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THE MODIFICATION OF ACTOMYOSIN BY α-ACTININ

IV. THE ROLE OF SULFHYDRYL GROUPS

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SUMMARY

A study has been made of the consequences of the substitution of about half of the sulfhydryl groups in myosin or actin (or both) by reaction with 2-aminoethyl isothiuronium (AET).

With respect to myosin, this substitution makes the ATPase activity specifically dependent on the presence of calcium ions, and independent of the suppression normally encountered at high ionic strength.

AET-substitution of myosin makes the resulting actomyosin unresponsive to α-actinin; that of actin does not affect the responses to α-actinin. Either substitution diminishes the binding of α-actinin to actomyosin. Neither substitution abolishes contractility as measured in terms of gel synaeresis.

INTRODUCTION

In this fourth of the present series of papers on the interactions among actin, α-actinin and myosin (*cf.* BRISKEY, SERAYDARIAN AND MOMMAERTS[1,2]; SERAYDARIAN, BRISKEY AND MOMMAERTS[3]), we wish to present observations suggesting an essential participation of sulfhydryl groups in these phenomena. As the main reagent for these studies, we selected 2-aminoethyl isothiuronium abbreviated as AET (DOHERTY AND SHAPIRO, see MORALES *et al.*[4]), because this had been found to exert some striking effects upon the ATPase activity of myosin[4,5]. This reagent is thought to act by formation of a disulfide bond, whereas the presence of the guanidino group will confer some additional specific features.

Abbreviations: AET, 2-aminoethyl isothiuronium; EGTA, ethylene glycol-bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid.

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METHODS

The same procedures are employed as in the 3 preceding papers[1-3]. Specifically, data will be presented on the course of the routine turbidity assay, gel synaeresis, actomyosin-ATPase, and ultracentrifugal sedimentation pattern.

Concerning the preparation of the proteins, we have learned that very good myosin samples are obtained by 3 purification steps, each involving dissolution in 0.3 M KCl followed by dilution to 0.23 M in order to precipitate contaminants, all in the cold, and that the stability of actomyosin is enhanced if it is reconstituted from its components and washed immediately after the final purification of its constituent proteins. Actin prepared by our standard method[6] frequently gives some precipitate with 3.3 M KCl, and hence this purification step was always used as described by SERAYDARIAN, BRISKEY AND MOMMAERTS[3].

Treatment of proteins with AET

This is based upon various procedures used by MORALES *et al.*[4,5], our treatment being standardized as follows. Each protein is brought to a concentration of 12 mg per ml, and about 5 ml of the solution is placed in a cellophane dialysis bag of 10 mm diameter. This bag is rotated at about 200 rev./min for 1 h in the cold in 100 ml of 1 mM AET in the solvent, 0.025 M alanine buffer (pH 9.5), with 0.1 M KCl in the case of F-actin and 0.4 M KCl, 0.1 M alanine (pH 9.5) for myosin or actomyosin. The bag is then stirred for another hour in 0.05 M Tris buffer (pH 7.5) without AET, and with the same amount of KCl as previously, after which the solution is ready for analysis and experimentation.

Determination of sulfhydryl groups

This is done with the method of BOYER[7] as modified by SELA, WHITE AND ANFINSEN[8]; for the details of our application to actin, see KATZ AND MOMMAERTS[9].

The reagent is prepared by dissolving about 12.5 mg of chloromercuric benzoate with the minimal amount of 0.1 M NaOH and dilution to 100 ml with buffer. The final concentration is determined spectrophotometrically at 232 mμ, where the molar extinction coefficient equals $1.69 \cdot 10^4$. A 1:3 dilution of the stock solution, about $1.1 \cdot 10^{-5}$ M, is added in graded increments of 0.05 ml to each of a series of tubes containing 0.5 mg of protein in a medium of 0.2 ml 1 M Tris buffer (pH 7.5), 0.75 ml 2 M KCl (in the case of myosin, 0.15 ml for actin) and water to a final volume of 3 ml. After standing at room temperature overnight, the solutions are read in a Beckman DU spectrophotometer at 255 mμ. The equivalence point is obtained as the quantity of added mercurial (abscissa) where the 2 straight lines on the plot of the absorbance change (ordinate) intersect. For the calculation of the sulfhydryl content, the molecular weight in the case of actin is taken to be 60000 as a rounded-off average between the original proposal for the molecular weight as 57000 (ref. 10) and some of the slightly higher values reported subsequently; the normal SH-content is then about 6.5–7.0 (refs. 11, 9, 7, 12), probably meaning 6 moles per lower or 7 moles per higher molecular weight entity. For myosin, the arbitrary figure of 100000 is used, in view of the persisting disagreement on the accurate value of the molecular weight. The normal SH-content is then about 7.4 (*e.g.* refs. 13, 14, 5, 23).

