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EFFECT OF α-ACTININ ON ACTIN VISCOSITY*

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SUMMARY

As determined by analytical ultracentrifugation, purified α-actinin does not form stable complexes with G-actin, myosin, tropomyosin, or the tropomyosin-troponin complex. However, α-actinin forms a stable complex with F-actin polymerized either in 100 mM KCl or in 2 mM $MgCl_2$ without KCl. Viscosity studies confirm that α-actinin interacts as strongly with Mg^{2+}-polymerized actin as it does with KCl-polymerized actin.

When measured at 0°, addition of 0.02–0.05 parts of purified α-actinin to 1 part of actin, by weight, causes a 4–5-fold increase in specific viscosity of F-actin. At 37°, 0.10–0.20 parts of purified α-actinin to 1 part of actin are required to cause a 2–3-fold increase in specific viscosity of F-actin. Addition of α-actinin above this level at 37° results in precipitation of F-actin. Tropomyosin (0.25 parts to 1 part actin, by weight) has no effect on the α-actinin-induced increase in F-actin viscosity at 0° but almost completely abolishes the effect of α-actinin on F-actin viscosity at 37°. The effects of temperature on the α-actinin-induced increase in F-actin viscosity are completely reversible.

Trypsin treatment of α-actinin–F-actin mixtures for 5 min at trypsin to actin ratios of 1:50, by weight, completely destroys the α-actinin-induced increase in F-actin viscosity at 37°. Subsequent incubation of the trypsin-treated mixture at 0°, however, shows the ability of α-actinin to increase F-actin viscosity at 0° is only partially destroyed by 15 min of trypsin digestion. Similar results are obtained by treating purified α-actinin with trypsin and then mixing the treated α-actinin with untreated actin. Neither temperature nor trypsin has any effect on viscosity of F-actin in the absence of α-actinin; hence, trypsin and temperature affect α-actinin and the α-actinin–F-actin interaction. The results are consistent with the hypothesis that at 0° α-actinin cross-links F-actin by binding strongly both along the length and at one end of the F-actin strand. At 37°, however, strong binding of α-actinin is restricted primarily to one end of the F-actin strand, and any weak binding that may occur along the length of the F-actin strand is abolished by tropomyosin.

Abbreviation: TAME, p-toluenesulfonyl-L-arginine methyl ester.

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INTRODUCTION

The discovery of α-actinin as a new myofibrillar protein that accelerates *in vitro* contractile responses of reconstituted actomyosin suspensions[1,2] was soon followed with the finding that α-actinin exerts its influence on actomyosin suspensions by combining with the actin part of the actin–myosin complex[1–3]. Early studies on α-actinin–actin mixtures[3] showed that high α-actinin to actin ratios led to precipitation of F-actin, whereas gelation occurred at low α-actinin to actin ratios. Subsequent viscosity studies suggested that interaction of α-actinin with actin was stronger at 0 than at 21° (ref. 4), and furthermore, that tropomyosin abolished the ability of α-actinin to induce a viscosity increase in actin[4,5]. Since it is known that tropomyosin also interacts with actin[6], this latter result intimated that α-actinin and tropomyosin competed for the same binding sites on actin[4,5]. The effect of tropomyosin on the α-actinin-induced increase in actin viscosity was more noticeable at 21 than at 4°; this gave additional credence to the idea that the α-actinin–actin interaction is stronger at 0 than at 21°. At the same time, DRABIKOWSKI AND NOWAK[4] found that α-actinin at 21° would not increase the viscosity of actin polymerized by 1 mM Mg^{2+}, but would more than triple the viscosity of actin polymerized by 100 mM KCl *plus* 1 mM Mg^{2+}.

These early studies on the α-actinin–actin interaction were done, for the most part, with α-actinin prepared according to the original method of EBASHI AND EBASHI[2], although DRABIKOWSKI *et al.*[5] used a preparation of 6-S α-actinin made according to the procedure of NONOMURA[7]. The results of these early studies were often quite variable, and DRABIKOWSKI AND NOWAK[4] suggested that this variability originated from heterogeneity in the α-actinin preparations available at that time. Recently, ROBSON *et al.*[8] have shown that these early α-actinin preparations may have contained less than 20 % of their protein as the active 6-S α-actinin species. By using a partially purified form of α-actinin released by tryptic digestion, GOLL *et al.*[9] found that the α-actinin-induced increase in actin viscosity was as great with actin polymerized by 1 mM Mg^{2+} as it was with actin polymerized by 100 mM KCl. Since a method is now available for preparation of highly purified α-actinin[8], we have carefully re-examined the effects of α-actinin on actin viscosity in an attempt to gain some insight into the nature of the α-actinin–actin interaction. Moreover, since BRISKEY *et al.*[10], using the early α-actinin preparations, found that α-actinin interacted with G-actin as well as with F-actin, whereas GOLL *et al.*[9] subsequently found no evidence for an interaction between α-actinin and G-actin, we have used purified α-actinin to determine whether α-actinin will complex with any myofibrillar protein other than F-actin. Our results show that purified α-actinin forms a stable complex only with F-actin among the myofibrillar proteins, but that α-actinin will interact as strongly with Mg^{2+}-polymerized actin as it does with KCl-polymerized actin.

MATERIALS AND METHODS

The back and leg muscles of rabbits were used for all experiments reported here. Rabbits were anesthesized with sodium pentobarbital and D-tubocurarine chloride[10], and the muscles handled as described by ARAKAWA *et al.*[11]. All preparations were done at 0–2° using precooled solutions. Double-deionized, distilled water that had been redistilled in glass and stored in polyethylene containers was used throughout.

