barrier to transformation into the stable polymorph II. As we have previously suggested, a function of 2-hydroxy fatty acids in cerebrosides may be to prevent a membrane-destructive hydration–dehydration cycle associated with metastability, particularly in the multilamellar myelin membrane, which possesses a high cerebroside content (Curatolo, 1982).

ACKNOWLEDGMENTS

We thank Dorsey Gibbes and Dr. Barry Sears for providing the psychosine and Dr. Donald Small of the Boston University School of Medicine for the use of the calorimeter. We also thank Charles Nolan of the Shriver Center for his expert technical assistance in the preparative HPLC isolation of cerebrosides.

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Covalent Complexes Formed between Plasma Gelsolin and Actin with a Zero-Length Cross-Linking Compound†

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Received March 13, 1985

ABSTRACT: Actin and plasma gelsolin were covalently cross-linked with the zero-length cross-linker 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. Two major intermolecularly linked products were identified on polyacrylamide gels. By use of ^{14}C-labeled actin and ^{125}I-labeled gelsolin, these were shown to be the 1:1 and 2:1 complexes of actin with gelsolin, respectively. The higher molecular weight complex predominated under all conditions tested including the presence and absence of Ca^{2+}. In titration experiments in which actin at different concentrations was reacted with a fixed concentration of gelsolin, end points were obtained for the formation of both cross-linked species at about two actins per gelsolin, implying that a 2:1 noncovalent complex is cross-linked. In 0.1 mM Ca^{2+}, the extent of cross-linking was independent of protein concentration down to 50 nM gelsolin. At low Ca^{2+} concentrations ($<10^{-8}$ M), the extent of cross-linking was very much reduced at micromolar gelsolin and fell to zero at about 100 nM gelsolin. The binding of actin to gelsolin to give a cross-linkable complex is therefore very strong at 0.1 mM Ca^{2+} but much weaker at low Ca^{2+} concentrations.

Control of the motile behavior of cells is dependent on the regulation of actin filament assembly and organization. A protein which is likely to be an important modulator of actin behavior in vivo is gelsolin, which solvates actin gels in the presence of Ca^{2+} (Yin & Stossel, 1979). It has been identified in a wide variety of cells and tissues (Yin et al., 1981a; Snabes et al., 1983) and is also found extracellularly, in plasma. The plasma and cytoplasmic forms of gelsolin are distinct but closely related polypeptides (Yin et al., 1984). Studies on the

†Supported by a project grant from the Medical Research Council (U.K.).

0006-2960/85/0424-6613$01.50/0

interactions of actin with plasma gelsolin may clarify the mechanisms of gelsolin action in cells.

Plasma gelsolin, a protein of M_r 92 000, has previously been called actin depolymerizing factor (Norberg et al., 1979; Harris & Gooch, 1981) or brevin (Harris & Schwartz, 1981). It severs actin filaments and binds to the "barbed" ends of the shortened filaments thus formed (Harris & Weeds, 1984), thereby increasing the critical concentration of actin. In addition, it accelerates the rate of G-actin assembly (Harris & Weeds, 1983; Lees et al., 1984), probably by interacting with actin monomers to form a stable nucleus. Because actin interacts with plasma gelsolin in the absence of Ca^{2+}, yet micromolar concentrations of Ca^{2+} stimulate nucleation, a model was proposed in which one actin is bound in a Ca^{2+}-independent manner, but addition of a second actin to form a nucleus requires Ca^{2+} (Harris & Weeds, 1983). To test this model, and obtain more information about the interactions of gelsolin with actin monomers, complex formation in mixtures of actin and gelsolin was studied by chemical cross-linkage. This paper characterizes the products formed by reacting actin and gelsolin with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC),[1] a "zero-length" cross-linking reagent which promotes the formation of peptide bonds between free amino and carboxyl groups in proteins (Hoare & Koshland, 1967).

Materials and Methods

Chemicals were of Analar grade or equivalent. Solutions were made with double-distilled water. EDC was obtained from Sigma Chemical Co., and a fresh stock solution in 0.1 M imidazole hydrochloride was prepared immediately before each experiment. Iodo[1-^{14}C]acetamide and radioiodinated Bolton–Hunter reagent [*N*-succinimidyl 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionate] were obtained from Amersham International.

