PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Regulation of Myosin Filament Assembly by Light-Chain Phosphorylation [and Discussion]

R. C. Smith, W. Z. Cande, R. Craig, P. J. Tooth, J. M. Scholey, J. Kendrick-Jones and N. Crawford

Phil. Trans. R. Soc. Lond. B 1983 **302**, 73-82
doi: 10.1098/rstb.1983.0039

Phil. Trans. R. Soc. Lond. B **302**, 73–82 (1983) [73]
Printed in Great Britain

Regulation of myosin filament assembly by light-chain phosphorylation

By R. C. Smith, W. Z. Cande, R. Craig, P. J. Tooth, J. M. Scholey and J. Kendrick-Jones

M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

[Plates 1 and 2]

Myosins isolated from vertebrate smooth muscles and non-muscle cells such as lymphocytes and platelets contain regulatory light chains (M_r = 20000), which are phosphorylated by a Ca^{2+}-calmodulin-dependent kinase and dephosphorylated by a Ca^{2+}-insensitive phosphatase. Phosphorylation of the regulatory light chains of these myosins *in vitro* regulates not only their interactions with actin but also their assembly into filaments. Under approximately physiological conditions (0.15 M NaCl, pH 7.0) stoichiometric levels of Mg-ATP disassemble these non-phosphorylated myosin filaments into species with sedimentation coefficients ($S^{\circ}_{20,w}$) of approximately 11*S*. Hydrodynamic and electron microscope observations have indicated that this 11*S* species is a monomer with a folded conformation (Trybus *et al.*, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6151 (1982)). Rotary shadowing reveals that the tails of disassembled gizzard and thymus myosins are folded twice at two hinge points to form a folded three-segment structure. Phosphorylation of the regulatory light chains of these myosins causes these folded 11*S* molecules to unfold into the conventional extended monomeric form (6*S*), which is able to assemble into filaments. Thus *in vitro* these myosin filaments can be assembled or disassembled by phosphorylation or dephosphorylation of their light chains. Whether these results have any relevance to the situation within *living* non-muscle and smooth muscle cells remains to be established.

Introduction

Muscular contraction and many types of motile activities are believed to be generated by the interaction of myosin with Mg-ATP and actin. We are interested in the mechanisms by which changes in the intracellular free calcium ion concentration ($[Ca^{2+}]$) control these interactions and thus regulate movement in vertebrate non-muscle and smooth muscle cells. Over the last decade, considerable progress has been made in characterizing myosins isolated from a variety of non-muscle and smooth muscle cells (Korn 1978; Hartshorne & Gorecka 1980; Small & Sobieszek 1980; Adelstein & Eisenberg 1980). It is now generally accepted that the interaction of myosin and actin in these cells as measured by the actin-activated myosin Mg-ATPase activity is regulated by the level of phosphorylation of the myosin regulatory light chains (M_r 20000) (Adelstein & Conti 1975; Adelstein & Eisenberg 1980; Hartshorne & Gorecka 1980; Small & Sobieszek 1980). It is envisaged that when the regulatory light chains are not phosphorylated, the actin-activated Mg-ATPase activity is very low. Upon stimulation, calcium activates a specific calmodulin-dependent kinase (Dabrowska *et al.* 1978; Adelstein & Klee 1981), which phosphorylates the light chains and thus initiates actin–myosin interaction. When the light chains are dephosphorylated by a Ca^{2+}-insensitive phosphatase, the actin-activated ATPase activity is again inhibited and relaxation ensues. It should be stressed that it is likely, especially in non-muscle cells, that additional complementary regulatory or modulatory mechanisms may be present for controlling actin–myosin interactions in these cells.

An exciting recent finding was the demonstration by Suzuki *et al.* (1978) that phosphorylation

of the regulatory light chains of vertebrate smooth-muscle myosin not only initiates myosin interaction with actin but also initiates the assembly of this myosin into filaments. Further studies have shown that light-chain phosphorylation *in vitro* controls the stability of myosin filaments prepared not only from vertebrate smooth muscle myosins (Suzuki *et al.* 1982; Trybus *et al.* 1982; Kendrick-Jones *et al.* 1983) but also from vertebrate non-muscle myosins (Scholey *et al.* 1980; Martin *et al.* 1981; Kendrick-Jones *et al.* 1982*b*). In this paper we shall describe our studies aimed at understanding how phosphorylation of the regulatory light chains of vertebrate non-muscle (thymus) and smooth muscle (gizzard) myosins regulates the assembly of these myosins into filaments.

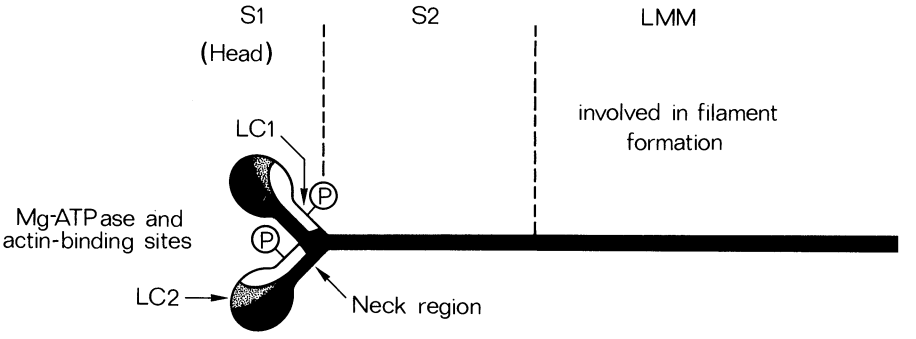

FIGURE 1. Diagrammatic representation of a myosin molecule illustrating the distribution of functional properties. Myosin is an asymmetric molecule consisting of two heavy chains (M_r *ca.* 200000) and two pairs of light chains of two types (M_r *ca.* 20000). The C-terminal halves of the heavy chains form the α-helical coiled-coil tail region (LMM and S2) and the N-terminal halves form the globular heads (S1). Each S1 contains one of each type of light chain, called regulatory (LC1, labelled with an encircled P: phosphorylatable) and essential (LC2) light chains. The precise structure and location of these light chains on S1 are not yet known.

