Shift of Binding Site at the Interface between Actin and Myosin†

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Received April 13, 1989; Revised Manuscript Received July 19, 1989

ABSTRACT: The molar ratio dependent change in the binding manner between actin and the lysine-rich sequence at the junction between 50K and 20K domains of subfragment 1 was studied by both protease digestion and cross-linking with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. The tryptic cleavage site at the function between 50K and 20K was found to be located between the third and fourth lysine residues in the lysine-rich sequence -KKGGKKK-. This site was not protected by actin when the molar ratio of actin to subfragment 1 was 1:1 but was protected at 2:1 and 3:1. The V_8 protease cleavage site of chicken subfragment 1 and the elastase cleavage site of rabbit subfragment 1 were found to be located four residues away from the N-terminus of the lysine-rich sequence. Unlike the tryptic cleavage site, this site was protected by actin more when the molar ratio of actin to subfragment 1 was 1:1 than when it was 2:1 and 3:1. To understand the reason for the opposite effect of the molar ratio observed at the middle of and at four residues away from the lysine-rich sequence, actual cross-linked residue(s) was (were) determined by subjecting cross-linked product to a protein sequencer. It was found that the cross-linked sites were mainly at the first and second lysine residues of the lysine-rich sequence when the molar ratio of actin to subfragment 1 was 1:1. Considering the previously reported result that all five lysine residues were cross-linked to actin when the molar ratio was 5:1 (Yamamoto, 1989), it is suggested that the cross-linked site spreads as the molar ratio increases. All these results suggested that the area or the number of residues participating in actin binding shifts when the molar ratio of actin to subfragment 1 is altered.

It is generally accepted that the contractile force is generated by the cyclic interaction of the subfragment 1 (S-1)[1] portion of myosin with actin and ATP. However, the molecular mechanism of the force generation is still not well understood. Recently, in vitro motility assay clearly demonstrated that S-1 alone is capable of moving actin filaments (Harada et al., 1987; Toyoshima et al., 1987). It is, therefore, suggested that the relative motion of domains in S-1 on binding actin is important for the force generation mechanism. The actin binding sites on S-1 have been studied by various techniques. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), the so-called zero-length cross-linker, cross-links actin to several sites on S-1 (Mornet et al., 1981b; Sutoh, 1983; Yamamoto & Sekine, 1983, 1986). There are at least two sites on the S-1 heavy chain which are cross-linked to actin by EDC. Trypsin cleaves the S-1 heavy chain at two sites and produces 26K, 50K, and 20K fragments in that order from the N-terminus (Balint et al., 1978; Lu et al., 1978). Sutoh suggested that one of the sites which is most readily cross-linked to actin is located near the amino end of the 20K fragment produced by trypsin (1983). Recently, it was demonstrated that the junctional region between the 50K and 20K fragments is the actual binding site of actin. Chaussepied and Morales (1988) synthesized the so-called anti-peptide which has negatively charged amino acids just complementary to the lysine-rich junctional sequence. The binding of the peptide did not affect the intrinsic ATPase activities but significantly reduced the affinity of S-1 to actin. The binding of actin to the junctional region was shown directly by sequence analysis of the region which was cross-linked to actin by EDC. When the junctional region cross-linked to actin was analyzed by a protein sequencer, cross-linked residues could be detected because the yield of the corrsponding amino acid decreased. From the yield of PTH-lysines in the junctional region, it was suggested that all five lysine residues participate in actin binding in the rigor complex (Yamamoto, 1989). Since the binding site is at the junction of the two domains, the relationship between the binding of actin to the site and the interdomain motion of S-1 is of interest. Therefore, the binding manner of actin to this site was investigated in detail.

It is known that the molar ratio of actin to S-1 affects the susceptibility of the 50K–20K junction to trypsin (Mornet et al., 1981a; Yamamoto & Sekine, 1986). The junction was attacked by trypsin when the molar ratio of actin to S-1 was 1:1, but the site was protected when the ratio was more than 2:1. The rate of the cross-linking reaction between actin and the junctional region of S-1 also depends on the molar ratio of actin to S-1 (Yamamoto & Sekine, 1986). The reaction rate is high when actin is in excess over S-1 but low in the 1:1 complex. The reason for the molar ratio dependent change in their interaction was investigated in this study, and it was found that the number of amino acid residues of S-1 participating in the actin binding changed when the molar ratio of actin to S-1 was altered.

