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Investigation, by Cross-Linking, of Conformational Changes in F-Actin during Its Interactions with Myosin†

Peter Knight* and Gerald Offer

ABSTRACT: The hypothesis that the subunits of F-actin rotate during interaction with myosin and ATP has been tested by using the specific cross-linking reagent *p*-phenylene-*N*,*N*′-bis(maleimide) (PM). The insertion of cross-links between F-actin subunits does not change the ability of the F-actin to activate the ATPase of either myosin subfragment-1 (S-1) or heavy meromyosin, and its ability to superprecipitate with myosin is unimpaired. We conclude that large-scale rotations of actin subunits are not required for activity. The cross-linking of F-actin by PM is, however, inhibited in a non-cooperative fashion by S-1 binding, suggesting that a small local change in actin structure may accompany the binding of S-1 or that S-1 sterically blocks the cross-linking by binding near the contact region between actin subunits.

The sliding of thick and thin filaments during muscle contraction is thought to be brought about by tilting of the myosin heads attached to actin (Huxley, 1969; Huxley & Simmons, 1971). Although in Huxley's original hypothesis the tilting was supposed to take place by rotation of the heads over the surface of the actin subunits, it is equally plausible that the contact between actin and myosin is unchanged and that tilting results from rotation of the actin subunits (Huxley, 1974).

The interactions between myosin and actin during the hydrolysis of ATP in solution are thought to mimic the cross-bridge cycle (Lymn & Taylor, 1971). Therefore, the hypothesis that actin subunits rotate during the cross-bridge cycle may be tested by modifying F-actin so as to prevent rotation of the subunits and then observing whether this impairs the ability of F-actin to activate myosin ATPase and to form a superprecipitating mixture. In an earlier paper (Knight & Offer, 1978), we showed that the reagent *p*-phenylene-*N*,*N*′-bis(maleimide) (PM)[1] forms cross-links between a cysteine

† From the Department of Biophysics, King's College, London WC2B 5RL, England (P.K. and G.O.), and the Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218 (P.K.). *Received September 26, 1979; revised manuscript received May 20, 1980.* This work was supported by a training award from the Medical Research Council of Great Britain and a fellowship from the Muscular Dystrophy Association of America to P.K. and in part by National Institutes of Health Grant AM04349 to Dr. W. F. Harrington.

* Address correspondence to this author. Present address: Department of Biophysics, King's College, London.

0006-2960/80/0419-4682$01.00/0

residue of one actin subunit and a lysine residue in a neighboring subunit. The presence of such links would be expected to restrain relative movements of the subunits severely. Nevertheless, as we will report in this paper, the ability of F-actin to activate myosin ATPase and to superprecipitate is essentially unimpaired by the cross-linking.

PM reacts rapidly with a cysteine residue on every actin subunit and then more slowly forms cross-links by reaction with a lysine residue of a neighboring subunit. The final extent of cross-linking depends on the rate of the cross-linking reaction compared to the rate of hydrolysis of the actin-bound reagent (Knight & Offer, 1978; Knight, 1979). Thus, the change in the rate of the cross-linking reaction that would result from a small change in the relative positions of the cysteine and lysine residues involved in the reaction should have a marked effect on the final extent of cross-linking. Study of the cross-linking reaction itself is thus a sensitive method for detecting changes in the contact region between actin subunits. We have used this approach to determine whether the binding of myosin heads alters the structure of F-actin, and we find that the cross-linking reaction was inhibited when each actin subunit was bound to myosin subfragment-1. Thus, a small conformational change may occur in an actin subunit when it binds myosin, or the myosin heads may sterically block the cross-linking reaction by binding close to the contact region between actin subunits.