Pig plasma gelsolin was prepared as described previously (Harris & Weeds, 1983) with published modifications (Harris & Weeds, 1984). It was 90–95% pure by densitometric analysis of Coomassie blue stained gels. For all quantitative work with ^{125}I-gelsolin, minor iodinated contaminants were removed by gel filtration on G-150 Sephadex.

Gelsolin was radioiodinated according to the method of Bolton & Hunter (1973). Gelsolin at about 4 μM was reacted in 0.1 M sodium borate, pH 8.4, and excess reagent was removed by gel filtration on G-10 Sephadex in 10 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, and 3 mM NaN_3 with gelatin added as a carrier protein at 1 mg·mL^{-1}. ^{125}I-Gelsolin was diluted at least 100-fold with unlabeled gelsolin before use.

Actin was prepared from an acetone powder of rabbit back and leg muscles (Taylor & Weeds, 1976). G-Actin was prepared by exhaustive dialysis of F-actin at less than 120 μM into 2 mM HEPES, pH 8.0, 0.2 mM ATP, 0.2 mM DTT, 0.1 mM $CaCl_2$, and 1 mM NaN_3. The resulting solution was centrifuged for 0.5 h in a batch rotor in a Beckman Airfuge to remove any residual filaments.

^{14}C-Actin. F-Actin (0.3 μmol) in 0.6 mL of 25 mM K_2HPO_4 and 0.1 mM DTT was mixed with 2.44 μmol of iodo[1-^{14}C]acetamide at 20.4 mCi·mmol^{-1} in 0.3 mL of 0.1 M sodium phosphate buffer, pH 8.6, and reacted for 3.5 h under nitrogen. The reaction was stopped with 20 μmol of DTT. The actin was diluted to 70 μM with a depolymerizing buffer (2 mM Tris-HCl, pH 8.0, 0.2 mM ATP, 0.2 mM DTT, 0.1 mM $CaCl_2$, and 1 mM NaN_3) and dialyzed exhaustively. The G-actin produced was centrifuged in a Beckman Airfuge and polymerized by addition of KCl to 100 mM and $MgCl_2$ to 2 mM. The F-actin was sedimented and resuspended in 5 mM Tris-HCl, pH 8.0, 0.5 mM ATP, 0.2 mM DTT, 100 mM KCl, 2 mM $MgCl_2$, and 3 mM NaN_3. It contained 0.87 mol of iodoacetamide per mole of actin monomer.

Protein concentrations were determined spectrophotometrically by using A_{290} = 0.63 for 1 mg·mL^{-1} actin (Lehrer & Kerwar, 1972) and A_{280} = 1.24 for 1 mg·mL^{-1} gelsolin (A. Weeds, personal communication).

SDS–PAGE was performed on slab gels (15 × 15 × 0.5 cm) using the discontinuous buffer system of Laemmli (1970). Gels were stained with Coomassie brilliant blue. Apparent molecular weights were estimated by using the following markers: α- spectrin, M_r 240 000; β-spectrin, M_r 220 000; ferritin half-unit, M_r 220 000; myosin heavy chain, M_r 210 000; *Escherichia coli* β-galactosidase, M_r 130 000; phosphorylase *b*, M_r 94 000; albumin, M_r 67 000; catalase, M_r 60 000. A regression line was calculated through these markers.

For autoradiography, ^{125}I-containing gels were dried and exposed to prefogged Kodak X-Omat S film. ^{14}C-containing gels were soaked in "Amplify" (Amersham International) according to the manufacturer's instructions, dried, and fluorographed with prefogged X-Omat XAR 5 film.

Intensities of Coomassie blue stained bands were determined with a Joyce-Loebl double-beam microdensitometer, with a red filter. Relative peak areas were evaluated either as the product of height and width (for symmetric peaks) or by cutting and weighing. Peak area varied linearly with the weight of protein over the full range studied, for both actin and gelsolin. The two proteins stained approximately equally on a weight basis; the ratio of actin to gelsolin stain per microgram of protein was 0.95.

^{14}C-Actin:^{125}I-Gelsolin Ratios. Cross-linked sample (conditions in Table I) containing about 0.3 mg of total protein was run on 7.5% SDS–PAGE gels. After the gel was stained, bands I and II were excised by hand, and the ^{125}I in each gel slice was quantitated in a Packard Auto Gamma scintillation counter. The gel slices were then solubilized in 1 mL of hydrogen peroxide (30% w/v) at 50 °C for 16 h. Ten milliliters of scintillant (Packard 299) was added, and ^{14}C was determined by counting in a Packard Tricarb liquid scintillation spectrometer. Corrections were made for overlap between ^{125}I and ^{14}C counts. The specific activities of gelsolin and actin were determined by excising bands containing known amounts (5–10 μg) of the pure products from gels and treating and counting exactly as for the samples.