Protein preparations

α-Actinin-free actin was made according to ARAKAWA *et al.*[11], and myosin was made according to the procedures outlined by SERAYDARIAN *et al*[12]. The procedures used for preparation of the tropomyosin–troponin complex and purified tropomyosin have also been described[13]. All tropomyosin–troponin preparations possessed Ca^{2+}-sensitizing activity as determined by both the ATPase and turbidity assays for this activity[14], whereas the tropomyosin preparations used in this study had no Ca^{2+}-sensitizing activity according to these same two criteria. All tropomyosin preparations used were homogeneous in the analytical ultracentrifuge. α-Actinin was extracted as described by ARAKAWA *et al.*[11] and was purified by two passages through a DEAE-cellulose column according to the procedure of ROBSON *et al.*[8]. These α-actinin preparations are homogeneous as demonstrated by analytical ultracentrifuge diagrams[8] and polyacrylamide (disc) electrophoresis patterns[15]. Protein concentrations were measured by the biuret method[16] as modified by ROBSON *et al*[17].

Trypsin digestion

Both trypsin and soybean trypsin inhibitor were the purest, salt-free preparations available from Sigma Chemical Co. Trypsin was dissolved at 2.0 mg/ml in 0.001 M HCl and then diluted to 1.0 mg/ml in 160 mM Tris–acetate buffer (pH 7.2) just before use. Stock trypsin solutions were stable for 3–4 months at 2°. Soybean trypsin inhibitor (4–10 mg/ml) was dissolved in water. Activity of trypsin preparations was routinely monitored by using *p*-toluenesulfonyl-L-arginine methyl ester (TAME) as a substrate[18]. All trypsin preparations used in this study had 12000–21000 TAME units of activity per mg (ref. 18). Experimental conditions during trypsin treatment will be given with the individual experiments.

Viscosity determinations

Ostwald viscometers with flow times between 65 and 70 sec for water at 37° were used. Specific viscosity was calculated by subtracting one from the ratio obtained by dividing flow times of protein solutions with flow times of the respective solvents. Actin concentration was 1.0 mg/ml in all experiments, and when it was added, tropomyosin concentration was maintained at 0.25 mg/ml (25 % of the actin present by weight). In the experiments reported in this paper, G actin, tropomyosin (if added), and α-actinin were mixed in the viscometer, and then actin was polymerized by addition of 100 mM $MgCl_2$ to a final concentration of 2 mM or by the addition of 2 M KCl to a final concentration of 100 mM. α-Actinin-free actin polymerized very slowly at 0°, even in the presence of α-actinin; thus, in all experiments to be done at 0°, actin was first polymerized by incubation at 37° for 15 min, after which the viscometer was immersed in a 0° bath for 15 min before measurements were initiated. We have found that the effect of α-actinin on actin viscosity can be changed reversibly by alternating the incubation temperature between 0° and 37°, so prior incubation at 37° did not change the effect of α-actinin on actin viscosity at 0°.

The effect of trypsin on the α-actinin–F-actin interaction was studied by using two different procedures. In the first procedure, a measured amount of trypsin was added directly to a viscometer containing the α-actinin–F-actin complex, and the time-course of viscosity changes was monitored. In the second procedure, α-actinin was treated for different periods of time with trypsin, the tryptic digestion was stop-

ped by soybean trypsin inhibitor, and the trypsin-treated α-actinin was then mixed with G-actin. The G-actin was polymerized, and the effect of the trypsin-treated α-actinin on the viscosity of F-actin was recorded. Other experimental details will be given with the individual experiments.

Other procedures

Analytical ultracentrifugation was done with a Spinco Model E analytical ultracentrifuge equipped with a phase plate and RTIC unit. Runs were done at 20° using Kel-F centerpieces, and plates were measured on a Nikon 6C profile projector.

RESULTS

Interaction of α-actinin with myofibrillar proteins other than F-actin

Before initiating an extensive study of the α-actinin–F-actin interaction, it seemed necessary to establish, by using purified α-actinin, whether α-actinin interacted with any myofibrillar protein other than F-actin. α-Actinin has a $s^{\circ}_{20,w}$ of 6.23 (ref. 8), which is distinctly different from the sedimentation coefficients of all other myofibrillar proteins except myosin; therefore, analytical ultracentrifugation of purified α-actinin in the presence of a second purified myofibrillar protein was chosen as a convenient and sensitive method for measuring the ability of α-actinin to complex with any of the other known myofibrillar proteins. Moreover, since the sedimentation coefficient of α-actinin is affected only slightly by concentration, it is also possible to study the α-actinin–myosin interaction by this procedure because at protein concentrations near 4–5 mg/ml, purified myosin will sediment more slowly than purified α-actinin. When using this experimental technique, a stable (*i.e.*, long lived) complex of α-actinin with the other myofibrillar protein in the cell would cause three changes in the sedimentation pattern: (1) the area under the schlieren boundary of the 6-S α-actinin species should diminish to the extent that α-actinin is bound to the second myofibrillar protein; (2) the area under the schlieren boundary of the second myofibrillar protein should diminish to the extent that it is bound to α-actinin; and (3) a new schlieren boundary representing the complex between α-actinin and the second myofibrillar protein should appear with a sedimentation coefficient different from that of either α-actinin or the second myofibrillar protein.

As determined by the analytical ultracentrifuge assay, purified α-actinin does not form a stable complex with G-actin, myosin, the tropomyosin–troponin complex, or tropomyosin (Fig. 1, Table I). Sedimentation patterns of mixtures of each of these four proteins with α-actinin contain only two boundaries, and the sedimentation coefficient of the faster of these two boundaries ranges from 4.5 to 5.2 S, depending on the solvent (Table I). Although the sedimentation rate of these faster boundaries is sometimes slightly lower than that of purified α-actinin when sedimented alone in the same solvents (Table I), SCHACHMAN[19] has indicated that protein sedimentation coefficients often change in an unpredictable fashion when two macromolecular species are sedimented together. Extensive experimentation at different α-actinin to companion protein ratios has shown that size of the 4.5–5.2-S boundaries varies directly with concentration of α-actinin in the cell, and that size of the companion protein boundaries does not change under these conditions. Furthermore, area under the 4.5–5.2-S boundaries is always between 85–100 % of the area expected for the amount of

