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Structure and Function of Myosin Subfragment 1 As Studied by Tryptic Digestion†

Tetsu Hozumi

ABSTRACT: Limited tryptic digestion of myosin subfragment 1 (S-1) was carried out under a high concentration of trypsin (weight ratio of trypsin to S-1 of 1:20) in the absence and presence of actin and/or ADP, and changes in the Mg^{2+}-ATPase activities of S-1 and acto-S-1, binding of S-1 to actin, and structure were followed during the course of proteolysis. Two of the three main products of lesser hydrolysis, the 50K and 27K fragments, converted into 45K and 22K fragments, respectively. The 20K fragment also degraded into smaller fragments but more slowly. When the acto-S-1 complex was digested, the 70K fragment degraded into 50K and 20K fragments (the 70K is a stable fragment under milder proteolysis). Addition of ADP accelerated every breakdown and was more effective for the breakdown of the 70K and 27K fragments. The 70K fragment was also converted into a 34K fragment when ADP was added to the acto-S-1 complex. In every case except the acto-S-1 complex without ADP, the Mg^{2+}-ATPase activities of S-1 and acto-S-1 and the binding of S-1 to actin decreased during the course of proteolysis. On the other hand, in the case of acto-S-1 without ADP, the Mg^{2+}-ATPase activity and the binding of S-1 to actin were protected from tryptic digestion, whereas the turbidity drastically fell to half of the initial value within 15 min of digestion and remained constant even after 60 min. The latter result suggests that large conformational changes were induced in the trypsinized S-1 by the binding of actin and may support the idea of two actin binding sites on S-1 [Mornet, D., Bertrand, R., Pantel, P., Audemard, E., & Kassab, R. (1981) *Nature (London) 292*, 301–306]. That is, we may speculate that on proteolysis of acto-S-1 one of the actin bonds was broken and one was retained. If so, the binding of actin to half of the sites of S-1 may be enough for actin activation.

It is generally believed that myosin subfragment 1 (S-1)[1] is the segment of the myosin molecule containing the active site for ATPase and also the site at which actin interacts. These sites are interacting with, but distinct from, each other (Barany & Barany, 1959; Highsmith, 1976). So, studies of S-1 conformational changes accompanying these interactions are highly significant. Here we pursue these studies using a method based on proteolysis. Balint et al. (1978) showed that limited tryptic proteolysis of the heavy chain of S-1 produced mainly three fragments (called 27K, 50K, and 20K). The 27K and 20K fragments contain the N and C termini of S-1 heavy chain, respectively (Lu et al., 1978). Mornet et al. (1979) and Yamamoto & Sekine (1979a,b) showed that the 50K/20K cut was abolished in the acto-S-1 complex and that the cut reduced the activation of S-1 Mg^{2+}-ATPase by actin (but not the Mg^{2+}-ATPase of S-1 alone). During the course of tryptic digestion of S-1, neither the binding property of S-1 to actin nor the Mg^{2+}-ATP-induced dissociation of the complex was significantly changed (Mornet et al., 1979). The three fragments have become a valuable frame work to which one can assign specific groups and functionalities. Szilagyi et al. (1979) showed that a photoaffinity analogue of ATP was specifically incorporated into the 27K fragment. It was found that both reactive thiols, SH_1 and SH_2, and the reactive lysyl residue resided on the 20K (Balint et al., 1978) and the 27K (Mornet et al., 1980; Miyanishi & Tonomura, 1981; Hozumi & Muhlrad, 1981) fragments, respectively. In previous papers, we reported the distributions of tryptophan (Hozumi, 1981) and cysteine (Hozumi, 1982) residues over the three fragments. Mornet et al. (1981b) showed that the 95K heavy chain of S-1 intimately contacts with two neighboring actin monomers; one monomer is bound to the 50K domain and the other to the 20K domain. Recently, we studied the proteolytic generation of the 27K fragment, i.e., the 27K/50K cut. It was found that the 27K fragment was generated by two routes proceeding in parallel; in one case, a 29.5K fragment was first produced and then degraded to 27K; in the other, a 27K fragment was directly produced without any precursor (Hozumi & Muhlrad, 1981). In addition, it was observed that

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[1] Abbreviations: S-1, myosin subfragment 1; TPCK-trypsin, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone treated trypsin; DACM, *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide; 1,5-IAEDANS, *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine; NaDodSO$_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; NMR, nuclear magnetic resonance.

