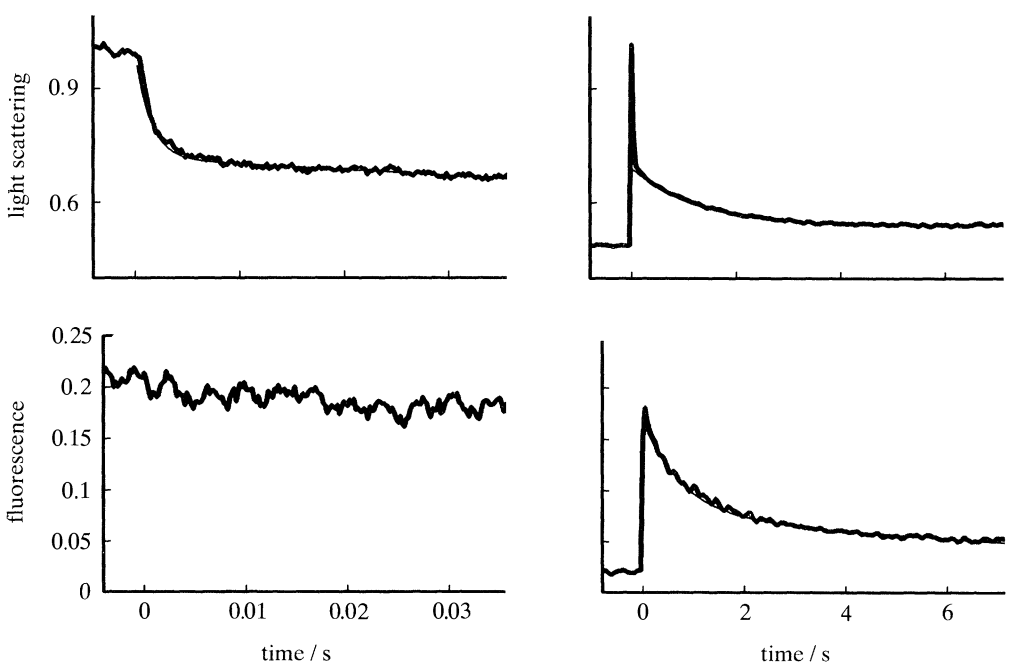

Figure 3. Displacement of mant.ADP from acto.S1 by addition of excess ATP. Acto.S1 (5 μM) and mant.ADP (100 μM) were mixed with 1 mM ATP in a stopped flow fluorimeter and the reaction monitored by 90° light scattering (410 nm) or by mant.ADP fluorescence (excitation 360 nm, emission 440 nm). The light scattering signal showed a biphasic dissociation of acto.S1 with $k_{obs} = 500\ s^{-1}$ and 0.5 s^{-1} and ratio of amplitudes, after correction for signal loss in the 2 ms dead time, of 10:1. The fluorescence signal showed no change on the fast timescale but did exhibit a fluorescence change at 0.5 s^{-1}. Doubling the [ATP] made no significant difference to the observed reactions. Mixing 1 mM ATP with acto.S1 in the absence of mant.ADP gave a light scattering change which was complete in the mixing time of the apparatus; i.e. dissociation greater than 1000 s^{-1}.

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should reduce the tension held as the force holding R states re-equilibrate with the low or zero force holding A state. An apparatus to test this prediction was built in Bristol, in collaboration with K. W. Ranatunga, and indeed an increase of pressure to 10 MPa was observed to induce a reversible 8% decline in the isometric tension (Geeves & Ranatunga 1987; Fortune *et al.* 1989). A series of control experiments were required to establish that this effect was due to pressure affecting the population of force holding crossbridges rather than on some other property of the organized muscle fibre. This is not the appropriate place to go through these in detail but control studies of the effect of pressure on both the structure and the tension held by muscle fibres in the relaxed (no actin or myosin interactions) and rigor (no nucleotide present) states are consistent with the A-to-R transition being perturbed in the contracting muscle fibre (Knight *et al.* 1990; Ranatunga *et al.* 1990).

