2-methyl-2,4-pentanediol (Pittz & Timasheff, 1978), where the water interaction parameter is as high as 1 g/g. Here the reason for the large water layer seems to be "salting out" of the probe by the charges on the surface of the molecule. For ethanol it is even possible that there might be some preferential binding. In the present analysis we have only measured samples up to 20% ethanol content, and in this case it is quite possible that some of the surface binds ethanol, while part of the rest is inaccessible. Further measurements with higher ethanol concentrations are therefore being planned. It is clear, though, that a molecular picture of these interactions cannot be obtained by any of the methods discussed in this paper and that only a three-dimensional analysis based on single crystal data might eventually give us this information.

Acknowledgments

We are indebted to Drs. B. Jacrot, P. Timmins, and R. Leberman for many useful discussions.

**Registry No.** Ribonuclease A, 9001-99-4; neutron, 12586-31-1.

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# Actin–Actin and Actin–Deoxyribonuclease I Contact Sites in the Actin Sequence[†]

Kazuo Sutoh

ABSTRACT: Actin subunits in F-actin were cross-linked with *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester (MBS). Peptide maps of the cross-linked actin dimer have revealed that the attachment sites of the MBS cross-link in actin are Cys-373 and a lysine residue in the CB-17 segment (Lys-191, Lys-213, or Lys-215). Since MBS spans ~8 Å, the result indicates that Cys-373 in an actin subunit is within the distance of ~8 Å from the lysine residue in the neighboring actin subunit. Therefore, it seems that Cys-373 and the lysine residue in the CB-17 segment are close to the regions of the actin–actin contact sites. The actin–DNase I complex was cross-linked with 1,5-difluoro-2,4-dinitrobenzene (FFD). Peptide maps of the actin–DNase I cross-linked complex have shown that the attachment site of the FFD cross-link in actin is in its CB-10 segment. The CB-10 segment of actin contains Lys-50, Lys-61, Lys-68, Tyr-53, and Tyr-69 as candidates for the attachment site. FFD can span only 3 Å, and therefore it is most likely that one of these residues is in the region of the binding site of DNase I in actin.

It has been well established that actin is present both in muscle and in nonmuscle cells. In muscles, actin molecules self-associate to form a stable filamentous structure (F-actin), in which actin subunits are arranged on two helical strands. For muscle contraction, many proteins must interact with F-actin. For example, ATP-dependent interaction of myosin with F-actin is the basis of muscle contraction and calcium-sensitive interactions of regulatory proteins such as troponin and tropomyosin with actin are essential for the contraction–relaxation cycle of muscles.

Unlike in muscles, F-actin is not a stable structure in nonmuscle cells. Many types of proteins interact with actin to control its higher order structure. Actin-depolymerizing factors such as DNase I (Lazarides & Lindberg, 1974; Hitchock et al., 1976) and profilin (Carlsson et al., 1976) are known to stabilize the monomeric form of actin by forming a 1:1 complex with actin monomer. Many other actin-binding proteins that regulate the higher order structure of actin have been isolated from various tissues and cells.

All the proteins capable of binding to actin would be expected to have specific binding sites in actin. Since primary sequences of actins from various sources (from *Physarum* plasmodia to rabbit skeletal muscle) are highly conserved (Elzinga et al., 1973; Collins & Elzinga, 1975; Vandekerckhove & Weber, 1978a–d), it seems likely that the surface of any actin is covered with a large number of areas responsible for binding these actin-binding proteins.

The studies in this and previous papers (Sutoh, 1982) were undertaken to identify these binding sites in the actin sequence. By employment of C-terminal labeling of actin, cross-linking of the labeled actin with myosin, and finally peptide maps of

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the cross-linked product, binding sites of myosin in the actin sequence have been identified (Sutoh, 1982). The experiments have shown that the N-terminal acidic segment of actin, spanning residues 1–12, contains a binding site for the myosin heavy chain while its C-terminal segment, spanning residues 355–374, has a binding site for the alkaline light chain 1.

The analytical method employed to identify the binding sites of myosin in the actin sequence was again used here to map the contact sites of actin subunits in F-actin and also the binding site of DNase I in actin.

