An Actin-Binding Site on the 20K Fragment of Myosin Subfragment 1†

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ABSTRACT: Myosin subfragment 1 (S1) was covalently labeled with a fluorescent dye, *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide (DACM), and then digested by trypsin to cleave S1 heavy chain into fragments. The DACM-labeled and trypsin-treated S1 was complexed with F-actin and treated with a zero-length cross-linker, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC). The cross-linking reaction generated a covalently linked complex of actin and the 20K fragment of S1 heavy chain, which exclusively incorporated the fluorescent dye, to form a fluorescent 65K cross-linked product. The 20K and 65K fluorescent peptides were isolated and purified and then subjected to cyanogen bromide and/or hydroxylamine cleavages. Mapping of fluorescent cleavage products on acrylamide gels revealed that the N-terminal 20 residues of the 20K fragment of S1 heavy chain contained a cross-linking site of actin.

Interaction of actin and myosin is a basic process of muscle contraction. In view of its biological importance, the interaction has been extensively studied by enzymatic, chemical, and physicochemical techniques. Among them, three-dimensional image reconstitution of electron micrographs of actin filaments decorated by myosin subfragment 1 (S1)[1] particles has provided a structural basis for understanding the molecular mechanism of the actin–myosin interaction (Moore et al., 1970; Toyoshima & Wakabayashi, 1979; Wakabayashi, 1980; Wakabayashi & Toyoshima, 1981; Taylor & Amos, 1981), though detailed three-dimensional structure of the actin–S1 complex has not yet been obtained because of ambiguity in assignment of reconstituted mass to the actin and S1 particles.

Another promising approach to understand the structural basis of the actin–myosin interaction is to determine primary sequences involved in the interaction of actin and myosin polypeptide chains, by employment of chemical analysis. By using a cross-linking reaction between actin and S1 induced by a zero-length cross-linker, 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (EDC), it has been shown that S1 heavy chain binds at and around some of the N-terminal acidic residues of actin at positions 1, 2, 3, 4, and 11 while alkaline light chain 1 binds at and around some of its C-terminal acidic residues at positions 360, 362, and 363 (Sutoh, 1982). Rather remarkable facts that acidic residues cluster at the N-terminal region of actin polypeptide and that the high number of amino acid exchanges in this acidic region during eukaryotic evolution have never destroyed its acidic nature (Lu & Elzinga, 1977; Vandekerckhove & Weber, 1978a,b,c) are understandable in the light of the above finding. Since acidic side chains of actin in its myosin-binding site are in direct contact with amino groups of myosin in its actin-binding site (Mornet et al., 1981b; Sutoh, 1982), ionic forces between these groups are expected to play an important role in the actin–myosin interaction. Carboxyl groups in the N-terminal segment of actin would be, therefore, essential for the interaction.

Although myosin-binding sites have been determined in the primary sequence of actin as above, their spacial locations on an actin particle are not available at present, since X-ray diffraction studies on crystals of the actin–DNase I complex have provided a three-dimensional structure of actin only at low resolution so far (Suck et al., 1981).

A similar approach to identify locations of actin-binding sites on the myosin polypeptide chain would be fruitful for understanding the molecular mechanism of the actin–myosin interaction. Mornet et al. (1981a,b) have already shown that two domains of S1 heavy chain, i.e., 20K and 50K segments of S1 heavy chain, contain actin-binding sites. In this paper, their work has been extended to locate one of the actin-binding sites along the amino acid sequence of the 20K fragment of S1 heavy chain (Gallagher & Elzinga, 1980) by employing chemical cross-linking of actin and S1 with EDC.

Materials and Methods

Preparations of Proteins. S1 was prepared according to Weeds & Taylor (1975) from rabbit skeletal muscle myosin. Actin was prepared according to the method of Spudich & Watt (1971).