Simple steady-state measurements only give a limited amount of information on the effect of pressure on the system. Far more information can be obtained by examining transient changes in tension. We built an adaptation of the pressure jump apparatus used for several years in Bristol (Davis & Gutfreund 1976) for examining pressure induced transients in protein–protein interactions (see figure 2) and used it to follow the tension transients following the release of 10 MPa pressure applied to single-skinned muscle fibres. Typical results are shown in figure 4 for an actively contracting muscle fibre. The relaxed fibre shows no change in measured tension even when passively stretched to hold a tension 30% of the active tension. The rigor fibre, also holding a tension of 30% of the active tension, shows a single-step decrease in tension in phase with the pressure change. This has been assigned to an effect of pressure on an elastic element in series with the crossbridge (Ranatunga *et al.* 1990). The active fibre with cycling crossbridges shows a complex response consisting of three phases (Fortune *et al.* 1991). The first is a rapid decrease in tension equivalent to the event seen in rigor fibres. This is followed by a two-phase recovery of tension at 15 s^{-1} and 2 s^{-1} with an amplitude of 8% of the steady ambient tension in each case. As these are only seen in the actively contracting muscle fibre they must be a property introduced by the cycling crossbridges. The result is compatible with pressure perturbing the fraction of crossbridges in the low force A and high force R states in the contracting muscle. Further information on the nature of the specific transitions comes from examination of the effect of P_i on the transients. Addition of P_i to a contracting muscle inhibits force production and this is believed to be due to simple product inhibition resulting in a lower occupancy of the force holding states (Cooke & Pate 1989). The only significant change in the pressure induced transients is that phase 2 the quick recovery event is accelerated while its normalized amplitude remains constant. Phases 1 and 3 are affected to a

