Actin–Fragmin Interactions As Revealed by Chemical Cross-Linking

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Received April 16, 1985

ABSTRACT: A one to one complex of actin and fragmin (a capping protein from *Physarum polycephalum* plasmodia) was cross-linked with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. The cross-linking reaction generated two cross-linked products with slightly different molecular weights (88 000 and 90 000) as major species. They were cross-linked products of one actin and one fragmin. The cross-linking site of fragmin in the actin sequence was determined by peptide mappings [Sutoh, K. (1982) *Biochemistry 21*, 3654–3661] after partial chemical cleavages of cross-linked products with hydroxylamine. The results indicated that the N-terminal segment of actin spanning residues 1–12 participated in cross-linking with fragmin. The cross-linker used in this study covalently bridges lysine side chains and side chains of acidic residues when they are in direct contact. Therefore, it seems that acidic residues in the N-terminal segment of actin (Asp-1, Glu-2, Asp-3, Glu-4, and Asp-11), at least some of them, are in the binding site of fragmin. It has already been shown that the same acidic segment of actin is in the binding site of myosin or depactin (an actin-depolymerizing protein isolated from starfish oocytes). We suggest that the unusual amino acid sequence of the N-terminal segment of actin makes its N-terminal region a favorable anchoring site for various types of actin-binding proteins.

Actin is one of the most abundant proteins in eukaryotic cells and plays essential roles in the contractile or cytoskeletal apparatus of these cells. Organization of actin molecules into higher order structures is dynamically regulated by various types of "actin-binding proteins". Surprisingly large numbers of actin-binding proteins have been isolated from various cells and tissues so far. They cross-link F-actin into bundles or networks, depolymerize it, or cap its ends. Although a wide variety of actin-binding proteins are present in all types of eukaryotic cells, actin itself is a very conservative protein in its primary sequence (Elzinga et al., 1973; Elzinga & Lu, 1976; Vandekerckhove & Weber, 1978a,b, 1984; Vandekerckhove et al., 1984). Thus, any actin can bind almost all actin-binding proteins isolated from various sources (there are some exceptions such as the actin–profilin interaction). It is very interesting to ask how the actin molecule can accommodate such a large number of actin-binding proteins. One approach to answer the question is to map binding sites of various actin-binding proteins in the primary sequence of actin.

We have recently developed a new technique to locate these binding sites in the actin sequence (Sutoh, 1982a, 1984; Sutoh & Mabuchi, 1984). So far, binding sites of three proteins were mapped in actin. In the actin–myosin rigor complex, two distinct segments of the myosin heavy chain are in contact with the N-terminal acidic segment of actin which spans residues 1–12 (Sutoh, 1982a,b, 1983). The C-terminal segment of actin which spans residues 356–375 participates in binding myosin light chain (Sutoh, 1982a). These N-terminal and C-terminal segments also participate in binding depactin (Sutoh & Mabuchi, 1984), an actin-depolymerizing protein isolated from starfish oocytes (Mabuchi, 1981, 1982, 1983). Another actin-depolymerizing protein. DNase I (Lazarides & Lindberg, 1974; Hitchcock et al., 1976), uses some residues in the actin segment CB 10 (residues 48–82) (Elzinga et al., 1973) as its binding site (Sutoh, 1984). It is noteworthy that myosin and depactin share the same binding site on the actin molecule, at least partially, in spite of the fact that their effects on actin are just the opposite; myosin accelerates actin polymerization while depactin depolymerizes F-actin.

In this study, we mapped a binding site of fragmin, a barbed end capping protein isolated from *Physarum polycephalum* plasmodia (Hasegawa et al., 1980; Sugino & Hatano, 1982). Surprisingly, the N-terminal acidic segment of actin was again identified as the binding site of fragmin. The results indicate that three proteins with different effects on actin share the same binding site on the actin molecule. It seems that the unusual sequence of the N-terminal segment of actin (very high proportion of acidic residues) makes this segment a favorable point of interactions with various types of actin-binding proteins.

Materials and Methods

Proteins and Reagents. Fragmin was prepared according to the method of Hasegawa et al. (1980) with modifications as follows. Actin–fragmin complex eluted from a hydroxyapatite column was dialyzed against 6 M urea, 10 mM NaCl, 20 mM imidazole, and 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)[1] (pH 7.0). The resulting solution was loaded on a DEAE-cellulose column (1.2 cm × 4 cm) equilibrated with the above solvent. Proteins were eluted with a linear gradient of NaCl from 10 to 60 mM (total 200 mL) and monitored by the absorbance at 280 nm. The first major peak corresponded to fragmin and the second one to actin. Fractions containing fragmin were collected and dialyzed against 10 mM Tris-HCl (pH 8.0). It was then

[1] Abbreviations: DACM, *N*-[7-(dimethylamino)-4-methyl-3-coumarinyl]maleimide; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide; BNPS-skatole, 2-[(2-nitrophenyl)sulfenyl]-3-bromoindolenine; CNBr, cyanogen bromide; $NaDodSO_4$, sodium dodecyl sulfate; DTT, dithiothreitol; Tris, tris(hydroxymethyl)aminomethane; S1, myosin subfragment 1; HPLC, high-performance liquid chromatography; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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concentrated by Ficoll 400 (Pharmacia). Actin was prepared from acetone powder of rabbit skeletal muscle according to the method of Spudich & Watt (1971).