DACM Labeling of S1 and Its Tryptic Cleavage. S1 (1 mg/mL) in 30 mM KCl, 20 mM Tris-HCl, and 1 mM MgADP (pH 8.0) was reacted with 1.3 equiv mol of *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide (DACM) over S1 for 3 min at 0 °C. The reaction was quenched by addition of 0.01 volume of 0.1 M *N*-acetylcysteine. The DACM-labeled S1 was then digested by trypsin (0.01 mg/mL) for 15 min at 25 °C. The reaction was terminated by the addition of excess trypsin inhibitor. The tryptic digestion cleaved the intact S1 heavy chain into fragments with apparent molecular weights of 20000, 25000, 50000, and 75000 (25K + 50K) (Mornet et al., 1979; Yamamoto & Sekine, 1979).

Cross-Linking of F-Actin and S1. F-actin (1 mg/mL) in 0.1 M NaCl, 10 mM imidazole, and 2 mM $MgCl_2$ (pH 7.0) was mixed with an equal volume of the DACM-labeled and trypsin-treated S1 (1 mg/mL) in the same solvent. After the mixture was gently stirred for 30 min at 25 °C, cross-linking reaction was initiated by addition of 0.05 volume of freshly prepared 0.1 M 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) in 0.1 M imidazole (pH 7.0). The cross-linking reaction was allowed to proceed for 1 h at 25 °C and then quenched by the addition of excess 2-mercaptoethanol.

Purification of Peptides. The cross-linked mixture was electrophoresed on $NaDodSO_4$ gels containing 12% acrylamide and 0.36% bis(acrylamide) (Laemmli, 1970). Fluorescent

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[1] Abbreviations: S1, myosin subfragment 1; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide: DACM, *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; $NaDodSO_4$, sodium dodecyl sulfate.

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bands (20K and 65K) were cut out under illumination with a UV lamp. The 20K and 65K peptides were then electrophoretically eluted out of gels into dialysis tubes attached to bottoms of short acrylamide gels. The collected peptides were again electrophoresed on $NaDodSO_4$ gels containing 12% acrylamide and 0.36% bis(acrylamide). The 20K and 65K fluorescent bands were cut out under illumination with a UV lamp and thoroughly washed with 20% methanol and then with 50% methanol. These gels were dried in vacuo.

Hydroxylamine Cleavage of Peptides and Electrophoresis of Cleavage Products. The dried gels containing the 20K or 65K peptide were soaked in freshly prepared 6 M guanidine hydrochloride–1 M hydroxylamine (pH 9.0). The cleavage reaction was allowed to proceed for 4 h at 45 °C (Sutoh, 1981). The resulting gels were washed with 20% methanol–1% 2-mercaptoethanol and then with 50% methanol. These gels were dried in vacuo.

The dried gels were soaked in 1% $NaDodSO_4$, 10 mM Tris-HCl, 2% 2-mercaptoethanol, and 10% glycerol (pH 8.0) for 4 h at 25 °C and directly layered on another slab $NaDodSO_4$ gel containing 12% acrylamide and 0.36% bis(acrylamide) with a stacking gel system (Laemmli, 1970). After electrophoresis, DACM fluorescence was detected by illuminating the gel with a UV lamp.

Cyanogen Bromide Cleavage of Peptides and Electrophoresis of Cleavage Products. The dried gels containing the 20K or 65K peptides were soaked in freshly prepared 0.2 M cyanogen bromide in 70% formic acid. The cleavage reaction was allowed to proceed for 18 h at 25 °C. These gels were thoroughly washed with 20% methanol–1% 2-mercaptoethanol and then with 50% methanol. They were dried in vacuo. Electrophoreses of cleavage products were carried out on a slab $NaDodSO_4$ gel containing 15% acrylamide and 0.45% bis(acrylamide) as above.