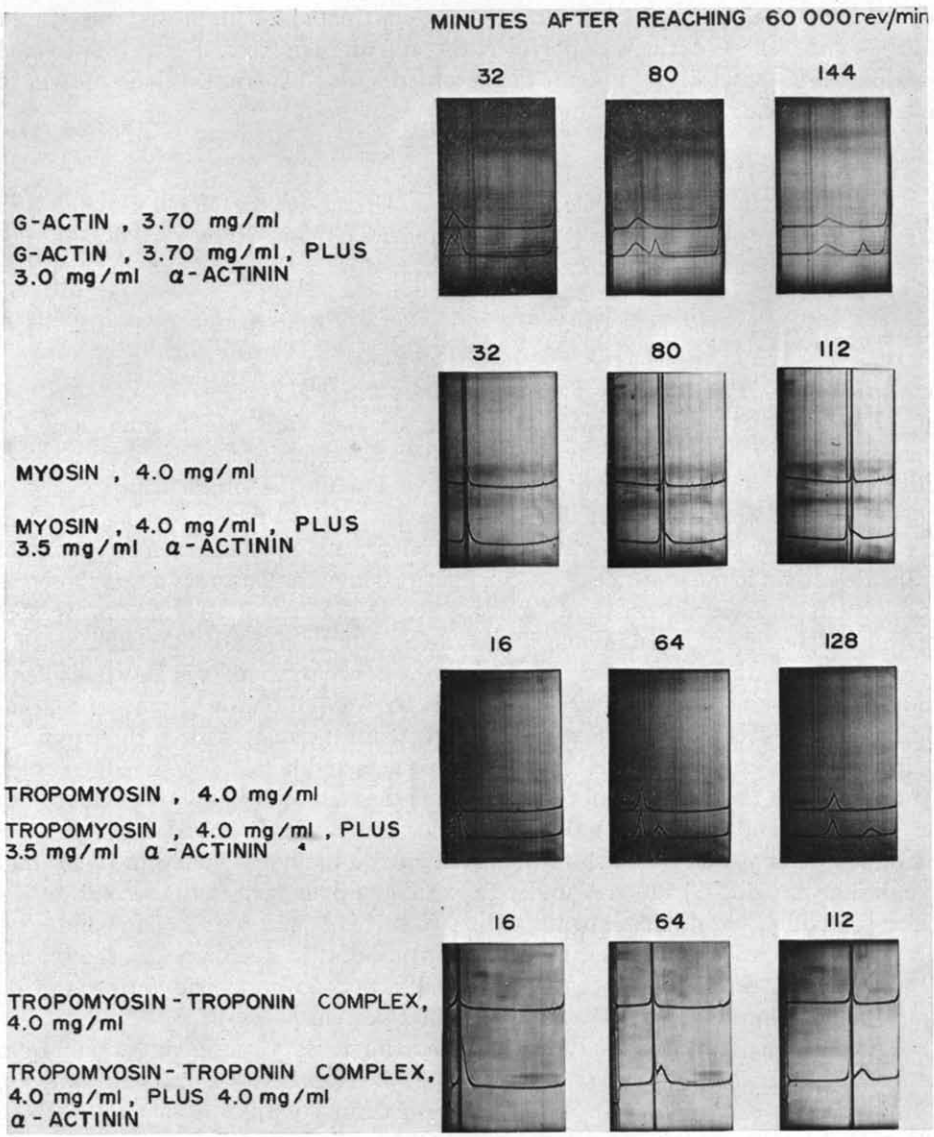

Fig. 1. Sedimentation patterns of mixtures of purified α-actinin and a second myofibrillar protein. α-Actinin was mixed with the myofibrillar protein indicated and the mixture sedimented. The proteins and protein concentrations are indicated. G-actin and the mixture of G-actin *plus* α-actinin were in 0.54 mM ATP, 0.14 mM $CaCl_2$, and 0.03% 2-mercaptoethanol; myosin and the mixture of myosin *plus* α-actinin were in 500 mM KCl, 50 mM Tris–acetate buffer (pH 7.5); tropomyosin, the mixture of tropomyosin *plus* α-actinin, the tropomyosin–troponin complex, and the mixture of the tropomyosin–troponin complex *plus* α-actinin were all in 100 mM KCl, 20 mM Tris–acetate buffer (pH 7.5). Phase plate angle, 70°; temperature, 20.0°.

α-actinin in the cell. Therefore, the decrease in sedimentation rate of α-actinin when centrifuged in the presence of myosin, tropomyosin, or the tropomyosin–troponin complex can probably be attributed to the increase in viscosity caused by addition of

TABLE I

OBSERVED SEDIMENTATION COEFFICIENTS OF BOUNDARIES IN MIXTURES OF α-ACTININ WITH OTHER MYOFIBRILLAR PROTEINS

α-Actinin and a second myofibrillar protein were sedimented both singly and when mixed together in the different solvents listed below. Sedimentation coefficients of the boundaries were calculated. Values in the table are averages of runs with at least three different protein preparations. All runs were made at 20.0° and protein concentrations of 2.0–4.5 mg/ml.

Solvent	*Proteins in cell*	*Observed sedimentation coefficient (Svedberg units)*	
		Fastest boundary	*Other boundary*
100 mM KCl, 20 mM Tris–acetate (pH 7.5)	α-Actinin	5.88	—
	Tropomyosin	—	2.81
	Tropomyosin and α-actinin	4.95	2.83
	Troponin–tropomyosin complex	—	5.15
	Troponin–tropomyosin complex and α-actinin	5.15	4.97
0.45 mM ATP, 0.25 mM $CaCl_2$, 0.05 % 2-mercaptoethanol	α-Actinin	4.56	—
	G-Actin	—	2.93
	G-Actin and α-actinin	4.72	2.93
500 mM KCl, 50 mM Tris–acetate (pH 7.5)	α-Actinin	5.17	—
	Myosin	—	4.56
	Myosin and α-actinin	4.51	4.25

these highly asymmetric molecules to the solution, and the 4.5–5.2-S boundaries in Fig. 1 can be identified as originating from sedimentation of α-actinin rather than sedimentation of α-actinin complexed with the companion protein. This conclusion together with the finding that sedimentation coefficient of the companion protein is not affected by sedimentation in the presence of α-actinin (Table I) indicates that no strong interaction, even of a rapidly dissociative nature, exists between α-actinin and the myofibrillar proteins tested in Fig. 1.