LOCATION OF THE REGULATORY LIGHT CHAINS ON MYOSIN

The myosins have the conventional myosin subunit structure shown in diagrammatic form in figure 1. Each myosin head contains a regulatory light chain (M_r 20000), which is specifically phosphorylated by light-chain kinase (Adelstein & Klee 1981). In the gizzard light chain it has been shown that a single serine residue (19 residues from the N terminus) is phosphorylated (Jakes *et al.* 1976). Structural evidence indicates that the regulatory light chain is located in the neck region of the head, possibly extending across the hinge region between the head and tail (S_1–S_2 junction) portions of the myosin molecule (Flicker *et al.* 1981; Vibert & Craig 1982; Kendrick-Jones *et al.* 1982*a*). An intriguing question is how does phosphorylation of the light chain, located in the neck region of the myosin, control not only the interaction of the myosin head with actin but also the interactions in the LMM region of the tail (figure 1), which are believed to be involved in filament formation?

THE EFFECT OF LIGHT-CHAIN PHOSPHORYLATION ON ACTIN–MYOSIN INTERACTION

Parallel studies were carried out on gizzard and thymus myosins so that the validity of the observations obtained with either myosin could be cross-checked. In all experiments the myosins behaved similarly, for example actin–myosin interaction as measured by the actin-activated Mg-ATPase activities of both myosins always required light-chain phosphorylation.

In no case have we observed a significant actin-activated Mg^{2+} ATPase activity when the myosins were non-phosphorylated. When the light chains are phosphorylated, the Mg^{2+} ATPase activities of both gizzard and thymus myosin are activated approximately 8 to 10-fold by actin (to *ca.* 160 and 100 nmol P_i mg^{-1} myosin min^{-1} respectively) (Scholey *et al.* 1982; Kendrick-Jones *et al.* 1983).

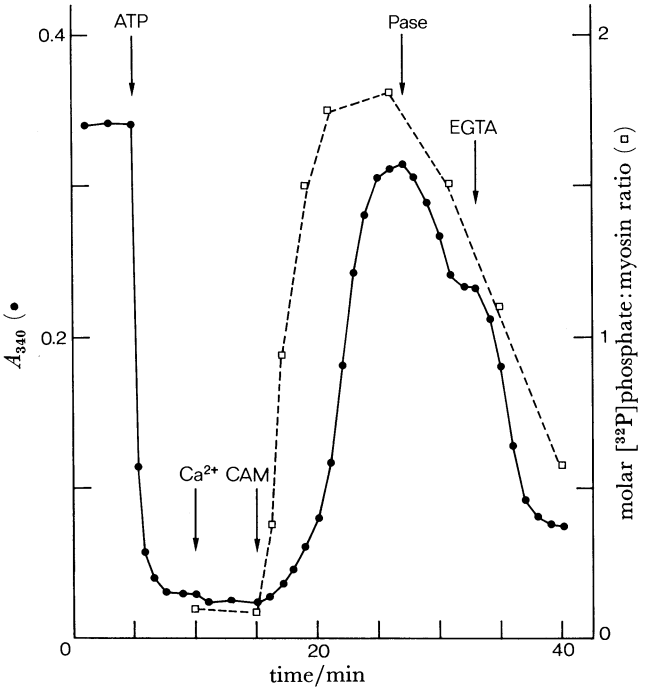

FIGURE 2. The reversible assembly of gizzard and thymus myosin filaments is controlled by phosphorylation–dephosphorylation of the regulatory light chains (M_r 20000). Non-phosphorylated gizzard myosin filaments (0.5 mg ml^{-1}) in 0.15 M NaCl, 0.2 mM EGTA, 5 mM Mg^{2+}, 25 mM imidazole buffer, pH 7.0, 0.25 mM dithiothreitol (DTT) were incubated at 20 °C with kinase (5 μg ml^{-1}), and the turbidity at 340 nm was recorded (Scholey *et al.* 1980). After 5 min 2.5 mM [γ-^{32}P]ATP (2.5 μCi μmol^{-1}) was added, and at 10 min, Ca^{2+} was added to a free concentration of 0.01 mM. At 15 min calmodulin (CAM) (10 μg ml^{-1}) was added and at 27 min gizzard phosphatase (Pase) (25 μg ml^{-1}) followed by EGTA to 0.2 mM were added. Samples were removed from the turbidity assays and the level of light-chain phosphorylation determined by ^{32}P incorporation with a Millipore filter assay (Scholey *et al.* 1982) and checked by glycerol–urea gel electrophoresis (Perrie & Perry 1970). Aliquots were also taken for negative straining and electron microscopy (figure 3) and dark-field microscopy to verify that the changes in turbidity were monitoring filament assembly and disassembly. Similar results were obtained with thymus myosin (see, for example, Scholey *et al.* 1982).

THE EFFECT OF LIGHT-CHAIN PHOSPHORYLATION ON THE STABILITY OF THE MYOSIN FILAMENTS

The stability of thymus and gizzard myosin filaments was routinely monitored by turbidity measurements by using an assay procedure based on that originally described by Suzuki *et al.* (1978) (figure 2). Non-phosphorylated gizzard or thymus myosins at 0.15 M NaCl, 5 mM $MgCl_2$ and pH 7.0 assemble into filaments, most of which resemble the side polar or mixed polarity filaments observed in previous ultrastructural studies (Craig & Megerman 1977; Hinssen *et al.* 1978). If ATP is added to a solution of these non-phosphorylated myosin filaments (0.5 mg ml^{-1}) incubated at 20 °C in the presence of light-chain kinase and EGTA, the turbidity drops rapidly (figure 2). The speed of turbidity decrease and the level to which it drops are dependent on

the purity and freshness of the myosin preparation, for example in figure 2, 2 min after ATP addition the turbidity level is roughly the same as that obtained if the filaments were dissolved in 0.6 M NaCl. The turbidity remains low, even after the addition of Ca^{2+} to 10 μM, but subsequent addition of calmodulin to activate the kinase leads to a steady increase in turbidity to a level roughly the same as that of the original filament solution. With gizzard myosin, light-chain phosphorylation increases rapidly and precedes the increase in turbidity (figure 2). Further additions of 1 mM ATP have no effect on the turbidity of this phosphorylated myosin solution. When gizzard phosphatase and then EGTA (which switches off the kinase) are added, there is a gradual decrease in turbidity and in the level of light-chain phosphorylation.