Results

Products of the Reaction of Gelsolin–Actin Mixtures with EDC. SDS–PAGE of mixtures of gelsolin and actin treated with EDC showed that two major cross-linked species were formed, designated I and II in order of increasing molecular weight (Figure 1a). Actin alone (F or G) showed little, if any, cross-linking. Gelsolin did not give rise to higher molecular weight bands with EDC. F- or G-actin mixed with gelsolin gave essentially identical cross-linking patterns, although more material was retained at the top of gels when F-actin was used (Figure 1c), and, therefore, most experiments were carried out with G-actin. However, after preliminary experiments, the salt concentration was raised to 50 mM to facilitate gelsolin solution; under this condition, G-actin would

[1] Abbreviations: EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; ^{14}C-actin, iodo[1-^{14}C]acetamide-labeled actin; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

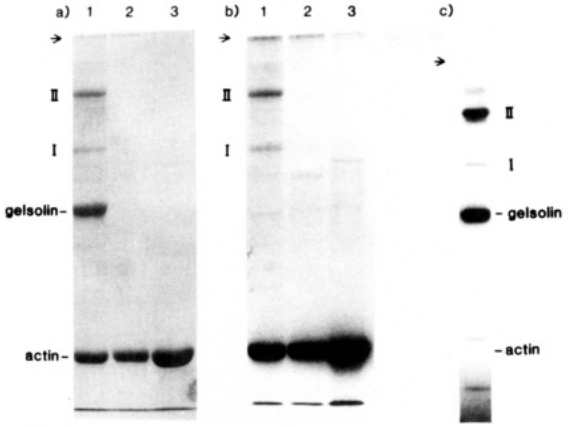

FIGURE 1: SDS–PAGE on 7.5% gels of actin and gelsolin cross-linked with 5 mM EDC for 1 h. (a) Coomassie blue stained gel: (1) ^{14}C-actin and gelsolin at approximately 2:1 molar ratio cross-linked in 50 mM NaCl, 30 μM ATP, 30 μM DTT, 0.1 mM $CaCl_2$, 5 mM imidazole hydrochloride, pH 7, and 1 mM NaN_3; (2) ^{14}C-actin treated with EDC as in (1); (3) 10 μg of ^{14}C-actin. (b) Fluorograph of samples shown in (a), 4-day exposure. (c) Autoradiograph of ^{125}I-gelsolin cross-linked to F-actin with EDC at a 2:1 gelsolin:actin mole ratio, other conditions as in (a). 170000 cpm loaded on gel, 24-h exposure. Arrows indicate tops of gels.

be expected to polymerize very slowly in the absence of gelsolin. To determine the components of bands I and II, cross-linking was carried out by using either ^{14}C-actin or ^{125}I-gelsolin. Fluorography of gels of ^{14}C-actin samples (Figure 1b) showed that both band I and band II were labeled and, therefore, that both contained actin. Similarly, autoradiography of gels of ^{125}I-gelsolin-containing cross-linked samples showed radioactivity in bands I and II (Figure 1c). Bands I and II therefore contained both actin and gelsolin.

Optimal conditions for cross-linking were determined. The dependence of formation of bands I and II on EDC concentration at constant time is shown in Figure 2A. Both bands increased to a plateau value at 5–10 mM EDC. Residual gelsolin, however, continued to decrease with increasing EDC concentration. This decrease correlated with the formation of additional cross-linked products which were seen as a diffuse complex of bands above band II, or as protein which did not enter the gel. To give an optimal balance between maximal production of bands I and II and minimal formation of high molecular weight species, 5 mM EDC was chosen as the standard condition for most experiments. Figure 2B shows the time dependence of formation of bands I and II at constant EDC concentration. Both increased approximately in parallel, to about 60 min. After this time, higher molecular weight products were formed. On hour was therefore chosen as the standard reaction time.

The proportion of gelsolin entering bands I and II was determined by using ^{125}I-gelsolin and cutting out and counting bands from an SDS gel. In a typical experiment using 5 mM EDC for 1 h, the relative distributions were the following: un-cross-linked gelsolin, 77%; band I, 8%; band II, 15%. The extent of formation of bands I and II was therefore very low, even under optimal conditions.