These results (Fig. 1 and Table I) obtained with highly purified α-actinin, confirm previous findings[5,10] obtained with partially purified α-actinin[10] or with NONOMURA's 6-S α-actinin[7] that α-actinin does not interact with myosin[10] or tropomyosin[5]. That α-actinin does not interact with G-actin or the tropomyosin–troponin complex, however, disagrees with earlier findings[5,10] obtained by using partially purified α-actinin[10] or 6-S α-actinin of NONOMURA[7]. Consequently, it now appears that some of the earlier results on interaction of α-actinin with other myofibrillar proteins were complicated by heterogeneity of the α-actinin preparations then available [5,7,10]. We conclude that purified α-actinin interacts strongly only with F-actin.

Effect of α-actinin on F-actin viscosity

The effect of α-actinin on F-actin viscosity under a variety of conditions is shown in Fig. 2. The results in Fig. 2 were obtained by polymerizing G-actin in the presence of α-actinin at 37° and then measuring viscosity of the resulting solution at either 0 or 37° as described in MATERIALS AND METHODS. We have confirmed the pre-

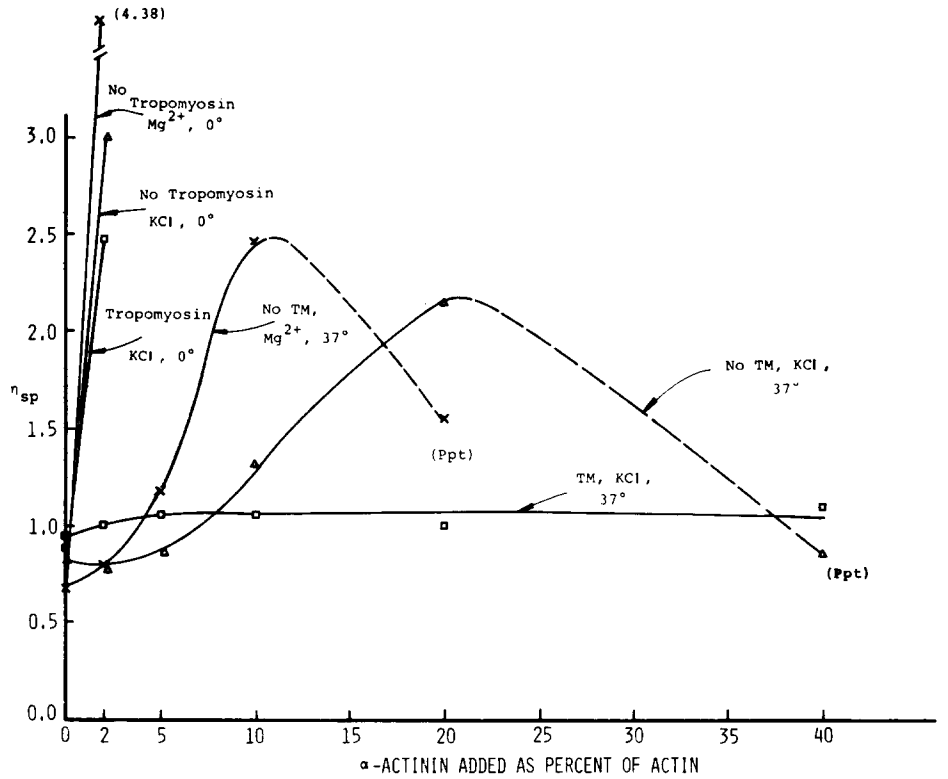

Fig. 2. Effect of α-actinin and temperature on viscosity of actin polymerized with KCl or $MgCl_2$ in the presence or absence of tropomyosin (TM). Conditions: 1 mg actin/ml, 100 mM KCl or 2 mM Mg_2Cl_2, as indicated, 20 mM Tris–acetate buffer (pH 7.5), 0.25 mg tropomyosin/ml when added, α-actinin indicated as percent of actin by weight. α-Actinin was mixed with G-actin at 37°, KCl or Mg_2Cl_2 added to polymerize the action, and after polymerizing 15 min at 37°, the viscometer was equilibrated at the temperature indicated, and the viscosity measured.

vious finding[9] that α-actinin causes a much larger increase in specific viscosity of F-actin when actin is polymerized in the presence of α-actinin than when α-actinin is added to pre-polymerized actin (*cf* results in Fig. 2 with those in Fig. 3 of ref. 15). It is also clear that α-actinin causes much larger increases in F-actin viscosity at 0 than it does at 37°. Addition of as little as 0.02 part of purified α-actinin to 1 part of G-actin causes the specific viscosity, after polymerization, to increase from near 0.75 to more than 3.0 (Fig. 2). Specific viscosity of α-actinin–actin mixtures at 0° could not be measured above α-actinin to F-actin ratios of 0.02–1.0, because the gels formed at these α-actinin to F-actin ratios would not flow through the viscometer capillaries.

It is also clear from Fig. 2 that when determined viscometrically, α-actinin will interact with actin polymerized by addition of $MgCl_2$ to a final concentration of 2 mM just as strongly as it interacts with actin polymerized by KCl. Indeed, at 37°, α-actinin appears to interact more strongly with Mg^{2+}-polymerized actin than with KCl-polymerized actin since the maximum viscosity response is reached at lower α-actinin to F-actin ratios with Mg^{2+}-polymerized actin than with KCl-polymerized actin. Although this difference is small, it has been consistently observed in our experiments.