To verify that the turbidity and light-chain phosphorylation measurements are monitoring the disassembly and assembly of the myosin filaments, samples were taken from the turbidity assay solutions and examined in the electron microscope after negative staining. When ATP is added to the non-phosphorylated filaments, they disappear and instead a dense amorphous background of dissolved protein is visible (figure 3*a*, *c*, plate 1). When the light chains are phosphorylated, this material is induced to assemble into filaments, which in gizzard myosin resemble the non-phosphorylated myosin filaments originally present (average length 1.58 ± 0.53 μm, $n = 50$) (Kendrick-Jones *et al.* 1983) (figure 3*b*). The reassembled thymus myosin filaments (figure 3*d*) are, however, considerably longer (1.1 ± 0.38 μm, $n = 256$) than those initially present (Scholey *et al.* 1981). Most of the filaments observed appear to be side-polar or mixed-polarity filaments and have crossbridges all along their lengths with no central bare zones but possibly with bare edges at their tips. These results have been confirmed by using dark field microscopy as an alternative procedure for monitoring myosin filament assembly (Kendrick-Jones *et al.* 1983). This method avoids many of the sampling problems inherent in any electron microscopical procedure and has the added advantage that one is able to observe individual myosin filaments continuously as they form.

Thus *in vitro* the stability of thymus and gizzard myosin filaments at approximately physiological conditions is dependent on the level of light-chain phosphorylation.

The structure of the disassembled myosins

When the non-phosphorylated thymus or gizzard myosin filaments are disassembled by stoichiometric levels of Mg-ATP, they disassemble into a single species with a sedimentation coefficient of *ca.* 11*S* (Suzuki *et al.* 1978; Kendrick-Jones *et al.* 1982*b*). Since monomeric myosin in high salt concentration sediments with a sedimentation coefficient of 6*S*, it was originally thought that this disassembled species might be a dimer (Suzuki *et al.* 1978; Kendrick-Jones *et al.* 1982*b*) and might be the antiparallel dimer that had been suggested as the building block

Description of plate 1

Figure 3. Electron micrographs of disassembled and reassembled gizzard (*a*, *b*) and thymus (*c*, *d*) myosin filaments. (*a*, *c*) The effect of the addition of ATP on non-phosphorylated gizzard and thymus myosin filaments respectively. Samples were taken from the turbidity assay (figure 2) 5 min after addition of ATP. Note the absence of filaments; there is instead an amorphous background of dissolved material. (*b*, *d*) Light-chain phosphorylation induces this disassembled material to reassemble into myosin filaments. Samples taken at time 25 min in figure 2. Samples (10 μl) of the myosin filament assay solutions described in figure 2 were taken at the required times and prepared for electron microscopy by the negative-staining procedure previously described (Scholey *et al.* 1980).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