Materials and Methods

Preparation of Proteins. Actin was prepared by the methods of either Spudich & Watt (1971) or Hitchcock (1973) from an acetone powder of rabbit skeletal muscle (Straub, 1942; Katz & Hall, 1963). Twice-precipitated rabbit myosin was prepared by a method similar to that of Perry (1955). Subfragment-1 was prepared by papain digestion of myosin using either the method of Margossian & Lowey (1973a) or a modified version of the myofibril digestion method of Cooke (1972). For the latter, 300 mL of washed myofibrils in 0.1 M KCl, 5 mM K_2HPO_4, 5 mM KH_2PO_4, and 1 mM $MgCl_2$ at approximately 15 mg/mL total protein was digested at 25 °C for 30 min by 0.011 mg/mL papain (Worthington; 19 units/mg dissolved in 10 mL of 10 mM cysteine and 10 mM EDTA, pH 6, prior to addition to fibrils). Digestion was terminated by 1 mM sodium iodoacetate. After three washes of the fibrils to remove peptide material and papain, the S-1 was released by the addition of 1 mM magnesium pyrophosphate with stirring for 20 min at 4 °C. The fibrils were sedimented at 13000*g*, and the supernatant was recentrifuged at 78000*g* for 3 h to sediment the appreciable quantities of thin filaments that are released. The supernatant was dialyzed vs. 20 mM imidazole–HCl at pH 7.0 and chromatographed on a 2.5 × 25 cm column of DEAE-cellulose (Whatman DE-52) equilibrated with 20 mM imidazole–HCl, pH 7.0, and eluted with a linear gradient from 0 to 0.3 M NaCl in 1 L of 20 mM imidazole–HCl, pH 7.0. The protein in the major peak was precipitated with 2.3 M ammonium sulfate at 0 °C and the precipitate dialyzed into 2 mM Tris-HCl, pH 8.1. The yield after clarification at 78000*g* for 90 min was about 250 mg. Heavy meromyosin was prepared by the method of Weeds & Taylor (1975).

Cross-Linking F-Actin with PM for S-1 ATPase Studies. F-Actin at about 4.5 mg/mL in 0.1 M KCl, 1 mM $MgCl_2$, 0.2 mM ATP, and 2.5 mM disodium tetraborate was reacted with a 2:1 molar ratio of PM to actin at pH 9.5 and 25 °C by the addition of 0.01 volume of 5 mg/mL PM dissolved in dimethylformamide. 2-Mercaptoethanol was added to a final concentration of 6 mM after 5 h, and excess reagents were removed by dialysis against 12.5 mM KCl, 1.0 mM $MgCl_2$, and 2.5 mM Tris-HCl, pH 7.5, at 4 °C.

Fractionation of Cross-Linked Actin. F-Actin at 2 mg/mL in 0.1 M KCl, 1 mM $MgCl_2$, and 10 mM disodium tetraborate was reacted with a 2:1 molar ratio of PM to actin at pH 9.1 and 25 °C. After 30 min of reaction the F-actin was sedimented by centrifugation. The pellets were homogenized in 2 mM Tris-HCl, 0.2 mM $CaCl_2$, and 0.2 mM ATP, pH 8.0, at 5 °C, dialyzed against the same buffer for 36 h to depolymerize the cross-linked material as much as possible, and then centrifuged at 130000*g* for 120 min. The supernatant, which could be shown by $NaDodSO_4$ gel electrophoresis to contain most of the un-cross-linked monomers of the original mixture, was repolymerized with 0.1 M KCl and 1 mM $MgCl_2$ and concentrated by centrifugation. These pellets were homogenized in a small volume of 10 mM imidazole–HCl, 10 mM KCl, and 0.6 mM $MgCl_2$, pH 7.2, at 5 °C to yield preparation A. The actin pellets from the 130000*g* centrifugation, when examined by $NaDodSO_4$ gel electrophoresis, could be shown to contain larger oligomers but were depleted of un-cross-linked monomers compared with the original mixture. For reduction of the monomer content still further, the pellets were homogenized in 2 mM Tris-HCl, 0.2 mM $CaCl_2$, and 0.2 mM ATP, pH 8.0, at 5 °C and dialyzed overnight against the same buffer. The actin was then diluted with an equal volume of water, thoroughly homogenized, and stirred for 2 h before centrifugation at 130000*g* for 120 min. The pellets (designated preparation B) were homogenized and, together with preparation A described above, were dialyzed against 10 mM imidazole–HCl, 10 mM KCl, and 0.6 mM $MgCl_2$, pH 7.2, at 5 °C. A control sample of actin treated with the same concentration of dimethylformamide as the cross-linked actin was carried through the same procedure as the preparation A.