RESULTS

Effect of AET upon myosin-ATPase under various conditions

Since the publications by MORALES and coll.[4,5] deal only with "myosin" (presumably a mixed actomyosin) at high ionic strength, it appears necessary first to describe the influence of AET under a greater variety of conditions. The results are shown in Figs. 1–3.

In curves 2 and 3 of Fig. 1 are given the ATPase of pure myosin as dependent upon the KCl concentration in presence of ethyleneglycol-bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) or $CaCl_2$. Descriptively, this can be regarded as showing (1) a weak activation by K^+, optimal at moderate ionic strength above which the activity declines because of the higher ionic concentration, specifically perhaps that of the disrupting anion (*cf.* WARREN, STOWRING AND MORALES[15]) (2) a much more effective activation by Ca^{2+}, declining at all investigated ionic strengths, probably for the same reason and perhaps also, at lower ionic strength, because of a competition between the weak activator, K^+, and the better activator, Ca^{2+}. Curves 1 and 4, similarly, show the reactions of AET-myosin. This shows (3) that without Ca^{2+} the activities are now much lower, and (4) with Ca^{2+} a much higher activity is displayed, which is not suppressed at high salt concentration. Thus, AET allows full expression of the K^+–Ca^{2+} activated ATPase and protects the enzyme against the salt-sensitivity as listed under (2).

In curves 2 and 3 of Fig. 2, we are dealing with normal actomyosin; the results are entirely similar to those of Fig. 1, although the activities without Ca^{2+} (curve 2) have been consistently lower. This seems paradoxical, since at the higher salt concen-

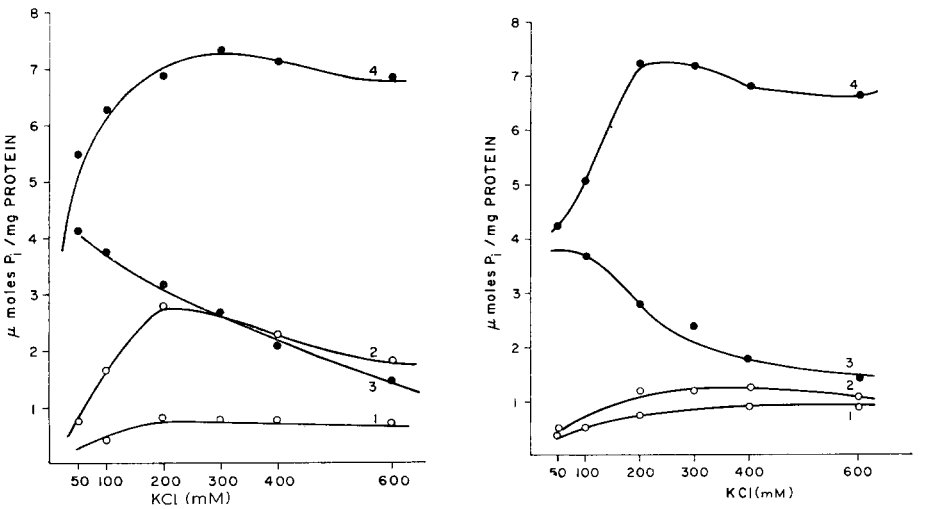

Fig. 1. The ATPase activity of myosin as a function of the KCl concentration. Protein concentration 1.0 mg per 10 ml in the presence of calcium, 1.8 mg per 10 ml in the presence of EGTA. Tris–acetate buffer, 50 mM (pH 8.0). ○, with $2 \cdot 10^{-3}$ M EGTA; ●, with $1 \cdot 10^{-3}$ M $CaCl_2$. Curves 1 and 4, AET-myosin; 2 and 3, normal myosin.