Materials and Methods

Proteins and Reagents. Myosin was prepared from rabbit back and chicken breast muscle according to Kielley and Bradley (1956). Actin was extracted from the acetone powder of rabbit skeletal muscle and purified according to Spudich and Watt (1971). S-1 was prepared by digesting myosin with chymotrypsin according to Weeds and Pope (1977). Chymotrypsin, elastase, *Staphylococcus aureus* V_8 protease, and trypsin were purchased from Sigma Chemical Co. EDC was

† This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan and by a Special Project Fund of The University of the Air.

[1] Abbreviations: S-1, subfragment 1; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PVDF, poly(vinylidene difluoride); ALC, alkali light chain.

0006-2960/90/0429-844$02.50/0

purchased from Dojin Chemical Co. *N*-[7-(Dimethylamino)-4-methyl-3-coumarinyl]maleimide was purchased from Wako Pure Chemical Co.

Cross-Linking of S-1 and Actin. Rabbit S-1 (1.2 mg/mL) and actin (0.44 mg/mL) were mixed in 60 mM KCl, 2 mM $MgCl_2$, and 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.0, and the solution was left for 30 min at 25 °C to hydrolyze ATP in the solution. The molar ratio of actin to S-1 was 1:1. Then, $^1/_{50}$th volume of 0.3 M EDC freshly dissolved in 0.2 M MOPS, pH 7.0, was added to the solution. The reaction was allowed to proceed for 60 min at 25 °C. The reaction was stopped by adding 2-mercaptoethanol to make final concentration of 50 mM.

Protease Digestion. Rabbit or chicken S-1 (1 mg/mL) and actin (0.36–1.1 mg/mL) were mixed in 60 mM KCl, 2 mM $MgCl_2$, and 20 mM MOPS, pH 7.0, and the solution was left for 30 min at 25 °C to hydrolyze ATP in the solution. Elastase, trypsin, or V_8 protease was added to the solution and allowed to digest at 25 °C. Weight ratios of elastase, trypsin, and V_8 to S-1 were 1:20, 1:50, and 1:50, respectively. Aliquots were withdrawn at every 20 min and mixed with an equal volume of hot SDS solution containing 4% SDS, 20% glycerol, 25 mM Tris-HCl, pH 6.8, and 10% 2-mercaptoethanol. The mixture was heated for 4 min in boiling water to stop the digestion. Elastase digestion of the EDC-treated 1:1 complex of actin and S-1 was done as follows. After the cross-linking reaction was stopped, the actin-S-1 solution was cooled on ice and washed with ATP to reduce the amount of un-cross-linked S-1. One-fourth volume of a cold solution containing 2 M KCl, 0.1 M MgATP, and 20 mM MOPS, pH 7.0, was added to the actin–S-1 solution, and the mixture was centrifuged immediately at 50 000 rpm for 1 h. The pellet was homogenized with a solution containing 100 mM KCl and 20 mM MOPS, pH 7.0, and the volume of the suspension was made equal to the initial actin–S-1 solution. SDS–polyacrylamide gel electrophoresis (PAGE) of the suspension revealed that about one-fifth of the S-1 heavy chain was cross-linked to actin and a nearly equal amount of un-cross-linked S-1 was contaminated in the suspension. The S-1 in the solution was digested with elastase at a weight ratio of elastase to S-1 of about 1:10 and 25 °C for 20–60 min. The digestion was stopped by 2 mM phenylmethanesulfonyl fluoride.

SDS–Polyacrylamide Gel Electrophoresis and Electroblotting of Protein Bands to the Membrane. SDS–polyacrylamide gel electrophoresis (PAGE) was done according to Laemmli and Favre (1973). Protein bands in the gel were electroblotted onto a poly(vinylidene difluoride) (PVDF) membrane (Immobilon, Millipore) according to Matsudaira (1987) using a semi-dry blotting apparatus (AE-6670P, Atto) at 3 mA/cm^2 for 90 min.