To determine the relative amounts of actin and gelsolin in bands I and II, ^{14}C-actin was cross-linked to ^{125}I-gelsolin; the resultant bands were excised from gels and counted (for details, see Materials and Methods). The results of two separate experiments are shown in Table I. The best integral values for actin to gelsolin ratios are 1 for band I and 2 for band II.

Molecular weight determinations on 6% SDS–PAGE gave values of 136000 ± 3000 for band I and 182000 ± 1000 for band II. Expected molecular weight values for complexes of

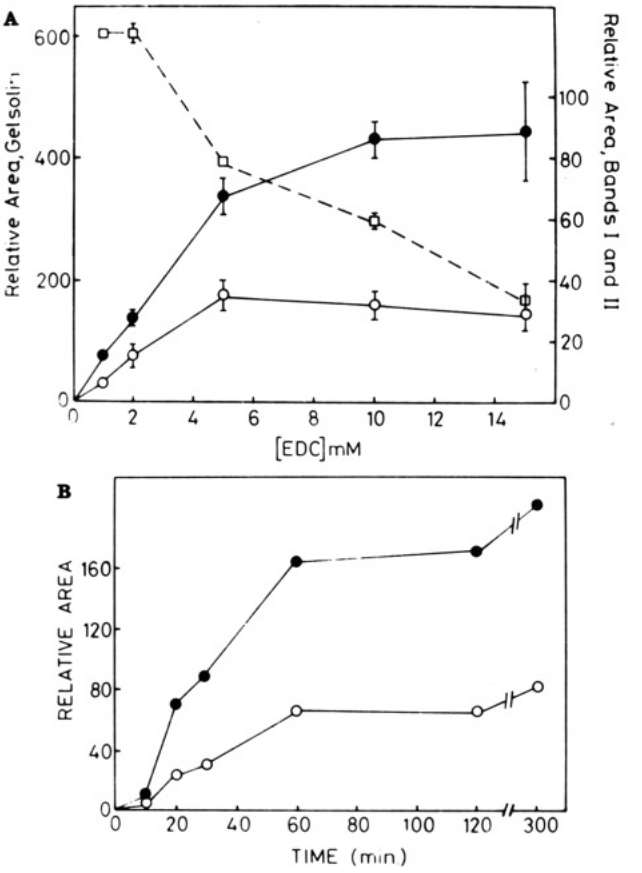

FIGURE 2: (A) Dependence of gelsolin–actin cross-linking on EDC concentration. G-actin at 5.3 μM mixed with gelsolin at 3 μM (buffer conditions as in Figure 1) and reacted with varying concentrations of EDC for 1 h at room temperature. Samples were then analyzed by SDS–PAGE and peaks quantitated by densitometry. (□) Gelsolin; (○) cross-linked band I; (●) band II. Error bars show standard deviations for four determinations. (B) Time dependence of gelsolin–actin cross-linking with EDC. G-Actin at 6.4 μM mixed with gelsolin at 6.4 μM (buffer conditions as in Figure 1) and cross-linked with 5 mM EDC for varying times at room temperature. Reactions were stopped by addition of gel sample buffer for SDS–PAGE and boiling. Samples were analyzed by SDS–PAGE and peaks quantitated by densitometry. (○) Cross-linked band I; (●) band II.

Table I: Mole Ratios of Actin to Gelsolin in EDC Cross-Linked Products[a]

	actin:gelsolin mole ratio	
expt	band I	band II
1	1.4	2.3
2a	1.0	1.6
2b	1.3	2.1
2c		2.1
mean ± SD	1.2 ± 0.2	2.03 ± 0.3

[a] Reaction conditions as in Figure 1. Experiments 1 and 2 are independent cross-linking experiments which used different preparations of gelsolin. Experiments 2a, 2b, and 2c refer to determinations carried out with different gels of the same cross-linked sample.

gelsolin with one actin or two actins are 134000 and 176000, respectively. These data therefore support the conclusion of the double-labeling experiment.

Actin–Gelsolin Complexes in Ca^{2+}. Cross-linking experiments were carried out in which fixed concentrations of gelsolin were titrated with increasing actin. Combined results from three such titrations are shown in Figure 3. In 0.1 mM Ca^{2+}, band II formation increased with actin concentration to a plateau, reaching saturation at about two actins per gelsolin (Figure 3). The 2:1 band (II) was the major cross-linked species, even at actin concentrations well below saturation.