Fig. 2. The ATPase activity of actomyosin; conditions and meaning of open and filled circles as in Fig. 1. Curves 1 and 4, actomyosin made of AET-myosin and normal actin; curves 2 and 3, normal actomyosin.

tration in the presence of ATP there should be no actomyosin, only myosin and actin. We have no supported explanation for this difference, except to suppose that at these high actin/myosin ratios some interaction persists even at high ionic strength, reminiscent of the experiments of NANNINGA[16] who spoke of a competition between actin and ATP for the myosin. In addition, there appears to be a second feature, which adds some specification: with EDTA, myosin displays a very strong activation[19,18] whereas actomyosin does so much less. It is possible that EGTA to a much weaker extent has a comparable effect, which would also explain the difference between the curves 2 in Figs. 2 and 3. The distinction between myosin and actomyosin in regard to EDTA activation (not illustrated) likewise persists at high ionic strengths where dissociation of the components would be thought to occur, so that the same consideration applied here too.

Turning to the AET-myosin–actin complex (curves 1 and 4), this is seen to be much the same as pure AET-myosin.

In Fig. 3, curves 2 and 3 refer to the myosin–AET-actin complex. This is much like ordinary actomyosin (Fig. 2, curves 2 and 3), except that the AET-substitution of actin causes an additional activation at low ionic strength. This will be documented in several ways further on. When actomyosin as such is AET-substituted (curves 1 and 4), the results are rather like those with AET-myosin–actin (see Fig. 2); however, the maximal activation reached is less than when myosin is singly exposed to the reagent, it must have been somewhat screened off by the presence of actin. With AET-myosin–AET-actin (not illustrated), the results are also dominated by the AET-myosin.

At the relatively high ionic strengths, all activities are strongly inhibited by Mg^{2+} (not illustrated), and thus the actomyosin–Mg–ATPase[19,20] is not in evidence here.

Effects of aging and of alkaline treatment

In general, all reductions of the number of sulfhydryl groups seem to act in the same direction. Upon aging either the myosin or the actin moieties, changes occur

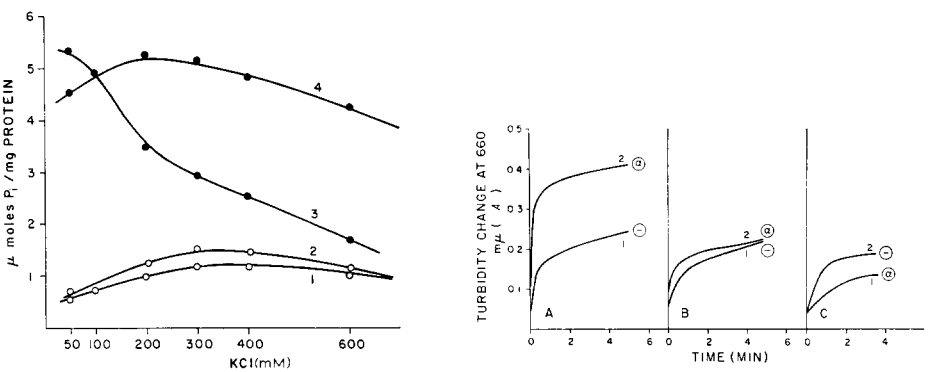

Fig. 3. The ATPase activity of actomyosin, as in Fig. 1. Curves 1 and 4, actomyosin treated with AET; curves 2 and 3, actomyosin made of myosin and AET-actin.

Fig. 4. Turbidity responses of normal actomyosin (A), and of actomyosin made of AET-treated myosin (2 examples in B and C) with and without α-actinin. Final concentration: 50 mM KCl; 1 mM $MgCl_2$; 1 mM ATP, 20 mM Tris–maleate (total) (pH 6.8); 0.8 mg actomyosin/ml; 0.24 mg α-actinin/ml. Temp. 27°.