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the binding of ATP and its derivatives affected the conformation of the 29.5K/27K region (Muhlrad & Hozumi, 1982).

In the present work, using a higher concentration of trypsin (weight ratio to S-1 of 1:20), we demonstrate a correlation between the losses of S-1 and acto-S-1 Mg^{2+}-ATPase activities, binding of S-1 to actin, and the nature of the proteolytic events on the structure of S-1 heavy chain during proteolysis. We also examine effects of actin and/or ADP on these losses. The 27K, 50K, and 20K fragments in the case of S-1 and the 70K fragment in the case of acto-S-1 (which are all stable fragments under milder concentration of trypsin) were degraded into smaller fragments. It was observed that addition of ADP accelerated every fragmentation and that a new fragment was produced by digestion of the acto-S-1–ADP complex, which is presumably different from the acto-S-1 rigor complex. Except in the acto-S-1 complex without ADP, the Mg^{2+}-ATPase activities of S-1 and acto-S-1 and the binding of S-1 to actin decreased during the course of tryptic digestion, presumably due to progressive destruction of S-1. However, a very interesting result was found in the case of acto-S-1 without ADP. After a 60-min digestion, the actin activation of S-1 Mg^{2+}-ATPase and the binding of S-1 to actin remained perfectly protected from proteolysis, but the turbidity decreased to half of the initial value. This behavior shows that large conformational changes occurred in the acto-S-1 structure during the tryptic digestion. Adopting the conclusion of Mornet et al. (1981b) that there are two actin binding sites on S-1, we speculate that in our case one of these bonds was broken and one was retained. If so, our results also show that S-1 ATPase is fully activated by actin when only half of the actin binding sites of S-1 are occupied by actin.

Materials and Methods

Chemicals. L-1-(Tosylamido)-2-phenylethyl chloromethyl ketone treated trypsin (TPCK-trypsin), soybean trypsin inhibitor, and α-chymotrypsin were from Worthington Co. ATP and ADP were from Sigma Chemical Co. *N*-(Iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (1,5-IAEDANS) and *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide (DACM) were purchased from Aldrich Chemical Co. and Wako Chemical Co., respectively. All other chemicals were of reagent grade.

Proteins. Myosin and actin from back and leg muscles of rabbits were prepared by the methods of Tonomura et al. (1966) and Spudich & Watt (1971), respectively. S-1 was prepared by digestion of myosin filaments with α-chymotrypsin (Weeds & Taylor, 1975) and purified by filtration through Sephacryl S-200 in 20 mM Tris-HCl, 0.1 mM dithiothreitol, and 0.1 mM NaN_3, pH 7.3. We calculated S-1 and actin concentrations from their absorbance, assuming absorption coefficients $A^{1\%}_{280nm}$ of 7.5 cm^{-1} (Wagner & Weeds, 1977) and $A^{1\%}_{290nm}$ of 6.3 cm^{-1} (Houk & Ue, 1974), respectively; in each case a light scattering correction at 330 nm was applied. We assumed that S-1 and actin monomer had molecular weights of 110K and 42K, respectively.

Tryptic Digestion of S-1 and Analysis of Separated S-1 Fragments. S-1 (2 mg/mL, 18.2 μM) was digested by one-twentieth of its concentration (w/w) of TPCK-trypsin in 50 mM Tris-HCl, 30 mM KCl, and 2 mM $MgCl_2$ at pH 7.5, 25 °C, in the absence and presence of F-actin (1.5 mg/mL, 35.7 μM) and/or ADP (2 mM) if not stated otherwise. The digestion was started by addition of trypsin. At prescribed time intervals, aliquots were withdrawn and added to an equal volume of a boiling solution containing 0.1 M Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate ($NaDodSO_4$), 2% β-mercaptoethanol, 20% glycerol, and 0.002% brom phenol blue and were incubated for 3 min. Samples were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1970). For ATPase activity and binding measurements, trypsin inhibitor was added at twice the trypsin concentration (w/w) to terminate the digestion. The cleavage fragments were separated by electrophoresis on a 7–18% polyacrylamide gradient slab gel. After electrophoretic separation, a gel slice containing a Coomassie blue stained band of the fragment of interest was extracted with 25% (v/v) pyridine–water for 5 h at 37 °C, and the fragment concentration was taken to be proportional to the 605-nm absorbance of the eluted dye. The myosin fragments of this size give absorbances that obey Beer's law (Fenner et al., 1975). Molecular weights of fragments were estimated by comparing electrophoretic mobilities to those of authentic marker proteins and myosin light chains of known molecular weight.