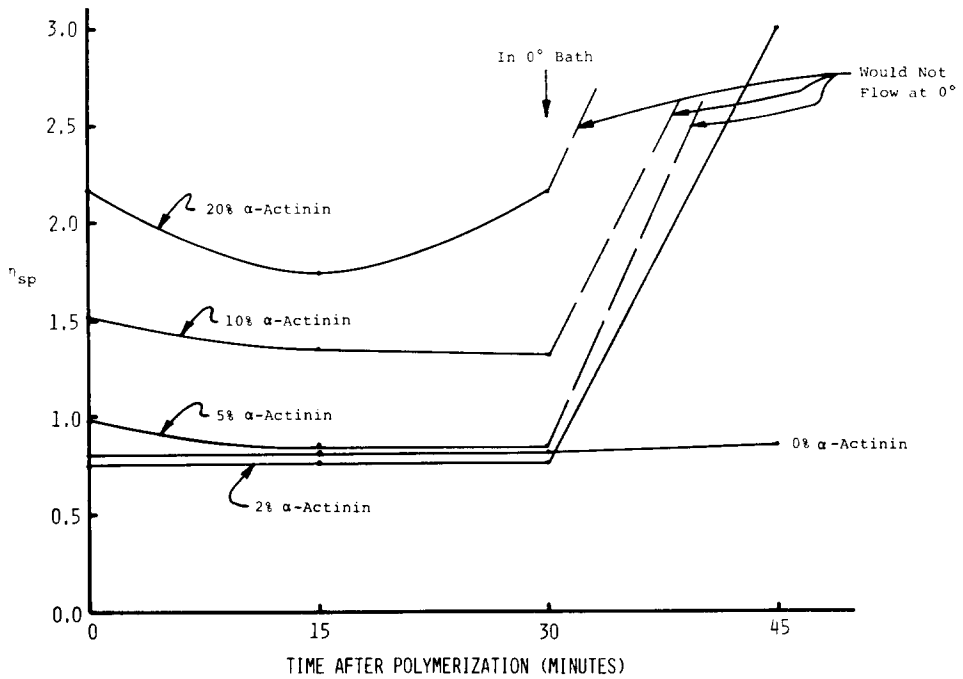

Fig. 3. Effect of α-actinin and temperature on viscosity of KCl-polymerized actin in the absence of tropomyosin. G-actin in the presence of α-actinin was polymerized at 37° by addition of KCl. Final concentrations: 1.0 mg actin/ml, α-actinin indicated as percent of actin by weight, 100 mM KCl, 20 mM Tris–acetate buffer (pH 7.5). At time indicated, the viscometer was taken from the 37° bath and placed in a 0° bath.

The third point that emerges from Fig. 2 is that tropomyosin has no effect on ability of α-actinin to increase F-actin viscosity at 0°, but almost completely prevents the effect of α-actinin on F-actin viscosity at 37°.

Since the results in Fig. 2 show that temperature has a large effect on ability of α-actinin to increase F-actin viscosity, a series of experiments were done in which viscosity of a given α-actinin–F-actin mixture was monitored as the temperature of the mixture was varied. These experiments showed that the effect of temperature on viscosity of α-actinin–F-actin mixtures was completely reversible (Figs. 3 and 4). Lowering the temperature from 37 to 0° caused 4-fold or greater increases in specific viscosities of α-actinin–F-actin mixtures (Figs. 3 and 4); raising the temperature back to 37° then caused specific viscosities of the mixtures to decrease to their previous levels (Fig. 4). These changes occurred both when tropomyosin was present (Fig. 4) and when it was absent (Fig. 3). The results in Figs. 3 and 4 also confirm the conclusion (Fig. 2) that tropomyosin prevents α-actinin from increasing F-actin viscosity at 37° but has no effect on ability of α-actinin to increase F-actin viscosity at 0°. Since specific viscosity of F-actin in the absence of α-actinin does not change as the temperature is lowered from 37 to 0° and then returned to 37° (Fig. 4), it is clear that temperature affects viscosity of α-actinin–F-actin mixtures by altering the α-actinin–F-actin interaction itself, and not by altering F-actin viscosity.