which are much like those to be described (they are not documented separately). Traces of heavy metal in the solutions can also cause this rapidly, myosin being more sensitive than actin. Finally, exposure to alkaline reaction may have such an influence. Since the AET-substitution is carried out in an alkaline medium, it was important to establish that actin, under exactly the same conditions but for the presence of AET, did not undergo the changes caused with that reagent. Myosin was more variable in that regard. Sometimes, alkaline exposure by itself caused changes in the same direction as those caused more completely by AET; in such cases, the free SH-contents had also measurably decreased, *e.g.* from 7.0 to about 5.0 moles per 10^5 g, whereas AET caused a further reduction to about 4 moles or less. In what follows, we shall limit ourselves to the more controlled modifications caused by AET. All descriptions now refer to low ionic strengths, 50 mM KCl, because it is known that at higher salt concentrations the α-actinin effects vanish. Perhaps this occurs to the extent that the actin–myosin interactions vanish for the case of the calcium-activated ATPase over a broad range of ionic strength[3]. For the magnesium-activated ATPase, the allowed range of KCl concentration is narrower[21], probably because the Mg^{2+} ceases to be an activator; in this case, a large excess of α-actinin is required to obtain an effect at all.

Reaction of myosin with AET

When AET-myosin is prepared as described, and is used to reconstitute actomyosin, it is found that at the ionic strengths used here the resulting ATPase is about the same as with untreated myosin in some cases (*cf.* Fig. 2), or moderately reduced in some others. This activity is not enhanced by α-actinin, while with normal acto-

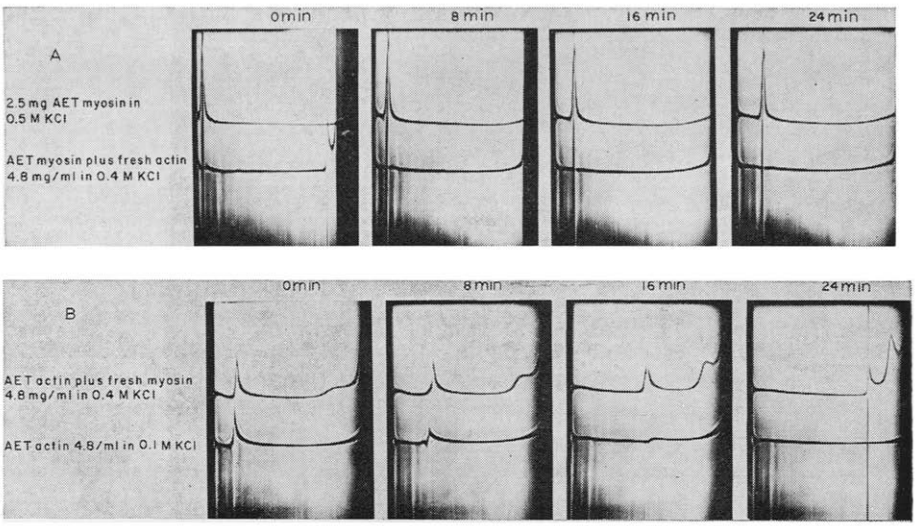

Fig. 5. A, Ultracentrifugal sedimentation of AET-myosin (upper traces), and of actomyosin made from this (lower traces) at various times (min) after reaching 59780 rev./min. In the latter case, sedimentation is so rapid that only the first frame shows a boundary, which has nearly reached the bottom. Ordinary actomyosin under these circumstances would be still in the upper half of the cell. B, Ultracentrifugal sedimentation of AET-actin (lower traces) and of actomyosin made from this (upper traces) at various times (min) after reaching 59780 rev./min. The actin boundary is normal, and so is the actomyosin but for a heavy component.

myosin a sizable activation was found. There is some variability in the outcome of the turbidity test (Fig. 4). Either (Fig. 4B as compared to 4A), this is about normal, or by itself somewhat less. With α-actinin, there is only a negligible activation (Fig. 4B) or none, or actually some inhibition (Fig. 4C) of the turbidity response. We must stress the rather undiscriminating quality of the test because, even when the recorded measurements are about normal, the visual appearance of the suspension has been markedly altered by the AET-treatment; often, the gel precipitates in the cuvette within 4 min. Such acto-AET-myosin still gives a seemingly normal gel synaeresis.

In the ultracentrifuge (Fig. 5A), AET-myosin is found to be normal. Actomyosin made from it (same figure, visible in the first exposure only) sediments extremely rapidly in one major boundary; its solution appears normal to visual inspection.

Neither the presence of α-actinin nor of 1 mM ATP (added at the start) protects the myosin against its reactions with AET, but actin does to some extent (*cf.* Fig. 3).