Fluorescence Labeling of S-1 and Actin. S-1 was labeled with 1,5-IAEDANS as previously described (Hozumi & Muhlrad, 1981). For reaction of G-actin with DACM, G-actin was incubated for 10 min with equimolar DACM in 2 mM Tris-HCl, 0.2 mM ATP, and 0.1 mM $CaCl_2$ at pH 8.0, 0 °C. The labeled G-actin was dialyzed against 50 mM Tris-HCl and 30 mM KCl at pH 7.5 to get labeled F-actin.

Determination of ATPase Activity. The Mg^{2+}-ATPase activities of S-1 and acto-S-1 were measured at 25 °C. The P_i liberated was measured by the method of Anner & Moosmayer (1975). For assays, the medium contained 0.03 mg/mL (0.3 μM) S-1, 0.3 mg/mL (7.1 μM) actin (if acto-S-1 ATPase), 50 mM Tris-HCl, 30 mM KCl, 2 mM MgCl, and 1 mM ATP at pH 7.5. Enzyme activities were expressed as percent of the control S-1 ATPase activity.

Measurement of Binding of S-1 to Actin. The binding of S-1 to actin during the tryptic digestion was measured by a sedimentation method. After incubation with actin for 5 min at 25 °C, the mixture was centrifuged (2 h, 45K rpm), and the supernatant was carefully removed. The concentration of protein in the supernatant was estimated by the Folin phenol method. In this study, there were corrections for the unpolymerized actin and originally denatured S-1 that sedimented individually. Also, the concentrations of trypsin and trypsin inhibitor were subtracted from the total protein measured in the supernatant. Conditions for sedimentation were 0.3 mg/mL (2.7 μM) S-1, 0.6 mg/mL (14.3 μM) actin, 50 mM Tris-HCl, 30 mM KCl, and 2 mM $MgCl_2$ at pH 7.5.

Turbidity Measurement. The effect of tryptic digestion on the turbidity following combination of S-1 and actin was measured at 350 nm. In the case of acto-S-1, the tryptic digestion was carried out in an optical cell, and the time course of turbidity change was followed by a recorder. In the case of S-1, trypsin inhibitor was added to terminate the digestion at prescribed time intervals, and the change of turbidity upon combination with actin was read. S-1 (2 mg/mL, 18.2 μM) was mixed with actin (1.5 mg/mL, 35.7 μM) in 50 mM Tris-HCl, 30 mM KCl, and 2 mM $MgCl_2$ at pH 7.5, 25 °C. Analyses were carried out after subtraction of the sum of turbidities of actin and S-1 measured individually.

Results and Discussion

Effect of Tryptic Digestion of S-1 on Structure. Limited tryptic digestion of S-1 in the absence and presence of actin and/or ADP was carried out under higher concentrations of trypsin (weight ratio to S-1 1:20). When a lower concentration of trypsin (weight ratio to S-1, 1:100) was used, the heavy chain of S-1 was cleaved into mainly three large fragments (27K, 50K, and 20K) (Balint et al., 1978; Mornet et al., 1979; Yamamoto & Sekine, 1979a) and into two stable fragments

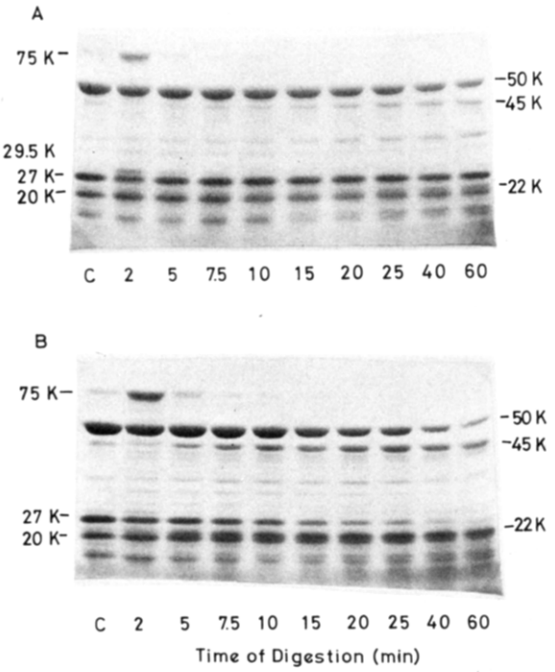

FIGURE 1: Electrophoretograms of S-1 digested by trypsin for different time intervals. Tryptic digestion was carried out as described under Materials and Methods in the absence (A) and presence (B) of ADP. 75 μg of S-1 was applied. (C) S-1 was digested with a trypsin to S-1 weight ratio of 1:100 for 45 min.