Because the conclusion that α-actinin interacts as strongly with Mg^{2+}-poly-

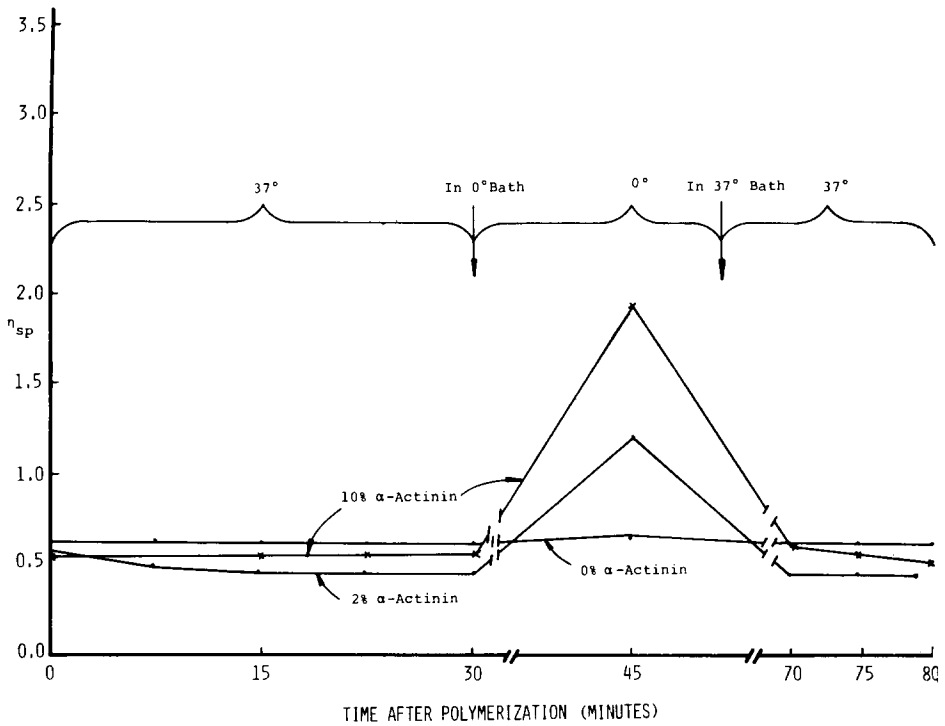

Fig. 4. Reversibility of the effect of temperature on the α-actinin-induced increase in viscosity of Mg^{2+}-polymerized actin in the presence of tropomyosin. G-actin in the presence of α-actinin and tropomyosin was polymerized at 37° by addition of $MgCl_2$. Final concentrations: 1.0 mg actin/ml, 0.25 mg tropomyosin/ml, α-actinin indicated as percent of actin by weight, 2 mM $MgCl_2$, 20 mM Tris–acetate buffer (pH 7.5). At times indicated, viscometer was placed in 0° or in 37° bath.

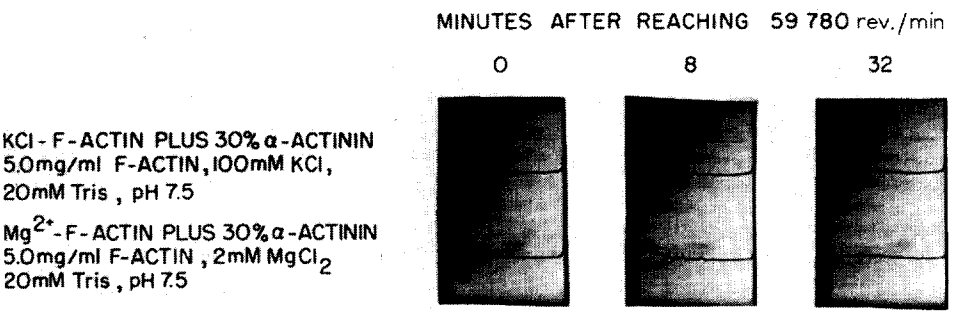

Fig. 5. Sedimentation patterns of the complexes of α-actinin with KCl-polymerized actin and with Mg^{2+}-polymerized actin. G-actin was polymerized by addition of KCl or $MgCl_2$ and then 0.3 part of α-actinin added to 1 part of F-actin by weight. Final conditions: the upper or wedge cell contained 5.0 mg F-actin and 1.5 mg α-actinin/ml in 100 mM KCl, 20 mM Tris–acetate buffer (pH 7.5); the lower or regular cell contained 5.0 mg F-actin and 1.5 mg α-actinin/ml in 2 mM $MgCl_2$, 20 mM Tris–acetate buffer (pH 7.5). Temperature, 20.0°; phase plate angle, 65°.

merized actin as with KCl-polymerized actin (Fig. 2) contradicts the earlier finding of DRABIKOWSKI AND NOWAK[4] that α-actinin does not interact with Mg^{2+}-polymerized actin, we obtained additional evidence on this question by comparing the sedimen-

tation pattern of α-actinin–Mg^{2+}-polymerized actin mixtures with the sedimentation pattern of α-actinin–KCl-polymerized actin mixtures (Fig. 5). For both kinds of actin, addition of α-actinin causes the rapidly sedimenting, hypersharp F-actin boundary to disappear, leaving only the α-actinin–F-actin complex, which sediments as heterogeneous aggregates without any visible boundary (0 min after reaching 59780 rev./min, Fig. 5). A very small excess of α-actinin, not bound by F-actin at 20° (see GOLL *et al.*[15] for stoichiometry of the α-actinin–F-actin interaction at different temperatures), can be detected 8 and 32 min after reaching 59780 rev./min (Fig. 5). The sedimentation coefficients of both these small boundaries were 6.2 S, confirming that they represent uncombined α-actinin. Since the areas under these two small boundaries are also identical (equivalent to 0.23 mg/ml in the upper pattern and 0.18 mg/ml in the lower pattern), the results of the ultracentrifugal experiments clearly confirm the conclusion that α-actinin interacts with Mg^{2+}-polymerized actin at least as well as it does with KCl-polymerized actin.

Effect of trypsin on α-actinin and the α-actinin–F-actin interaction

GOLL *et al.*[9] showed that very brief trypsin digestion of myofibrils caused release of α-actinin, presumably from the Z-disk because trypsin removes Z-disks, and that this effect of trypsin on myofibrils was paralleled by a remarkable ability of trypsin to destroy the α-actinin-induced increase in F-actin viscosity at 27°. Since our measurements with purified α-actinin showed that the α-actinin-induced increase in F-actin

