Ca\textsuperscript{2+}-dependent Actin-binding Phosphoprotein in Physarum polycephalum

SUBUNIT b IS A DNase I-BINDING AND F-ACTIN CAPPING PROTEIN*

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Physarum contains at least four proteins of 42,000 daltons which are functionally distinguishable from each other (1–5). The major protein is actin which forms filaments by self assembly. The other three proteins, i.e. fragmin, Cap 42 (a), and Cap 42 (b), do not form filaments by themselves but regulate actin polymerization in the following manner. Fragmin is a Ca\textsuperscript{2+}-dependent F-actin severing protein which forms a 1:1 complex with actin monomer and causes a rapid fragmentation of actin filaments only in the presence of Ca\textsuperscript{2+} (1–3). Cap 42 (a) and Cap 42 (b) are two distinct subunits of a Ca\textsuperscript{2+}-dependent F-actin capping protein called Cap 42 (a + b) which caps or binds to the fast growing end of actin filaments, blocks actin polymerization at this end, and induces rapid depolymerization of the filaments at the opposite end (4, 5). Cap 42 (a + b) requires Ca\textsuperscript{2+} for its capping activity only when Cap 42 (b) is phosphorylated (5). When Cap 42 (a) and Cap 42 (b) are separated from each other in the presence of 7 M urea, the F-actin capping activity resides in Cap 42 (a) but not in Cap 42 (b) (5). Cap 42 (a) alone requires Ca\textsuperscript{2+} for its capping activity (5). However, unlike fragmin, Cap 42 (a) has no F-actin severing activity (5). Cap 42 (b) is a phosphoprotein whose phosphorylation is completely inhibited by an equimolar complex of Cap 42 (a) and actin only in the presence of Ca\textsuperscript{2+} (4). Neither Cap 42 (a), actin, or fragmin are phosphorylated by Cap 42 (b) kinase (4). When Cap 42 (b) is depolymerized, the capping activity of Cap 42 (a + b) becomes Ca\textsuperscript{2+}-independent, indicating that Cap 42 (a) requires two alternative activators, i.e. Cap 42 (b) or the depolymerized Cap 42 (b), for its capping activity (5).

Since actin was identified as a DNase I inhibitor and forms a tight 1:1 complex with DNase I (6, 7), DNase I-agarose has been widely used for the rapid purification of actin from a variety of organisms (8–10). Here we provide evidence for the occurrence of a second DNase I-binding protein in Physarum, i.e. Cap 42 (b), which is distinct from actin. DNase I completely inhibited the phosphorylation of Cap 42 (b) but in a Ca\textsuperscript{2+}-independent manner. Cap 42 (b) itself bound to DNase I-agarose even in the absence of Cap 42 (a) and actin. In addition, we show that Cap 42 (b) alone has an F-actin capping activity which becomes Ca\textsuperscript{2+}-dependent when Cap 42 (b) is phosphorylated.

MATERIALS AND METHODS

Cell Culture—Microplasmodia of Physarum polycephalum Colonia CL were used as the source for Cap 42 (a + b), Cap 42 (b) kinase, and actin preparations as previously described (4).

Buffers—AM buffer contained 30 mM Tris-HCl, pH 7.5, 40 mM \textsuperscript{32}P\textsubscript{ATP}, 5 mM Mg\textsuperscript{2+}, 1 mM dithiothreitol, and 15% sucrose. HC 10 buffer contained 10 mM Hepes-NaOH, pH 8.0, and 10 mM CaCl\textsubscript{2}. HC 100 buffer contained 100 mM Hepes-NaOH, pH 8.0, and 100 mM CaCl\textsubscript{2}. TEDA buffer contained 10 mM Tris-Cl, pH 7.5, 1 mM dithiothreitol, and 0.02% NaN\textsubscript{3}. TDSA buffer contained 15% sucrose in TDA buffer. TDS buffer contained 10 mM Tris-Cl, pH 7.5, 1 mM dithiothreitol, and 0.1 M NaCl. TEDA buffer contained 1 mM EGTA in TDA buffer.