1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) was purchased from Nakarai Chemical Co. (Kyoto, Japan). *N*-[7-(Dimethylamino)-4-methyl-3-coumarinyl]maleimide (DACM) was from Wako Chemical Co. (Osaka, Japan). BNPS-skatole was from Pierce Chemical Co. (Rockford, IL).

Cross-Linking of the Actin–Fragmin Complex. G-Actin (4 mg/mL) in 2 mM Tris-HCl, 0.2 mM ATP, and 0.2 mM $CaCl_2$ (pH 8.0) was labeled with DACM in a molar ratio of 0.8:1.0 (DACM/actin) for 5 min at 0 °C. After the labeling reaction was quenched with DTT (final concentration 1 mM), the DACM-labeled actin was mixed with fragmin in a molar ratio of 1:1. The final solution contained DACM–actin (0.2 mg/mL) and fragmin (0.2 mg/mL) in 50 mM NaCl, 20 mM imidazole, and 0.1 mM $CaCl_2$ (pH 7.0). The mixture was incubated for 1 h at 0 °C before the addition of EDC. Formation of the actin–fragmin complex was checked by high-performance liquid chromatography (HPLC). Then the cross-linking reaction was initiated by the addition of 0.1 volume of 0.2 M EDC in 0.1 M imidazole (pH 7.0). The cross-linking reaction was allowed to proceed for 20 or 40 min at 25 °C. It was quenched by the addition of excess 2-mercaptoethanol.

HPLC Analysis. HPLC analysis was carried out with a G3000SW column (Toyo-Soda). Elution was carried out with 0.5 M ammonium acetate. Proteins were monitored by the absorption at 280 nm and by the fluorescence of DACM at the same time. Apparent molecular weights were estimated by the use of standard proteins with known molecular weights.

Chemical Cleavages and Electrophoresis of Purified Peptides. After the cross-linking reaction with EDC, the actin–fragmin solution was electrophoresed in the presence of $NaDodSO_4$ on acrylamide gels [10% acrylamide–0.3% bis(acrylamide)]. Major cross-linked products (88K and 90K peptides) and 42K actin were identified by illuminating gels with a UV lamp (356 nm). Corresponding fluorescent bands were cut out from these gels. They were washed with 50% methanol and then 100% methanol and dried in vacuo.

Chemical cleavages of purified peptides trapped in these dried gels were carried out as previously described (Sutoh, 1982a,b, 1983, 1984; Sutoh & Mabuchi, 1984). Hydroxylamine cleavages were, however, carried out in the absence of guanidine hydrochloride. After these chemical cleavages, gels were again washed with 50% and 100% methanol sequentially and then dried in vacuo. Dried gels were then soaked in 2% $NaDodSO_4$, 10 mM Tris-HCl, 2% 2-mercaptoethanol, and 10% glycerol (pH 8.0) at 37 °C for 1 h. They were directly put on acrylamide gels for electrophoresis. After electrophoresis, fluorescent fragments were visualized by illumination with a UV lamp (356 nm).

Chemical Cleavages and Electrophoresis of Fragmin. Fragmin was electrophoresed on acrylamide gels [15% acrylamide–0.45% bis(acrylamide)] in the presence of $NaDodSO_4$ (2 or 0.5 μg per well). Lanes containing fragmin were cut out from gels, washed with 50% and 100% methanol sequentially, and then dried in vacuo. These dried gels were treated with CNBr, BNPS-skatole, and hydroxylamine as described previously. After chemical cleavages, they were again washed with 50% and 100% methanol sequentially and then dried. The dried gels were soaked in 2% $NaDodSO_4$, 10 mM Tris-HCl, 2% 2-mercaptoethanol, and 10% glycerol (pH 8.0). Electrophoresis was carried out on acrylamide gels [15% acrylamide–0.45% bis(acrylamide)].