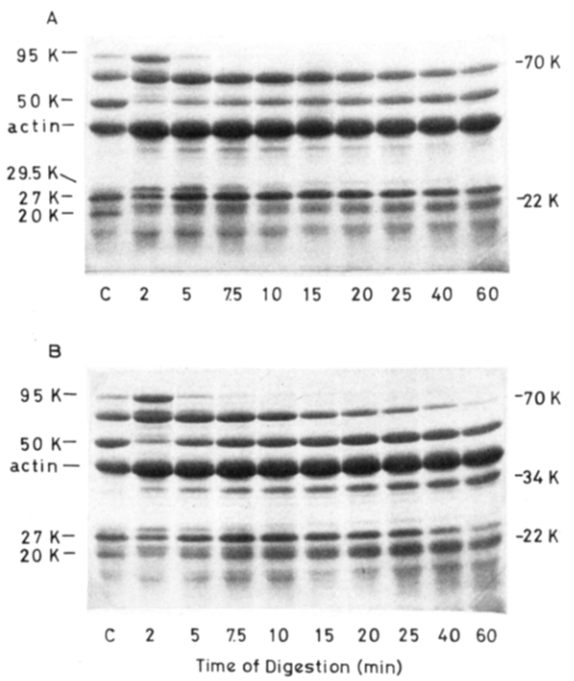

FIGURE 2: Electrophoretograms of acto-S-1 digested by trypsin for different time intervals in the absence (A) and presence (B) of ADP. (C) Mixtures of S-1 and acto-S-1 that were digested with a trypsin to S-1 weight ratio of 1:100 for 45 min, respectively.

(70K and 27K) in the presence of actin (Mornet et al., 1979; Yamamoto & Sekine, 1979a). When proteolysis was effected at higher trypsin, the previously stable fragments were entirely converted into smaller fragments. The 27K and 50K fragments were converted into 22K and 45K fragments, respectively (Figures 1A and 4A), and the 70K fragment into the 50K and 20K fragments (Figures 2A and 4B). Although initially it appears stable, it is revealed that the 20K fragment also converted (though more slowly) into smaller fragments when S-1 was made fluorescent by a specific reaction with 1,5-IAEDANS at the SH_1 thiol group on the 20K fragment (Balint et al., 1978) (Figure 3). Addition of ADP to the medium accelerated the further degradations observed at high trypsin ratios. Especially, ADP strongly enhanced the degradation of the 27K and 70K fragments (Figures 1B, 2B, and 4). In the case of acto-S-1, addition of ADP produced a new 34K fragment (Figures 2B and 4B), which probably arose from the 70K and/or 50K fragments, because F-actin is known to be refractory to proteolysis (Jacobson & Rosenbusch, 1976) and because the fragment had no fluorescence when 1,5-IAEDANS-labeled S-1 was used (Figure 3B). It has been reported that from the proteolysis of G-actin by either trypsin or chymotrypsin a fragment of 34K containing reactive Cys-373 was isolated (Jacobson & Rosenbusch, 1976). However, this is not the origin of our 34K fragment, because our fragment had no fluorescence when DACM-labeled F-actin was used. So, the 34K fragment must arise from the 50K parent fragment.

In a previous paper (Hozumi & Muhlrad, 1981), it was found that the 27K fragment was generated by two routes proceeding in parallel; in one route a 29.5K fragment was first produced and then degraded to a 27K fragment; in the other, a 27K fragment was directly produced without any precursor. Also, it was shown that ADP suppressed the route with the 29.5K precursor and that actin greatly decreased the suppressing effect (Muhlrad & Hozumi, 1982). In the present work, we confirmed these effects and, in addition, found that ADP accelerated the breakdown of 27K into a 22K fragment. This effect might be expected, since the 27K fragment contains a part of the nucleotide binding site (Szilagyi et al., 1979). ADP also accelerated the degradation of other fragments, so it is very probable that ADP binding induces a conformational change not only locally but propagated over some distance in the structure of S-1.