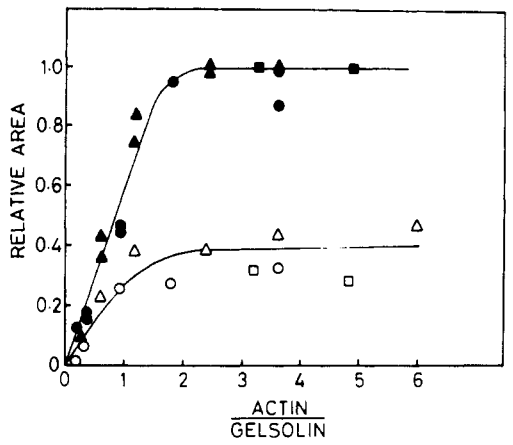

FIGURE 3: Titrations of gelsolin with actin. Formation of band II. Gelsolin at constant concentration mixed with varying concentrations of G-actin (buffer conditions as in Figure 1) and cross-linked with 5 mM EDC for 1 h at room temperature. Samples were analyzed by SDS–PAGE, and the extent of cross-linking was quantitated by densitometry. Peak areas are expressed relative to the maximum in Ca^{2+} as 1.0. Open symbols, 0.5 mM EGTA; closed symbols, 0.1 mM Ca^{2+}: (△, ▲) gelsolin, 5 μM; (○, ●) gelsolin, 3 μM; (□, ■) gelsolin, 0.7 μM, 50 μM $MgCl_2$ included in buffer.

Table II: Ratios of Band II to Band I at Constant Gelsolin Concentration and Variable Actin Concentration[a]

actin:gelsolin mole ratio	band II:band I ratio in 0.1 mM Ca^{2+}	0.5 mM EGTA
0.24	2.00	
0.60	2.58	4.05
1.20	2.37	3.99
2.40	2.40	4.57
3.60	2.49	4.00
6.00		4.90
mean ± SD	2.37 ± 0.22	4.30 ± 0.40

[a] Buffer and cross-linking conditions as Figure 1, except for substitution of EGTA for $CaCl_2$ where indicated; 5 μM gelsolin was used.

Furthermore, the ratio of band II to band I remained constant (Table II). Because band I is weakly stained, its densitometry data are more scattered. Nevertheless, a plateau was reached at between 1.5 and 2 actins per gelsolin.

These titrations suggest that bands I and II are both derived from a 2:1 complex. To test this further, cross-linked proteins were gel filtered on Sephadex G150 in 5 mM imidazole hydrochloride, pH 7, 100 mM NaCl, and 0.1 mM $CaCl_2$. A single peak eluted to the high molecular weight side of the elution position for native gelsolin. SDS–PAGE analysis showed that both bands I and II were present across this peak. Furthermore, the ratio of II to I (3.9 ± 0.2) did not change significantly across the peak. Thus, in Ca^{2+} only complexes of gelsolin with actin dimers form. Band I, the 1:1 cross-linked complex, is an SDS–PAGE artifact due to incomplete cross-linkage of the ternary complex.

To estimate the affinity of gelsolin for actin in the 1:2 complex, the proteins were cross-linked over a wide range of concentrations. Figure 4 shows the amount of band II obtained relative to total gelsolin. In 0.1 mM $CaCl_2$, band II formation is essentially unchanged over a gelsolin concentration range from 50 nM to 5 μM. Band I (data not shown) also showed no significant variation with protein concentration. This suggests that the native complex from which bands I and II are obtained has a K_d well below 50 nM.

The effect of adding successive aliquots of fresh EDC during reaction was tested; 1:1 molar ratio mixtures of actin and gelsolin were reacted as follows: (1) control, 5 mM EDC for 1 h; (2) EDC to 5 mM added at 0, 15, 30, and 45 min, total