Reaction of actin with AET

This substitution does not seem to have been studied previously, but proceeds in a manner comparable to that with myosin. Again, about half of the sulfhydryl groups disappear under the conditions of the reaction. In the case of G-actin, the reaction does not interfere with subsequent polymerization; and with F-actin, it causes no depolymerization.

In comparison with the ATPase of untreated actomyosin (Fig. 6A) it is seen that an actomyosin with AET-actin has an ATPase activity exceeding that of normal actomyosin (see also Fig. 3 compared with Fig. 2), but that the addition of α-actinin still gives rise to further activation (Fig. 6B). For the turbidity test (Fig. 6C giving the responses of normal actomyosin for comparison), it is seen from Fig. 6D that actomyosin so treated also gives a normal or moderately enhanced turbidity increase, and that the addition of the actinin causes further enhancement, to a variable degree. Actomyosin so modified still shows gel synaeresis.

When actin is reacted with AET while in the monomeric form and then polymerized, the resultant actomyosin shows the same behavior as when the F-form had been exposed to AET.

AET-treated actin appears to show normal ultracentrifugal sedimentation, and combines with normal myosin to form a modified actomyosin which has an additional heavy component (Fig. 5B). This actomyosin solution is exceedingly viscous, as can be established by visual inspection.

Reaction of actomyosin with AET and other combinations

When actomyosin as such is treated with AET, the resultant effects are closer to those when the actin was treated than when the myosin alone has been reacted. In the ATPase test (Fig. 7A), the treated actomyosin is more active than normal, and addition of α-actinin causes a further enhancement. The turbidity test (Fig. 7B) could be described in the same terms. The remaining possibility of activation by α-actinin in the ATPase assay suggests that myosin is markedly protected against AET when combined with actin. Treatment of actomyosin with AET has no significant influence on its ultracentrifugal sedimentation pattern.

Other experiments, not described in detail, in which proteins are exposed to AET in the presence of other constituents, show that neither ATP nor α-actinin protect myosin against AET, and the effect on actin is partially protected by α-actinin.

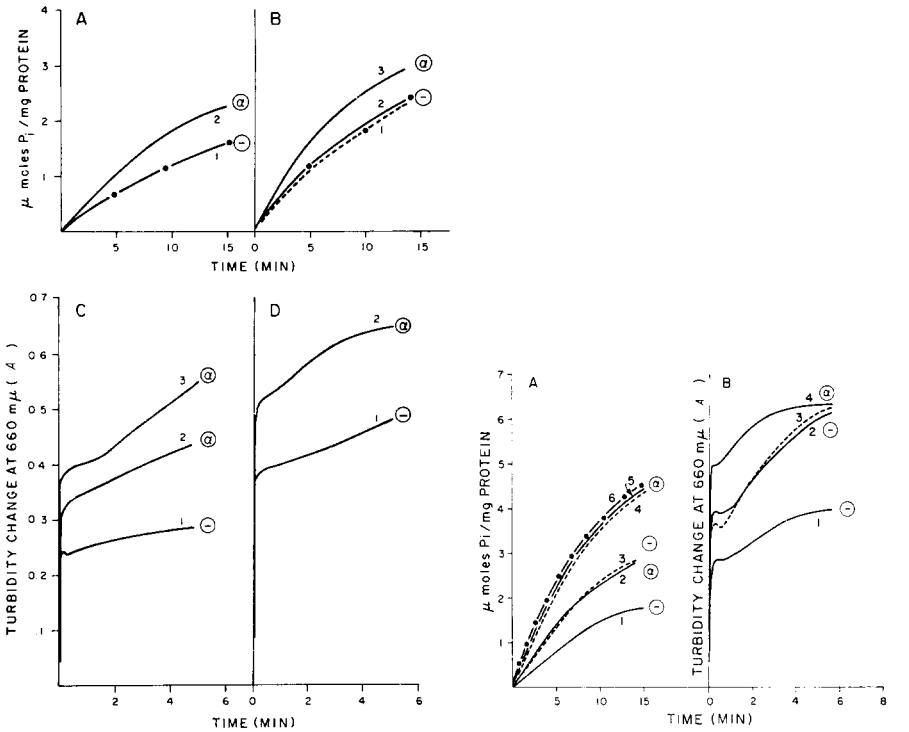

Fig. 6. ATPase and turbidity responses of normal actomyosin and actomyosin made with AET-treated actin. A, ATPase activity of normal actomyosin with and without α-actinin. B, curves 2 and 3, same for myosin with AET-actin; curve 1 is the same as 2, but in the presence of $2.5 \cdot 10^{-5}$ M EGTA. C, turbidity responses of normal actomyosin without (1) and with 20% (2) and 30% (3) α-actinin. D, same for myosin with AET-actin, with and without 30% α-actinin. Conditions as in Fig. 4 for the turbidity tests. In the ATPase assays (at 27°), the concentrations are 0.18 mg actomyosin with or without 0.054 mg α-actinin per ml in 50 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, Tris, acetate (50 mM) at pH 6.8.