Extraction of 22K and 22K–Actin from the Polyacrylamide Gel. Since the amount of S-1 cross-linked to actin was small when the 1:1 complex was treated with EDC (Yamamoto & Sekine, 1986), 22K and 22K–actin produced by elastase digestion were separated by SDS–PAGE, extracted from the gel, and concentrated. To extract 22K and 22K–actin from the gel, the fluorescent thiol reagent *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide was added to the actin–S-1 solution digested with elastase at a molar amount nearly equal to the sum of actin and S-1. The reagent was allowed to react for 15 h at 4 °C. Since the reagent reacts mainly with actin and the 22K fragment of S-1, we can observe the bands of 22K and 22K–actin under UV illumination. The gel portion containing the 22K and 22K–actin was excised, and proteins were extracted from the gel and were concentrated by using an apparatus which utilizes a glass filter modified specifically by the author (Yamamoto, 1987). The 22K and 22K–actin were subjected to electrophoresis separately and were blotted onto a PVDF membrane for the sequence analysis.

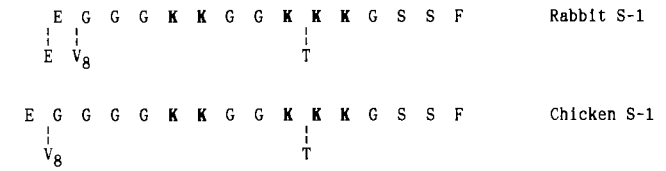

FIGURE 1: Protease cleavage sites of the 50K–20K junctional region of rabbit and chicken S-1's. E, V_8, and T indicate the sites cleaved by elastase, V_8 protease, and trypsin, respectively.

Protein Sequencing. Proteins on PVDF membrane were stained with Coomassie Brilliant Blue and a band of protein was excised with clean scissors. The membrane was cut into small pieces and placed on a polybrene conditioned glass fiber filter in a cartridge block of a pulsed liquid phase protein sequencer (Model 477A, Applied Biosystems) with on-line PTH analyzer (Model 120A).

Results and Discussion

Protease Cleavage Sites on the 50K–20K Junction. The 50K–20K junctional region is very susceptible to various proteases (Applegate & Reisler, 1983; Mornet et al., 1984). Before studying the effect of the molar ratio of actin to S-1 on the cleavage of the 50K-20K junctional region by elastase, trypsin, and V_8 protease, the cleavage sites by these proteases were determined by analyzing the amino end sequence of resulted 20K–22K fragments. As previously reported, the 22K fragment of rabbit S-1 produced by elastase has the amino end sequence of EGGGKKGGKKKG-- (Yamamoto, 1989). On the other hand, chicken S-1 was quite resistant to elastase digestion. When a large amount of elastase was added, chicken S-1 was degraded into a small peptide. Therefore, the 22K fragment was not obtained from chicken S-1. Although chicken S-1 has a very similar sequence at the junction from Glu-630 (EGGGGKKGGKKKGSSF--; Maita et al. 1987), its tertial structure may be somewhat different from that of rabbit, and the region is less susceptible to elastase. A 20K fragment was produced by trypsin from both rabbit and chicken S-1's. The fragment was separated by SDS–PAGE, electroblotted onto a PVDF membrane, and subjected to the protein sequencer. Amino end sequences of the two 20K fragments were the same, KKGSSF--. The cleavage site was, therefore, between the third lysine (K_3) and fourth lysine (K_4) in the lysine-rich sequence (Figure 1). Maita et al. (1987) suggested that the amino end sequence of chicken 20K fragment was KGSSF--. Since the whole 20K band was analyzed in this study, our sample might contain more than one peptide. However, if a fragment having the amino end sequence of KGSSF-- is contaminated in our 20K, it can be noticed from the yield of PTH-Gly in the second Edman degradation cycle. The amount of PTH-Gly in the second Edman degradation cycle was less than one-fifth that of PTH-Lys under our standard conditions. The amount of PTH-Gly in the second Edman degradation cycle increased when S-1 was digested with a high concentration of trypsin (1/20 w/w) for a long time (60 min). The cleavage, therefore, occurs mainly at a site between K_3 and K_4 under our standard conditions.