Preparation of Hemoglobin-Agarose—200 mg of hemoglobin (Sigma, H-2000) was covalently coupled with 20 ml of Affi-Gel 10 (Bio-Rad) in HC 100 buffer according to the manufacturer’s manual.

Preparation of a Protease-free DNase I—Since DNase I from Sigma (D-4763) contains a high protease activity that cleaved actin and Cap 42 (b), 10 ml of DNase I solution (20 mg/ml) in HC 10 buffer were passed through a hemoglobin-agarose column (20 ml) and sub-

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DNase I-Agarose Affinity Chromatography of Cap 42 (a + b) and Cap 42 (b) Kinase—After precipitation with 3.0 M ammonium sulfate and dialysis against TDSA buffer containing 2 mM MgCl₂, the 150 mM NaCl eluate from DEAE-cellulose, containing Cap 42 (a + b) and Cap 42 (b) kinase, was further fractionated on 25 ml of DNase I-agarose equilibrated with TDA buffer containing 2 mM MgCl₂. Most of the Cap 42 (b) kinase activity was eluted with 1.0 M NaCl, and all of Cap 42 (a + b) was subsequently eluted with 8.8 M formamide in the above buffer. Separation of Cap 42 (a) and Cap 42 (b) by Hydroxylapatite Chromatography—The 8.8 M formamide eluate from DNase I-agarose, containing Cap 42 (a) and Cap 42 (b), was further fractionated on hydroxylapatite equilibrated with TDA buffer containing 8.8 M formamide by a stepwise elution with 75 mM, 150 mM, and 300 mM NaCl in TEDA buffer as described by Maruta et al. (4). All the Cap 42 (a + b) and the Cap 42 (b) kinase activity were recovered in the 150 mM NaCl eluate, whereas all the actin was recovered in the 300 mM NaCl eluate.

Separation of Cap 42 (a + b) and Cap 42 (b) Kinase from Actin—400 g of microplasmodia were extracted by 800 ml of sucrose buffer by a stepwise procedure as described for the preparation of hemoglobin-agarose. Separation of Cap 42 (a + b) and Cap 42 (b) Kinase from Actin—400 g of microplasmodia were extracted by 800 ml of sucrose buffer by a stepwise procedure as described for the preparation of hemoglobin-agarose. The sucrose extract was then fractionated on 300 ml (bed volume) of DEAE-cellulose equilibrated with TEDA buffer by a stepwise elution with 75 mM, 150 mM, and 300 mM NaCl in TEDA buffer as described by Maruta et al. (4). All the Cap 42 (a + b) and the Cap 42 (b) kinase activity were recovered in the 150 mM NaCl eluate, whereas all the actin was recovered in the 300 mM NaCl eluate.

DNase I-Agarose Affinity Chromatography of Cap 42 (a + b) and Cap 42 (b) Kinase—After precipitation with 3.0 M ammonium sulfate and dialysis against TDSA buffer containing 2 mM MgCl₂, the 150 mM NaCl eluate from DEAE-cellulose, containing Cap 42 (a + b) and Cap 42 (b) kinase, was further fractionated on 25 ml of DNase I-agarose equilibrated with TDA buffer containing 2 mM MgCl₂. Most of the Cap 42 (b) kinase activity was eluted with 1.0 M NaCl, and all of Cap 42 (a + b) was subsequently eluted with 8.8 M formamide in the above buffer. Separation of Cap 42 (a) and Cap 42 (b) by Hydroxylapatite Chromatography—The 8.8 M formamide eluate from DNase I-agarose, containing Cap 42 (a) and Cap 42 (b), was further fractionated on hydroxylapatite equilibrated with TDA buffer containing 8.8 M formamide by a stepwise elution with 75 mM, 150 mM, and 300 mM phosphate in the above buffer. Most of Cap 42 (b) was recovered in the 75 mM phosphate eluate, whereas most of Cap 42 (a) was recovered in the 150 mM phosphate eluate. Both fractions were subsequently dialyzed against TDSA buffer and kept on ice.