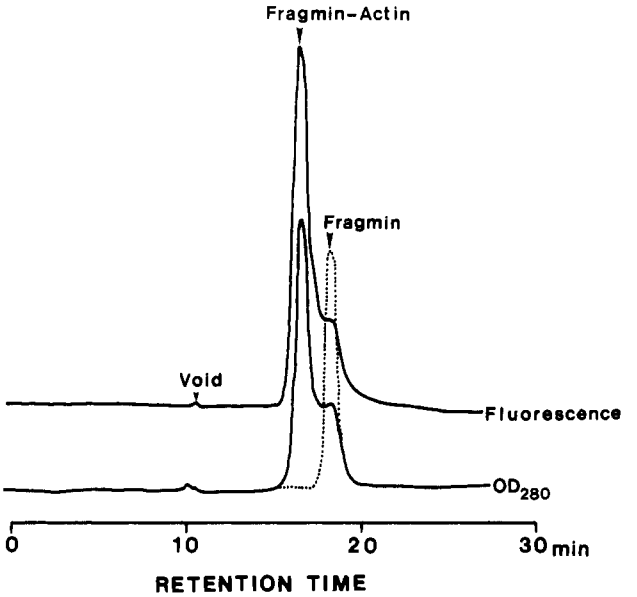

FIGURE 1: HPLC analysis of the DACM–actin–fragmin complex. Fragmin alone (10 μg) or a mixture of fragmin (10 μg) and DACM–actin (10 μg) in 50 mM NaCl, 20 mM imidazole, and 0.1 mM $CaCl_2$ (pH 7.0) was loaded on an HPLC column (G3000SW, Toyo-Soda). Proteins were eluted with 0.5 M ammonium acetate and monitored by the absorption at 280 nm (marked as "OD_{280}") and by the fluorescence of the DACM label (marked as "fluorescence"). Elution profiles of fragmin (···) and the mixture of fragmin and DACM–actin (—) are shown. Peaks corresponding to fragmin alone and the actin–fragmin complex are marked in the elution profiles.

Miscellaneous Procedures. $NaDodSO_4$ gel electrophoresis was carried out according to the method of Laemmli (1970). Peptides were visualized either by UV illumination or by Coomassie staining.

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

RESULTS

Cross-Linking of the Actin–Fragmin Complex with EDC. Cys-374 of actin was selectively labeled with the fluorescent dye DACM (Sutoh, 1982a) to introduce the C-terminal label into the actin molecule. The DACM-labeled actin was then mixed with fragmin in a molar ratio of 1:1 in 50 mM NaCl, 20 mM imidazole, and 0.1 mM $CaCl_2$ (pH 7.0) to form the actin–fragmin complex. Complex formation between DACM–actin and fragmin was detected by HPLC. When fragmin alone was loaded on a HPLC column (G3000SW, Toyo-Soda), only a single peak with an apparent molecular weight of 40 000 was observed (Figure 1). When the mixture of DACM–actin and fragmin was loaded on the same column, a new major peak with an apparent molecular weight of 80 000 was detected by the absorption at 280 nm and by the fluorescence of DACM (Figure 1). The new 80K peak was detected only when calcium ions were included in the actin–fragmin mixture, indicating that the peak actually corresponded to the actin–fragmin complex (Hasegawa et al., 1980). Considering its apparent molecular weight, we concluded that the 80K complex contained one actin and one fragmin. It must be noted that no actin–fragmin complex larger than 80 kilodaltons was observed under the present conditions.

The actin–fragmin complex was treated with EDC (20 mM) to introduce cross-links between fragmin and DACM-labeled actin at their interface. The resulting cross-linked products were analyzed by $NaDodSO_4$–acrylamide gel electrophoresis as shown in Figure 2. Before EDC treatment, only a single band was observed on gels by UV illumination (lane A) or by