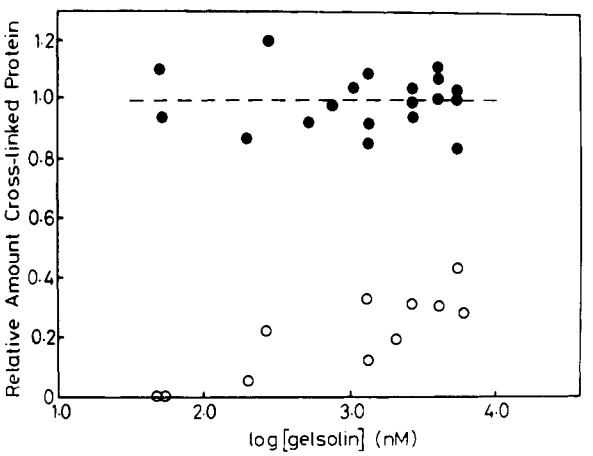

FIGURE 4: Dependence of the extent of band II formation on protein concentration. G-Actin and gelsolin were mixed at a 1:1 molar ratio and cross-linked with 5 mM EDC for 1 h at room temperature. Buffer conditions were as in Figure 1, except that 50 μM $MgCl_2$ was included in all samples and 0.5 mM EGTA was substituted for 0.1 mM $CaCl_2$ as indicated. Reaction was stopped in concentrated samples by gel sample buffer, and boiling, but for dilute samples, 2-mercaptoethanol was added to 50 mM before concentration with Amicon "Centricon 30" tubes and subsequent preparation for SDS–PAGE. The extent of cross-linking relative to total gelsolin in each samples was determined by gel densitometry and is expressed in arbitrary units, relative to the mean in Ca^{2+} as 1.0. (○) 0.5 mM EGTA; (●) 0.1 mM $CaCl_2$.

reaction time 1 h. SDS–PAGE showed little increase in the total amount of bands I and II formed but an undesirable amount of minor cross-linked species in sample 2. The ratios of bands II to I were 2.6 in the control and 3.0 in sample 2, a scarcely significant difference. The failure to achieve a higher level of cross-linking, or a greater proportion of band II, is not, therefore, due to shortage of cross-linker.

Cross-Linkage in the Absence of Ca^{2+}. When gelsolin was titrated with actin in EGTA, a plateau was reached at only about a third as much cross-linking as in Ca^{2+} but at about the same ratio of actin to gelsolin (i.e., 2:1) (Figure 3). The extent of cross-linking was not dependent on the presence or absence of an actin-stabilizing cation (50 μM $MgCl_2$) in EGTA. Band I was present at lower levels than band II over the whole range of actin concentrations tested (Table II). The ratio of II to I was always higher in EGTA than in Ca^{2+}; in three experiments (including that of Table II), the values were 4.3, 4.3, and 3.4 in EGTA and 2.4, 2.4, and 3.2 in Ca^{2+}, respectively; in the last example, the values are not significantly different for the two conditions.

The dependence of cross-linking on protein concentration also differed in the absence of Ca^{2+} (Figure 4). Formation of band II fell with protein concentration over the concentration range tested, and *no* cross-linking was observed below 100 nM gelsolin. This observation was tested particularly carefully; the two points on the abscissa in Figures 4 were obtained in independent experiments, and no cross-linked bands were visible on the gel. This implies that the association of gelsolin and actin into a cross-linkable complex is weaker in the absence of Ca^{2+}. So far as it could be determined, the behavior of band I paralleled that of band II in this experiment.

DISCUSSION

The major products of the reaction of gelsolin–actin complexes with the zero-length cross-linker EDC contain one gelsolin and one actin (band I) or one gelsolin and two actins (band II), respectively. Titration of gelsolin with actin shows that maximal production of both bands is obtained at greater than two actins per gelsolin, which implies that both are formed

by cross-linking within a native 2:1 complex. In Ca^{2+}, actins in the complex bind to gelsolin with high affinity; the extent of cross-linking is unaltered by reducing the gelsolin concentration to 50 nM, implying that the dissociation constants are less than this value. Bands I and II are both derived from the same noncovalent complex, and not from 1:1 and 2:1 complexes, respectively, because the ratio of the two bands is constant across a peak of gel-filtered, cross-linked complex. Similar, but un-cross-linked complexes, isolated by gel filtration in the presence of Ca^{2+}, have been shown to contain one gelsolin to two actins by densitometry of SDS–polyacrylamide gels (Weeds et al., 1985). Fluorescence titrations of plasma gelsolin with pyrenyl-G-actin also show an end point at 2:1 (Lees et al., 1984; Doi & Frieden, 1984); cytoplasmic gelsolins from macrophage (Yin et al., 1981b), platelets (Markey et al., 1982; Bryan & Kurth, 1984), and smooth muscle (Hinssen et al., 1984) similarly complex with two actin monomers in the presence of Ca^{2+}.