## Materials and Methods

*Proteins and Chemicals.* Actin was prepared according to the method of Spudich & Watt (1971). Electrophoretically purified DNase I was purchased from Sigma Chemical Co. and used without further purification. MBS[1] (*m*-maleimidobenzoyl *N*-hydroxysuccinimide ester) and FFD (1,5-difluoro-2,4-dinitrobenzene) were purchased from Pierce Chemical Co. DACM [*N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide] was purchased from Wako Chemical Co.

*Cross-Linking of F-Actin with MBS.* G-Actin (2 mg/mL) in 2 mM imidazole, 0.1 mM ATP, and 0.1 mM $CaCl_2$ (pH 7.0) was modified with DACM in a molar ratio of 1.0:0.5 (actin monomer:DACM) for 3 min at 0 °C. The labeling reaction was quenched by addition of 0.01 volume of 100 mM *N*-acetylcysteine. Under the reaction conditions, 40–45% of total Cys-373 thiols were selectively labeled with the fluorescent dye (Sutoh, 1982). G-Actin partially labeled with DACM as above was then polymerized to form F-actin by addition of an equal volume of 0.2 M NaCl, 40 mM imidazole, and 4 mM $MgCl_2$ (pH 7.0). The resulting F-actin was dialyzed against 0.1 M NaCl, 20 mM triethanolamine hydrochloride and 2 mM $MgCl_2$ (pH 8.0).

To the DACM-labeled F-actin (1 mg/mL) was added MBS in a molar ratio of 1.0:0.6 or 1.0:1.2 (actin monomer:MBS). The cross-linking reaction was allowed to proceed for 3 h at 25 °C and then quenched by addition of excess 2-mercaptoethanol.

In some cases, F-actin without prior modification by DACM was cross-linked with MBS under the same conditions as above, with a 1:1 actin monomer:MBS molar ratio.

*Cross-Linking of the Actin–DNase I Complex with FFD.* G-Actin was modified with DACM in a molar ratio of 1:1 (actin monomer:DACM) under the same reaction conditions as above. The DACM-labeled G-actin (2 mg/mL) in 2 mM imidazole, 0.1 mM ATP, and 0.1 mM $CaCl_2$ (pH 7.0) was mixed with DNase I (2 mg/mL) in 5 mM imidazole (pH 7.0) containing 10 μg/mL leupeptin in a molar ratio of 1.0:1.1 (actin monomer:DNase I). Without the protease inhibitor (leupeptin), DNase I was readily cleaved into smaller fragments by endogenous proteases. After the mixture was incubated for 1 h at 0 °C, a portion was analyzed by HPLC using a column TSK G3000SW. Formation of the 1:1 complex of actin and DNase I was confirmed by the elution profile [elution solvent: 0.1 M NaCl and 20 mM imidazole (pH 7.0)]. Then, 0.1 volume of 1 M triethanolamine hydrochloride (pH 8.5) was added to the mixture, and the cross-linking reaction was initiated by addition of 0.1 volume of 1 mg/mL FFD (1,5-difluoro-2,4-dinitrobenzene) in DMF. The cross-linking reaction was allowed to proceed for 2 h at 25 °C and then quenched by addition of excess 2-mercaptoethanol.

*Purification and Chemical Cleavages of Fluorescent Peptides.* Cross-linked products were electrophoresed on acrylamide gels [10% acrylamide–0.3% bis(acrylamide)] in the presence of $NaDodSO_4$ (Laemmli, 1970). After electrophoresis, fluorescent cross-linked peptides as well as the fluorescent actin monomer were cut out from these gels under illumination by a UV lamp. These gels were then washed with 50% methanol several times and dried in vacuo.

These dried gels were soaked in 20 mM CNBr–70% formic acid. The cleavage reaction by CNBr was allowed to proceed for 2 h at 37 °C and then quenched by washing these gels with 50% methanol–5% 2-mercaptoethanol. The gels were finally washed with 50% methanol and dried in vacuo.

For BNPS-skatole cleavages, dried gels were treated as previously described (Sutoh, 1983).