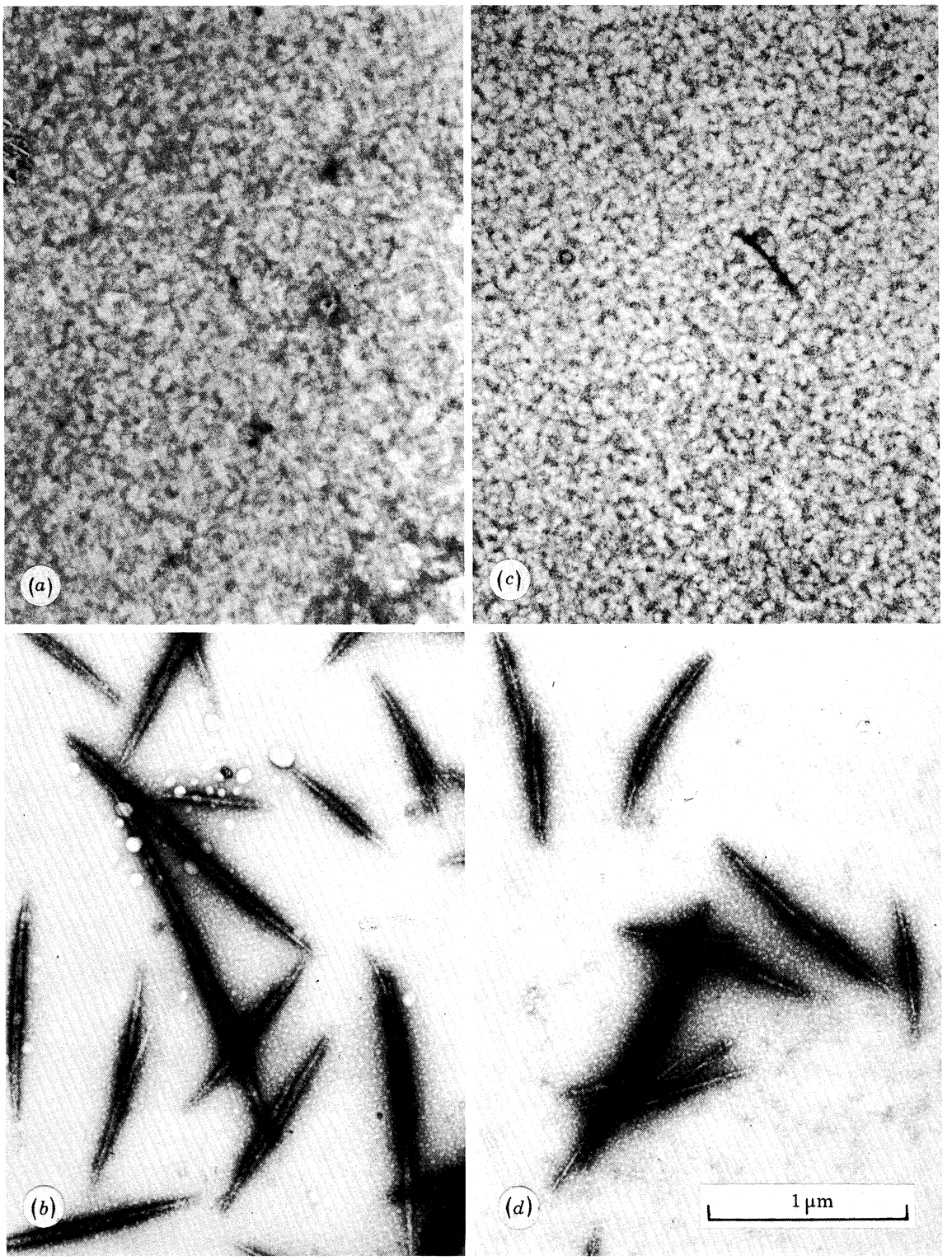

FIGURE 3. For description see opposite.

(*Facing p.* 76)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

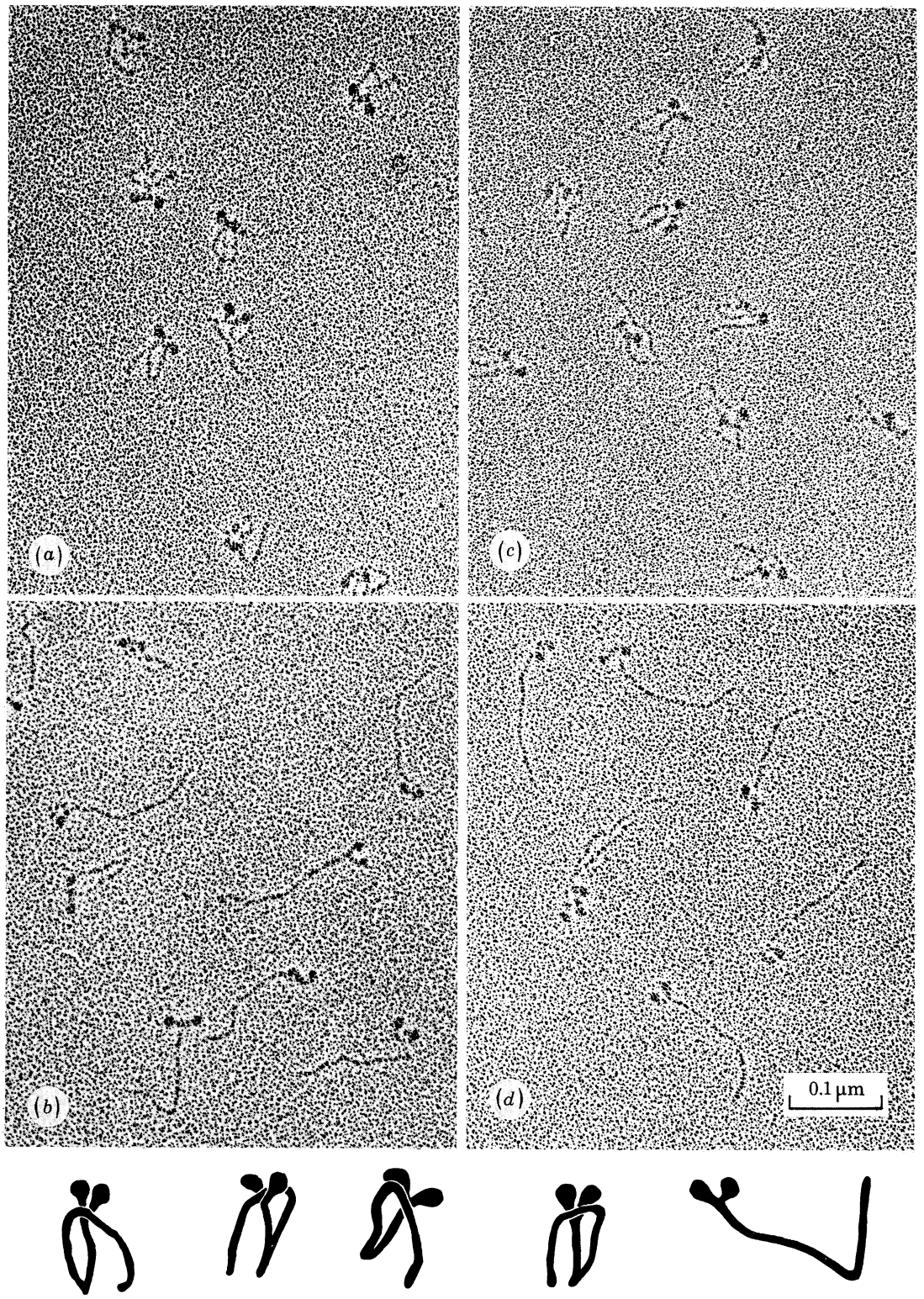

FIGURE 4. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

for the assembly of smooth muscle and non-muscle myosin filaments (Craig & Megerman 1977; Hinssen *et al.* 1978). It has recently been shown, however, that the disassembled *ca.* 11*S* smooth muscle myosin is monomeric (Trybus *et al.* 1982; Suzuki *et al.* 1982), and the elevated sedimentation coefficient (11*S* instead of normal monomer 6*S*) appears to be due to a major conformational change in the myosin molecule which reduces its frictional coefficient, i.e. the myosin tail is folded (Trybus *et al.* 1982; Onishi & Wakabayashi 1982). If disassembled *ca.* 11*S* thymus and gizzard myosin molecules are examined in the electron microscope after rotary shadowing, they are seen to have a folded structure (figure 4*a*, *c*, plate 2) (Craig *et al.* 1983). When non-phosphorylated thymus and gizzard myosin filaments are disassembled by Mg-ATP (figure 2), one observes that generally more than 95% of the thymus myosin and more than 80% of the gizzard myosin molecules are in a folded state. The molecules are folded twice, at two hinge regions situated approximately one-third and two-thirds of the way along the tail so that the tail is folded into three segments (see tracings at the bottom of figure 4). The essential feature of these folded molecules seems to be the binding of the myosin tail in the region of the distal hinge to the neck region of the myosin heads, near where the regulatory light chains are believed to be located (Flicker *et al.* 1981; Vibert & Craig 1982; Kendrick-Jones *et al.* 1982*a*). This binding of the tail to the neck appears to maintain the folded state because there is apparently no specific charge attraction between the different segments of the myosin tail: they are generally seen clearly separated from each other. Onishi & Wakabayashi (1982) observed a similar arrangement, which they describe as 'intramolecular hairpin loops' in the tails of their folded molecules of gizzard myosin.