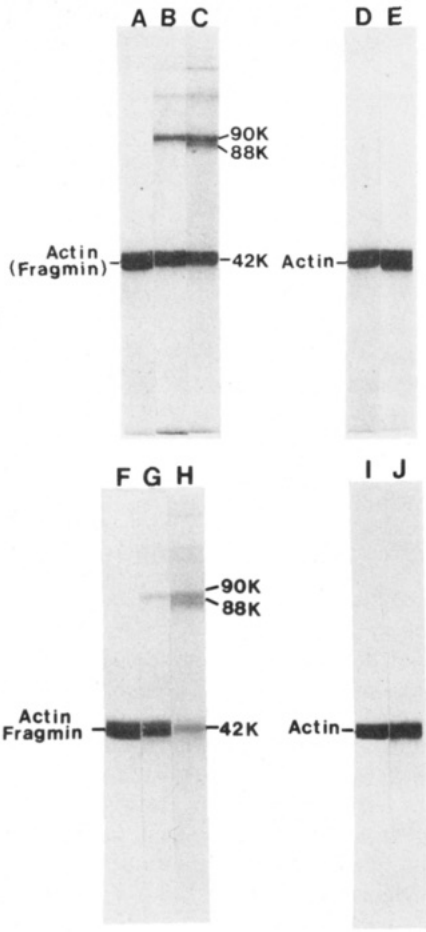

FIGURE 2: Cross-linking of the DACM–actin–fragmin complex with EDC. The mixture of fragmin (0.2 mg/mL) and DACM–actin (0.2 mg/mL) or DACM–actin alone (0.2 mg/mL) was treated with 20 mM EDC in 50 mM NaCl, 20 mM imidazole, and 0.1 mM $CaCl_2$ (pH 7.0). After electrophoresis, peptides were visualized either by illuminating the gel with a UV lamp (lanes A–E) or by Coomassie staining (lanes F–J). Peptides carrying the C-terminus of the actin polypeptide were selectively visualized by the former procedure. Lanes A and F, mixture of fragmin and DACM-labeled actin before EDC treatment; lanes B and G, after EDC treatment for 20 min; lanes C and H, after EDC treatment for 40 min; lanes D and I, DACM–actin alone before EDC treatment; lanes E and J, DACM–actin after EDC treatment of 40 min. Note that fluorescent bands are observed as black bands on a white background since negatives of the gel picture were reversed for easier observation. All pictures of fluorescent gel patterns shown later were processed in the same way.

Coomassie staining (lane F), since fragmin migrated very closely to actin under the present conditions. After EDC treatment, however, closely spaced cross-linked bands with apparent molecular weights of 88 000 and 90 000 were detected on gels both by Coomassie staining (lanes G and H) and by UV illumination (lanes B and C). The appearance of these cross-linked products was accompanied by the concomitant loss of DACM–actin and fragmin (lanes B, C, G, and H). Small amounts of cross-linked products with higher molecular weights were also detected on those gels. Since the 88K and 90K products were never generated when DACM–actin alone was treated with EDC (lanes E and J), these peptides cannot be homodimers of actin. Considering their apparent molecular weights, we concluded that the 88K and 90K peptides were the cross-linked products of one DACM–actin and one fragmin.

Hydroxylamine Cleavages of Fluorescent Peptides. We mapped the cross-linking site of fragmin in the actin sequence using the fluorescent 88K and 90K cross-linked products. These fluorescent peptides and the 42K peptide (DACM–actin plus fragmin) were electrophoretically purified (Materials and Methods). These peptides were then subjected to various chemical cleavages as previously described (Sutoh, 1982a,b, 1983, 1984; Sutoh & Mabuchi, 1984).

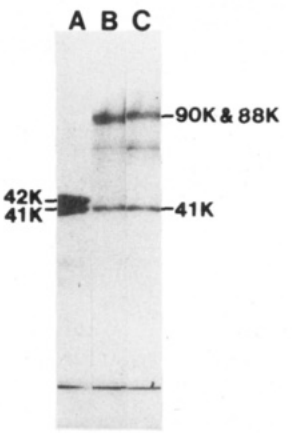

FIGURE 3: Hydroxylamine cleavages of the 42K, 88K, and 90K fluorescent peptides. The peptides were digested with 1 M hydroxylamine (pH 9.0) at 45 °C for 4 h. Resulting fluorescent fragments were analyzed on an acrylamide gel [12.5% acrylamide–0.375% bis(acrylamide)]. The fluorescent fragments containing the C-terminus of the actin polypeptide were visualized by illuminating the gel with a UV lamp. Lane A, fluorescent fragments generated from the control 42K peptide; lane B, fluorescent fragments generated from the 88K cross-linked peptide; lane C, fluorescent fragment generated from the 90K cross-linked peptide.

At first, these fluorescent peptides were subjected to hydroxylamine cleavages. When cleavage products of the control 42K peptide (DACM–actin plus fragmin) were analyzed on an acrylamide gel, it was observed that a fluorescent fragment with an apparent molecular weight of 41 000 was released (lane A in Figure 3), consistent with previous results (Sutoh, 1982a, 1984; Sutoh & Mabuchi, 1984). At positions 12–13, actin contains one Asn–Gly bond (Elzinga et al., 1973), which was very susceptible to the hydroxylamine treatment (Bornstein & Balian, 1977; Sutoh, 1981, 1982a, 1984; Sutoh & Mabuchi, 1984). The 41K fluorescent fragment released from the fluorescent 42K peptide was thus the actin fragment spanning residues 13–375. Under the present cleavage conditions, about 40% of the Asn–Gly bond was cleaved. When the fluorescent 88K and 90K peptides were subjected to the same hydroxylamine treatment, fragment patterns from these fluorescent products were very similar to each other (lanes B and C in Figure 3). It was also observed that the fluorescent 41K fragment was released from both peptides (lanes B and C in Figure 3). Yields of the 41K fragment from the 88K and 90K peptides (30–40%) were similar to that from the control 42K peptide. Since the 41K fluorescent fragment generated from the 88K and 90K peptides was the free actin fragment spanning residues 13–375 as shown below, we concluded that the major cross-linking site of fragmin in the actin sequence was outside the 13–375 segment, i.e., within the N-terminal segment spanning residues 1–12. The N-terminal segment participated in cross-linking with fragmin to generate both the 88K and 90K products.