The demonstrated existence of ternary complexes (Tonomura & Morita, 1960; Beinfeld & Martonosi, 1975; Highsmith, 1976) such as actin–S-1–ADP confirms the early assertion (Barany & Barany, 1959) that S-1 has two spatially distinct sites. There is, however, intersite communication, since the binding of ADP reduces the affinity of actin to S-1, and vice versa (Szent-Gyorgyi, 1947; Kiely & Martonosi, 1968). The mechanism of intersite communication is still unclear. One possibility is communication by means of propagated structural distortion (Morales et al., 1982). Segmental flexibility of myosin has already been directly shown (Mendelson et al., 1973; Highsmith et al., 1977). It is a feature of myosin related to its function and was first inferred from the proteolytic susceptibility that generates the segments (Lowey et al., 1969). So, because various proteolytic enzymes also first cut myosin at the same two places, it must be thought the these places are noncompact regions and therefore potentially flexible. The tryptic digestion of S-1 shows that the S-1 heavy chain is actually a three-region complex made up of the three fragments, 27K, 50K, and 20K, which are covalently connected by two protease-sensitive places. Here it was shown that within the three fragments there are additional protease-sensitive, less compact "hinges". It was observed that when both actin and ADP bound a new 34K fragment is produced; this suggests that actin and ADP together induced a different conformation of S-1 than did actin alone. These changes may be manifestations of an intersite "signaling system" (Morales & Botts, 1979). From a recent NMR investigation on myosin and S-1, it was concluded that there is an S-1 region exhibiting much internal motion and that this motion is quenched by actin binding (Highsmith et al., 1979). These observations too may

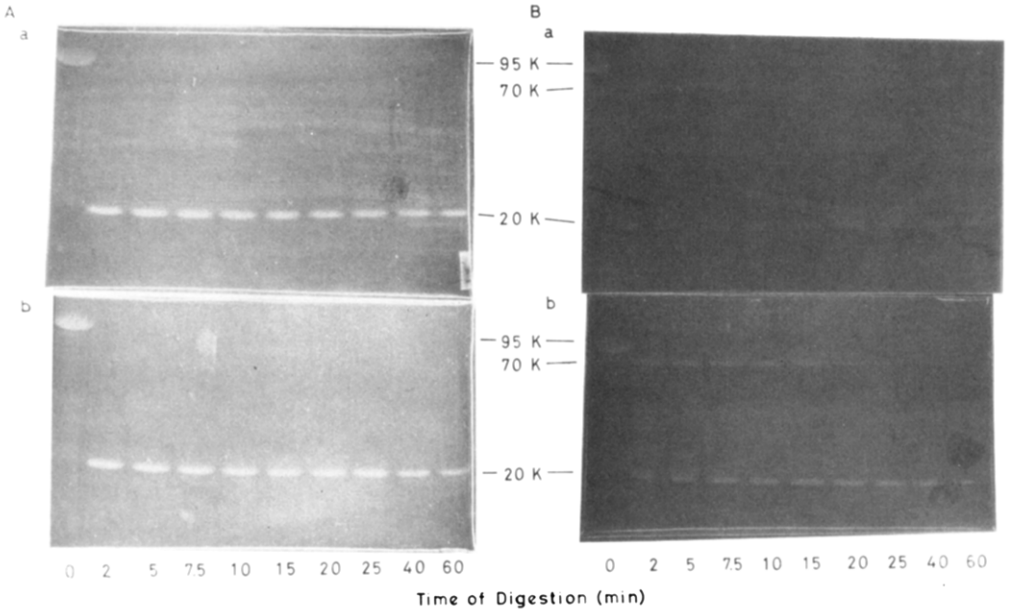

FIGURE 3: Electrophoretograms of S-1 (A) and acto-S-1 (B) digested by trypsin for different time intervals when 1,5-IAEDANS-labeled S-1 was used in the absence (a) and presence (b) of ADP. Fluorescence of 1,5-IAEDANS incorporated in SH_1 was detected by illuminating a $NaDodSO_4$ gel with a UV lamp.