Effect of S-1 on the Cross-Linking of F-Actin with PM. The stoichiometry of the effect was examined as follows. One-milliliter portions of F-actin in 0.1 M KCl, 1.0 mM $MgCl_2$, and 2.5 mM disodium tetraborate, pH 9.1, at 25 °C were reacted for 30 s with 1.25 mol of PM per mol of actin followed by the addition of 1 mL of S-1. The final actin concentration was 0.94 mg/mL, and the S-1 concentration was varied to cover the range 0–1.5 mol/mol of actin subunit. After about 90 min of reaction, the mixtures were centrifuged at low speed to remove air bubbles and their absorbance at 400 nm was determined to assess acto-S-1 binding. Samples were then subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis to determine the extent of cross-linking.

The effect of S-1 on the time course of cross-linking of F-actin by PM was examined by adding equimolar S-1 to an F-actin solution 30 s after the addition of PM and then quenching 1-mL samples with 10 μL of 2-mercaptoethanol at known times.

In both experiments, the mean number of cross-links per actin subunit of each sample was determined by densitometry of polyacrylamide–$NaDodSO_4$ gels, as described previously (Knight & Offer, 1978).

ATPase Measurements. A Radiometer pH stat assembly, modified as described by Offer et al. (1972), was employed for the acto-S-1 ATPase studies. A 10 mM NaOH solution was used as titrant with a nitrogen atmosphere. The reaction was initiated by adding S-1.

[1] Abbreviations used: PM, *p*-phenylene-*N*,*N*′-bis(maleimide); S-1, myosin subfragment-1; HMM, heavy meromyosin; $NaDodSO_4$, sodium dodecyl sulfate.

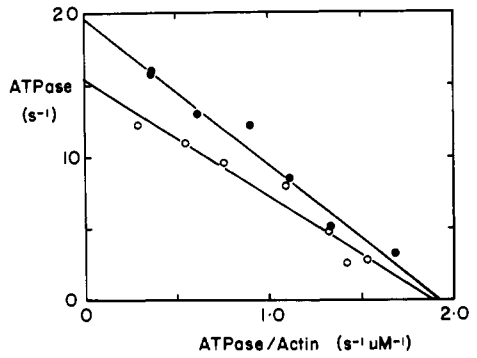

FIGURE 1: Activation of S-1 ATPase by F-actin and cross-linked F-actin. Assays were performed in 10 mM KCl, 0.8 mM $MgCl_2$, 2.0 mM Tris-HCl, and 0.5 mM ATP, pH 7.0, at 25 °C with 2–45 μM F-actin (●) or cross-linked F-actin (○). 0.2–2 nmol of S-1 was added to start the reaction. Lines of best fit were calculated by the method of least squares.

For the acto-HMM ATPase studies, 1-mL aliquots from an initial 5-mL reaction mixture were quenched at 2-min intervals with 1 mL of 20% trichloroacetic acid and the supernatant obtained by centrifugation was assayed for inorganic phosphate by the method of Fiske & Subbarow (1925) using *p*-(methylamino)phenol sulfate ("Elon", Eastman-Kodak, Ltd.) as reducing agent.

Assay of Actomyosin Superprecipitation. A Cary 15 double-beam recording spectrophotometer was used to record light scattering changes during the superprecipitation of actomyosin. The conditions of the assay were essentially as described by Margossian & Lowey (1973b).