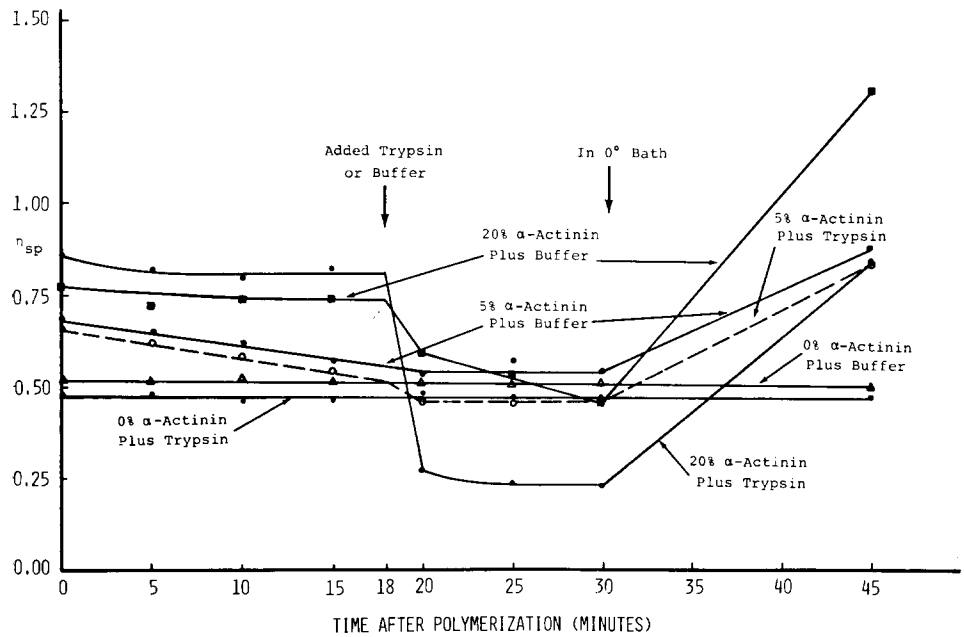

Fig. 6. Effect of trypsin on viscosity of the α-actinin–F-actin complex. G-actin in the presence of α-actinin was polymerized at 37° by addition of $MgCl_2$ and at the time indicated, 1 part of trypsin (0.1 ml) to 50 parts of actin, by weight, or 0.1 ml of buffer was added. Final concentrations: 1.0 mg actin/ml, α-actinin indicated as percent of actin by weight, 2 mM $MgCl_2$, 20 mM Tris–acetate buffer (pH 7.5). At the time indicated, viscometer was taken from the 37° bath and placed in a 0° bath.

viscosity is very dependent upon temperature, we have studied the effect of trypsin on viscosity of α-actinin–F-actin mixtures at both 37 and 0° (Fig. 6). The experiments in Fig. 6 were done by polymerizing α-actinin–G-actin mixtures at 37°, measuring the viscosity of these mixtures for 18 min after polymerization, and then adding trypsin directly to the α-actinin–F-actin complex in the viscometer. Under these conditions, trypsin has a remarkable ability to decrease the specific viscosity of α-actinin–F-actin mixtures (Fig. 6). At high α-actinin–actin ratios, trypsin lowers the viscosity of α-actinin–F-actin mixtures below the viscosity of trypsin-treated F-actin alone (the 20% α-actinin *plus* trypsin curve in Fig. 6). This surprising result may indicate that interaction with α-actinin alters the structure of the F-actin polymer so as to make it more vulnerable to trypsin than it is in the absence of α-actinin. The possibilty that the α-actinin–F-actin interaction may cause a structural change in these proteins and thereby increase their susceptibility to trypsin has been suggested previously[9]. Immersing the α-actinin–F-actin mixture in a 0° bath 12 min after initiation of trypsin treatment results in an immediate increase in specific viscosity (Fig. 6). However, viscosity of the trypsin-treated mixtures, particularly at α-actinin to F-actin ratios of 0.20 to 1, increased much less after incubation at 0° than did viscosity of the untreated control mixtures (Fig. 6). Hence, 12 min of trypsin treatment lowers, but does not completely eliminate, the α-actinin-induced viscosity increase at 0°. As shown in Fig. 6, trypsin treatment at 37° had very little effect on viscosity of F-actin alone,

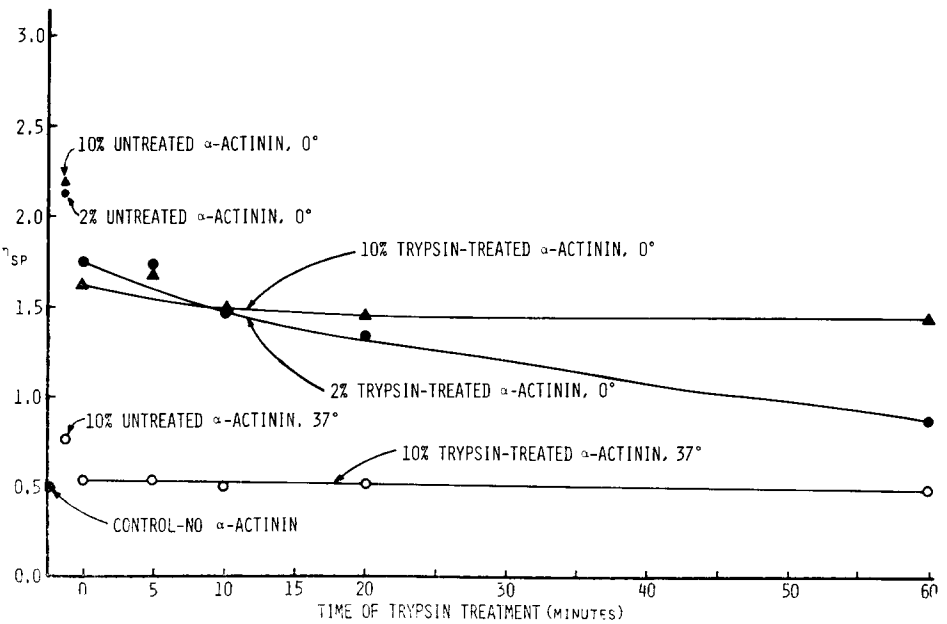

Fig. 7. Effect of trypsin-treated α-actinin on viscosity of actin. G-actin in the presence of trypsin-treated or untreated α-actinin was polymerized at 37° by addition of $MgCl_2$. Final concentrations in viscosity assay: 1 mg actin/ml, α-actinin indicated as percent of actin by weight, 2 mM $MgCl_2$, 20 mM Tris–acetate buffer (pH 7.5). All samples were polymerized at 37° but some were measured at 37° and some were measured at 0°. Conditions for trypsin treatment of α-actinin: 102 mM KCl, 51 mM Tris–acetate buffer (pH 8.0), 1.0 mg α-actinin/ml, 1 part of trypsin to 50 parts of α-actinin, by weight, 25.0°.

so the effect of trypsin on viscosity of α-actinin–F-actin mixtures may be ascribed to an effect of trypsin on the α-actinin–F-actin interaction.