V_8 protease produced 22K fragment from both rabbit and chicken S-1's. Amino end sequences of rabbit and chicken 22K fragments were GGGKKG-- and GGGGKK--, respectively. Chicken S-1 was less susceptible to V_8 protease than rabbit S-1. The difference in the susceptibility to V_8 protease

also seems to reflect the structural difference in this region between rabbit and chicken S-1's as observed by elastase digestion. It was sometimes observed that the 22K band became a very close doublet. When the doublet was sequenced together, a sequence of A^A/pAPA-- was observed together with the sequence of 22K mentioned above. This sequence corresponds to the alanine-proline-rich sequence of alkali light chain 1 (ALC1; Frank & Weed, 1974; Maita et al., 1981). It was reported that ALC1 is degraded to 17K by V_8 protease (Chaussepied et al., 1983). Our result suggests that ALC1 is degraded at first to 22K and then to 17K. Since there is no acidic amino acid in the N-terminal region of ALC1, we could not explain why V_8 protease cleaves this region. One possible explanation is that our V_8 protease is contaminated by a small amount of other protease and ALC1 is very susceptible to the protease. The cleavage sites at the junctional region are summarized in Figure 1.

Effect of the Molar Ratio of Actin to S-1 on the Cleavage of the 50K–20K Junctional Region. It is known that the molar ratio of actin to rabbit S-1 affects the cleavage of the 50K–20K junction by trypsin (Mornet et al., 1981m; Yamamoto & Sekine, 1986). The same effect of the molar ratio on the tryptic attack was also observed in chicken S-1. As seen in Figure 2b, lanes 1–3, production of 20K fragment was observed at a molar ratio of actin to S-1 of 1:1 (lane 1), but there is little 20K fragment at the molar ratio of 2:1 or 3:1 (lanes 2 and 3). The 20K fragment produced in the presence of actin at a molar ratio of 1:1 had the same amino end sequence, KKGSSF--. Therefore, the site between K_3 and K_4 in the 50K–20K junctional region was protected by the binding of actin when actin is in excess (more than 2-fold molar excess). The molar ratio of actin to S-1 exerted a quite different effect on the cleavage of the junctional region by V_8 protease. Cleavage of the junctional region of rabbit S-1 by V_8 protease was completely blocked by actin, and the effect was independent of the molar ratio (from 1:1 to 3:1, Figure 2a, lanes 5–7). The band with an apparent molecular weight of 22K is not a fragment of heavy chain but that of ALC1 was judged from its amino end sequence (A^A/pAPA--). On the other hand, the cleavage of chicken S-1 by V_8 protease was affected by the molar ratio. The 22K fragment of the heavy chain appeared just above the band of degraded product of ALC1 (Figure 2b, lanes 4–6). However, an increase in the molar ratio of actin to S-1 rather decreased the protective effect of actin. The amount of 22K fragment produced at a molar ratio of 2:1 or 3:1 was about 3 times that produced at 1:1 (Figure 2b, lanes 4–6). This change can be noticed also from the increase in the amount of 76K (26K + 50K) fragment in the digest at molar ratios of 2:1 and 3:1.