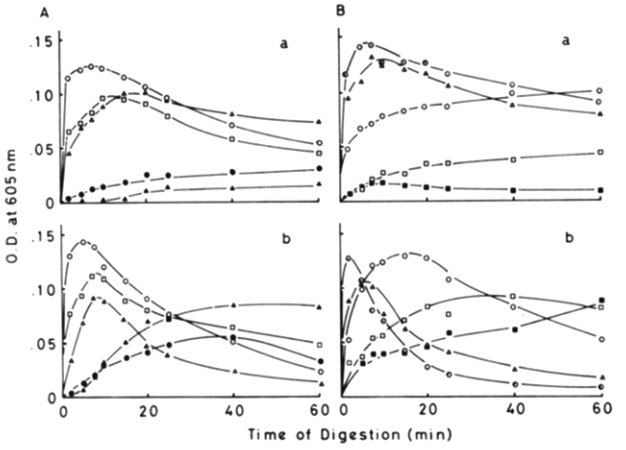

FIGURE 4: Estimation of band intensities present in $NaDodSO_4$ slab gels of S-1 (A) and acto-S-1 (B) during tryptic digestion in the absence of (a) and presence (b) of ADP. 40 μg of S-1 was applied to gels. Details are as under Materials and Methods. The band concentrations are expressed with value of OD_{605nm}: (⊙) 70K; (○) 50K; (●) 45K; (■) 34K; (△) 27K; (▲) 22K; (□) 20K.

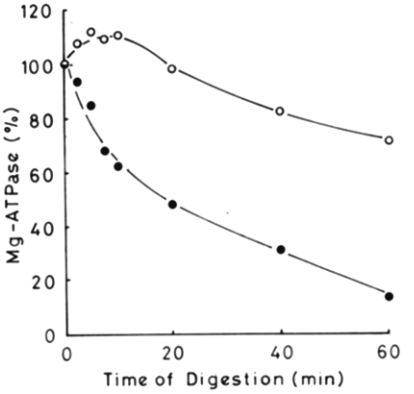

FIGURE 5: Time course of changes in Mg^{2+}-ATPase activity of S-1 during tryptic digestion of S-1 in the absence (○) and presence (●) of ADP. Percent is expressed with percent of the original S-1 ATPase. The original S-1 ATPase activity was 0.024 μmol/(min·mg).

be manifestations of the signaling system.

Effect of Tryptic Digestion of S-1 on Its Mg^{2+}-ATPase Activity. The various ATPase activities of S-1 alone were not affected by the digestion under the milder concentration of trypsin (Mornet et al., 1979). But inactivation of S-1 Mg^{2+}-ATPase activity upon trypsin treatment was observed under a higher concentration of trypsin (Figure 5). When S-1 was digested without ADP, its Mg^{2+}-ATPase activity was slightly activated over the first 15 min; after that, it started to decrease progressively, reaching about 50% of the control value after 60 min. The ATPase inactivation was enhanced by addition of ADP; the enhancement was evident immediately after the start of the reaction. A similar drop of the K^+-ATPase activity has been reported under similar conditions (Kassab et al., 1981). These inactivations must be due to further destruction of S-1. As a matter of course, the inhibition of actin-stimulated ATPase was also observed in both cases (Figure 6B).

In the case of acto-S-1 without ADP, the actin-stimulated ATPase was completely conserved [just as when using a weight ratio of trypsin to S-1 of 1:100 (Mornet et al., 1979)] even after 60 min of digestion (Figure 6B). However, upon addition of ADP the protective effect of actin from tryptic digestion disappeared within a few minutes of reaction. A similar effect of ADP on the ATPase activity of acto-S-1 during the tryptic digestion was also observed with a milder concentration of trypsin (Mornet et al., 1981a). The observed effect of ADP on the digestion of acto-S-1 could be due to dissociation from actin by ADP. However, it was observed by sedimentation and turbidity methods that only about 10% of the S-1 dissociated from actin upon addition of ADP. And, also, a new 34K fragment was produced when acto-S-1 was digested with ADP but not when S-1 was digested with ADP. So, it is thought that upon addition of ADP to acto-S-1 a ternary complex was formed in which the S-1 is in a different conformation (and thus has a different proteolytic susceptibility) than it was when it was alone or when it was bound only to actin.

Mornet et al. (1979) and Yamamoto & Sekine (1979b) noted that the 50K/20K cut [or more precisely the degradation of an initial 22K precursor into the stable 20K fragment (Mornet et al., 1981a)] seemed to abolish actin activation of S-1 Mg^{2+}-ATPase. More recently, Botts et al. (1982) found,