In the second method used to study the effect of trypsin on the α-actinin–F-actin interaction, purified α-actinin was treated with trypsin for different periods of time at 25°, the digestion was stopped by addition of soybean trypsin inhibitor, the treated α-actinin was mixed with G-actin, and the resulting mixture was polymerized. Untreated α-actinin samples were made by stirring α-actinin at 25° for 60 min, and then adding pre-mixed soybean trypsin inhibitor and trypsin. Such untreated samples had already lost some of their ability to increase F-actin viscosity (Fig. 7). This indicates that α-actinin is partially denatured by exposure to 25° for 1 h at pH 7.6. ARAKAWA *et al.*[11] have previously suggested that α-actinin is not stable to extraction at room temperature. Very brief trypsin treatment causes almost complete loss of α-actinin's ability to increase F-actin viscosity at 37°, but only slowly affects α-actinin's ability to increase F-actin viscosity at 0° (Fig. 7). Even after 60 min of trypsin digestion, 0.02 part of α-actinin to 1 part of F-actin doubles the specific viscosity of F-actin (Fig. 7). The specific viscosity of F-actin mixed with α-actinin that had been treated with trypsin for 60 min was never less than that of F-actin alone at 37°, even though the previous line of experimentation has shown that trypsin added directly to the α-actinin–F-actin complex at 37° caused viscosity of the complex to decrease below that of F-actin alone (*cf.* Figs. 6 and 7). This result supports the suggestion that interaction between α-actinin and F-actin renders one or both these proteins more susceptible to tryptic hydrolysis than it normally is.

DISCUSSION

This study, using highly purified α-actinin, clearly shows that α-actinin does not form stable complexes with G-actin, myosin, tropomyosin, or the tropomyosin–troponin complex. Earlier studies reporting an interaction between α-actinin and G-actin[10], or between α-actinin and the tropomyosin–troponin complex[5], were probably complicated by heterogeneity of the α-actinin preparations then available. On the other hand, α-actinin will form stable complexes with F-actin under a variety of conditions, and regardless of whether the actin has been polymerized by 2 mM $MgCl_2$ or 100 mM KCl. This latter finding confirms the earlier results of GOLL *et al.*[9] who used a partially purified form of α-actinin, but disagrees with the results of DRABIKOWSKI AND NOWAK[4] who used the early procedures for preparing α-actinin. We have, however, confirmed the finding of DRABIKOWKSI AND NOWAK[4] that interaction of α-actinin with F-actin is much stronger at 0° than it is at higher temperatures. Indeed, our viscosity studies with highly purified α-actinin show that interaction between α-actinin and F-actin at 0° differs from the interaction between these proteins at 37° in several ways: (1) interaction of α-actinin with F-actin produces a much larger viscosity increase at 0 than it does at 37°; (2) tropomyosin has no detectable effect on ability of α-actinin to increase F-actin viscosity at 0° but nearly eliminates this ability at 37°; and (3) when measured viscometrically, trypsin treatment of either α-actinin alone or the α-actinin–F-actin complex directly does not affect the α-actinin–F-actin complex formed at 0° nearly as much as it affects the α-actinin–F-actin complex formed at 37°.

Although these viscosity results enable us to clearly differentiate between the

α-actinin–F-actin interaction at 0° and the α-actinin–F-actin interaction at 37°, they do not, in themselves, afford a clear insight into the molecular nature of the α-actinin–F-actin interaction at these two temperatures. Flow-birefringence studies[3] done at 18° with early α-actinin preparations indicated that addition of α-actinin caused the apparent mean particle length in F-actin solutions to decrease from 5.6 to 4.3 μm. The relationship of this finding to mechanism of the α-actinin–F-actin interaction has remained unclear. BRISKEY *et al*[10] have suggested that since α-actinin seems to cross-link actin, α-actinin may be a constituent of the Z-disk, for only here does cross-linkage of actin occur *in vivo*. End-to-end cross-linking of F-actin strands by α-actinin, as implied by the suggestion of BRISKEY *et al*[10], should double the apparent mean particle length in F-actin solutions. Our viscosity results clearly show that such end-to-end cross-linking of F-actin strands does not occur at 37°, a temperature near the *in vivo* temperature of most mammalian muscles. On the other hand, the available evidence[9,20,21] suggests that α-actinin is, in fact, located at the Z-line.

These ostensibly paradoxical results can be explained by considering the recent finding[8] that F-actin will quantitatively bind 41 % of its weight of α-actinin at 0°. This stoichiometric ratio for α-actinin binding to F-actin is just enough to place one α-actinin molecule on every turn of the F-actin double helix[8]. Binding of α-actinin along the entire length of the F-actin strand as well as to one end of the strand affords ample opportunity for formation of an extensive, three-dimensional network in α-actinin–F-actin mixtures at 0°, and also explains the earlier flow-birefrigence results showing that apparent mean particle length of F-actin decreases upon addition of α-actinin. If, on the other hand, α-actinin binding at 37° is restricted to one end of the F-actin strand, with only very limited or weak binding occurring along the length of the strand, there would be little formation of a three-dimensional network, and α-actinin would have a much smaller effect on F-actin viscosity. Additionally, tropomyosin, which binds along the length of the F-actin strand[22], may be expected to completely eliminate any limited or weak α-actinin binding occurring along the length of the F-actin strand at 37°. Very brief tryptic digestion may also cause conformational changes in α-actinin that would destroy its ability to bind along the length of F-actin strands at 37° without destroying its ability to bind to this same area at 0°. Since our sedimentation patterns show that α-actinin molecules do not bind strongly to one another[8] and since the probability is very low that the ends of two actin strands in an F-actin solution would lie close enough to permit simultaneous binding to the same α-actinin molecule, α-actinin at 37° would bind to, and thereby block, the end of each F-actin strand before extensive cross-linking of these strands could occur. These circumstances would completely account for our viscosity results at 37°. Other experimental evidence recently obtained in our laboratory[15] directly supports this mechanism for α-actinin binding to F-actin at 37°.

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