Double Cleavage of Peptides with Cyanogen Bromide and Hydroxylamine. Fluorescent peptides trapped in acrylamide gels were cleaved by cyanogen bromide as above. After electrophoresis, the resulting gels were washed and then dried in vacuo. They were then soaked in freshly prepared 6 M guanidine hydrochloride–1 M hydroxylamine (pH 9.0), and the cleavage reaction was carried out as above. After the hydroxylamine cleavage, the gels were thoroughly washed with 20% methanol–1% 2-mercaptoethanol and then with 50% methanol. They were dried in vacuo. The resulting dried gels were soaked in 1% $NaDodSO_4$, 10 mM Tris-HCl, 2% 2-mercaptoethanol, and 10% glycerol (pH 8.0), and the fluorescent bands were cut out. They were directly layered on a $NaDodSO_4$ gel containing 15% acrylamide, 0.45% bis(acrylamide), and 6 M urea. After electrophoresis in the presence of $NaDodSO_4$, DACM fluorescence was detected by illuminating the gel with a UV lamp.

Electrophoresis. Acrylamide gel electrophoresis in the presence of $NaDodSO_4$ was carried out according to Laemmli (1970). Apparent molecular weights of various peptides were estimated according to the method of Weber & Osborn (1969).

Results

DACM Labeling of Reactive Thiols in S1. It has been shown that two reactive thiols in S1 heavy chain, SH_1 and SH_2 (Sekine & Kielley, 1962; Sekine et al., 1962), are selectively labeled with a fluorescent dye, *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide (DACM), when S1 is reacted with a less than stoichiometric amount of the dye over the thiols in low ionic strength solvent in the presence of MgADP (Sutoh, 1981). In the present study, S1 was reacted with 1.3 equiv mol of DACM over S1 to label these thiols. The

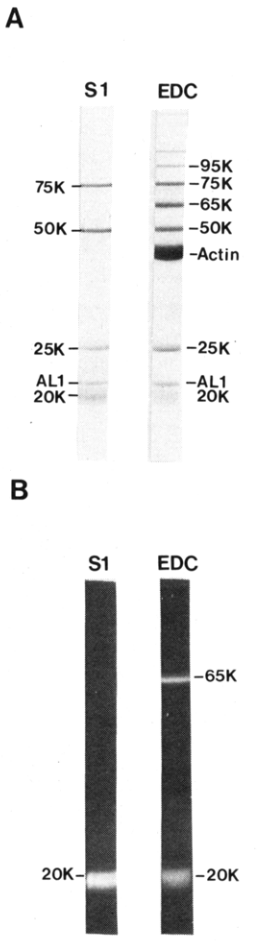

FIGURE 1: Cross-linking of F-actin and the DACM-labeled and trypsin-treated S1. (A) Gels were stained with Coomassie Blue after electrophoresis. Lane S1 is the DACM-labeled and trypsin-treated S1. Lane EDC is a mixture of F-actin and the DACM-labeled and trypsin-treated S1 that was cross-linked with EDC. Cross-linking conditions: protein concentrations 0.5 mg/mL of F-actin and 0.5 mg/mL of S1; concentration of cross-linker 5 mM; solvent 0.1 M NaCl, 10 mM imidazole, and 2 mM $MgCl_2$ (pH 7.0); reaction time 1 h; temperature 25 °C. Electrophoresis was carried out on $NaDodSO_4$ gels containing 10% acrylamide and 0.3% bis(acrylamide). (B) Same gels as in (A) were illuminated with a UV lamp before Coomassie Blue staining to detect DACM fluorescence.

DACM-labeled S1 thus prepared was digested by trypsin to cleave S1 heavy chain into fragments (20K, 25K, and 50K) (Mornet et al., 1979; Yamamoto & Sekine, 1979) as shown in Figure 1A (75K peptide observed in the figure is a digestion intermediate, 25K + 50K). After tryptic digestion, DACM fluorescence was exclusively found in the 20K fragment as shown in Figure 1B, consistent with previous observation (Sutoh, 1981). The fluorescent 20K fragment was isolated and purified electrophoretically and then treated with hydroxylamine as described under Materials and Methods. Hyroxylamine cleaved the 20K fragment at the Asn–Gly (Asn-57–Gly-58) bond (Bornstein & Balian, 1977; Sutoh, 1981) present between Cys-56 (SH_2) and Cys-66 (SH_1) along the 20K peptide (Elzinga & Collins, 1977; Gallagher & Elzinga, 1980) as schematically shown in Figure 2 (throughout this paper, residues are numbered according to their positions in the 20K fragment, not to those in the whole heavy chain), to yield the 13K peptide containing SH_1 and the 7K peptide containing SH_2 (Sutoh, 1981). As shown in Figure 3 (lane A), the resulting 13K peptide was much more fluorescent than the 7K peptide (fluorescent intensity of the 7K peptide was about one-tenth of that of the 13K peptide), indicating that