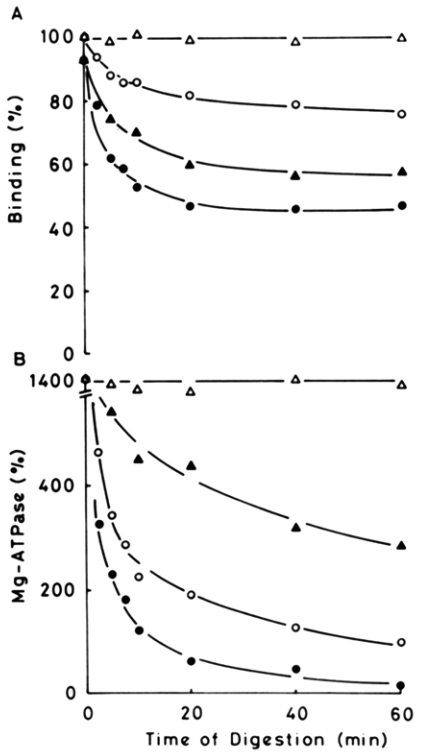

FIGURE 6: Time course of changes in binding of S-1 to actin (A) and Mg^{2+}-ATPase activity of acto-S-1 (B) during tryptic digestion of S-1 and acto-S1. Digestion conditions: (○) S-1 without ADP; (●) S-1 with ADP; (△) acto-S-1 without ADP; (▲) acto-S-1 with ADP. (A) (●) and (▲) contain 0.3 mM ADP. Percent is expressed as percent of the control binding. (B) Percent is expressed with percent of the original S-1 ATPase. The original acto-S-1 ATPase activity was 0.34 μmol/(min·mg).

however, that what the cut does is to reduce actin affinity; for at a saturating [actin], the S-1 Mg^{2+}-ATPase is fully restored. Our results also would be inconsistent with the assumption that the cut directly impairs the activation mechanism. In our case, the Mg^{2+}-ATPase activity of acto-S-1 was perfectly protected from the tryptic digestion, even though the 70K fragment converted into 50K and 20K fragments (not into 50K and 22K fragments; see Figure 7). Of course, we were using saturating [actin], so our observation would also be adequately explained by the contentions of Botts et al. (1982).

Effect of Tryptic Digestion of S-1 on Binding of S-1 to Actin. During the course of milder tryptic digestion of S-1 alone, neither the ability of S-1 to bind to actin nor the ability to be dissociated from actin by Mg^{2+}-ATP seem altered, although the actin-activated ATPase is progressively lost (Mornet et al., 1979). When S-1 is treated with trypsin at a weight ratio to S-1 of 1:20, its binding to actin is clearly and progressively lost, reaching about 75% of the control value after a 60-min digestion (Figure 6A). Upon addition of ADP, this drop is strongly enhanced, and after a 10-min digestion only 50% of the original binding was observed. When the acto-S-1 complex is digested, its binding property seems perfectly protected from proteolysis, at least at saturating [actin]. However, this protection is removed by addition of ADP; a drop in the binding is observed within a few minutes of digestion and reaches about 55% of the control value after 60 min. In each case, addition of Mg^{2+}-ATP induced dissociation of the complex, and the extent of dissociation of S-1 from the complex by ADP is about 10% of the bound S-1 when the complex is formed.

Change in Turbidity of Acto-S-1 Complex during the Course of Tryptic Digestion. S-1 and actin bind firmly on

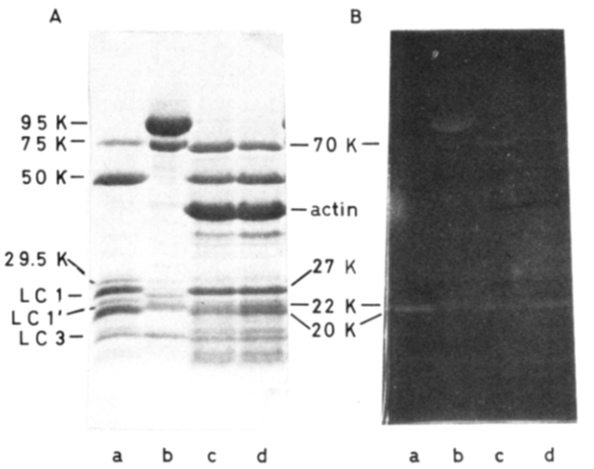

FIGURE 7: Comparison of fragments around 20K using different concentrations of trypsin: (A) protein banding pattern after staining with Coomassie blue; (B) location on gel of fluorescent products. (a) S-1 digested by 1:100 trypsin for 10 min; (b) S-1 digested by 1:100 trypsin for 5 min (Mornet et al., 1981a); (c and d) acto-S-1 digested by 1:20 trypsin for 10 min; (c) without ADP; (d) with ADP. LC 1 (light chain 1), 25K; LC 1′, 23.5K; LC 3, 16K. The 22K peptide in (b) is different from that in (c) or (d), because the 22K in (c) or (d) had no fluorescence.