When the regulatory light chains on these folded molecules are phosphorylated by light-chain kinase (see figure 2), the tails are released from the neck and unfold to produce extended molecules (figure 4*b*, *d*). Frequently, especially with gizzard myosin, the distal hinge can still be seen (see tracings in figure 4). There is a reasonably good correlation between the level of light-chain phosphorylation and the number of extended molecules for both thymus and gizzard myosins. The observation that filament assembly lags behind light-chain phosphorylation (see figure 2, where at 18 min the light chains are 80% phosphorylated but filament assembly as measured by turbidity is only 20%) allows us to see the unfolded molecules before they are assembled into filaments.

How does Mg-ATP's binding to these non-phosphorylated myosins cause filament disassembly?

The observation that stoichiometric amounts of Mg-ATP cause non-phosphorylated myosin filaments to disassemble, whereas filaments formed from gizzard myosin rod subfragments are

Description of plate 2

Figure 4. Rotary-shadowed thymus (*a*, *b*) and gizzard myosin (*c*, *d*) molecules. (*a*, *c*) Disassembled 11*S* non-phosphorylated thymus and gizzard myosin molecules respectively. (*b*, *d*) Molecules in an assembly assay similar to that shown in figure 2, where filament assembly as measured by turbidity is low but the light chains are 70–90% phosphorylated. Samples (50 µl) of myosin were removed from the turbidity assay solutions when required and diluted into 450 µl 0.15 M NaCl, 0.2 mM EGTA in 50% glycerol (by volume). The specimens were rotary shadowed as described previously (Craig *et al.* 1983). Most folded molecules observed show myosin tail association with the myosin head or neck. Shown are tracings of two typical thymus and two gizzard folded myosin molecules respectively. Also shown is a phosphorylated gizzard myosin molecule showing a bend at the distal hinge.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

stable in 1 mM Mg-ATP (Scholey *et al.* 1980) suggests that the Mg-ATP binding site is located in the head region of the myosin. The obvious candidate with the required affinity is the Mg-ATPase site. ATP seems to be essential for *complete* filament disassembly, although it remains to be established whether both binding and hydrolysis are required (Suzuki *et al.* 1978; Kendrick-Jones *et al.* 1982*b*; Trybus *et al.* 1982; Onishi 1982). How Mg-ATP's binding to the Mg-ATPase active sites on these non-phosphorylated myosin heads induces the gross conformational change in the whole myosin molecule, which not only leads to filament disassembly but also inhibits interaction with actin, and how phosphorylation of the light chains located in the neck region reverses these changes, is at present not understood. However, one could speculate that the following simple model might operate. If one assumes that the effects of Mg-ATP's binding to these myosins are qualitatively similar to those observed in vertebrate striated myosin (Taylor 1979) then the changes in the non-phosphorylated (inhibited) head induced by ATP's binding might generate a 'sticky patch' in the neck region, which could trap the mobile 'flexible' tail and form the folded 11*S* state. The net effect of trapping monomer in this folded 11*S* form would be to displace the filament ⇋ monomer equilibrium to the right by removing 6*S* monomers, and thus filament disassembly would occur (figure 5). When the regulatory light chains in the neck region are phosphorylated, the 'sticky patch' is eliminated and the tails unfold to form 6*S* monomers, which readily reassemble into filaments.

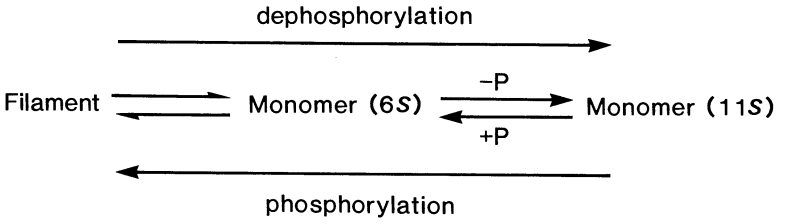

FIGURE 5. A speculative model for myosin filament disassembly and reassembly regulated by light-chain phosphorylation and dephosphorylation. Mg-ATP binding to non-phosphorylated myosin induces the formation of folded 11*S* monomers, which would promote filament disassembly by removing 6*S* monomers and thus displacing the filament–6*S*-monomer equilibrium. Phosphorylation of the light chains by unfolding the molecules to 6*S* monomers would induce filament reassembly. A similar mechanism has also been proposed by Onishi &Wakabayashi (1982).

DO THESE STUDIES *IN VITRO* HAVE ANY RELEVANCE TO THE STATE OF MYOSIN ASSEMBLY IN LIVING VERTEBRATE NON-MUSCLE AND SMOOTH MUSCLE CELLS?