*Cleavage of the DACM-Labeled Actin with NTCB.* The DACM-labeled actin (2 mg/mL) was dialyzed against 6 M urea and 0.1 M Tris-HCl (pH 8.0). It was mixed with 0.05 volume of 0.2 M NTCB [2-nitro-5-(thiocyano)benzoic acid] in DMF. After 30-min incubation at 25 °C, 0.1 volume of acetic acid was added to the reaction mixture. The resulting solution was dialyzed against 8 M urea and 0.05% acetic acid. The cleavage reaction was initiated by addition of 0.4 M $NaHCO_3$–$Na_2CO_3$ (pH 9.3) to the dialyzed sample. The cleavage reaction was allowed to proceed for 2 h at 37 °C and then quenched by addition of excess 2-mercaptoethanol.

*Miscellaneous Procedures.* Acrylamide gel electrophoresis was carried out according to the method of Laemmli (1970).

HPLC analysis was carried out on a TSK G3000SW column (Toyo Soda Manufacturing Co.) using 0.1 M NaCl and 20 mM imidazole (pH 7.0) as a solvent. Proteins were monitored by the absorbance at 280 nm. The apparent molecular weight of the actin–DNase I complex was estimated from elution volumes of standard proteins with known molecular weights.

Protein concentrations were determined by the method of Lowry et al. (1951).

## Results

*Identification of the Cysteine Residue Involved in the Actin–Actin Cross-Linking.* When F-actin was treated with a cross-linking reagent MBS in a molar ratio of 1:1 (actin monomer:MBS), covalently linked oligomers were generated as revealed by the $NaDodSO_4$–acrylamide gel electrophoresis. On the gel, monomer, dimer, and trimer bands split into doublets, though intensities of the major bands were much higher than those of the minor ones. The pattern of these cross-linked species on the gel was very similar to that observed previously for the cross-linking of F-actin with *p*-phenylenebis(maleimide) (*p*-PDM) (Mockrin & Korn, 1981).

The cross-linker MBS has a maleimide group on one end and an active ester on the other as functional groups. Since the maleimide group reacts with a cysteine residue to form a covalent bond, it is most likely that a cysteine residue in actin is involved in the cross-link between actin subunits. The most likely candidate is Cys-373, since this thiol is selectively labeled with various maleimide reagents such as *N*-ethylmaleimide (Collins & Elzinga, 1975) or DACM (Sutoh, 1982). In fact, when all Cys-373 thiols were labeled with DACM prior to the cross-linking reaction with MBS, no cross-linked species was detected on the $NaDodSO_4$–acrylamide gel (lane D in Figure 1), indicating that Cys-373 actually is the attachment site of

[1] Abbreviations: MBS, *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester; FFD, 1,5-difluoro-2,4-dinitrobenzene; DACM, *N*-[7-(dimethylamino)-4-methyl-5-coumarinyl]maleimide; NTCB, 2-nitro-5-(thiocyano)benzoic acid; CNBr, cyanogen bromide; BNPS-skatole, 2-[(2-nitrophenyl)sulfenyl]-3-methyl-3-bromoindolenine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; $NaDodSO_4$, sodium dodecyl sulfate; DMF, *N,N*-dimethylformamide; HPLC, high-performance liquid chromatography; *p*-PDM, *p*-phenylenebis(maleimide).

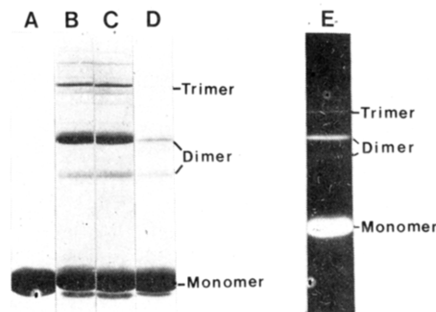


FIGURE 1: Cross-linking of the DACM-labeled actin subunits with MBS. (Lanes A–C) Actin was partially labeled with DACM: (lane A) before cross-linking with MBS; (lane B) after cross-linking with MBS (actin monomer:MBS = 1.0:0.6); (lane C) after cross-linking with MBS (actin monomer:MBS = 1.0:1.2). (Lane D) Actin was fully labeled with DACM prior to the MBS treatment (actin monomer:MBS = 1:1). Lanes A–D show gels stained with Coomassie Blue. (Lane E) The gel shown in lane B was illuminated with a UV lamp before staining it with Coomassie Blue to detect fluorescence of DACM covalently linked to Cys-373. Cross-linking conditions were as follows: F-actin, 1 mg/mL; solvent, 0.1 M NaCl, 20 mM triethanolamine hydrochloride, and 2 mM $MgCl_2$ (pH 8.0); reaction time, 3 h; temperature, 25 °C.