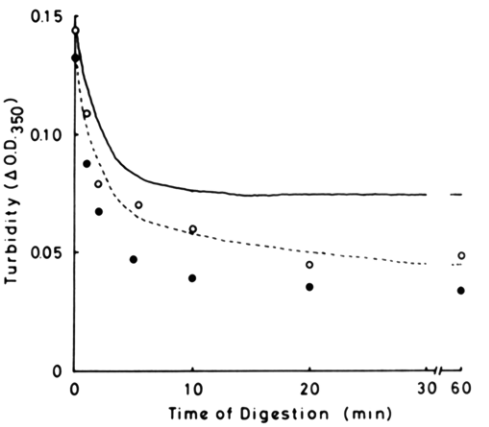

FIGURE 8: Time course of change in turbidity of acto-S-1 during tryptic digestion of S-1 and acto-S-1. Digestion conditions: (○) S-1 without ADP; (●) S-1 with ADP; (—) acto-S-1 without ADP; (---) acto-S-1 with ADP. (●) and (---) contain 2 mM ADP.

forming the rigor complex in the absence of ATP. The turbidity of the rigor complex is appreciably larger than that of ATP-dissociated acto-S-1 solution. The effect of tryptic digestion on the turbidity change was measured as an empirical indicator of structural change in the acto-S-1 complex during the course of proteolysis (Figure 8). In the case of S-1 alone, the turbidity upon adding actin dropped steeply to more than 50% of the control after a 5-min digestion and decreased slowly after. In the presence of ADP, a similar but slightly enhanced drop was observed. When the acto-S-1 complex was digested without ADP, a similar large drop in the turbidity (to about 50% of the control) was also observed initially, but after that, the turbidity remained constant, even after 60 min of digestion. Addition of ADP to the acto-S-1 complex enhanced the early drop in the turbidity and induced a progressive decrease after that. These results showed that tryptic digestion induced large conformational changes in the acto-S-1 structure including the inability of S-1 to form a complex. Remarkably, in the case of the acto-S-1 complex without ADP, all the S-1 is still bound to actin, even though the turbidity of the complex is reduced to half of that of the control after 60 min of digestion. Special interest is attached to the case in which acto-S-1 was digested

at high trypsin. In this process it appears that despite its partial proteolysis S-1 remained attached to actin, at least during the first hour of digestion. This is shown by the fact that its actin-activated Mg^{2+}-ATPase remained constant during that period and also by the fact that sedimentation during the period brought down practically all the S-1. Yet during that period the turbidity of the system was at least 50% less than that of the control. So, something happened, but it was not dissociation. As we have acknowledged, turbidity (especially of a multicomponent system) has to be regarded as simply an empirical indicator of structural events, but in very general terms, the intensity of scattered light depends on particle number and on particle shape. If in our process particle number does not increase (S-1's are not released from the complex), it is possible that what changes is the shape of the attached S-1. Adopting the conclusion of Mornet et al. (1981b), we could speculate further the proteolysis compels S-1 to give up one of its two contacts with actin (thus changing its shape) but that the other contact (responsible for actin activation of its ATPase) is retained. Under these circumstances a change in S-1–actin affinity should occur, but with our present methods and under saturating [actin], such a change would go undetected.

When the present paper was being prepared in a revised form, Borovikov et al. (1982) also suggested the presence of two actin binding sites on S-1 and speculated that actin binding to one of the sites is regulated by the blocking of one of the sites with 5,5′-dithiobis(2-nitrobenzoic acid) (Nbs_2) on the light chain and depended on Ca^{2+} concentration. Their suggestion supports the present observations, because it is generally accepted that this light chain has no effect on the actin activation of S-1 Mg^{2+}-ATPase even in the absence of Ca^{2+} in the skeletal muscle.

Added in Proof

At the suggestion of D. Mornet, we investigated cutting of actin–ADP (no S-1) and found some evidence of a 34K fragment. As stated in the text, such a cut would not be expected from actin alone. Thus the inference made above ("the conformation of S-1 in actin–S-1–ADP is different. So a different cut is made") should be changed to the conformation of actin in actin–S-1–ADP is different, etc.

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Registry No. ATPase, 9000-83-3; ADP, 58-64-0; trypsin, 9002-07-7.

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