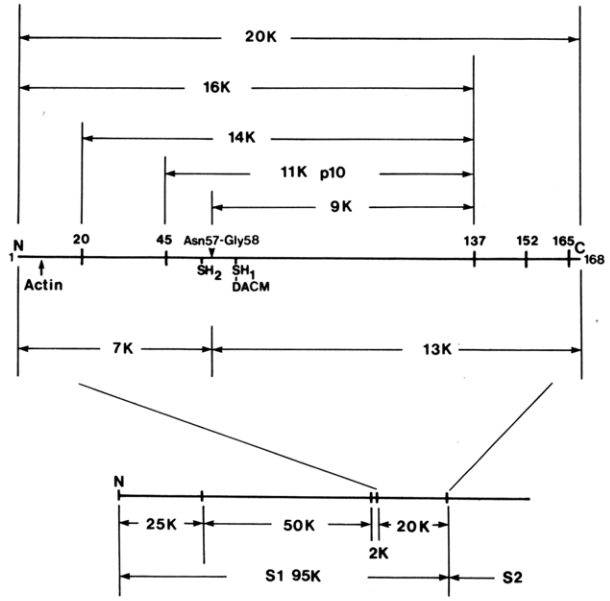

FIGURE 2: Locations of various peptide fragments on myosin heavy chain. The 95K S1 heavy chain is cleaved into fragments (25K, 50K, 2K, and 20K) by trypsin. Among these fragments, amino acid sequence of the 20K fragment has been determined (Gallagher & Elzinga, 1980). Positions of methionine residues (residues 20, 45, 137, 152, and 165) on the 20K fragment are indicated [at position of 152, both methionine and isoleucine have been found (Gallagher & Elzinga, 1980)]. Locations of two reactive cysteines [Cys-56 (SH_2) and Cys-66 (SH_1)] and the Asn–Gly bond (Asn-57–Gly-58) are also indicated. Notice that actin binds at the N-terminal segment of the 20K fragment (residues 1–20).

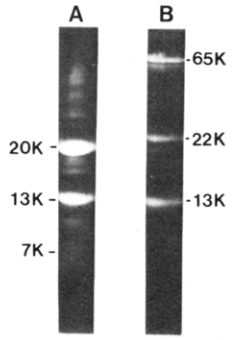

FIGURE 3: Hydroxylamine cleavage of fluorescent 20K and 65K peptides: (A) cleavage product of control non-cross-linked 20K peptide; (B) cleavage product of 65K cross-linked peptide. Conditions for hydroxylamine cleavage: solvent 6 M guanidine hydrochloride–1 M hydroxyalmine (pH 9.0); reaction time 4 h; temperature 45 °C. Electrophoresis was carried out on a slab $NaDodSO_4$ gel containing 12% acrylamide and 0.36% bis(acrylamide). After electrophoresis, DACM fluorescence was detected by illuminating the gel with a UV lamp.

SH_1 thiols covalently incorporated a major fraction of DACM while SH_2 thiols were only slightly labeled with the dye under the present experimental conditions.

Cross-Linking of Actin and DACM-Labeled S1. The rigor complex of F-actin and the DACM-labeled and trypsin-treated S1 (see Materials and Methods) was cross-linked with the zero-length cross-linker EDC. As shown in Figure 1A, the cross-linking reaction generated various types of cross-linked products. Among them, the fluorescent 65K product (Figure 1B) has been already shown to be a cross-linked product of actin and the 20K fragment of S1 heavy chain (Mornet et al., 1981a,b; Sutoh, 1982). The fluorescent 65K product and the non-cross-linked 20K fragment were isolated and purified electrophoretically and then subjected to chemical cleavage reactions.