Fig. 7. ATPase and turbidity responses of normal actomyosin, and actomyosin treated as such with AET. Conditions as in Figs. 4 and 6. A, ATPase activity. Curves 1–2 normal actomyosin with and without α-actinin. The other curves refer to AET-treated actomyosin; curve 3, without α-actinin; curve 4, with, in the presence of $2.5 \cdot 10^{-5}$ EGTA; curve 5, with α-actinin; curve 6, with α-actinin, in the presence of $5 \cdot 10^{-5}$ M $CaCl_2$. B, turbidity responses. Curve 1, normal actomyosin, no α-actinin; curve 2, AET-treated actomyosin; curve 3, as 2, with $2.5 \cdot 10^{-5}$ M EGTA; as 2, with α-actinin.

When both actin and myosin are reacted with AET and then combined to form actomyosin, the product behaves like actomyosin made from AET-myosin and normal actin (not illustrated). Hence, the inhibitory phenomena due to the modification of myosin overrule the activating effects caused by the modification of actin and conversely it appears that actin protects myosin against some of the consequences of the reaction. We finally mention that α-actinin itself is not inactivated by AET.

The binding of α-actinin to modified actomyosin

We shall describe this on the basis of the test introduced by BRISKEY, SERAYDARIAN AND MOMMAERTS[2], in which α-actinin is added to actomyosin, and the supernatant solution, after removal of the actomyosin by centrifugation, is mixed with normal fresh actomyosin in order to test whether it still contains α-actinin. Actomyosin was prepared fresh from the constituent proteins kept on ice for 30 min, diluted with water to 0.05 M KCl and centrifuged, washed once with 0.05 M KCl, and resuspended in 0.4 M KCl (11.3 mg/ml); α-actinin was 7.0 mg per ml.

For the interaction, 20 mg of actomyosin was treated with various amounts of α-actinin (4, 2 and 1 mg) with enough 2 M KCl and water to a final volume of 2.6 ml (0.35 M KCl final concentration), kept on ice at 0° for 30 min diluted with 18.2 ml cold water (to 0.044 M KCl), and centrifuged for 20 min at 3000 rev./min. Part of the supernatant solution was used to assay for remaining α-actinin, but this experiment suffers from the high dilution of the proteins; therefore, the rest was treated with solid $(NH_4)_2SO_4$ (22.0 g per 100 ml) to precipitate the remaining α-actinin. This was left standing on ice for 60 min and then centrifuged for 30 min at 3000 rev./min. The precipitate was dissolved in 2 ml of 1 mM $NaHCO_3$ and dialyzed overnight in 1 mM $NaHCO_3$. The dialyzed solution was assayed for α-actinin activity by adding to normal actomyosin in the turbidity assay system and ATPase activity.

Results are given in Fig. 8; A shows the turbidity responses of actomyosin in this instance, as such and with several doses of α-actinin; B shows that the supernatant solutions remaining after incubation of normal actomyosin with α-actinin contain no significant amount of α-actinin unless an excess had been used; C shows that, upon incubation with acto-AET-myosin, the supernatant solution contains detectable amounts of α-actinin, *e.g.* 7.5% α-actinin remaining largely unbound. This experiment, which has been repeated with some variations, and also in terms of the ATPase assay (Fig. 9) shows that AET-substitution of the myosin markedly diminishes the α-actinin binding by the resulting actomyosin. These experiments were done without concentrating the α-actinin, if remaining, from the supernatant