The reason for the difference between rabbit and chicken S-1's was further investigated. Since the number of glycine residues between glutamic acid and the first lysine residue (K_1e of the lysine-rich sequence is 3 in rabbit and 4 in chicken (Figure 1), the distance from the actin–S-1 interaction site (lysine-rich region; Chaussepied & Morales, 1988; Yamamoto, 1989) may cause this difference. Therefore, the effect of the molar ratio on the cleavage of rabbit S-1 by elastase was studied because the distance from K_1 to its cleavage site in rabbit is the same as that from K_1 to the site attacked by V_8 protease in chicken on the primary structure (Figure 1). As can be seen in Figure 2a, lanes 8–10, cleavage by elastase was observed at molar ratios of 2:1 and 3:1 but only slightly at the molar ratio of 1:1. Since the so-called zero-length cross-linker EDC cross-links actin to the lysine residues in the junctional region (Yamamoto, 1989), it is likely that the binding of actin

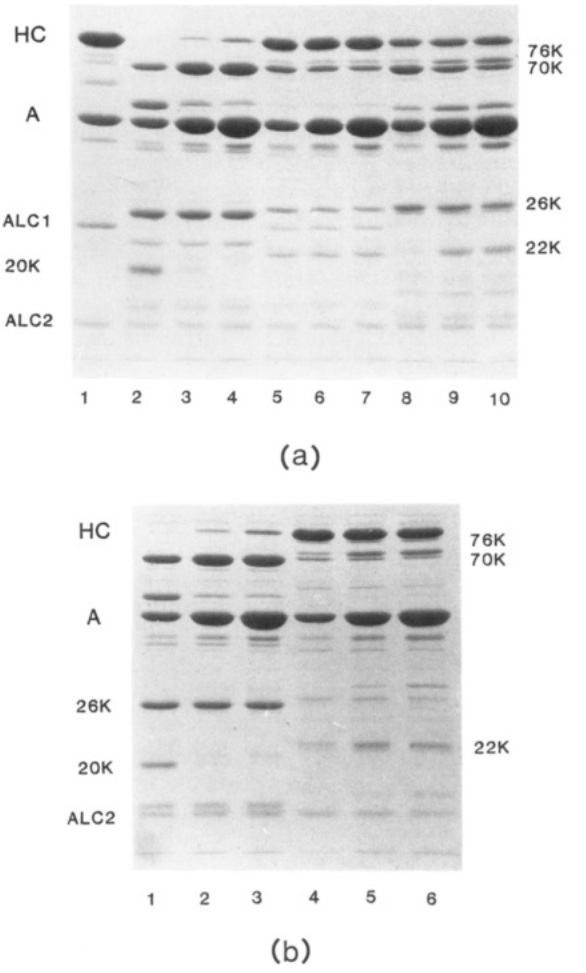

FIGURE 2: Effect of the molar ratio of actin to S-1 on the cleavage of the 50K–20K junctional region by proteases. (a) Actin and rabbit S-1 were mixed at a molar ratio of 1:1 to 3:1 and were digested with trypsin (lanes 2–4), V_8 protease (lanes 5–7), and elastase (lanes 8–10) for 40 min at 25 °C. Undigested 1:1 complex of S-1 and actin is shown in lane 1. (b) Actin and chicken S-1 were mixed at a molar ratio of 1:1 to 3:1 and were digested with trypsin (lanes 1–3) and V_8 protease (lanes 4–6) for 40 min at 25 °c. HC, A, and ALC mean S-1 heavy chain, actin, and alkali light chain, respectively.

to S-1 sterically interferes with the attack of proteases to this region. A change in the molar ratio of actin to S-1 seems to alter the binding manner of actin to this region. It was noticed that, as the molar ratio increased, a site in the middle of the lysine-rich sequence (between K_3 and K_4; attacked by trypsin) was protected but a site four residues away from K_1 to the N-terminus became less protected (Figure 2). Considering the position of these cleavage sites, it seems that actin shifts its binding site on S-1 in a direction from the N-terminal side to the C-terminal side of the junctional region. The minimum number of residues covered by actin is about 9 (from the elastase cleavage site to the tryptic cleavage site in rabbit, from the V_8 cleavage site to the tryptic cleavage site in chicken). Since the V_8 cleavage site of rabbit S-1 which is only one residue away from the elastase cleavage site was blocked by actin at all molar ratios tested, this shift of actin binding site may not be so large.