Determination of Protein Concentration. Actin concentrations were measured by the biuret method (Gornall et al., 1949) standardized with unmodified G-actin for which a specific absorption coefficient of 0.63 L g^{-1} cm^{-1} at 290 nm was taken (Lehrer & Kerwar, 1972). The specific absorption coefficient at 280 nm for S-1 was taken as 0.8 L g^{-1} cm^{-1} (Margossian & Lowey, 1973a), for HMM as 0.56 L g^{-1} cm^{-1} (S. Himmelfarb, personal communication), and for myosin as 0.52 L g^{-1} cm^{-1} (Godfrey & Harrington, 1970). The molecular weight of actin was taken as 42000 (Collins & Elzinga, 1975), of S-1 as 120000 (Lowey et al., 1969), and of HMM prepared by α-chymotryptic digestion as 350000 (S. Himmelfarb, personal communication).

Polyacrylamide–NaDodSO$_4$ Gel Electrophoresis. Electrophoresis was performed on 4% polyacrylamide gels by the method of Weber & Osborn (1969). Gels were stained with Coomassie brilliant blue G-250 or R-250 and destained by diffusion into 5% methanol and 7% acetic acid (v/v), and the amount of staining was quantitated by densitometry. Relative peak areas were determined by planimetry or by excision and weighing of each peak.

Electron Microscopy. Negative staining with unbuffered 1% uranyl acetate was performed as described by Huxley (1963) by using a carbon support film on copper grids. Grids were examined in a Philips EM200 instrument operated at 80 kV.

Results

Activation of S-1 and HMM ATPase by Cross-Linked F-Actin. The activation of S-1 ATPase by F-actin and by F-actin cross-linked with PM was compared over the range 2–45 μM F-actin at low ionic strength. Moos (1972) showed that the acto-S-1 ATPase has a hyperbolic dependence on actin concentration. The data can therefore be fitted by an equation of the Michaelis–Menten type. Figure 1 shows an analysis of the data using the linear form of the Michaelis–Menten equation $v = -K_m(v/s) + V_{max}$ (Hofstee, 1959). The extrapolated ATPase rate at infinite actin concentration, V_{max}, was 20 s^{-1} for F-actin and 15 s^{-1} for cross-linked F-actin, compared with 0.05 s^{-1} for S-1 alone under these conditions. Modification of the subunits of F-actin has therefore reduced only slightly its ability to activate S-1 ATPase. The Michaelis constant, K_m, was 10 μM for F-actin and 8 μM for cross-linked F-actin. Quantitative analysis of the extent of cross-linking showed that a mean of 0.49 cross-links/actin subunit was present in the cross-linked sample and that only 25% of the actin subunits were not involved in cross-linked complexes. If the cross-linked subunits were inactive so that the observed activation of the S-1 ATPase by the cross-linked actin was due solely to the un-cross-linked subunits, we would have observed a fourfold increase in the K_m as a result of cross-linking. In fact, a 20% decrease in K_m was measured, suggesting that the cross-linked subunits were also participating in the acto-S-1 ATPase.

Table I: HMM ATPase Activation by Cross-Linked F-Actin Compared to the Extent of Cross-Linking

F-actin type	% monomer	% subunits cross-linked to: one other	% subunits cross-linked to: two others[a]	V_{max} (s^{-1})[b]	K_m (μM)[b]
unmodified	100	0	0	8.4	7.5
preparation A[c]	32	54	14	6.8	4.4
preparation B[c]	8.4	52	39	6.6	4.1

[a] Computed as $\Sigma[(\text{mass fraction of } n\text{-mer}) \times [(n-2)/n]]$ from densitometer traces. [b] ATPase was measured in 5 mM KCl, 0.3 mM $MgCl_2$, 5 mM imidazole–HCl, and 2 mM MgATP, pH 6.8, at 25 °C (ionic strength = 18 mM) by using 0.1 μM HMM and 1–40 μM F-actin. Values for V_{max} (expressed as molecules of ATP split per second per head) and K_m (in micromolar actin) were obtained by a nonlinear least-squares fitting program, assuming Michaelis–Menten kinetics. [c] See Materials and Methods for preparation.