The extent of cross-linking to form bands I and II is low; only about 20% of the gelsolin is incorporated into bands I and II under optimal conditions, even with successive additions of EDC. The cross-linking of two polypeptides with EDC requires the very close apposition of amino and carboxyl groups, which might occur only in a minor conformational state. Successful cross-linking would be expected to trap this state, thus displacing the conformational equilibrium so that quite extensive cross-linking might ensue, but perhaps slowly. Since attempts to improve interchain bonding with successive EDC additions failed, it is likely that there are unfavorable competing reactions.

The cross-linking experiments show that the nature of the interaction between actin and gelsolin in Ca^{2+} dependent. In EGTA, the same pattern of cross-linking is obtained as in Ca^{2+}, that is, bands I and with band II predominating. The binding which gives rise to a cross-linkable complex is weaker, since cross-linking is abolished at low gelsolin concentrations (less than 100 nM). The simplest interpretation is that the same complex is formed between gelsolin and actin in the absence as in the presence of Ca^{2+}, but with lower affinity. However, the major product of approximately stoichiometric mixtures of the two proteins in EGTA has been shown by gel filtration to be a 1:1 complex (Weeds et al., 1985). Cross-linking of this binary complex would be expected to yield preominantly band I. Instead, band II was always formed at 3–5 times the amount of band I. If each band was formed from the equivalent native complex (ternary for band II and binary for band I), two predictions can be made. (1) The ratio of band I to band II should increase with decreasing actin concentration at constant gelsolin concentration. Instead, Table II shows that the ratio is approximately constant, even at substoichiometric actin concentrations. (2) The ratio of band I to band II would increase with decreasing gelsolin concentration (if the second actin bound more weakly than the first). However, in the experiment of Figure 4, both bands diminished to zero with decreasing gelsolin concentration.

The simplest conclusion from these data is that the EGTA-stable binary complex, demonstrated by gel filtration, does not cross-link with EDC. Only actin and gelsolin in the ternary complex cross-link, to give mixtures of bands I and II. Thus, cross-linkage is supposed to be a specialized reaction which does not reflect the prevalence of native complexes. This model is consistent with the data for the titration of gelsolin with actin in Ca^{2+} (Figure 3 and Table II). If addition of actins to gelsolin in Ca^{2+} is not cooperative, at very low actin:gelsolin ratios most of the complexes in solution would be 1:1 rather than 2:1. However, the ratio of band II to band I is independent of the actin%gelsolin ratio, even with gelsolin is present in large excess. Thus, either the cross-linking detects only the 2:1 complex or actin binding to gelsolin is cooperative; i.e., 2:1 is formed in preference to 1:1.

At low Ca^{2+} concentrations, the extent of formation of bands I and II reaches saturation at only about a third of the level obtained in 0.1 mM calcium (Figure 3). Such intermolecular cross-linking as is observed seems likely to be due to weak formation of the ternary complex, and, indeed, cross-linking may, by displacing an unfavorable equilibrium, exaggerate the formation of this minor speecies. It is not clear why band II formation does not increase, even very gradually, with actin concentration, as low levels of binding due to a high K_d should be overcome at higher protein concentrations. The apparent saturation of the reaction at a low level could be due to even more unfavorable competition from intrapolypeptide reactions than in Ca^{2+}.

Since successive EDC additions failed to convert band I into band II, it is possible that band I is an alternative to rather than a precursor of II. That is, formation of one peptide bond to give I may stabilize a conformation which excludes the formation of a second bond; formation of II must then be postulated to occur by the rapid formation of two cross-links, effective simultaneously, in a different conformational isomer.

Complex formation by plasma gelsolin contrasts in some respects with that by the cytoplasmic gelsolins, which do not interact with G-actin in the absence of Ca^{2+} (Yin et al., 1981b; Bryan & Kurth, 1984; Kurth & Bryan, 1984). However, the binary complex of platelet gelsolin with actin, once formed in Ca^{2+}, behaves similarly to the equivalent plasma gelsolin complex in that it cannot be dissociated by EGTA (Bryan & Kurth, 1984). The formation of an EGTA complex is therefore a property of both cytoplasmic and extracellular gelsolins. Both platelet and plasma gelsolin binary complexes require Ca^{2+} for the addition of a second actin (Bryan & Kurth, 1984; Weeds et al., 1985).

ACKNOWLEDGMENTS

I thank Drs. R. T. Tregear and A. G. Weeds for their helpful comments on the manuscript and B. J. Pope for his assistance with the radioiodination of gelsolin.