Further Purification of Cap 42 (b) Kinase—The 1.0 M NaCl eluate from DNase I-agarose, containing the Cap 42 (b) kinase, was further fractionated on Sephadex G-150 (superfine, 1.6 × 60 cm, Pharmacia) equilibrated with TDA buffer containing 0.1 M NaCl, 1 mM EGTA, and 2 mM MgCl₂. Most of Cap 42 (b) kinase activity was recovered in the protein fractions with a native molecular mass of approximately 35,000 daltons.

Purification of Phyrasm Actin—Actin in the 300 mM NaCl eluate from DEAE-cellulose was precipitated with 3.0 M ammonium sulfate in the presence of 2 mM MgCl₂-ATP and 0.2 mM CaCl₂ after two cycles of polymerization-depolymerization, the actin was further purified by gel filtration on Sephadex G-150 (4). The final actin preparation was at least 99% pure as judged by SDS-polyacrylamide gel electrophoresis and did not contain any detectable F-actin capping activity.

Assay for Cap 42 (b) Kinase Activity—10 µg of purified Cap 42 (b) were incubated with various kinase fractions at 35°C in AM buffer, and the incorporation of ³²P into a trichloroacetic acid-insoluble fraction was measured by the filter paper assay as previously described (4).

Assay for F-actin Capping Activity—The falling ball viscometer was used to monitor the F-actin capping activity of Cap 42 (a) and/or Cap 42 (b) which reduces the low shear viscosity of rabbit skeletal muscle actin (0.5 mg/ml) solutions as previously described (4). Short fragments of S-1 decorated and fixed actin filaments were used as nuclei to test for actin filament growth in order to determine which end of the actin filaments is capped by Cap 42 (b) according to the procedure described previously (5).

Polyacrylamide Gel Electrophoresis—SDS-polyacrylamide slab gels were prepared according to Laemmli (11), and stained with Coomassie blue R-250 (Gibco) according to Fairbanks et al. (12).

RESULTS
DNase I Inhibits the Phosphorylation of Cap 42 (b)—We have previously shown that (i) Cap 42 (a + b) is a heterodimer of two subunits (a and b), named Cap 42 (a) and Cap 42 (b), respectively, (ii) only Cap 42 (b) is phosphorylated at 2 threonine residues by a specific protein kinase partially purified from Phyrasm, and (iii) the phosphorylation of Cap 42 (b) is inhibited by a tertiary complex of Cap 42 (a), actin, and Ca²⁺ (see Ref. 4). As shown in Fig. 1, DNase I from bovine pancreas also inhibited the phosphorylation of Cap 42 (b) in Cap 42 (a + b) but in a Ca²⁺-independent manner. Since 1 or 2 mol of DNase I/mol of Cap 42 (a + b) were required for the complete inhibition of Cap 42 (b) phosphorylation, it is conceivable that the inhibition is due to the binding of DNase I to Cap 42 (a + b) rather than to Cap 42 (b) kinase.

DNase I-Agarose Affinity Chromatography of Cap 42 (a + b) and Cap 42 (b) Kinase—We used DNase I-agarose affinity chromatography to determine if Cap 42 (a + b) or Cap 42 (b) kinase bind to DNase I, and to purify both proteins. The new procedure for the rapid purification of these proteins is summarized below. The sucrose extract of Phyrasm was first fractionated on DEAE-cellulose to separate Cap 42 (a + b) from actin in the presence of EGTA, for the following two reasons: (i) actin also forms an equilibrium tight complex with DNase I, and (ii) in the presence of Ca²⁺, actin forms another equilibrium complex with Cap 42 (a + b) and completely inhibits the phosphorylation of Cap 42 (b). Most of Cap 42 (a + b) and Cap 42 (b) kinase were eluted with 150 mM NaCl from DEAE-cellulose. As previously shown, the 150 mM NaCl eluate contained no actin, since Cap 42 (b) phosphorylation of this fraction was not affected by Ca²⁺ unless actin was added (see Ref. 4). Instead, all the actin was eluted with 300 mM NaCl. Cap 42 (a + b) plus Cap 42 (b) kinase fractions from DEAE-cellulose were then further fractionated on DNase I-agarose. Although a small portion of Cap 42 (b) kinase activity appeared in the flow through fractions, all the F-actin capping activity and most of the Cap 42 (b) kinase activity were bound to DNase I-agarose (Figs. 2 and 3). The bound Cap 42 (b) kinase activity was subsequently eluted by 1.0 M NaCl (Fig. 3). Cap 42 (a + b), like actin, was only tightly bound to DNase I-agarose and was not eluted by 1.0 M NaCl (Fig. 2). However, 8.8 M formamide eluted almost exclusively Cap 42 (a + b) in addition to a trace amount of several other peptides (Figs. 2 and 4). The results from DNase I-agarose affinity chromatography confirmed that, in addition to actin, Cap 42 (a + b) is a DNase I-binding protein which is able to form a very tight complex with DNase I. Furthermore, Cap 42 (b) kinase itself was unable to bind to DNase I-agarose in the absence of Cap 42 (a + b) (data not shown), indicating that the kinase is not bound directly to DNase I but to Cap 42 (a + b).