Alternatively, the cross-linked subunits might act as competitive inhibitors of the ATPase, for instance, by reversibly associating with the S-1 but not promoting product release. In that case we would expect to observe a decrease in V_{max} and possibly also in K_m, the magnitude of the changes depending on the relative amounts of cross-linked and un-cross-linked subunits and on their relative association constants for S-1. For an examination of whether such competitive inhibition was present, a sample of cross-linked F-actin was separated into two fractions, one containing most of the un-cross-linked subunits and the other containing most of the multiply cross-linked subunits. Table I shows that the fractions were very similar indeed in both the V_{max} and the K_m of their activation of HMM ATPase, despite a fourfold difference in content of un-cross-linked subunits and an almost threefold difference in content of multiply cross-linked subunits. Both fractions were slightly less active than the unmodified F-actin, indicating that it is modification of every subunit with PM, rather than the introduction of cross-links, which causes the slight reduction in V_{max} and K_m. We conclude that the cross-linked subunits do not act as competitive inhibitors but are instead potent activators of both S-1 and HMM ATPase.

Superprecipitation of Cross-Linked F-Actin with Myosin. The superprecipitation of actomyosin solutions has been taken as a model for muscle contraction (Szent-Györgyi, 1951). The precipitate is formed by the clumping of synthetic myosin and actin filaments [see, e.g., Nonomura & Ebashi (1974)]. It is presumed that the movement of the filaments that is required for this clumping is produced by the same movement of myosin

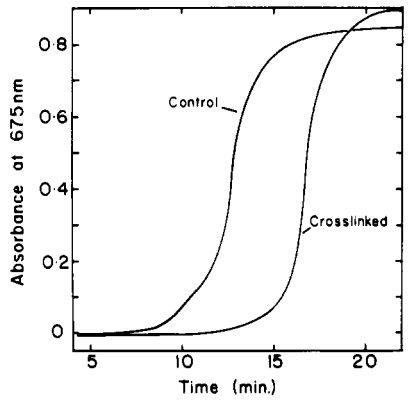

FIGURE 2: Superprecipitation of myosin with F-actin and cross-linked F-actin. Myosin (in 0.5 M KCl) was mixed with F-actin in a 10-mm path length cuvette to yield a mixture of 1.3 mg/mL myosin and 0.33 mg/mL F-actin in 0.1 M KCl, 0.3 mM $MgCl_2$, 1.7 mM sodium borate, and 3.3 mM potassium phosphate, pH 7.2, at 24 °C. At zero time, 1.7 mM MgATP was mixed into the actomyosin gel and the absorbance at 675 nm recorded.

heads attached to F-actin that occurs in muscle contraction. Although we have shown that cross-linked F-actin can activate the ATPase effectively, this does not rule out the possibility that large-scale rotations of actin subunits are required for movement of myosin heads bound to actin but not for ATPase activation. Therefore, a mixture of cross-linked F-actin with myosin was tested by observation and by measurement of light scattering for its ability to superprecipitate. An actomyosin gel at 1.6 mg/mL had an absorbance of 0.15 and cleared completely on mixing with ATP under the ionic conditions used. The sharp rise in absorbance shown in Figure 2 for both F-actin and cross-linked F-actin resulted from the formation of a flocculent precipitate which contracted away from the walls and stood as a square pillar in the center of the cuvette, leaving a clear liquid surrounding it. The gel state was not regained, and the mixture had a low viscosity.