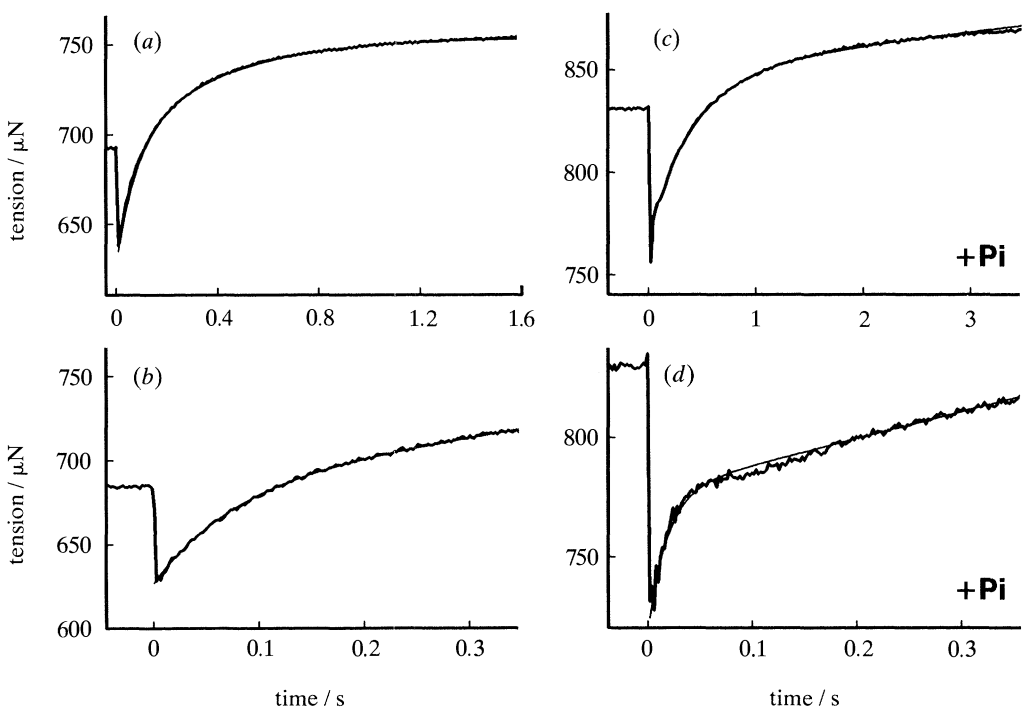

Figure 4. Pressure induced tension transients in activated skinned rabbit psoas fibres. Observed changes in isometric tension following a rapid (1 ms) release of 10 MPa pressure applied to a single-skinned muscle fibre. In (*a*) the transient observed is shown with a double exponential fit superimposed ($1/\tau_2 = 13.8\ s^{-1}$, $1/\tau_3 = 2.68\ s^{-1}$) and in (*b*) a similar transient is shown with faster data collection and a single exponential fit superimposed ($1/\tau_2 = 13.9\ s^{-1}$). In (*c*) and (*d*) the measurements are repeated on a different fibre in the presence of 10 mM P_i. A single exponential fit to the later part of the transient in (*c*) gives $1/\tau_3 = 2.32\ s^{-1}$ and in (*d*) $1/\tau_2 = 55\ s^{-1}$. Conditions: ionic strength 0.2 M, pH 7.0, 12°C. Full experimental details are provided by Fortune *et al.* (1991).

much smaller extent. The relaxation time of phase 2 is hyperbolically dependent upon $[P_i]$ suggesting that a two-step P_i dissociation process is being perturbed

$$\underset{\text{low force}}{\text{A—M.D.P}_i} \rightleftharpoons \underset{\text{high force}}{\text{A.M.D.P}_i} \overset{P_i}{\rightleftharpoons} \underset{\text{high force}}{\text{A.M.D.}}$$

A similar result has been discussed by in the paper by Homsher & Millar (1990) in which the transient decrease in force was characterized following rapid liberation of P_i in contracting muscle fibre. The suggestion that force generation is coupled to P_i release has a long history and is not a novel aspect of this work. What is novel is that a pressure sensitive transition between two attached crossbridge states has been identified which is coupled to both P_i release and the force generating event.

Summarizing the above section it can be stated that we have identified an isomerization of the actomyosin complex which couples events at the nucleotide binding site with events at the actin–myosin interface. The transition is required to accelerate the myosin ATPase rate, it represents a substantial conformational change of the complex and a similar pressure sensitive transition has been identified in a contracting muscle fibre which is coupled to both force generation and to P_i release.

4. CONTROL OF ACTIN MYOSIN INTERACTIONS BY THE TROPOMYOSIN COMPLEX

A further complexity of this nucleotide coupled interaction of actin and myosin is the question of the control of the interaction. Muscle contraction is stimulated by the release of calcium from the intercellular stores. Calcium binds to troponin (Tn) which induces a change in the conformation of the Tm.Tn complex. Tm interacts with seven adjacent actins (A_7Tm.Tn) in the thin filament and the calcium induced structural change in Tm.Tn allows myosin to interact with actin within the group of seven. In the original formulation of the model of control it was envisaged that Tm blocked any interaction between actin and S1 in the absence of Ca (Haselgrove 1972; Huxley 1972). More recent information has suggested a much more subtle interplay between Ca binding to Tn and nucleotide binding to myosin. Tn.Tm could operate, for example by inhibiting P_i release (Chalovich *et al.* 1981). We thought that an alternate possibility would be that it could operate by controlling the A-to-R transition. If this transition is controlled then both tension generation and ATPase activity are controlled.

The model we developed (Geeves & Halsall 1987) is based on the earlier work of Hill *et al.* (1980) and states that the thin filament exists in two conformation the open and closed states and the fraction of A_7TM.Tn units in each state depends upon the calcium concentration. Myosin can bind to actin in either the on or off state to form an A-type association but only the on state allows the formation of the high force R conformation. The equilibrium between the two states of the thin filament is not a simple on off switch but one of rapidly interconverting conformations. The binding of an S1 to form an R state is not prevented but the final equilibrium position is determined by the relative values of K_{II} for the A-to-R transition and

$K_T(=[A_7\text{Tm.Tn}_{open}]/[A_7\text{Tm.Tn}_{closed}])$.