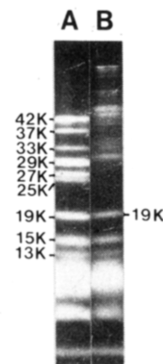


FIGURE 2: CNBr cleavages of the fluorescent actin monomer and dimer: (lane A) fluorescent fragments generated from the actin monomer; (lane B) fluorescent fragments generated from the actin dimer. Products cleaved from the fluorescent monomer or dimer with CNBr were electrophoresed on $NaDodSO_4$–acrylamide gels containing 15% acrylamide–0.45% bis(acrylamide). Fluorescent fragments were visualized by illuminating gels with a UV lamp.

the MBS cross-link that bridges two actin subunits.

*Identification of the Lysine Residue Involved in the Actin–Actin Cross-Linking.* The active ester of MBS is expected to react with a lysine residue. The lysine residue involved in the actin–actin cross-linking was identified by means of peptide maps as previously described (Sutoh, 1982).

G-Actin was reacted with DACM in a molar ratio of 1.0:0.5 (actin monomer:DACM). Under the condition, 40–45% of total Cys-373 thiols were labeled with the fluorescent dye. G-Actin was only partially labeled here, because full labeling of Cys-373 resulted in total supression of the cross-linking reaction as shown above. The partially labeled G-actin was polymerized, and then the resulting F-actin was treated with MBS in a molar ratio of 1.0:0.6 (actin monomer:MBS). The cross-linking reaction generated covalently linked dimer, trimer, and a small amount of higher oligomers (lane B in Figure 1). Higher concentration of MBS [molar ratio 1:0:1.2 (actin monomer:MBS)] resulted in the same pattern of cross-linked species (lane C in Figure 1). The major actin dimer, which was fluorescent (lane E in Figure 1), was isolated electrophoretically and then subjected to the CNBr fragmentation to identify the attachment site of the MBS cross-link.

The fluorescent actin dimer contains two types of actin polypeptides. One type of actin polypeptide (DACM chain) has the DACM label in its Cys-373 while Cys-373 of the other polypeptide (MBS chain) is occupied with the maleimide group of the MBS cross-link. A lysine residue in the DACM chain is bridged to Cys-373 in the MBS chain through the MBS cross-link. Since fluorescent CNBr fragments generated from the fluorescent actin dimer necessarily contain the C terminus of the DACM chain, location of the lysine residue involved in the cross-linking can be determined by examining these fluorescent fragments as previously described (Sutoh, 1982).

The CNBr fragmentation of the major fluorescent dimer generated several discrete fluorescent fragments as shown in Figure 2 (lane B). Comparison of these fluorescent bands to those generated by the CNBr fragmentation of the fluorescent actin monomer (lane A in Figure 2) revealed that fluorescent fragments of the monomer and those of the dimer were in alignment up to the 19K fragment (apparent molecular weights of these fluorescent fragments were estimated from their mobilities and are shown in the figure). The 25K fragment and fragments with higher molecular weights observed for the control monomer were completely missing in fragmentation products of the dimer. This result indicates that the attachment site of the MBS cross-link is outside the C-terminal 19K segment but inside the C-terminal 25K segment.

On the basis of their apparent molecular weights, the 19K and 25K fragments were tentatively identified as fragments spanning residues 191–374 and residues 177–374, respectively (Sutoh, 1982). However, agreement of their apparent molecular weights with those calculated from the actin sequence is not good enough to identify these fragments unequivocally. Since estimation of the apparent molecular weight of a peptide by the $NaDodSO_4$–acrylamide gel electrophoresis appreciably depends on the choice of standard proteins, it is necessary to employ another approach to assign these fluorescent fragments in the actin sequence.