Separation of Cap 42 (a) and Cap 42 (b) by Hydroxylapatite Chromatography—In order to remove trace amounts of contaminating proteins from the Cap 42 (a + b) fraction eluting with 8.8 M formamide from the DNase I-agarose, Cap 42 (a + b) was further purified on hydroxylapatite by a stepwise

FIG. 1. Inhibition of Cap 42 (a + b) phosphorylation by DNase I. After addition of DNase I at various concentrations, Cap 42 (a + b) (200 µg/ml) was phosphorylated by Cap 42 (b) kinase (20 µg/ml) at 35°C for 10 min in the presence or absence of Ca²⁺. 0.2 mM Ca²⁺; 0.1 mM EGTA. The 100% corresponds to 1.9 mol of phosphate/mole of Cap 42 (b). TCA, trichloroacetic acid.
by 11 times) were used to be assayed for the F-actin capping activity in the presence of that reduces the low shear viscosity of F-actin solutions in the presence of 0.2 mM Ca²⁺ (●). The flow through fractions (A), 1.0 M NaCl eluates (B), and 8.8 M formamide eluates (C) were pooled separately for further analysis.

Localization of Cap 42 (b) kinase activity in fractions eluting from DNase I-agarose. The pooled fractions A, B, and C (see Fig. 2) from DNase I-agarose (10 μl of each) were incubated at 35 °C for 15 min with or without Cap 42 (b) to be assayed for Cap 42 (b) kinase activity as described previously (4). The phosphorylation of Cap 42 (b) was detected by autoradiography after SDS-polyacrylamide gel electrophoresis of the phosphorylated samples. Lanes 1 to 4, in the presence of Cap 42 (b); Lanes 5 to 7, in the absence of Cap 42 (b). Lane 1, control; Lane 2, plus fraction A; Lane 3, plus fraction B; Lane 4, plus fraction C; Lane 5, fraction A alone; Lane 6, fraction B alone; Lane 7, fraction C alone. Cap 42 (b) alone (Lane 1) was only slightly phosphorylated by an endogenous kinase. However, Cap 42 (b) was highly phosphorylated by fraction B eluting with 1.0 M NaCl (Lane 3). Fraction A (Lane 2) also phosphorylated Cap 42 (b) but to a lesser extent. A minor phosphopeptide of 37,000 Da (Lane 3) was a proteolytic degradation product of Cap 42 (b) as previously shown (4).

elution with 75, 150, and 300 mM phosphate. As previously shown (see Ref. 4), Cap 42 (a + b) was almost quantitatively recovered in the 150 mM phosphate eluate if the protein was not treated with 8.8 M formamide. However, when treated with 8.8 M formamide during DNase I-agarose chromatography, Cap 42 (a + b) was dissociated into its subunits. Consequently, Cap 42 (b) eluted with 75 mM phosphate and Cap 42 (a) eluted with 150 mM phosphate from hydroxylapatite (Fig. 4).