This leads to a cooperative binding of S1 or S1 nucleotides to actin as shown in figure 5. The point to note here is that the titration is cooperative both in the presence and absence of Ca as noticed previously by Greene (1982). This confirms the observation of Lehrer & Morris (1982) that in the absence of myosin the filament remains predominantly in the off state. Analysis of these binding curves using our model requires only three constants; K_I & K_{II} and K_T. K_I and K_{II} can be determined in the presence of Ca as previously shown for acto.S1 in the absence of Tm.Tn. The results show that Tm and Tn make no significant difference to the values obtained. These values are independent of the control model used. Analysis of the titration curves then yields a value of K_T in the presence and absence of Ca. The values used to fit the curves are shown in table 3 and give K_T 0.2 and 0.02 in the presence and absence of Ca (McKillop & Geeves 1991). Titrations over a range of conditions, which vary the values of K_I and K_{II} by more than a factor of 10, give the same value of K_T suggesting that the model is an adequate description of the system.

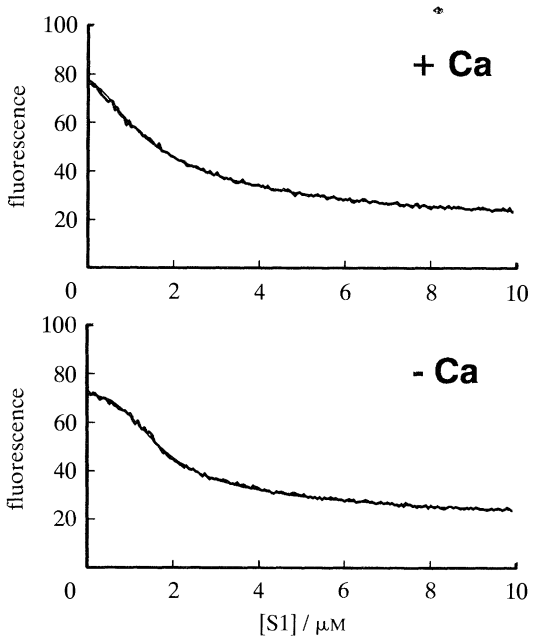

Figure 5. Fluorescence titrations of pyrene-actin and Tm.Tn with S1 in the presence of ADP. Fluorescence was continuously monitored (ex 365 nm, em 407 nm) as S1 was titrated into a stirred cuvette either in the presence of calcium (1 mM $CaCl_2$) or in its absence (1 mM EGTA). A best fit to the model of Geeves & Halsall (1987) is superimposed with the parameters given in table 3. Conditions: ionic strength 0.175 M, pH 7.0, 20°C; 0.5 μM actin, 0.3 μM Tm.Tn and a hexokinase ATP scavenging system.

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Table 3. *Equilibrium constants for the interaction between reconstituted thin filaments and S1*

S1 complex	K_I (M^{-1})	K_{II}	K_T +Ca	K_T −Ca
S1	1.3×10^5	190	0.17	0.01
S1.SO_4	2.1×10^4	280	0.20	0.01
S1.PO_4	2.1×10^4	75	0.33	0.025
S1.ADP	3.7×10^4	18	0.33	0.02

(Conditions: ionic strength 0.175 M, pH 7.0, 20°C. From McKillop & Geeves (1991).)

This model predicts that Tm.Tn controls the acto.S1 interaction not by directly changing the rate at which the A and R states interconvert but by controlling when the transition is allowed to take place. This can be tested by returning to the muscle fibre experiments and measure the relaxation time of phase 2, which we have shown measures the rate of interconverting the A-M.D.P_i and A.M.D.P_i states. The results show that when [Ca] is reduced such that tension is less than 20% of the maximum neither the observed relaxation time nor the normalized amplitude of phase II of the transient are changed. A similar observation has been reported by Millar & Homsher (1991) who observed P_i induced tension transients as a function of [Ca].