Here we have to consider the possibility that the 41K fragment released from the 88K and 90K peptides actually is not the free 13–375 fragment of actin but a cross-linked product between the 13–375 fragment and a small fragmin fragment. If the small fragment of fragmin was cross-linked to the 13–375 fragment of actin to generate the fluorescent 41K peptide observed on the gel (lanes B and C in Figure 3), we also would observe the 42K fluorescent band which corresponded to the cross-linked product of the 42K intact actin

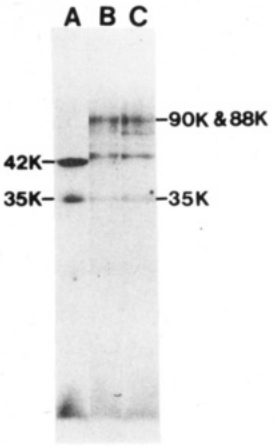

FIGURE 4: BNPS-skatole cleavages of the 42K, 88K, and 90K fluorescent peptides. The peptides were digested with BNPS-skatole (0.7 mg/mL) in 60% acetic acid at 37 °C for 4 h. Resulting fluorescent fragments were analyzed on an acrylamide gel [15% acrylamide–0.45% bis(acrylamide)]. The fluorescent fragments containing the C-terminus of the actin polypeptide were visualized with a UV lamp. Lane A, fluorescent fragments generated from the control 42K peptide; lane B, fluorescent fragments generated from the 88K cross-linked peptide; lane C, fluorescent fragments generated from the 90K cross-linked peptide.

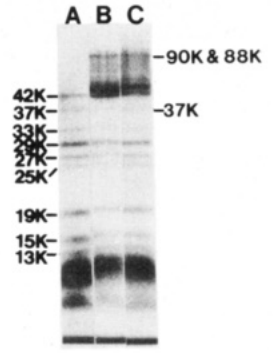

FIGURE 5: CNBr cleavages of the 42K, 88K, and 90K fluorescent peptides. The peptides were treated with 20 mM CNBr in 70% formic acid at 37 °C for 1 h. Resulting fluorescent fragments were analyzed on an acrylamide gel [15% acrylamide–0.45% bis(acrylamide)] containing 6 M urea. The fluorescent fragments carrying the C-terminus of the actin polypeptide were visualized with a UV lamp. Lane A, fluorescent fragments generated from the control 42K peptide; lane B, fluorescent fragments generated from the 88K cross-linked peptide; lane C, fluorescent fragments generated from the 90K cross-linked peptide.

and the small fragmin fragment. The intensity ratio of the 41K and 42K bands would be similar to that observed in lane A. As shown in Figure 3, this is not the case. Hydroxylamine treatment of the 88K and 90K fluorescent peptides never generated the 42K fluorescent fragments. Thus, we concluded that the 41K fluorescent fragment released from the 88K and 90K peptides was actually the free 13–375 actin fragment.

Besides the 41K fluorescent fragment, hydroxylamine cleavage of the 88K and 90K peptides generated a fluorescent fragment with an apparent molecular weight of 65 000 (a fluorescent band between the 41K fragment and the 88K or 90K peptide in lanes B and C in Figure 3). Since the cross-linking site of fragmin was in the N-terminal region of actin and since the fluorescent label was introduced in its C-terminus, the 65K fluorescent fragment was the cross-linked peptide between intact actin and a fragmin fragment. When fragmin was treated with hydroxylamine under the same conditions where the 88K and 90K peptides were digested, several fragments were generated as shown in Figure 6 (lane B), and a fragment with an apparent molecular weight of 25 000 was actually detected as indicated on the gel.