It is clear from Figure 2 that myosin superprecipitates with cross-linked F-actin with the same time course and to the same extent as with unmodified F-actin. The difference between the two traces is the length of the clear period between the addition of ATP and the onset of turbidity accompanying superprecipitation. The clear period corresponds to the duration of the steady state of the actomyosin ATPase, so the difference between the traces suggests that cross-linked F-actin is not quite as effective an activator of myosin ATPase as unmodified F-actin. The duration of the clear period can be used to calculate an approximate average turnover rate for the actomyosin ATPase during the steady state. For unmodified F-actin this rate is 0.4 mol of ATP per s per mol of heads and for cross-linked F-actin it is 0.3 mol of ATP per s per mol of heads. A more detailed study would be required to establish the origin of this small difference; the present data, however, show clearly that cross-linking F-actin does not affect the formation of superprecipitates with myosin.

Effect of S-1 on the Cross-Linking of F-Actin by PM. PM was allowed to react monofunctionally with the F-actin before varying amounts of S-1 were added: Figure 3 shows that this F-actin can still bind 1 mol of S-1 per mol of subunit since the turbidity of the mixtures reaches a plateau at an equimolar ratio of S-1 to actin. The interaction of S-1 with F-actin produces a marked decrease in the extent of cross-linking, which reaches a minimum at equimolar amounts of S-1 and actin. Since the decrease in linear, each S-1 exerts an equal inhibitory effect on the cross-linking reaction. The relative amounts of the cross-linked actin species in $NaDodSO_4$ gels

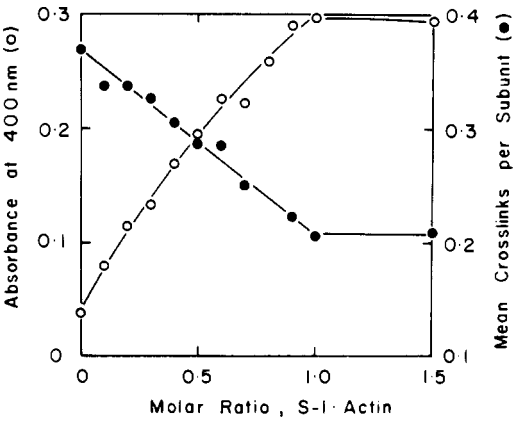

FIGURE 3: Inhibition by S-1 of F-actin cross-linking. 44 μM actin in 0.1 M KCl, 1.0 mM $MgCl_2$, and 2.5 mM sodium borate, pH 9.1, at 25 °C was reacted with 56 μM PM. After 30 s, an equal volume of S-1 was added in the same buffer to give the molar ratios shown. After 90 min the binding ratio of S-1 to F-actin was determined by absorbance at 400 nm (○). The extent of cross-linking was determined by densitometry of 4% polyacrylamide–$NaDodSO_4$ gels (●).

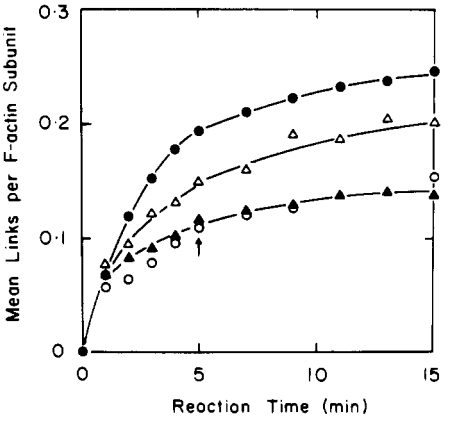

FIGURE 4: Time course of actin cross-linking in the presence of S-1. 24 μM actin in 0.1 M KCl, 1.0 mM $MgCl_2$, and 2.5 mM sodium borate, pH 9.1, at 25 °C was reacted with 30 μM PM. After 30 s of reaction, an equal volume of this solvent containing either no addition (●), or 26 μM S-1 (to give almost equimolar S-1 and actin) (▲), or 26 μM S-1 and 1.5 mM sodium pyrophosphate (△), or 26 μM S-1 (○) was added. To this last mixture was added sodium pyrophosphate after 5 min (arrow) to give a final concentration of 0.75 mM. Observation of the absorbance of the acto-S-1 mixture showed that 80% of the S-1 was dissociated by the addition of 0.75 mM pyrophosphate. The reaction in 1-mL aliquots was quenched with 10 μL of 2-mercaptoethanol. The extent of cross-linking was determined from densitometer scans of 4% polyacrylamide–$NaDodSO_4$ gels.