In summary what we have demonstrated is that a 'simple' two-state model of actin myosin interaction is sufficient to account for a large body of information on the interaction of these proteins in solution and in contracting muscle fibres. We have identified a conformation change of the complex which is influenced by both the nucleotide which is bound at the myosin active site and by the interaction with other thin filament proteins. One state has a high ATPase activity can generate or hold mechanical force and the other has a low ATPase activity and cannot hold such a force. These two states may be seen as similar to the two states of the 'switching' GTPase proteins or the ATP linked ion pumping proteins.

The ideas and experimental work described here are the development of a series of collaborations with colleagues, primarily in Bristol, over several years. Most of their names appear as coauthors on the papers cited from my laboratory. But I would like to acknowledge in particular the collaboration of Professor H. Gutfreund who has been a constant source of inspiration and encouragement during this work and K. W. Ranatunga who has been central in developing the methodology of examining the effects of hydrostatic pressure on muscle fibres.

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Discussion

G. F. Elliott (*Open University Research Unit, Boars Hill, Oxford, U.K.*). Dr Geeves attributes the volume change associated with the A-to-R transition to a conformational change of the actomyosin complex. Are there no large volume changes associated with changes in ionization?

M. A. Geeves. Yes, ionization changes are associated with large volume changes as are changes in the exposure of hydrophobic side groups to bulk water. We interpret the volume change as a protein conformation change which results in an increase in the number of charged or hydrophobic groups exposed to water.

G. F. Elliot. So the volume change could be due to a change in the ionization of myosin of the type which we have proposed?

M. A. Geeves. That is a possibility, however we have so far not been able to identify a significant volume change in the interaction of ADP or ADP and phosphate with myosin subfragment 1.

J. A. Sleep. Dr Geeves' experiment elegantly demonstrates the lack of Ca^{2+} dependence of the relaxation of tension after a release of pressure and this is clearly consistent with Ca^{2+} controlling recruitment of actin. I wondered whether he had any comments on how his model could accommodate Brenner's observation that the increase in tension after a ramp-restretch cycle was very dependent upon Ca^{2+} concentration. In his model, Ca^{2+} controlled the rate of a reaction not the recruitment of actin.

M. A. Geeves. There is a difference in these results but the measurements are quite different. In our experiments the muscle fibre is in a stable steady state which is then perturbed by a rapid pressure change. The perturbation involves only 5–10% of the cross-bridges which are holding force in the steady state. In Brenner's experiments the force recovery returns from essentially zero measured force to the steady isometric level. So we are probably measuring different events.

J. A. Sleep. What Brenner reported was a calcium-dependent single exponential rate of tension recovery. The model Dr Geeves proposes does not allow for such a process.

M. A. Geeves. I think the process observed by Brenner is likely to be limited by crossbridge recruitment.

J. A. Sleep. Would not crossbridge recruitment result in a lag phase in Brenner's measurements?

M. A. Geeves. I think it would depend upon the details of the model used.

R. Goody (*Max-Planck-Institut für medizinische Forschung, Heidelberg, F.R.G.*) I would like to ask the question which Ed Taylor would have asked if he were here. Is there a problem with the rate of mantADP release from your A state being as low as $0.5\ s^{-1}$?

M. A. Geeves. Yes there is a problem. What we measure is the net rate of mantADP release from all the routes available to it. This means that the A-M.mantADP to A-M.mantADP transition itself cannot be greater than $0.5\ s^{-1}$. This transition for ADP (rather than mantADP) has been measured directly and is $\approx 4\ s^{-1}$. So the rate is slower for the

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mantADP analogue. As you know although mant nucleotides are very good analogues of ATP and ADP, one difference is that the rate of mantADP release from myosin subfragment 1 is 10% of that of ADP. So a slower rate of the transition above is not inconsistent. However we should go back and try to measure it directly.

P. L. Dutton (*Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, U.S.A.*). Why is cooperativity needed?

M. A. Geeves. The usual reason is to give a finer degree of control of an interaction than is possible from normal hyperbolic binding. In this case the cooperativity results from Ca^{2+} binding to a single troponin regulating the interaction of myosin with seven actin sites.

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