BNPS-skatole Cleavages of Fluorescent Peptides. The 42K, 88K, and 90K peptides were partially cleaved with BNPS-skatole, to which peptide bonds containing tryptophan residues are very susceptible (Fontana, 1972). When cleavage products of the control 42K peptide were analyzed on an acrylamide gel, it was observed that a rather diffuse fluorescent band with an apparent molecular weight of 35 000 was generated together with small fluorescent fragments migrating at the buffer front (lane A in Figure 4). Since actin contains four Trp residues (Trp-79, Trp-86, Trp-340, and Trp-356) (Elzinga et al., 1973), it was very likely that the 35K fluorescent fragment corresponded to the actin fragments spanning residues 80–375 and 87–375 (Sutoh, 1984; Sutoh & Mabuchi, 1984). When the 88K and 90K peptides were cleaved by BNPS-skatole, fragment patterns from these fluorescent peptides were very similar to each other. The 35K fluorescent fragment was again released from both peptides (lanes B and C in Figure 4). By the same argument as above, we concluded that the 35K fluorescent fragment was the free 80–375 or 87–375 actin fragment since no fluorescent band was detected at the 42-kilodalton position on lanes B and C in Figure 4. The result led us to conclude that the cross-linking site of fragmin was outside the 80–375 or 87–375 actin segment, i.e., within the N-terminal segment spanning residues 1–79 or 1–86, consistent with the above result that the actin segment spanning residues 1–12 participated in cross-linking with fragmin.

Although the 42K fluorescent peptide was not released from the 88K and 90K peptides, a fluorescent fragment with a molecular weight slightly larger than 42K (about 44 000) was generated from both of these peptides. Since the cross-linking site of fragmin was located within the N-terminal segment of actin and since the fluorescent dye was linked to its C-terminal region, it seemed that the fluorescent fragment was the cross-linked peptide of intact actin and a small fragmin fragment. Actually, BNPS-skatole cleavage of fragmin generated two fragments with apparent molecular weights of 41K and 40K together with other minor fragments as shown in Figure 6 (lane C; these 41K and 40K fluorescent fragments are marked). In other words, BNPS-skatole cleavage generated small (1K and 2K) fragments from its N- and/or C-terminus. It seemed that the fluorescent fragment observed just above the 42K position in lanes B and C in Figure 4 was the cross-linked product of intact actin and one of these small fragmin fragments generated form its N- and/or C-terminus.

Cyanogen Bromide Cleavages of Fluorescent Peptides. When the control 42K peptide was digested with CNBr, ladderlike fluorescent bands were observed on acrylamide gel as shown in Figure 5 (lane A). Each of these fluorescent fragments has been located along the polypeptide chain of actin (Sutoh, 1982a, 1984). When the 88K and 90K peptides were cleaved with CNBr, the ladderlike fluorescent bands were again generated (lanes B and C in Figure 5). These fragment patterns were very similar to each other. Comparison of these fluorescent patterns (lane A vs. lanes B and C) revealed that they completely coincided with each other up to the 37K band. However, it was also observed that fluorescent fragments migrating to the 42K position were released from the 88K and 90K peptides (lanes B and C in Figure 5).

Since the above result was rather puzzling, CNBr cleavage products of fragmin were analyzed on an acrylamide gel as shown in Figure 6 (lane D). Under the cleavage conditions where actin was rather extensively cleaved (see lane A in Figure 5), no cleavage of fragmin was detected at first sight. However, it was observed that after prolonged electrophoresis

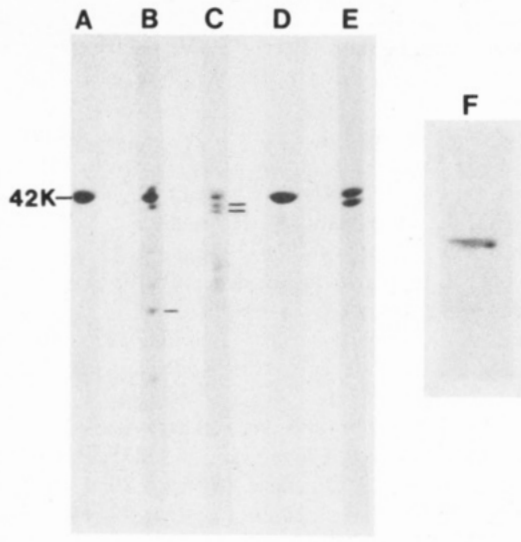

FIGURE 6: Chemical cleavages of fragmin. Fragmin trapped in an acrylamide gel was treated with hydroxylamine, BNPS-skatole, or CNBr under the conditions shown in Figures 3–5. Cleavage products were analyzed on acrylamide gels [15% acrylamide–0.45% bis-(acrylamide)]. Peptides were stained with Coomassie Blue. Lane A, intact fragmin (2 μg); lane B, fragmin treated with hydroxylamine (the 25K fragment is marked on the gel); lane C, fragmin treated with BNPS-skatole (the 41K and 40K fragments are marked on the gel); lane D, fragmin treated with CNBr; lane E, actin treated with hydroxylamine as a control; lane F, an enlarged picture of the doublet bands of fragmin (0.5 μg) treated with CNBr. Note that the 42K fragmin band in lane D split into the doublet bands with similar intensities on this gel.