Registry No. EDC, 1892-57-5; Ca, 7440-70-2.

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Essential Light Chain Exchange in Scallop Myosin†

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Received February 8, 1985; Revised Manuscript Received May 20, 1985

ABSTRACT: The exchange of essential light chains (SH-LCs) of scallop myosin was followed with the aid of scallop SH-LC alkylated with ^{14}C-labeled iodoacetate. More than 70% of the SH-LCs were exchanged in myosin preparations that were desensitized by removal of both regulatory light chains (R-LCs) with ethylenediaminetetraacetic acid (EDTA) treatment. Although desensitized myosin solubilized with 0.6 M NaCl or with 10 mM adenosine 5′-triphosphate (ATP) in the absence of salt equilibrated rapidly with SH-LCs even in the cold, exchange in myosin filaments required elevated temperatures. Equilibration of the SH-LCs in desensitized preparations did not depend on ATP or magnesium ions but was significantly accelerated by actin. The desensitized myosin preparations containing alkylated SH-LCs (~1 mol of thiol alkylated/mol of SH-LC) readily recombined with R-LCs. The preparations regained fully the calcium dependence of the actin-activated magnesium adenosinetriphosphatase (Mg-ATPase), contained R-LCs and SH-LCs in equimolar amounts, and had an ATPase activity similar to that of untreated myosin preparations. R-LCs interfered with the equilibration of the SH-LCs. In intact myosin preparations, the exchange of SH-LCs was slow and was frequently associated with the dissociation of the R-LCs. The blocking action of the R-LC on SH-LC exchange agrees with evidence showing that the two light chain types interact and suggests that parts of the SH-LC may lie between the R-LC and the heavy chain of myosin.

Contraction of molluscan muscles is regulated by myosin, and activation results from the binding of calcium directly to myosin (Kendrick-Jones et al., 1970). The myosin subunits responsible for regulation are the regulatory light chains (R-LCs)[1] and the essential light chains (SH-LCs). Since the R-LCs of scallop myosin can be removed reversibly without loss of contractile activity, it was possible to establish directly that R-LCs are required for regulation: their removal abolishes completely the calcium sensitivity of the actin-activated ATPase or of tension generation (Szent-Györgyi et al., 1973; Simmons & Szent-Györgyi, 1978; Chantler & Szent-Györgyi, 1980).

The suggestion that the SH-LCs are also regulatory subunits is based on findings that antibody specific to the SH-LC desensitizes scallop myosin and that in its presence the actin-activated Mg-ATPase of myosin no longer requires calcium (Wallimann & Szent-Györgyi, 1981). The close proximity of R-LCs and SH-LCs in scallop myosin is indicated by several lines of evidence. Fab fragments of antibodies specific to R-LCs bind to the same region of the myosin molecule as the ones specific to the SH-LCs (Flicker et al., 1983), antibody specific to SH-LC interferes with the binding of R-LC to myosin (Wallimann & Szent-Györgyi, 1981), and the binding site for both R-LCs and SH-LCs is on the same proteolytic fragment of M_r approximately 14000 of the myosin heavy chain (Szentkiralyi, 1984) probably corresponding to the neck region of the myosin molecule (Bagshaw, 1977; Winkelman et al., 1984). Cross-linking studies have shown that SH-LCs and R-LCs overlap at least along half of their lengths and are separated by a distance of less than 9 Å (Wallimann et al., 1982). An interaction between R-LCs and SH-LCs in myosin is suggested by the observation that the R-LCs protect the SH-LCs from papain digestion (Stafford et al., 1979) and prevent the thiol groups of the SH-LC from reacting with thiol reagents (Hardwicke et al., 1982).

†This research was supported by grants from the U.S. Public Health Service (AM15963) and the Muscular Dystrophy Association (to A. G.S.-G.) and a fellowship from the Muscular Dystrophy Association (to G.A.).

[1] Abbreviations: R-LC(s), myosin regulatory light chain(s); SH-LC(s), scallop myosin essential light chain(s); A1 and A2, vertebrate myosin alkali light chains 1 and 2; S1, myosin subfragment 1; S2, myosin subfragment 2; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; DTE, dithioerythritol; Na-DodSO$_4$, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; HMM, heavy meromyosin; ATPase, adenosinetriphosphatase; Mg-ATPase, magnesium adenosinetriphosphatase.

0006-2960/85/0424-6618$01.50/0