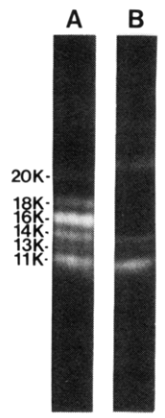

FIGURE 4: Partial cyanogen bromide cleavage of fluorescent 20K and 65K peptides: (A) cleavage product of control non-cross-linked 20K peptide; (B) cleavage product of 65K cross-linked peptide. Conditions for cyanogen bromide cleavage: solvent 0.2 M cyanogen bromide in 70% formic acid; reaction time 18 h; temperature, 25 °C. Electrophoresis was carried out on a slab $NaDodSO_4$ gel containing 15% acrylamide and 0.45% bis(acrylamide). After electrophoresis, DACM fluorescence was detected by illuminating the gel with a UV lamp. Notice that the 16K and 18K fluorescent peptides were missing in lane B.

Hydroxylamine Cleavage. When the fluorescent 65K product purified to homogeneity was subjected to hydroxylamine cleavage, a fluorescent peptide with apparent molecular weight of 13 000 was generated as shown in Figure 3 (lane B). Since hydroxylamine cleaves the actin peptide into two fragments (41K and 2 K) (Sutoh, 1982) and the 20K fragment into two fragments (13K and 7K) (lane A in Figure 3), the 13K fluorescent peptide originating from the cross-linked product of actin and the 20K fragment is unequivocally assigned as the free 13K peptide spanning residues 58–168 of the 20K fragment (Figure 2). The relative amount of fluorescence detected in the 13K band in lane A or B in Figure 3 was very similar to each other, indicating that the 65K product released the 13K peptide of residues 58–168 once the Asn–Gly bond in the 20K fragment was cleaved. Thus it is concluded that the cross-linking site of actin on the 20K fragment is located in its N-terminal segment of residues 1–57 (Figure 2).

The conclusion is consistent with the observation that the fluorescent 7K peptide detected in a cleavage mixture of the non-cross-linked 20K fragment (lane A in Figure 3) is completely missing in that of the 65K product (lane B in Figure 3), though quantitative argument about the 7K peptide is not possible because of its weak fluorescence intensity and its diffuse shape.

Besides the 13K fluorescent peptide, hydroxylamine cleavage of the fluorescent 65K product yielded a fluorescent peptide with an apparent molecular weight of 22 000 as shown in lane B in Figure 3. The 22K peptide is most likely to be a covalently linked complex of the intact 20K fragment and the 2K peptide originating from the N-terminal region of actin (Sutoh, 1982).

Cyanogen Bromide Cleavage. The fluorescent 20K fragment purified to homogeneity was subjected to partial cyanogen bromide cleavage (see Materials and Methods). When the resulting peptides were electrophoresed on a $NaDodSO_4$ gel, at least five discrete fluorescent bands were detected below the intact 20K band as shown in lane A in Figure 4. Their apparent molecular weights were estimated to be 18 000, 16 000, 14 000, 13 000, and 11 000. The smallest 11K peptide is the "p10" peptide (Elzinga & Collins, 1977), which spans residues 46–137 (Figure 2) (Gallagher & Elzinga, 1980).

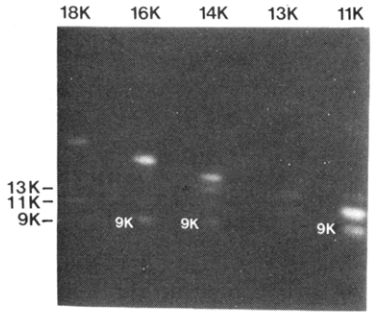

FIGURE 5: Double cleavages of 20K fragment with cyanogen bromide and hydroxylamine. Fluorescent cyanogen bromide peptides of the 20K fragment (18K, 16K, 14K, 13K, and 11K) were digested by hydroxylamine. Conditions for cleavage reactions were the same as in Figures 3 and 4. Resulting peptides were electrophoresed on a slab $NaDodSO_4$ gel containing 15% acrylamide, 0.45% bis(acrylamide), and 6 M urea. After electrophoresis, DACM fluorescence was detected by illuminating the gel with a UV lamp. The peptide containing residues 46–137 was denoted as 9K.