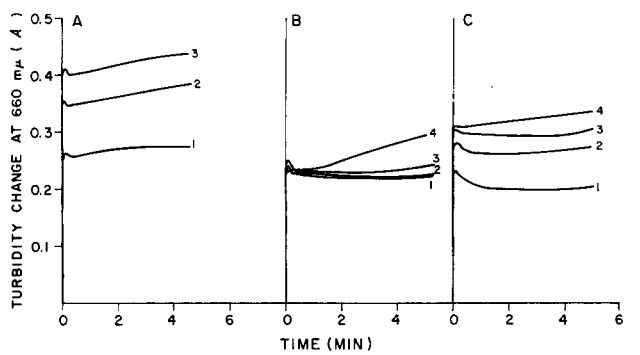

Fig. 8. Test for α-actinin binding by actomyosin and actomyosin made from AET-myosin and normal actin. Turbidity assays, conditions as in Fig. 4. Amount of supernatant used in B and C 1 ml. A, turbidity response of normal actomyosin without (1) and with 7.5% (2) and 15% (3) α-actinin. B, with supernatant solution left after the precipitation of normal actomyosin with 25% (4); 15% (3); 7.5% (2) α-actinin, (1) actomyosin alone; C, with supernatant solution left after the precipitation of actin–AET-myosin with 25% (4); 15% (3); 7.5% (2) α-actinin, (1) actomyosin alone. The rapid upstrokes of the curves start at orbinate values below 0.1, *cf.* Fig. 6C.

solutions. In Fig. 10, we summarize the results of a larger series of experiments in which, in some instances, the α-actinin was concentrated by precipitation. This composite experiment gives a convincing demonstration that AET-myosin–actin binds α-actinin much less than actomyosin does, if at all. The same applies to the myosin–AET-actin complex (not illustrated).

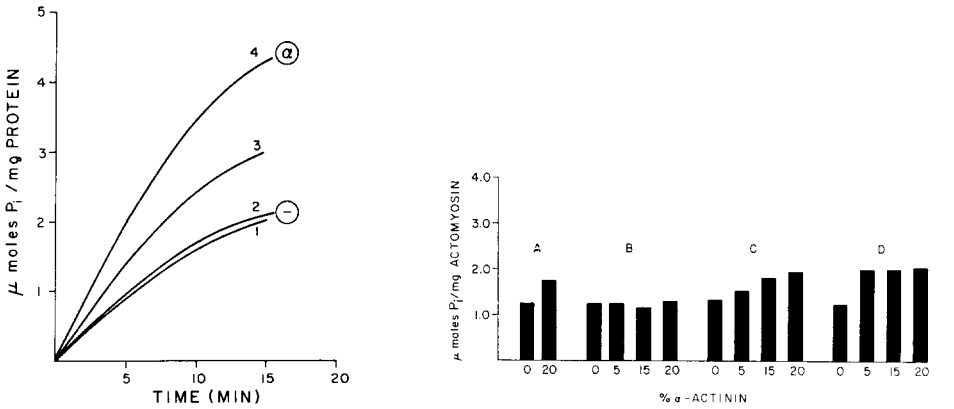

Fig. 9. Test for α-actinin binding by actomyosin made from AET-myosin and normal actin. ATPase assays, conditions as in Fig. 6, performed with normal actomyosin as test substance. Curve 1, with supernatant solution left after the precipitation of normal actomyosin with α-actinin; no remaining activator in this supernatant, all is bound to the actomyosin; curve 2, actomyosin without additions; curve 3, with supernatant solution remaining after the precipitation of AET-myosin and normal actin with α-actinin; curve 4, with α-actinin in same amount.

Fig. 10. Test for α-actinin-binding by actomyosin and acto-AET-myosin, ATPase assays, conditions as in Fig. 6, 15 min incubation. A, ATPase assay of normal actomyosin with and without α-actinin; B, with supernatant solution left after the precipitate of normal actomyosin with different quantities of α-actinin; C, with supernatant solution left after the precipitation of acto-AET-myosin with different quantities of α-actinin; D, same as in (C); α-actinin was precipitated from supernatants by adding solid $(NH_4)_2SO_4$ (22 g per 100 ml). The precipitate was dissolved in 1 mM $NaHCO_3$ and used in the assay.

Effects of calcium and of EGTA

A number of experiments were performed to see whether AET-treatment of the myosin was influenced by Ca^{2+} or EGTA. Examples are contained in Fig. 7. In no instance did treatment with AET induce a calcium requirement (see DISCUSSION).