with loading a smaller amount of fragmin (0.5 μg per lane), the 42K fragmin band split into a doublet with similar intensities (lane F in Figure 6). This result indicated that CNBr cut off small peptide(s) from the N- and/or C-terminus of fragmin. The apparent resistance of the fragmin polypeptide against the CNBr digestion is consistent with its amino acid composition; it contains only 3 methionine residues (Hasegawa et al., 1980) while actin contains 16 residues (Elzinga et al., 1973). Since the hydroxylamine and BNPS-skatole cleavages clearly showed that the major cross-linking site of fragmin was located in the N-terminal segment of actin which spans residues 1–12, the above results seemed to imply that the fluorescent fragments migrating at the 42K position (lanes B and C in Figure 5) were actually the cross-linked complex of intact actin and small fragmin fragment(s) cleaved by CNBr. This is consistent with the above notion that the small fragmin fragment cleaved off from its C- or N-terminus by BNPS-skatole participated in cross-linking with actin.

Discussion

Employing a newly developed technique, we have recently identified binding sites of various "actin-binding proteins" in the actin sequence (Sutoh, 1982a, 1984; Sutoh & Mabuchi, 1984) (Figure 7). In this paper, we employed the same technique to identify the binding site of fragmin, a capping protein isolated from *Physarum polycephalum* plasmodia (Hasegawa et al., 1980; Sugino & Hatano, 1982), in the actin sequence. After EDC cross-linking of the actin–fragmin complex, cross-linking site(s) of fragmin in the actin sequence was (were) mapped by partial chemial cleavages of the two types of cross-linked products (the 88K and 90K peptides). The peptide mappings revealed that the N-terminal segment of actin which spans residues 1–12 participated in cross-linking with fragmin.

Generally speaking, the fact that polypeptide segments of two interacting proteins are cross-linked with each other does not necessarily mean the direct contact of these segments. For the case of EDC cross-linking, however, it seems that the cross-linked segments are in contact with each other since the cross-linker covalently bridges lysine side chains to acidic side chains of aspartic and glutamic acids only when they are in direct contact. Thus, it seems that the N-terminal segment of actin which spans residues 1–12 is actually in direct contact with fragmin in the actin–fragmin complex.

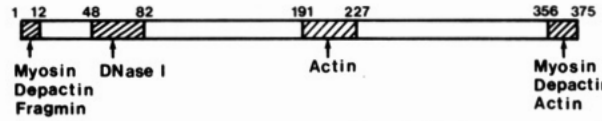

FIGURE 7: Interaction map of actin with actin-binding proteins. The horizontal bar represents the actin polypeptide. Residue numbers are shown above the bar. Hatched areas are actin segments which have been identified as binding sites of various actin-binding proteins.

It would be noteworthy that the same acidic N-terminal segment of actin is a part of binding sites of other actin-binding proteins (Figure 7) (Sutoh, 1982a, 1984; Sutoh & Mabuchi, 1984). Two segments of the heavy chain of myosin subfragment 1 (S1) spanning 18K–20K and 27K–35K regions from its C-terminus are in contact with the actin segment in the actin–S1 rigor complex (Sutoh, 1982a,b, 1983). The N-terminal actin segment is also in contact with depactin, an actin-depolymerizing protein isolated from starfish oocytes (Mabuchi, 1981, 1983), in the actin–depactin complex (Sutoh & Mabuchi, 1984). It is well-knwon that myosin accelerates actin polymerization. On the other hand, depactin rapidly depolymerizes F-actin by binding with F-actin and actively sequestering actin subunits (Mabuchi, 1981, 1982, 1983). Fragmin is another type of actin-binding protein; it caps the barbed end of F-actin (Sugino & Hatano, 1982). It is surprising that proteins with such different effects on actin organization share, at least partially, the same binding site on actin.

The N-terminal segment of actin contains five acidic residues (Asp-1, Glu-2, Asp-3, Glu-4, and Asp-11) but no basic residue (Elzinga et al., 1973; Vandekerckhove & Weber, 1978a). Therefore, a very large gain of free energy is expected when an actin-binding protein binds to this acidic region of actin through ionic interactions. The unusual amino acid sequence of the N-terminal actin segment (the high proportion of acidic residues) seems to make this region a favorable interaction site with various actin-binding proteins. In this context, it must be noted that actins from various sources maintain the acidic nature of their N-terminal segment even though this segment carries a disproportionately high number of amino acid exchanges (Vandekerckhove & Weber, 1978a,b, 1984; Vandekerckhove et al., 1984).