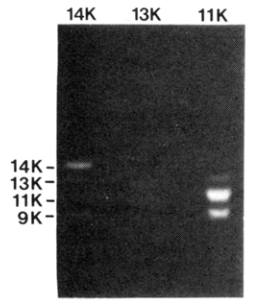

FIGURE 6: Double cleavages of 65K fluorescent cross-linked product with cyanogen bromide and hydroxylamine. Fluorescent cyanogen bromide peptides of the 65K product (14K, 13K, and 11K) were digested by hydroxylamine. Conditions for cleavage reactions were the same as in Figures 3 and 4. Resulting peptides were electrophoresed as in Figure 5. After electrophoresis, DACM fluorescence was detected by illuminating the gel with a UV lamp. Notice that the 9K fluorescent peptide was released from the 14K and 11K cyanogen bromide peptides on the hydroxylamine treatment. The 13K cyanogen bromide peptide was contaminated by the 14K and 11K peptides.

Some of these fluorescent peptides were assigned in the known amino acid sequence of the 20K fragment (Gallagher & Elzinga, 1980), by employment of double cleavages of the fragment with cyanogen bromide and with hydroxylamine. Cyanogen bromide peptides were electrophoresed on a $NaDodSO_4$ gel, and the resulting gel was soaked in hydroxylamine (see Materials and Methods) to cleave the Asn–Gly bond in situ. After the hydroxylamine treatment, these fluorescent cyanogen bromide peptides (18K, 16K, 14K, 13K, and 11K) were electophoresed on another $NaDodSO_4$ gel. As shown in Figure 5, the hydroxylamine cleavage of cyanogen bromide peptides yielded three types of fluorescent fragments with apparent molecular weights of 13 000, 11 000, and 9 000. Among these fragments, the 9K peptide (marked as 9K in the figure) is unequivocally assigned as a peptide fragment spanning residues 58–137, since the peptide is generated by the hydroxylamine cleavage of the 11K (p10) peptide (residues 46–137) at the Asn-57–Gly-58 bond as shown in Figure 5 (lane 11K).

The 14K and 16K cyanogen bromide peptides also released the 9K hydroxylamine fragment. Besides the 9K peptide, and 14K cyanogen bromide peptide generated the 13K and 11K hydroxylamine fragments as well (lane 14K in Figure 5). Thus, the 14K cyanogen bromide peptide seems to be a mixture of peptides with similar molecular weights. Although the 16K cyanogen bromide peptide seems to be a mixture of peptides as well, one type of peptide generating the 9K hydroxylamine fragment is a major species in the mixture (lane 16K in Figure 5). Since cyanogen bromide peptide releasing the 9K hydroxylamine fragment necessarily contain the fragment (residues 58–137) as their C-terminal segment, the 16K and 14K cyanogen bromide peptides generating the 9K hydroxylamine fragment are assigned as residues 1–137 and residues 21–137, respectively (Figure 2).

Cyanogen bromide cleavage of the 65K cross-linked product generated the 14K, 13K, and 11K fluorescent peptides while the 18K and 16K peptides were virtually missing in cleavage products as shown in Figure 4 (lane B). The 11K cyanogen bromide peptide originating from the 65K product released the 9K fluorescent fragment on the hydroxylamine treatment, indicating that the 11K peptide was residues 46–137 of the 20K fragment of S1 heavy chain (lane 11K in Figure 6). The 14K cyanogen bromide peptide originating from the 65K product released the 9K fragment as well as the 13K and 11K fragments of the hydroxylamine treatment (lane 14K in Figure 6), indicating that the 14K peptide contained a peptide spanning residues 21–137 of the 20K fragment of S1 heavy chain. These results show that cyanogen bromide cleavage of the 65K cross-linked product yields a peptide fragment spanning residues 21–137 (M_r 14K) and one spanning residues 46–137 (M_r 11K). It is, therefore, most likely that actin is cross-linked with a segment spanning residues 1–137 (M_r 16K) but not with a segment spanning residues 21–137 (M_r 14K). Thus, it is concluded that the cross-linking site of actin on the 20K fragment of S1 heavy chain is located in its N-terminal 20 residues (Figure 2).