DNase I Binds to Cap 42 (b) and Not to Cap 42 (a)—The direct binding of DNase I to Cap 42 (b) was shown in the following two ways: (i) the phosphorylation of Cap 42 (b) was almost completely inhibited by DNase I even in the absence of Cap 42 (a) (Fig. 5), and (ii) Cap 42 (b) alone bound to DNase I-agarose, whereas Cap 42 (a) alone did not bind at all under the same conditions (Fig. 6).

Cap 42 (b) Caps the Fast Growing End of Actin Filaments—We have previously shown that both Cap 42 (a + b) and Cap 42 (a) cap the fast growing end of actin filaments and block actin polymerization at this end (see Ref. 5). Using the same electron microscope technique which allows demonstrating of filament growth at the ends of S-1 decorated actin filament fragments, we found that in the presence of Cap 42 (b), actin polymerization occurred exclusively at the "pointed" slow growing end of actin filaments, and in no case at the "barbed"
described in the legend to Fig. 2. All of the Cap 42 (a) was recovered in flow through fractions, whereas all of the Cap 42 (b) was recovered in the 8.8 M formamide eluate.

fast growing end of the filaments (Fig. 7, b and c). This result indicates that Cap 42 (b), like Cap 42 (a), caps the fast growing end of actin filaments and blocks actin polymerization at the same end.

Effects of Phosphorylation on the F-actin Capping Activity of Cap 42 (b)—It has previously been shown that Cap 42 (a + b) requires Ca\(^{2+}\) for its F-actin capping activity when Cap 42 (b) is phosphorylated, and that its Ca\(^{2+}\) dependency is lost if Cap 42 (b) is dephosphorylated. Moreover, when separated from Cap 42 (b) in the presence of urea, Cap 42 (a) alone requires Ca\(^{2+}\) for its capping activity but Cap 42 (b) completely loses its capping activity. In contrast, when separated from Cap 42 (a) in the presence of 8.8 M formamide, Cap 42 (b) was fully active in capping actin filaments, and did not require Ca\(^{2+}\) for its capping activity (Fig. 8A). However, when phosphorylated, its capping activity became Ca\(^{2+}\)-dependent (Fig. 8B). Cap 42 (a) also required Ca\(^{2+}\) for its capping activity (Fig. 9A) even when separated from Cap 42 (b) in the presence of 8.8 M formamide. Interestingly, the maximum specific activities of both Cap 42 (a) and Cap 42 (b) were almost the same as that of Cap 42 (a + b) (Fig. 9B).

Further Evidence That Cap 42 (b) Is Not Identical with Actin—After prolonged storage, G-actin from both Physarum and rabbit skeletal muscle usually loses the ability to form filaments. Even in the presence of Ca\(^{2+}\), an aged and unpolymerizable preparation of Physarum actin showed no significant F-actin capping activity, when compared with Cap 42 (b) under the same conditions (data not shown). This result supported the functional differences between Cap 42 (b) and a denatured form of Physarum actin.

Actin from vertebrate smooth muscle and a few other sources was shown to be phosphorylated by the catalytic subunit of cAMP-dependent protein kinase (13). However, the catalytic subunit of cAMP-dependent protein kinase phosphorylated only the denatured (aged or digested), unpolymerizable actin preparations from Physarum or rabbit skeletal muscle and not the native, polymerizable actin (Fig. 10). Interestingly, the same kinase was unable to phosphorylate Cap 42 (b) (fresh or aged) (see Fig. 19), although the same preparation of Cap 42 (b) was fully phosphorylated by a specific Cap 42 (b) kinase from Physarum. These data have excluded the possibility that Cap 42 (b) is identical with this aged, unpolymerizable, form of actin.

DISCUSSION

In this paper we have shown that Cap 42 (b), i.e. subunit b of Cap 42 (a + b), is a DNase I-binding protein as well as an
were assayed in parallel in the presence or absence of Ca\textsuperscript{2+} under the phosphate eluate from hydroxylapatite) and Cap 42 (a 0.1 mM EGTA

capping activity of Cap 42 (b) with those of Cap 42 (a)
+ 0.1 mM EGTA

cGAP 42 (a, b) were separated by SDS-polyacrylamide gel electrophoresis, and the radioactivity of each protein band was visualized by autoradiography of the stained gel. A to F, Coomassie blue staining; a to f, autoradiogram. A to C or a to c, in the presence of the catalytic subunit of cAMP-dependent protein kinase; D to F or d to f, in the presence of Cap 42 (b) kinase. It should be noted that the catalytic subunit of cAMP-dependent protein kinase phosphorylated only the aged preparation of Physarum actin (c), whereas the Cap 42 (b) kinase phosphorylated only Cap 42 (b) (e). A faint band of 42,000 daltons just above the Cap 42 (b) band (B and E) indicates Cap 42 (a) as a slight contaminant in this preparation.