Fragmin is a barbed end capping protein (Sugino & Hatano, 1982). Therefore, it is expected that its binding site is located on the barbed end of the actin molecule. The N-terminal segment of actin identified as a part of the binding site of fragmin would not be on the barbed end, however. Three-dimensional reconstitution of electron microscopic images of the actin–S1 rigor complex showed that the binding site of S1 was located on the outer surface of the F-actin helix (Toyoshima & Wakabayashi, 1985a,b). Since the N-terminal segment of actin is in the actin–S1 interface (Sutoh, 1982a), the segment seems to be on the outer surface of the helix. Thus, the binding site of fragmin might cover a fairly large area on the actin molcule, extending from its barbed end to the N-terminal region. Further studies would be necessary to map the barbed end of the actin molecule.

Examinations of cleavage products of fragmin with

BNPS-skatole and CNBr revealed that both of the chemical cleavages generated small fragments from the N- and/or C-terminus of fragmin. Since cross-linked complexes of the intact actin polypeptide and these small fragmin fragments were detected in the cleavage products of the 88K and 90K peptides, it was likely that the N- or C-terminal segment cleaved off by the BNPS-skatole or CNBr cleavage participated in cross-linking with the N-terminal segment of actin. The size of the N- or C-terminal segment of fragmin participating in cross-linking would be rather small, considering the fact that the CNBr cleavage product of fragmin migrated very closely to the intact fragmin polypeptide.

The cross-linking reaction of the actin–fragmin complex with EDC generated two types of the one to one cross-linked products, i.e., the 88K and 90K peptides. Similar results were obtained when the actin–S1 rigor complex was cross-linked with EDC (Sutoh, 1983). The cross-linked products were observed as closely spaced doublet bands on a $NaDodSO_4$ gel. One product had an apparent molecular weight of 165 000 while the other hand of molecular weight of 175 000. Peptide mappings revealed that the 165K product was generated by cross-linking the N-terminal segment of actin with the 18K–20K region of the S1 heavy chain while the 175K product was from the cross-linking of the actin segment with the 27K–35K region of the heavy chain. Different positions of cross-linking points in these cross-linked products resulted in different mobilities on the $NaDodSO_4$ gel, even though they had the same molecular weight. Thus, it is possible that EDC cross-linking of the actin–fragmin complex generates two types of cross-linked products with different positions of cross-linking points. The other possible cause for the 88K–90K doublet is that EDC cross-linking generated intramolecular cross-link(s) in actin and/or fragmin. Considering the similarity between fragmentation patterns of the 88K and 90K products with three different chemicals, the latter is more likely for the case of actin–fragmin cross-linking.

References

Bornstein, P., & Balian, G. (1977) *Methods Enzymol. 47*, 132–143.

Elzinga, M., & Lu, R. C. (1976) *Contractile Systems in Non-Muscle Tissues* (Perry, S. V., Margareth, A., & Adelstein, R. S., Eds.) pp 29–37, North-Holland, Amsterdam.

Elzinga, M., Collins, J. H., Kuhel, W. M., & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2687–2691.

Fontana, A. (1972) *Methods Enzymol. 25*, 419–423.

Hasegawa, T., Takahashi, S., Hayashi, H., & Hatano, S. (1980) *Biochemistry 19*, 2677–2683.

Hitchcock, S. E., Carlsson, L., & Lindberg, U. (1976) *Cell (Cambridge, Mass.) 7*, 531–542.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lazarides, E., & Lindberg, U. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4742–4746.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randoll, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Mabuchi, I. (1981) *J. Biochem. (Tokyo) 89*, 1341–1344.

Mabuchi, I. (1982) *J. Biochem. (Tokyo) 92*, 1439–1447.

Mabuchi, I. (1983) *J. Cell Biol. 97*, 1612–1621.

Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.

Sugino, H., & Hatano, S. (1982) *Cell Motil. 2*, 457–470.

Sutoh, K. (1981) *Biochemistry 20*, 3281–3285.

Sutoh, K. (1982a) *Biochemistry 21*, 3654–3661.

Sutoh, K. (1982b) *Biochemistry 21*, 4800–4804.

Sutoh, K. (1983) *Biochemistry 22*, 1579–1585.

Sutoh, K. (1984) *Biochemistry 23*, 1942–1946.

Sutoh, K., & Mabuchi, I. (1984) *Biochemistry 23*, 6757–6761.

Toyoshima, C., & Wakabayashi, T. (1985a) *J. Biochem. (Tokyo) 97*, 219–243.

Toyoshima, C., & Wakabayashi, T. (1985b) *J. Biochem. (Tokyo) 97*, 244–263.

Vandekerckhove, J., & Weber, K. (1978a) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1106–1110.

Vandekerckhove, J., & Weber, K. (1978b) *Eur. J. Biochem. 90*, 451–462.

Vandekerckhove, J., & Weber, K. (1984) *J. Mol. Biol. 179*, 391–413.

Vandekerckhove, J., Lal, A., & Korn, E. D. (1984) *J. Mol. Biol. 172*, 141–147.