Discussion

When a rigor complex of F-actin and trypsin-treated S1 was cross-linked with the zero-length cross-linker EDC, the 20K and 50K fragments of S1 heavy chain as well as alkaline light chain 1 were cross-linked with actin, indicating that these heavy-chain fragments and alkaline light chain 1 contain actin-binding sites in their sequences (Sutoh, 1982). In this paper, one of these actin-binding sites has been located along the amino acid sequence of the 20K fragment of S1 heavy chain, by following similar methods used for locating myosin-binding sites on the actin sequence (Sutoh, 1982).

Cross-linking of F-actin and the DACM-labeled and trypsin-treated S1 generated the 65K fluorescent cross-linked product, which was a covalently linked complex of actin and the DACM-labeled 20K fragment of S1 heavy chain. The 65K product released the 13K peptide spanning residues 58–168 of the 20K fragment once the Asn–Gly bond was cleaved by hydroxylamine (Bornstein & Balian, 1977; Sutoh, 1981), indicating that a cross-linking site of actin on the 20K fragment was located in its N-terminal 57 residues. Furthermore, on the basis experiments of double cleavages of the 65K cross-linked product with cyanogen bromide and hydroxylamine, it has been shown that the N-terminal 20 residues of the 20K fragment of S1 heavy chain contain a cross-linking site of actin.

Mornet et al. (1981b) showed that the cross-linking reaction between actin and S1 induced by EDC involved carboxyl groups of actin and amino groups of S1. It has been shown, in fact, that acidic residues in cluster on the actin sequence are cross-linking sites of myosin heavy and light chains (Sutoh, 1982). Close examination of the amino acid sequence of the 20K fragment of S1 heavy chain (Gallagher & Elzinga, 1980) has revealed that its N-terminal segment containing residues 1–20 is rather basic. It contains three lysine residues and one

arginine residue while it only contains one acidic residue (glutamic acid). Since EDC cross-links carboxyl groups with amino groups, the cross-linking site(s) of actin on the segment may be some of these lysine side chains. Since carboxyl and amino groups are in direct contact at the site, it seems likely that the N-terminal segment of actin (the myosin-binding site) binds to the N-terminal segment of the 20K fragment of S1 heavy chain (the actin-binding site) through ionic interactions.

It was previously shown that a junction between the 20K and 50K segments of S1 heavy chain was readily cleaved by trypsin in the absence of actin, while it was protected against the tryptic attack when S1 was complexed with actin to form a rigor complex (Yamamoto & Sekine, 1979; Mornet et al., 1979, 1981a,b). The protecting effect of actin would result from a steric hindrance by actin, which binds close to the junction. The finding that the tryptic digestion of the junction between the 20K segment and the 50K segment results in a complete loss of actin-activated ATPase activity of S1 without affecting other ATPase activities (Yamamoto & Sekine, 1979; Mornet et al., 1979, 1981a) is also consistent with the notion that actin binds to the N-terminal region of the 20K segment of S1 heavy chain. The tryptic cleavage of S1 heavy chain at site(s) close to the actin-binding site might drastically change the way S1 interacts with actin during ATP hydrolysis by the actin–S1 complex. In this regard, it should be noted that Mornet et al. (1981a) have recently shown the presence of a peptide segment (M_r 2000) connecting the 20K and 50K segments of S1 heavy chain.

The actin-binding site located for a rigor complex of actin and S1 as above might be relevant to other actin–S1 intermediates postulated in cyclic hydrolysis of ATP by the actin–S1 complex [for example, see Lymn & Taylor (1971)], considering the recent observation that a complex of actin and S1 cross-linked with each other with EDC in its rigor state exhibits highly accelerated ATPase activity (Mornet et al., 1981b). Further work would be required, however, to determine whether the same binding sites are maintained on actin and S1 molecules during a cycle of ATP hydrolysis.

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