Cleavage of the DACM-labeled actin with 2-nitro-5-(thiocyano)benzoic acid (NTCB) was empoyed for the purpose. NTCB selectively reacts with cysteine residues and cleaves peptide chains (Jacobson et al., 1973; Degani & Patchoronik, 1974). When the DACM-labeled actin was treated with NTCB to cleave the actin polypeptide at cysteine residues (Cys-10, Cys-217, Cys-256, and Cys-284), three fluorescent fragments were generated (lane B in Figure 3). From their apparent molecular weights, these fluorescent fragments were identified as fragments spanning residues 217–374, residues 256–374, and residues 284–374, respectively. The product of cleavage at Cys-10 would comigrate with intact actin under the present electrophoresis conditions.

By aligning the fluorescent CNBr fragments and the fluorescent NTCB fragments as shown in Figure 3 (lanes A and B), it is evident that the methionine residue generating the 19K fragment resides between Cys-217 and Cys-256. The methionine residue is therefore identified as Met-227. Thus, the 19K fragment spans residues 228–374. The molecular weight of the fragment is calculated to be 16400 from the actin sequence (Elzinga et al., 1973). On the basis of the same reasoning, the 25K fragment is identified as a fragment spanning residues 191–374.

The result that the lysine residue involved in the cross-linking is located inside the C-terminal 25K segment but outside the C-terminal 19K segment thus means that the lysine residue resides in a segment spanning residues 191–227, i.e., the CB-17 segment (Elzinga et al., 1973). The CB-17 segment of actin

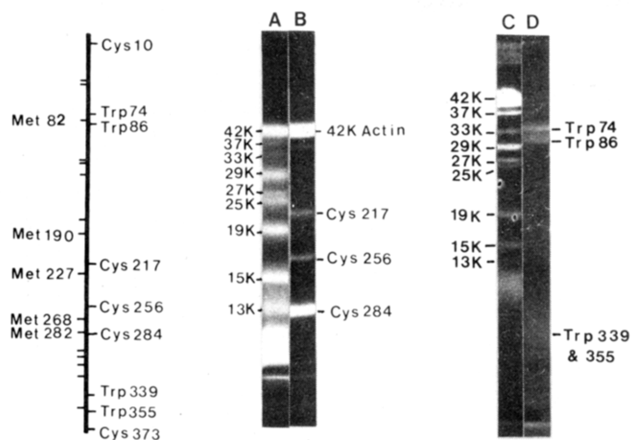


FIGURE 3: Assignment of fluorescent CNBr fragments in the actin sequence: (lane A) fluorescent fragments generated by the CNBr cleavage; (lane B) fluorescent fragments generated by the NTCB cleavage; (lane C) fluorescent fragments generated by the CNBr cleavage; (lane D) fluorescent fragments generated by the BNPS-skatole cleavage. Electrophoresis was carried out on $NaDodSO_4$–acrylamide gels containing 12.5% acrylamide–0.4% bis(acrylamide)–6 M urea (lanes A and B) or 15% acrylamide–0.4% bis(acrylamide) (lanes C and D). Fluorescent fragments were visualized by illuminating gels with a UV lamp. The solid vertical bar represents the actin polypeptide chain, on which locations of methionine (Met), cysteine (Cys), and tryptophan (Trp) residues are indicated.

contains three lysine residues (Lys-191, Lys-213, and Lys-215) (Elzinga et al., 1973), which are candidates for the attachment site of the MBS cross-link. Thus, it is concluded that in F-actin the distance between Cys-373 of one actin subunit and the lysine residue of the neighboring actin subunit is ~8 Å, which is the length between two functional groups of MBS.

*Cross-Linking of the Actin–DNase I Complex with FFD.* G-Actin was labeled with DACM in a molar ratio of 1:1 (actin monomer:DACM). The DACM-labeled actin in 2 mM imidazole, 0.1 mM ATP, and 0.1 mM $CaCl_2$ (pH 7.0) was mixed with DNase I in 5 mM imidazole (pH 7.0) in a molar ratio of 1.0:1.1 (actin monomer:DNase I). When the mixture was examined by HPLC (TSK G3000SW column), ~90% of total proteins were eluted as a peak with apparent molecular weight of 77 000, indicating that actin and DNase I formed a 1:1 complex as previously reported (Hitchcock et al., 1976).