of mixtures containing less than 1 mol of S-1 per mol of actin were close to those expected from a random insertion of cross-links [see Knight & Offer (1978)], showing that S-1 binds randomly along F-actin. The electrophoretic gels showed no evidence of cross-links between S-1 and actin, so the inhibition observed is not due to competition with actin–S-1 cross-linking.

For an investigation of the mechanism of the inhibition, the time course of cross-linking in the presence of S-1 was examined. We found that cross-linking continues at a reduced rate when S-1 is bound to F-actin, but the time courses of the reactions are otherwise similar (Figure 4). This suggests that the rate of hydrolysis of the cross-linking reagent bound to actin is unaffected by the binding of S-1, but the rate of cross-linking is decreased (Knight & Offer, 1978). In support of this interpretation, we found that if the S-1 is partially dissociated from the F-actin by the inclusion of pyrophosphate in the mixture, then the rate of cross-linking is increased

toward that of F-actin alone, but if the addition of pyrophosphate is delayed for 5 min, during which time most of the PM would be expected to have hydrolyzed, the rate of cross-linking indeed remains low (Figure 4).

The reduction in the rate of cross-linking could be caused either by a steric blockage by S-1 of the cross-linking sites on F-actin or by a small conformational change in the F-actin subunit. If S-1 sterically blocks cross-linking, the cross-links could affect the geometry of binding of S-1. In the electron microscope, the characteristic "arrowhead" appearance of F-actin decorated by S-1 depends on a rather specific orientation of the S-1 attached to each F-actin subunit (Moore et al., 1970) and might therefore be sensitive to such a change in S-1 binding geometry. However, the appearance of cross-linked F-actin decorated by S-1 was found to be indistinguishable from the structure obtained by using unmodified F-actin. However, we would not necessarily detect a small change in geometry, so from this result we cannot decide whether the inhibition of cross-linking arises from steric blocking by S-1 or from a conformational change in the F-actin.

Discussion

We have shown that the introduction of links between the subunits of F-actin has very little effect on the activation of the ATPase of S-1, HMM, and myosin. An analysis of the kinetics of activation of S-1 and HMM ATPases showed that a small decrease (up to 28%) in both V_{max} and K_m accompanied reaction with PM but that there was no correlation between activating ability and the extent of cross-linking. A similar small reduction in activating ability was noted by Stone et al. (1970) following reaction of F-actin with a maleimide spin-label. We conclude, therefore, that it is either the modification of the cysteine residue or the incorporation of new bulk in its vicinity which causes the change in activation and that the insertion of cross-links between the subunits has no additional effect.

There have been earlier reports on the activity of cross-linked F-actin. Poo & Hartshorne (1976) used glutaraldehyde to cross-link F-actin and found, at a single actin concentration, that the modified actin was a more potent activator of S-1 ATPase than unmodified F-actin, but they did not determine whether this was due to a change in V_{max} or K_m. Gadasi et al. (1974) showed that glutaraldehyde treatment reduced the ability of F-actin to superprecipitate with myosin and reported that the actomyosin ATPase was unchanged. Glutaraldehyde is a nonspecific reagent and at the concentration used in both studies (0.1 M) will presumably have modified many amino acid residues aside from those involved in cross-links. The changes in the properties of F-actin that resulted from glutaraldehyde treatment may therefore be unrelated to the introduction of cross-links. The present work shows that cross-links can be inserted into F-actin without impairing its ability to interact with myosin filaments and generate the force required to produce superprecipitation. Before the significance of these results can be assessed, we need to consider to what extent the cross-links inserted have prevented the putative movements of the subunits. As described earlier (Knight & Offer, 1978), only one cysteine residue of every actin subunit reacts with PM, forming cross-links by reaction with probably only one particular lysine residue of a neighboring subunit. F-Actin can be considered to consist of two chains of subunits wound together. Because it is probable that only a single species of cross-link is produced by PM, the cross-links are formed either within or between the chains, but not both. Therefore, every link will have an equal restraining effect. We have not been able to determine which of the two patterns of cross-linking has occurred, so we cannot be precise in our knowledge of the restraint produced by the cross-links.