- Fig. 9. F-actin capping activity of Cap 42 (a) and Cap 42 (a + b) in the presence or absence of Ca\textsuperscript{2+}. To compare the F-actin capping activity of Cap 42 (b) with those of Cap 42 (a) (A) (=150 mM phosphate eluate from hydroxylapatite) and Cap 42 (a + b) (B) (= 8.8 M formamide eluate from DNase I-agarose), all three samples were assayed in parallel in the presence or absence of Ca\textsuperscript{2+} under the same conditions as described in the legend to Fig. 8. 0.02 mM Ca\textsuperscript{2+}, 1 mM EGTA.

F-actin capping protein. Although both Physarum actin and Cap 42 (b) are able to bind to DNase I and have practically indistinguishable molecular masses of 42,000 daltons, Cap 42 (b) is clearly distinguished from native (= polymerizable) actin because (i) Cap 42 (b) is unable to form a filament by self-assembly, and (ii) the Cap 42 (b) kinase does not phosphorylate actin regardless of whether the latter is native or denatured.

Theoretically, actin could be denatured and become unpolymerizable in a number of different ways during its storage, preparation, and even within cells. Hence, the question has been raised if Cap 42 (b) is one of these denatured forms of actin. However, there is no way of knowing precisely which type of denaturation of actin, if any, might create Cap 42 (b) until a conversion of actin to Cap 42 (b) could be demonstrated in vitro under defined conditions. Since the preparation of Cap 42 (b) was carried out mostly at 0 °C, we simply asked whether actin could be converted to Cap 42 (b) under identical conditions, e.g., by prolonged storage of actin at 0 °C. This treatment always creates a denatured, nonpolymerizable, form of actin which becomes phosphorylatable by cAMP-dependent protein kinase. However, unlike Cap 42 (b), the aged (or denatured), nonpolymerizable, actin is unable to cap the fast growing end of actin filaments and is not phosphorylated by Cap 42 (b) kinase. Furthermore, Cap 42 (b) is never phosphorylated by cAMP-dependent protein kinase, nor loses its capping activity even after prolonged storage. Therefore, we can exclude the possibility that Cap 42 (b) is identical with this aged (or denatured), nonpolymerizable form of actin. Although also highly unlikely, however, we can not absolutely exclude the possibility that actin has been converted into Cap 42 (b) in vivo by a post-translational modification(s). This modification, however, can not be simply an in vivo phosphorylation, for example, because the treatment of Cap 42 (b) by a variety of protein phosphatases does not restore any polymerizability.

It was previously shown that F-actin capping activity resides in Cap 42 (a) but not in Cap 42 (b) when the two subunits of Cap 42 (a + b) are separated from each other in the presence of 7 M urea (5). As shown in this paper, however, when Cap 42 (a + b) is dissociated into its subunits by 8.8 M formamide, both Cap 42 (a) and Cap 42 (b) show almost the same level of F-actin capping activity. These results indicate that F-actin capping activity of Cap 42 (b) but not of Cap 42 (a) is completely lost if they are treated with 7 M urea instead of 8.8 M formamide. Concerning the possible similarity between actin and Cap 42 (b) or between fragmin and Cap 42 (a) in respect of their stability in formamide and urea, it is of interest to note that the polymerizability of actin is rapidly lost in 7 M urea but retained in 8.8 M formamide (9), whereas both F-actin capping and severing activities of fragmin are quite stable even in the presence of 7 M urea (1–3).