Concluding remarks. The experiments of Figs. 1–3 were done at pH 8.0 in view of the conditions chosen by Morales and coll.[4,5]. They are, therefore, not strictly comparable to the other assays in this and the preceding papers. There is, however, no great difference in rate between these two pH values (*cf.* MOMMAERTS AND GREEN[22], Fig. 7) and the α-actinin effects also allow some leeway in the choice of pH (ref. 3); the results of Figs. 1–3 at 0.1 M KCl are in keeping with those at pH 7.0 in the latter paper.

DISCUSSION

It is admitted that the experiments here described represent only a very first exploration of an approach that can be developed with endless variation. Many different chemical modifications of the sulfhydryl groups could be attempted, and

they could be executed so as to accomplish lesser or greater degrees of substitution, and also while attempting to protect certain specific active groups against alteration (*cf.* STRACHER AND DREIZEN[23]). This investigation remained restricted to the reaction of about half of the sulfhydryl groups of myosin, actin, or both, with AET. However, when about the same fraction of these groups is lost coincident with aging, similar modifications occur. Thus, our findings have at least some general validity. On the other hand, our substitutions are not as specifically defined as those developed especially by STRACHER[24] for myosin. With both actin and myosin, the reaction with AET leads to a determinable loss of sulfhydryl groups, and the most likely explanation is an autoxidative formation of disulfide bonds between protein-SH and AET. That this proceeds within an hour, as compared to a week for the autoxidation of protein-SH only, is not astonishing, since just this rapid reactivity is a feature of the AET reaction. Thus, we accept the explanation forwarded by MORALES AND HOTTA[5] in preference over the possibilities discussed earlier by MORALES *et al.*[4] when no SH-titrations had been applied to the problem.

Considering first the effect of AET substitution of myosin, singly or in combination with actin, this could be described (*cf.* KIELLEY AND BRADLEY[25]; MORALES *et al.*[4]) as having changed myosin into an ATPase which nearly lost its K^+-activation and has become Ca^{2+}-dependent. Thus, we have tested whether its physical-chemical reactions might also have become dependent upon trace amounts of Ca^{2+}, as is known to be the case in "natural actomyosin" (*cf.* WEBER[26]) due to the presence of troponin[27–29]. As described (Fig. 7), this was not found. We point out that in the case of the physiological regulations we are dealing with trace quantities affecting the Mg^{2+}-activated actomyosin-ATPase, whereas in Figs. 1–3 we referred to much higher activating concentrations of Ca^{2+} such as those studied by BAILEY[30], MOMMAERTS AND SERAYDARIAN[31] and, in more detail, by MOMMAERTS AND GREEN[22] for myosin and by TONOMURA and coll.[32,33] for actomyosin. Also, it is now known that the effect of trace amounts of calcium is upon the troponin factor, and is not a property of actomyosin itself[34]. Thus, the 2 properties are not related. Other implications of the findings were already mentioned in the experimental section.

With respect to the turbidity responses of actomyosin at low ionic strength, the results can be summarized as follows. When the myosin moiety is AET-substituted, the ATPase and turbidity response are not much altered, but all activation by α-actinin has been eliminated. Such an actomyosin has a lesser binding affinity toward α-actinin, which may or may not be the explanation; but it is a remarkable phenomenon because myosin is not the site of that binding, thus it must be able to exert a modifying influence upon actin in this regard. When the actin moiety is AET-substituted, there is a rise in the ATPase activity (at low ionic strength only) and of the turbidity response, and both processes are still activated by α-actinin. Both modified actomyosins still display a seemingly normal gel synaeresis.

Both these findings, that of the influence of AET substitution of myosin upon the α-actinin effect which is not located within the myosin molecule but in the actin moiety[35,2] and of the AET substitution of actin upon the ATPase of which it is not a part, are indicative of mutual interactions among the three proteins of a complex character. We observe that the effects of troponin–tropomyosin are to be looked upon in the same manner, but while there the functional role is obvious, in the present case it is not. So far, the findings are not explainable as to their mechanisms. They

are likely to be of interest, if only because the distinct properties of actomyosin- or myofibrillar ATPase, as compared to myosin-ATPase, indicate profound interactions between the two proteins. The natural ATPase activity, and presumably the chemomechanical transduction process, are the result of this specific interaction rather than a property of one or the other protein separately.

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