Cross-Linked Lysine Residues in the 1:1 Complex of Actin and S-1. It was previously suggested that, in the rigor complex at a molar ratio of actin to S-1 of 5:1, all five lysine residues in the junctional region participated in the interaction with actin (Yamamoto, 1989). Participation of the lysine residues in the interaction with actin was suggested by the decrease in the yield of PTH-lysine in the sequence analysis of 22K–

Table I: Comparison of Yields of PTH-Lysines and PTH-Glycines in 22K–Actin with Those in Free 22K[a]

	rel values[c] to yields of corresponding PTH-amino acids in free 22K in same digest for sample			
amino acid	1	2	3	average
K_1[b]	63.1	70.2	57.3	63.5 ± 5.3
K_2	65.7	73.8	64.2	67.9 ± 4.2
G_1	98.5	95.4	91.2	95.0 ± 3.0
G_2	95.7	88.4	96.8	93.6 ± 3.7
K_3	80.6	88.4	74.6	77.0 ± 2.6
K_4	97.1	102	99.2	99.3 ± 1.9
K_5	86.5	83.2	85.6	85.1 ± 1.4
G_3	103	99.9	111	104 ± 4.6

[a] S-1 and actin were cross-linked with EDC at a molar ratio of 1:1. [b] K_1, K_2, ..., G_3 represent the 5th lysine, 6th lysine, ..., 12th glycine in the sequence EGGGKKGGKKKG--, respectively. [c] The yields of K_1 to G_3 were expressed at first as normalized values to the averaged value of the first three glycines in the sequence, and then these values were compared between 22K–actin and 22K. Values in the table are the percent yields to those of corresponding residues in free 22K.

actin cross-linked with EDC. Since the cross-linked residue does not yield a PTH-amino acid in the corresponding Edman degradation cycle, the extent of cross-linking to actin can be estimated. It was suggested that 30–45% of each lysine residue was cross-linked to actin and the total decrease in the lysine residues due to the cross-linking was about two out of five (Yamamoto, 1989). By use of the same method, the binding manner of actin and S-1 in the 1:1 complex was investigated. S-1 and actin were mixed at a molar ratio of 1:1 and were cross-linked with EDC. The cross-linked complex was cleaved with elastase as described under Materials and Methods. Elastase cleaves the S-1 heavy chain at a site just upstream of the cross-linked site and produces 22K–actin. Resulted 22K and 22K–actin were separated by SDS–PAGE, extracted from the gel, concentrated, and electroblotted onto a PVDF membrane. The amino end sequence of the 22K fragment of rabbit skeletal S-1 is EGGGKKGGKKKG-- (Yamamoto, 1989). Since the N-terminus of actin is acetylated, the protein sequencer analyzes only the 22K fragment in 22K–actin. To compare the yield of PTH-lysine in 22K–actin with that in the un-cross-linked 22K quantitatively, the yield of the first three glycines (from second to fourth of 22K) was averaged, and the relative yield of the rest to that averaged value was calculated at first. These normalized yields were then compared between 22K–actin and un-cross-linked 22K. To reduce the possible artifact due to chemical reaction other than cross-linking, the yield of PTH-lysines in the 22K–actin was compared with that in the un-cross-linked 22K *in the same digest*. This 22K was produced not only from the un-cross-linked S-1 but also from the S-1 cross-linked to actin at the 50K fragment. Results of three experiments are shown in Table I. Values are the percent yields of residues in 22K–actin to those of corresponding residues in the un-cross-linked 22K. It is seen that a decrease in the yield of the first two lysine residues (K_1 and K_2, fifth and sixth residues of 22K) is most prominent (30–40%), and that of other lysine residues (K_3–K_5, 9th–11th residues of 22K) is small. There is a tendency that the decrease in the yield is large in K_1 and small in K_5. The total amount of the decrease in lysine residues was about one out of five. This result was quite different from that obtained for the 22K–actin produced by EDC at a molar ratio of actin to S-1 of 5:1 (Yamamoto, 1989). In that case, a decrease in the yields of K_2 and K_3 was most prominent, but other lysine residues decreased to a similar extent, and the total amount decreased was about two out of five. These results suggest that lysine residues participating in the binding of actin are restricted mainly to the first two lysine residues, K_1 and K_2, when the molar ratio of actin to S-1 is 1:1 but the participating residues spread to all (K_1–K_5) when the molar ratio of actin to S-1 increases. The molar ratio dependent change in the binding manner of actin and S-1 suggested by these results agrees very well with that suggested by the result of protease digestion. Both results strongly suggest that the actin binding site shifts depending on the molar ratio of actin to S-1.