We have found that Cap 42 (b) required Ca\textsuperscript{2+} for its F-actin capping activity when phosphorylated, but that the F-actin capping activity becomes Ca\textsuperscript{2+}-independent when it is dephosphorylated. This phosphorylation-dependent change in the Ca\textsuperscript{2+}-requirement of Cap 42 (b) for its capping activity appears to be the same whether Cap 42 (b) is complexed with Cap 42 (a) or not. Since Cap 42 (a), Cap 42 (b), and the equimolar complex show almost the same specific F-actin capping activities in the presence of Ca\textsuperscript{2+}, it is conceivable that both Cap 42 (a) and Cap 42 (b) are equally active in the complex. However, if we assume that in the dephosphorylated
state and in the absence of Ca\(^{2+}\), the F-actin capping activities of both Cap 42 (a) and Cap 42 (b) are independently expressed in the complex, we would expect that Cap 42 (a) is inactive and only Cap 42 (b) is active, resulting in half of the specific F-actin capping activity of the complex, in comparison with the activity expressed in the presence of Ca\(^{2+}\). In fact, however, the F-actin capping activity of the dephosphorylated complex is absolutely Ca\(^{2+}\) independent. This suggests the activation of the F-actin capping activity of one subunit by the other in the following two alternative ways: (i) dephosphorylated Cap 42 (b) fully activates the F-actin capping activity of Cap 42 (a) even in the absence of Ca\(^{2+}\) as we previously suggested (5) or (ii) Cap 42 (a) stimulates 2-fold the F-actin capping activity of dephosphorylated Cap 42 (b) at least in the absence of Ca\(^{2+}\), probably due to an increase in the affinity of the latter to actin.

It is also worth-while to note that Cap 42 (a) forms a very tight (1:1) complex with Cap 42 (b) (4, 5) as fragmin does with actin (1–3). However, Cap 42 (a) never formed such a tight complex with actin nor did fragmin with Cap 42 (b). This clearly indicates that Cap 42 (a) is not identical with fragmin nor Cap 42 (b) with actin, respectively. On the other hand, however, our preliminary data obtained from immunological analysis, tryptic peptide maps, and direct photoaffinity labeling by ATP of three of these four proteins of 42,000 daltons strongly suggest that Cap 42 (a) is, structurally and functionally, closely related to fragmin, while Cap 42 (b) is closely related to actin (14). Moreover, in order to establish the possible close relationships among these four proteins in Physarum, one should determine the primary sequences of fragmin, Cap 42 (a), and Cap 42 (b) in comparison with the known sequence of Physarum actin (15).

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REFERENCES

1. Hasegawa, T., Takahashi, S., Hayashi, H., and Hatano, S. (1979) Biochemistry 18, 2677–2683
2. Hinssen, H. (1981) Eur. J. Cell Biol. 23, 225–233
3. Hinssen, H. (1981) Eur. J. Cell Biol. 23, 234–240
4. Maruta, H., Isenberg, G., Schreckenbach, T., Hallmann, R., Risse, G., Shibayama, T., and Hesse, J. (1983) J. Biol. Chem. 258, 10144–10150
5. Maruta, H., and Isenberg, G. (1983) J. Biol. Chem. 258, 10151–10158
6. Lazarides, E., and Lindberg, U. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4742–4746
7. Mannherz, H. G., Barriargton Leigh, J., Leberman, R., and Pfang, H. (1975) FEBS Lett. 60, 34–38
8. Lindberg, U., and Eriksson, S. (1971) Eur. J. Biochem. 18, 474–479
9. Zechel, K. (1980) Eur. J. Biochem. 110, 337–341
10. Zechel, K. (1980) Eur. J. Biochem. 110, 343–348
11. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
12. Fairbanks, G., Steck, T. L., and Wallach, D. F. (1971) Biochemistry 10, 2606–2617
13. Walsh, M. P., Hinkins, S., and Hartshorne, D. J. (1981) Biochem. Biophys. Res. Commun. 102, 149–157
14. Maruta, H., Baltes, W., Knoerzer, W., and Isenberg, G. (1983) J. Cell Biol. 97, 373a
15. Vandekerckhove, J., and Weber, K. (1978) Nature (Lond.) 276, 720–721

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