The actin–DNase I complex was cross-linked with 1,5-difluoro-2,4-dinitrobenzene (FFD), and the cross-linked products were analyzed on a $NaDodSO_4$–acrylamide gel. As shown in Figure 4, the cross-linking reaction generated a major product with an apparent molecular weight of 72 000 together with a minor product with an apparent molecular weight of 75 000. When the fluorescence of DACM covalently linked to Cys-373 of actin was examined on the gel, it was observed that these cross-linked products were fluorescent, though the fluorescent intensity of the minor product was very weak (lane 2B in Figure 4).

*Identification of the Cross-Linking Site of DNase I in the Actin Sequence.* The major cross-linked complex of the DACM-labeled actin and DNase I was electrophoretically purified and cleaved with CNBr. The CNBr fragmentation generated several discrete fluorescent fragments as shown in Figure 5 (lane B). When these fluorescent bands were compared to those generated by the CNBr cleavage of the fluorescent actin monomer (lane A in Figure 5), it was found that these fluorescent bands were in alignment with each other up to the 33K fluorescent fragment. The 37K fluorescent fragment detected in the cleavage products of the actin monomer was completely missing in those of the actin–DNase I complex. Since the fluorescent label was covalently attached

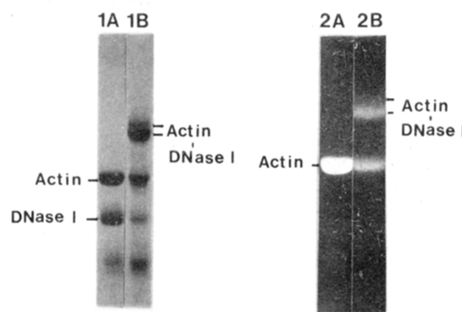


FIGURE 4: Cross-linking of actin and DNase I with FFD: (lanes 1A and 2A) the mixture of actin and DNase I before the cross-linking reaction with FFD; (lanes 1B and 2B) after the cross-linking reaction. Polypeptides were detected either by illuminating gels with a UV lamp (lanes 2A and 2B) or by staining them with Coomassie Blue (lanes 1A and 1B). Minor bands detected at low molecular weight region on these gels seem to correspond to fragments of DNase I generated by endogenous proteases, since intensities of these bands significantly increased when protease inhibitor was not included in the mixture during the cross-linking reaction.

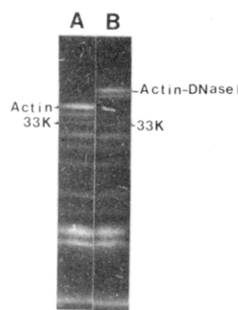


FIGURE 5: CNBr cleavages of the fluorescent actin monomer and actin–DNase I complex: (lane A) fluorescent fragments generated from the actin monomer; (lane B) fluorescent fragments generated from the actin–DNase I complex. Electrophoresis was carried out on $NaDodSO_4$–acrylamide gels containing 12.5% acrylamide–0.4% bis(acrylamide)–6 M urea. The fluorescence of DACM covalently linked to Cys-373 of actin was detected by illuminating gels with a UV lamp.

to Cys-373 of actin and served as its C-terminal label, the result indicates that the cross-linking site of DNase I in actin resides outside its C-terminal 33K segment but inside its C-terminal 37K segment.

The 33K fluorescent fragment was previously identified as a fragment spanning residues 83–374 on the basis of its apparent molecular weight (Sutoh, 1982). This assignment was supported by cleavage of the DACM-labeled actin with BNPS-skatole, which selectively cleaves a polypeptide chain at tryptophan residues (Fontana, 1972). As shown in Figure 3 (lane D), the BNPS-skatole treatment of the DACM-labeled actin generated doublet fluorescent bands with apparent molecular weights close to 35 000 and a diffuse low molecular weight band. Since actin contains four tryptophan residues (Trp-74, Trp-86, Trp-339, and Trp-355), it is likely that the doublet fluorescent bands were generated by cleavage of the DACM-labeled actin at Trp-74 and Trp-86. A diffuse fluorescent band would be generated by cleavages of actin at Trp-339 and Trp-355. By alignment of fluorescent fragments of the DACM-labeled actin generated with BNPS-skatole and those with CNBr (lanes C and D in Figure 3), it is evident that the methionine residue generating the 33K fragment resides between Trp-74 and Trp-86. Thus, the methionine is unequivocally identified as Met-82. This result confirms the previous assignment of the 33K fragment as a fragment spanning residues 83–374 (Sutoh, 1982). The 37K fragment is a fragment spanning residues 45–374 or residues 48–374

(Sutoh, 1982). The fact that the cross-linking site of DNase I in actin is located inside the 37K segment but outside the 33K segment indicates that the site is in the segment spanning residues 48–82, i.e., the CB-10 segment (Elzinga et al., 1973).