The results from studies *in vitro* suggest that in resting non-muscle cells and relaxed smooth muscles the myosin exists in a folded monomeric form that is inhibited from assembling into filaments and interacting with actin by the non-phosphorylated light chains (figure 6). Upon activation, Ca^{2+} binds to calmodulin and activates the kinase, which phosphorylates the light chains and removes their inhibitory effect. The tails can now unfold and the myosin can assemble into filaments that interact with actin to generate the force for movement. Such a scheme may be relevant to those motile activities in non-muscle cells, such as cell locomotion, endocytosis and cytokinesis, that occur in different places and at different times during the cell's life cycle and therefore the regulated assembly and disassembly of actin and myosin filaments may be an essential requirement for such activities. The disassembled myosin with its compact folded structure would facilitate the movement of myosin around the cell to a site where at a specific time it could be activated by light-chain phosphorylation to assemble into filaments with

the correct geometry to interact with actin to produce the required movement. At present, however, although myosin has been localized in non-muscle cells at the level of resolution of the electron microscope by using immunocytochemical techniques (Drenckhahn & Gröschel-Stewart 1980; Herman & Pollard 1981) its exact molecular organization *in vivo* at rest or during any motile activity has not been established. In vertebrate smooth muscles, it is difficult to understand the physiological advantage to be gained from regulating the assembly and disassembly of myosin filaments during the contraction–relaxation cycle. In fact, the most recent ultrastructural studies have suggested that in smooth muscles the myosin is organized into stable filaments and arranged in mini-sarcomeres or contractile units (Somlyo 1980; Small & Sobieszek 1980). Moreover, electron microscopy of rapidly frozen vascular smooth

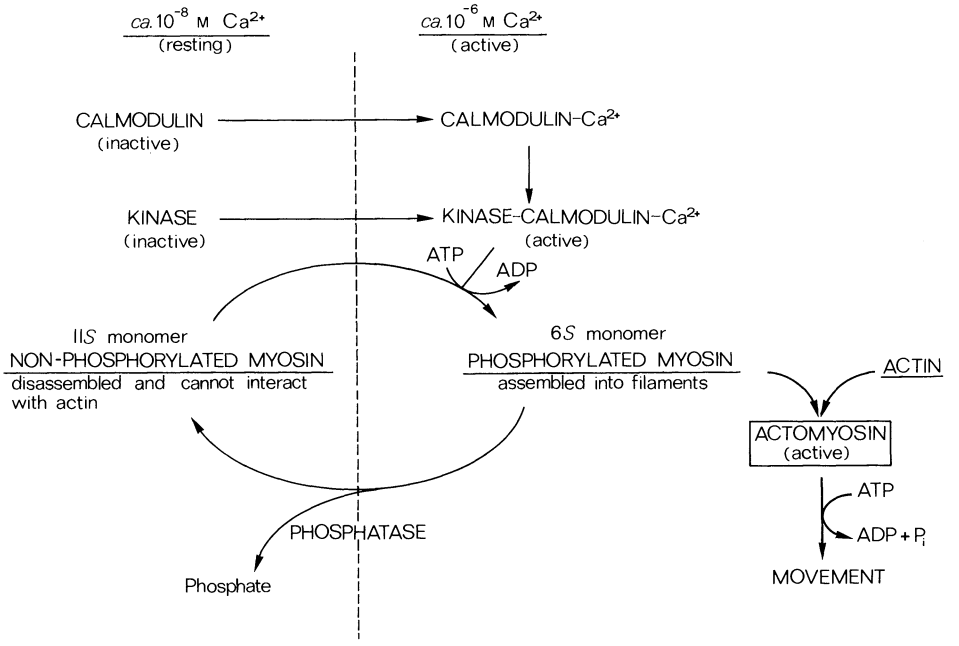

FIGURE 6. A possible scheme for the regulation of movement in vertebrate smooth muscles and non-muscle cells mediated by light-chain phosphorylation and dephosphorylation. This scheme is based on those proposed by Adelstein & Eisenberg (1980), Hartshorne & Gorecka (1980) and Small & Sobieszek (1980) for the regulation of actin–myosin interaction in vertebrate smooth muscle.

muscle has shown that myosin filaments are present under relaxing conditions even though the myosin light chains are completely non-phosphorylated. The discrepancies between these ultrastructural observations and the *in vitro* results with purified smooth muscle myosins are as yet difficult to explain and may be due to conditions or components present in the muscle that are missing or altered in the studies *in vitro*. It is possible that myosin filaments could be stabilized when the muscle relaxes by core or capping proteins that bind to the myosin filament or by the influence of the arrays of actin filaments that surround each myosin filament *in vivo*. It is more likely, however, that the effect of light-chain phosphorylation on myosin filament stability in living smooth muscles may be more subtle than the simple scheme shown in figure 6, which was proposed on the basis of the studies *in vitro*. Thus the role of light-chain phosphorylation *in vivo* may be to control the flexibility of the myosin molecules within the filament so that on activation, phosphorylation of the light chains by altering the molecular

packing may tighten the filament structure and thus confer an increased order on the cross-bridge arrays.

In conclusion, our studies *in vitro* have demonstrated that light-chain phosphorylation represents a plausible mechanism for assembling vertebrate non-muscle and smooth muscle myosin into filaments under conditions close to those believed to exist in living cells. To determine whether these findings have any relevance to the situation in living vertebrate non-muscle and smooth muscle cells we need to be able to measure and correlate myosin filament assembly (or 'flexibility') with altering levels of light-chain phosphorylation within a variety of non-muscle and smooth muscle cells during rest and different states or types of contractile activities.

We wish to thank Dr Hugh Huxley, F.R.S., for his continued interest and support. J.M.S. and R.C. held M.R.C. postdoctoral fellowships, W.Z.C. was supported by a Scholar in Cancer Grant from the American Cancer Society and R.C.S. holds an M.R.C. research studentship.

References

Adelstein, R. S. & Conti, M. A. 1975 Phosphorylation of platelet myosin increases actin-activated myosin ATPase activity. *Nature, Lond.* **256**, 597–598.

Adelstein, R. S. & Eisenberg, E. 1980 Regulation and kinetics of the actin–myosin–ATP interaction. *A. Rev. Biochem.* **49**, 921–956.

Adelstein, R. S. & Klee, C. B. 1981 Purification and characterisation of smooth muscle myosin light chain kinase. *J. biol. Chem.* **256**, 7501–7509.

Craig, R. & Megerman, J. 1977 Assembly of smooth muscle myosin into side-polar filaments. *J. Cell Biol.* **75**, 990–996.

Craig, R., Smith, R. C. & Kendrick-Jones, J. 1983 Light chain phosphorylation controls the conformation of vertebrate nonmuscle and smooth muscle myosin molecules. *Nature, Lond.* (Submitted.)