If rotation of an actin subunit attached to a myosin head were to bring about relative movement of the thick and thin filaments, we would expect the axis of rotation to lie in a plane approximately perpendicular to the axis of the thin filament. An actin subunit cross-linked to two neighbors could not have both links lying in this plane. It is very likely that neither link would lie in this plane (as for instance, when the cross-links join members of the same chain of subunits). Thus, such subunits would be severely restrained. Actin subunits linked only to one neighbor might experience less restraint but only in the case that the cross-links were fortuitously coincident with the axis of rotation could the freedom of movement approach that of a non-cross-linked subunit. This coincidence could in any case apply to only half the singly cross-linked subunits. Thus, we expect the cross-links to be a significant impediment to large-scale subunit rotations. We therefore conclude that large-scale rotations of actin subunits with the F-actin polymer do not seem to be necessary for effective interaction with myosin.

It is necessary to stress that we are examining here the possibility of large (perhaps 45°) transient rotations of actin subunits. Very much smaller relative movements of subunits, or distortions of subunit contacts, such as presumably occur during flexion of thin filaments in solution (Yanagida & Oosawa, 1978; Nagashima & Asakura, 1980; Newman & Carlson, 1980), might not be restrained by the insertion of a species of cross-link which almost completely blocked subunit rotations.

The additional possibility that movement occurs only in the part of the actin subunit that combines with the myosin head would not be expected to be tested by the effects of the intermolecular cross-links produced by PM, because the contacts between subunits may remain essentially unchanged during the movement. Recent data from saturation transfer electron paramagnetic resonance measurements of spin-labeled F-actin can be interpreted as suggesting that there may indeed be flexibility in part of the actin subunit and that the myosin head binds to this part of the subunit (Thomas et al., 1979). Since the spin-label used by Thomas et al. (1979) was attached to the same cysteine residue of actin as that with which PM reacts, it is possible that the cross-links introduced by PM might have reduced the movements they observed. Our finding that cross-linked F-actin is a potent activator of myosin ATPase would then argue against large-scale movements of this part of the actin subunit during interaction with myosin. As Thomas et al. (1979) point out, however, there are several alternative interpretations of the origins of their data; for instance, the movement of the spin-label could arise from torsional movements of small sections of the F-actin structure. The possibility of localized conformational changes within each actin subunit therefore remains. It is pertinent to this question that we have observed that when S-1 binds to F-actin it inhibits the insertion of cross-links by PM that has already reacted monofunctionally with actin. There is no evidence for cooperativity in this effect, suggesting that each S-1 affects the formation of a single cross-link and therefore has only a very local effect on the structure of the polymer. A fluorescent derivative of maleimide, which presumably reacted at the same site on F-actin as PM, was used by Sekine et al. (1974) to investigate changes in the structure of the F-actin subunit on binding HMM. Their data suggested that the fluorophore moved into a less polar environment when HMM bound to

labeled F-actin. Our data show that water is not completely excluded from the vicinity of the maleimide binding site when S-1 binds, since hydrolysis of the unreacted end of PM continues; however, the binding of S-1 may have caused the actin-bound PM to move away from the lysine residue with which it would otherwise react, thereby inhibiting cross-linking, and in that sense our work parallels that of Sekine et al. (1974). Our data show that cross-linking can be a sensitive probe for changes in the structure of F-actin.

Acknowledgments

We are grateful to Dr. W. F. Harrington, Dr. S. J. Lovell, and Dr. H. White for helpful discussions.

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