FFD reacts with lysine or tyrosine residue to form a covalent bond. The CB-10 segment of actin contains three lysine and two tyrosine residues (Elzinga et al., 1973). They are Lys-50, Lys-61, Lys-68, Tyr-53, and Tyr-69. One of them would be the attachment site of the FFD cross-link that bridges DNase I to actin.

## Discussion

It has been previously shown that actin subunits in F-actin are cross-linked with *p*-phenylenebis(maleimide) (*p*-PDM) to form covalently linked dimer and higher oligomers (Knight & Offer, 1978; Mockrin & Korn, 1981). The cross-linking reagent used in the present study, i.e., *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester (MBS), which spans a shorter distance than *p*-PDM, also cross-linked actin subunits very effectively. On an acrylamide gel, the cross-linked dimer and trimer showed doublet bands as previously reported for the cross-linking of F-actin with *p*-PDM (Mockrin & Korn, 1981), though intensities of the minor bands were much weaker than those of the major bands. These minor bands may represent cross-linked species that contain both intersubunit and intrasubunit cross-links (Mockrin & Korn, 1981), since the monomer actin showed doublet bands as well upon the MBS treatment. No characterization has been carried out on these minor species because their yield was very low.

The major actin dimer was used to identify the sites of attachment of the MBS cross-link in the actin sequence. Peptide maps of the fluorescent cross-linked dimer have revealed that a lysine residue in the CB-17 segment is the attachment site of the active ester group of the MBS cross-link. Furthermore, selective labeling of Cys-373 has revealed that this thiol is the attachment site of the maleimide group of the cross-link. Thus, it is concluded that the distance between Cys-373 of an actin subunit in F-actin and a lysine residue in the CB-17 segment (Lys-191, Lys-213, or Lys-215) (Elzinga et al., 1973) of the neighboring subunit is ~8 Å, which is the distance the MBS cross-link can span.

The facts that Cys-373 is readily modified with various thiol reagents in F-actin and that G-actin whose Cys-373 is labeled with a bulky thiol reagent such as pyrenylmaleimide can polymerize with almost identical kinetics as native G-actin (Kouyama & Mihashi, 1981) indicate that this thiol is not in the region of the actin–actin contact. However, the finding that Cys-373 and a lysine residue in the CB-17 segment in the neighboring actin subunits are located rather closely suggests that these residues are close to the regions that face toward neighboring subunits in F-actin.

It seems possible that the MBS cross-link bridges two actin subunits along the same strand in the double helices of F-actin, since Cys-373 is located outside of F-actin as revealed by fluorescence energy transfer experiments (Taylor et al., 1981). Recent three-dimensional reconstitution studies on the actin paracrystal (Smith et al., 1983) have revealed, however, that the prinicipal intersubunit contacts follow the genetic helix and that the contacts along the same strand of the two long-pitch helices are less significant. Therefore, the actin–actin contact sites close to Cys-373 and the lysine residue in the CB-17 segment may not significantly contribute to the helical association of actin subunits into F-actin.

The FFD treatment of the actin–DNase I complex (Lazarides & Lindberg, 1974; Hitchcock et al., 1976) generated two types of cross-linked products of actin and DNase I with different mobilities on a $NaDodSO_4$–arylamide gel. The result might indicate that there are at least two types of contacts between actin and DNase I. Since the yield of the minor cross-linked product was too low, only the major cross-linked product was analyzed by peptide maps. The experiments have shown that a residue in the CB-10 segment of actin (Lys-50, Lys-61, Lys-68, Tyr-53, or Tyr-69) is the attachment site of the FFD cross-link that covalently bridges actin and DNase I. Since the FFD cross-link can span only 3 Å, it is most likely that the residue in the CB-10 segment of actin is actually in the region of the binding site of DNase I.

**Registry No.** DNase, 9003-98-9; MBS, 58626-38-3; FFD, 327-92-4; Cys, 52-90-4; Lys, 56-87-1.

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