Dabrowska, R., Sherry, J. M. F., Aromatorio, D. K. & Hartshorne, D. J. 1978 Modulator protein as a component of the myosin light chain kinase from chicken gizzard. *Biochemistry, Wash.* **17**, 253–258.

Drenckhahn, D. & Gröschel-Stewart, U. 1980 Localisation of myosin, actin and tropomyosin in rat intestinal epithelium: immunohistochemical studies at light and electron microscope levels. *J. Cell Biol.* **86**, 475–482.

Flicker, P., Walliman, T. & Vibert, P. 1981 Location of regulatory light chains in scallop myosin. *Biophys. J.* **33**, 279a.

Hartshorne, D. J. & Gorecka, A. 1980 The biochemistry of the contractile proteins of smooth muscle. In *Handbook of physiology*, sect. 2 (*The cardiovascular System*), vol. 2 (*Vascular smooth muscle*) (ed. D. F. Bohr *et al.*), pp. 93–120. Bethesda, Maryland: American Physiological Society.

Herman, I. M. & Pollard, T. D. 1981 Electron microscopic localization of cytoplasmic myosin with ferritin-labelled antibodies. *J. Cell Biol.* **88**, 346.

Hinssen, H., D'Haese, J., Small, J. V. & Sobieszek, A. 1978 Mode of filament assembly of myosins from muscle and nonmuscle cells. *J. ultrastr. Res.* **64**, 282–302.

Jakes, R., Northrop, F. & Kendrick-Jones, J. 1976 Calcium binding regions of myosin regulatory light chains. *FEBS Lett.* **70**, 229–234.

Kendrick-Jones, J., Jakes, R., Tooth, P. J., Craig, R. & Scholey, J. M. 1982*a* Role of myosin light chains in the regulation of contractile activity. In *Basic biology of muscles: a comparative approach* (ed. B. M. Twarog, R. J. C. Levine & M. M. Bewey), pp. 255–272. New York: Raven Press.

Kendrick-Jones, J., Tooth, P. J., Taylor, K. A. & Scholey, J. M. 1982*b* Regulation of myosin filament assembly by light chain phosphorylation. *Cold Spring Harb. Symp. quant. Biol.* **46**, 929–938.

Kendrick-Jones, J., Cande, W. Z., Tooth, P. J., Smith, R. C. & Scholey, J. M. 1983 Studies on the effect of phosphorylation of the 20,000 M_r light chain of vertebrate smooth muscle myosin. *J. molec. Biol.* (In the press.)

Korn, E. D. 1978 Biochemistry of actomyosin-dependent cell motility. *Proc. natn. Acad. Sci. U.S.A.* **75**, 588–599.

Martin, F., Gabrion, J. & Cavadore, J.-C. 1981 Thyroid myosin filament assembly–disassembly is controlled by myosin light chain phosphorylation–dephosphorylation. *FEBS Lett.* **131**, 235–238.

Onishi, H. 1982 Possible role of ATP in dimerisation of myosin molecules from chicken gizzard muscle. I. *J. Biochem., Tokyo* **91**, 157–166.

Onishi, H. & Wakabayashi, T. 1982 Electron microscopic studies of myosin molecules from chicken gizzard muscle. *J. Biochem., Tokyo* **92**, 871–879.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Perrie, N. T. & Perry, S. V. 1970 An electrophoretic study of the low molecular weight components of myosin. *Biochem. J.* **119**, 31–38.
Scholey, J. M., Smith, R. C., Drenckhahn, D., Gröschel-Stewart, U. & Kendrick-Jones, J. 1982 Thymus myosin. *J. biol. Chem.* **257**, 7737–7745.
Scholey, J. M., Taylor, K. A. & Kendrick-Jones, J. 1980 Regulation of nonmuscle myosin assembly by calmodulin-dependent light chain kinase. *Nature, Lond.* **287**, 233–235.
Scholey, J. M., Taylor, K. A. & Kendrick-Jones, J. 1981 The role of myosin light chains in regulating actin–myosin interaction. *Biochimie* **63**, 255–271.
Small, J. V. & Sobieszek, A. 1980 The contractile apparatus of smooth muscle. *Int. Rev. Cytol.* **64**, 241–306.
Sobieszek, A. & Small, J. V. 1976 Myosin-linked calcium regulation in vertebrate smooth muscle. *J. molec. Biol.* **102**, 75–92.
Somlyo, A. V. 1980 Ultrastructure of vascular smooth muscle. In *Handbook of physiology*, sect. 2 (*The cardiovascular system*), vol. 2 (*Vascular smooth muscle*) (ed. D. R. Bohr *et al.*), pp. 33–67. Bethesda, Maryland: American Physiological Society.
Somlyo, A. V., Butler, T. M., Bond, M. & Somlyo, A. P. 1981 Myosin filaments have nonphosphorylated light chains in relaxed smooth muscle. *Nature, Lond.* **294**, 567–569.
Suzuki, H., Kamata, T., Onishi, H. & Watanabe, S. 1982 ATP induced reversible change in the conformation of chicken gizzard myosin and HMM. *J. Biochem., Tokyo* **91**, 1699–1705.
Suzuki, H., Onishi, H., Takahashi, K. & Watanabe, S. 1978 Structure and function of chicken gizzard myosin. *J. Biochem., Tokyo* **84**, 1529–1542.
Taylor, E. W. 1979 Mechanism of actomyosin ATPase and the problem of muscle contraction. *CRC crit. Rev. Biochem.* **6**, 103–164.
Trybus, K. M., Huiatt, T. W. & Lowey, S. 1982 A bent monomeric conformation of myosin from smooth muscle. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6151–6155.
Vibert, P. & Craig, R. 1982 3D-reconstruction of thin filaments decorated with a Ca^{++}-regulated myosin. *J. molec. Biol.* **157**, 299–319.