Since the cooperative binding of S-1 to F-actin is observed in the electron microscope (Craig et al., 1980), the molar ratio does not represent the actual S-1 density on F-actin. However, as the molar ratio of actin to S-1 increases, the number of unoccupied actin molecules in the S-1-decorated F-actin should increase. Such unoccupied space on the decorated filament will allow S-1 to change its binding angle to the axis of the actin filament or rotate around its binding site with some freedom, and this freedom will increase as the molar ratio of actin to S-1 increases. The reason why the cross-linked site at a molar ratio of 5:1 was not restricted to one or two specific residues but distributed to all five residues (Yamamoto, 1989) can be explained by this freedom at the interface between S-1 and actin. This freedom increases the number of lysine residues which can interact with actin and, therefore, enhances the rate to be cross-linked to actin.

Ando and Scales (1985) reported that the rate of bundle formation of decorated actin filaments depends on the molar ratio of actin to S-1. The rate becomes maximum at a molar ratio of actin to S-1 of 7:1 to 6:1. This ratio of actin to S-1 is similar to the ratio which gave a maximum rate of cross-linking (Yamamoto & Sekine, 1986). The bundle formation through S-1 may also require some freedom of S-1 motion. It should be noted that the high rate of cross-linking between actin and S-1 is not due to the bundle formation because the molar ratio dependence of the cross-linking reaction was observed even at low ionic strength (Yamamoto & Sekine, 1986) where bundle formation was not observed (Ando & Scales, 1985). Both freedom of S-1 motion and neutralizing some electrostatic repulsive force by salt seem to be required to form bundles of decorated actin filaments.

The rate of the cross-linking reaction reaches a maximum at a molar ratio of 5:1 (Yamamoto & Sekine, 1986) but the protective effect on tryptic cleavage reaches a maximum at a molar ratio of 2:1. The difference in the molar ratio which exerts a maximum effect in the two cases is probably due to the difference in the position in the lysine-rich sequence of the junctional region. As the molar ratio increases to 5:1, the residues participating in the binding with actin spread gradually to K_5, but the ratio of 2:1 is probably enough to sterically block the tryptic attach on the site located between K_3 and K_4.

It was reported that only a very small amount of cross-linked product was formed between the lysine-rich sequence and actin in the ternary complex S-1–ATP–actin and that the cross-linked site in such a cross-linked product was restricted almost to K_1 (Arata, 1986; Yamamoto, 1989). Recently, Duong and Reiser (1989) reported that ATP abolishes the protective effect of actin to tryptic attack when actin was cross-linked to a site(s) on the 50K fragment but it did not when actin was cross-linked to the junctional region. It is suggested, therefore, that in the ternary complex, actin binds mainly to a site (sites) other than the junctional region of S-1 and interacts infrequently at the edge (K_1) of the lysine-rich sequence. The transition from ternary complex to rigor complex with the release of ADP and P_i is generally believed to be the force generation step. Since the binding site in rigor is at the

junction between the 50K and 20K domains, this change in the binding state may alter the interdomain distance in S-1 and cause change in its shape and binding angle to the filament axis of F-actin. Present study also showed that, even in the absence of ATP (rigor complex), the S-1–actin interface can take different binding states depending on the molar ratio of actin to S-1. As judged from the structural arrangement and the molar ratio of actin to myosin in muscle, the myosin head seems to interact with F-actin with considerable freedom of motion as in the actin–S-1 complex at a molar ratio of 5:1. It should be noted, therefore, that the binding manner of actin and S-1 deduced from the three-dimensional reconstruction of F-actin fully decorated with S-1 may not represent the true rigor state of actin and S-1.

ACKNOWLEDGMENTS

I thank Drs. H. Noda and K. Maruyama for their encouragement and discussion.

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