Discussion

N. Crawford (*Royal College of Surgeons, London, U.K.*). About 10 years ago we observed (Cove & Crawford 1975; Harris & Crawford 1975) a similar finding of the coexistence of low and high $S^{\circ}_{20,w}$ species of myosin in blood platelet preparations. Thick-filament forms of myosin are rarely, if ever, seen in the cytoplasm of resting platelets. In our naïvety at that time, and without the elegant electron microscopy that Dr Kendrick-Jones has presented, we put forward the view that the high $S^{\circ}_{20,w}$ myosin may be a dimer and that this could perhaps act as a nucleating site for thick filament assembly when the platelet is activated by a haemostatic agent. However, one other feature of our study was that the equilibrium between the two myosin forms could be shifted in the direction of the high $S^{\circ}_{20,w}$ species in the presence of actin, ATP and Mg^{2+}.

Has Dr Kendrick-Jones observed a similar effect with his two forms of myosin? What really perplexes me is that, if what we observed earlier was not really dimerization but the formation of a folded form of myosin monomer with a high $S^{\circ}_{20,w}$ value (and the electron micrographs are quite convincing), then with so much actin around in the platelet cytosol (molar actin : myosin ratio over 100 : 1) this folded form will predominate. The studies in this paper show that it is incapable of forming stable thick filaments. How then can an actomyosin complex form for force generation? Does Dr Kendrick-Jones think that the light-chain phosphorylation is adequate to neutralize this actin effect completely?

References

Cove, D. H. & Crawford, N. 1975 Platelet contractile proteins: separation and characterisation of the actin and myosin-like components. *J. mechanochem. Cell Motility* **3**, 123–133.
Harris, G. L. A. & Crawford, N. 1975 The identification and subcellular localisation of thrombosthenin 'M', the myosin-like component of pig platelets. *J. mechanochem. Cell Motility* **3**, 134–145.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

J. Kendrick-Jones. We have not looked at the effect of actin on the formation of our folded myosin molecules. We observe with purified thymus and gizzard myosins, at roughly physiological ionic strength and pH, that ATP and Mg^{2+} alone induce these non-phosphorylated myosins to form folded molecules, i.e. the folded molecules are formed under relaxing conditions, where actin and myosin do not interact. Phosphorylation of these folded myosin molecules causes them to unfold and form stable filaments that interact with actin. Studies *in vitro* therefore show that myosin phosphorylation regulates not only myosin interaction with actin but also the stability of the myosin filaments.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(a)

(c)

(b)

(d)

1 μm

FIGURE 3. For description see opposite.

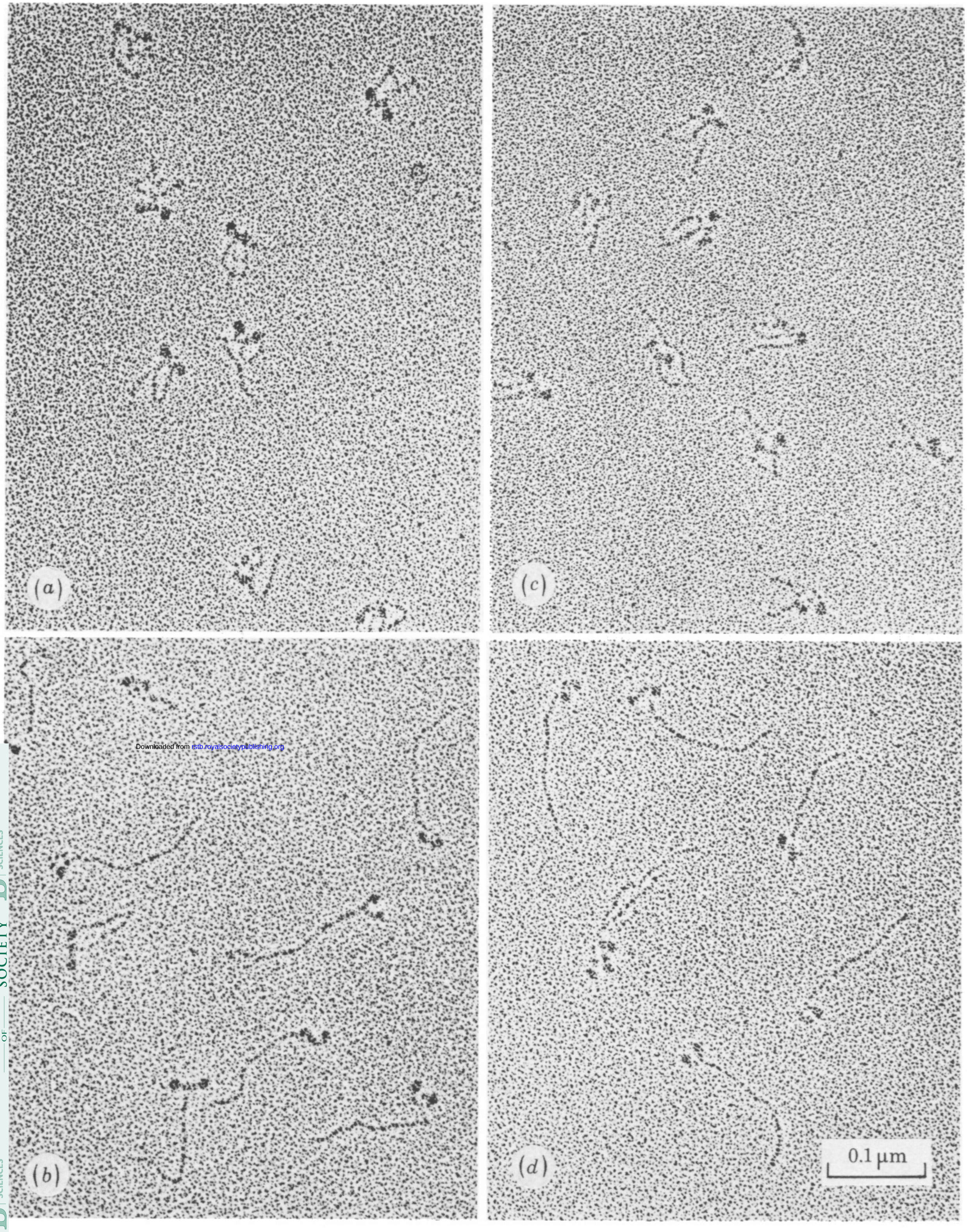

FIGURE 4. For description see opposite.