Gelsolin Inhibits Nucleotide Exchange from Actin

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ABSTRACT: The effects of platelet gelsolin on the state and exchangeability of the nucleotide bound to skeletal muscle actin monomer have been investigated. In the presence of Ca^{2+}, a stable ternary complex consisting of two actins and one gelsolin is formed. Removal of Ca^{2+} from this species results in the formation of a highly stable binary gelsolin–actin complex. The interaction of gelsolin with actin monomer has no effect on the virtually negligible [<0.01 mol of $P_i \cdot h^{-1} \cdot (\text{mol of actin})^{-1}$] intrinsic ATPase activity of actin monomer (in the absence of Mg^{2+}). A single molecule of ATP is bound to the binary complex while two molecules of ATP are bound to the actins within the ternary complex. The ATP within the binary complex is nonexchangeable, and only one of the two ATP molecules in the ternary complex is exchangeable. In the latter case the rate constant for this nucleotide exchange is decreased compared to that for free actin monomer. These results demonstrate the nonequivalence of actin monomers within the ternary complex. The involvement of these oligomeric complexes of gelsolin and actin in the expression of the activity(ies) of gelsolin is discussed.

The protein actin is involved in a wide range of cellular activities, and the regulation of its structure is probably crucial to the normal expression of those activities [see reviews by Clarke and Spudich (1977), Pollard (1981), Buckley (1981), and Korn (1982)]. Gelsolin, a protein initially disovered in macrophages but now shown to be present in a wide range of mammalian cells, has been implicated in the regulation of actin polymer formation in vivo as a consequence of its effects on the ability of actin to polymerize in vitro (Yin & Stossel, 1979; Yin et al., 1980, 1981a,b; Wang & Bryan, 1981; Tellam & Frieden, 1982). Gelsolin has three major activities. First, gelsolin accelerates the initial rate of salt-induced actin polymerization from actin monomer (nucleation activity). Second, gelsolin binds to the normally fast-growing end of an actin filament and inhibits further elongation of the filament at that end (capping activity). Third, gelsolin has the ability to sever actin filaments (severing activity). Further, the severing and nucleating activities of gelsolin are greatly enhanced in the presence of micromolar or greater concentrations of calcium ions (Yin et al., 1980; Wang & Bryan, 1981; Harris & Weeds, 1983; Janmey et al., 1985).

Actin monomer has a single nucleotide binding site, which is typicaly occupied by ATP. In association with actin polymerization, but not as a prerequisite for it, is the hydrolysis of this bound ATP to give actin polymer bound ADP and free inorganic phosphate [see review by Korn (1982)]. The state and exchangeability of the nucleotide bound to monomeric and polymeric actin are important determinants in the rate and final extent of actin polymerization and in the exchange of actin monomers with the ends of actin filaments (Lal et al., 1984; Pantaloni et al., 1984). Thus, it is important to determine the effect of actin regulatory proteins, like gelsolin, on the state and exchangeability of the nucleotide bound to actin. The results reported herein demonstrate that gelsolin effects nucleotide exchange from actin monomer while having little effect on the small intrinsic ATPase activity of actin monomer (in the absence of Mg^{2+}). These results are discussed in terms of the regulation of actin polymer formation by gelsolin.

Experimental Procedures

Proteins. Rabbit skeletal muscle actin was isolated and purified according to the procedure described by Pardee and Spudich (1982) with the gel filtration (Sephadex G-150) modification of MacLean-Fletcher and Pollard (1980). Initially actin monomer was in a buffer consisting of 2 mM Tris, 200 μM ATP, 200 μM $CaCl_2$, and 1.5 mM NaN_3, pH 8.0 (G buffer),[1] and was subsequently dialyzed (three changes, 2 days, 4 °C) exchaustively against 2 mM Tris, 50 μM ATP, 50 μM $CaCl_2$, and 1.5 mM NaN_3, pH 8.0 (A buffer). This lengthy dialysis period is needed to ensure the equilibration of ATP solutions. At the end of this period, the actin monomer was fully capable of Mg^{2+}-induced polymerization (Tellam & Turner, 1984). Actin monomer was centrifuged (30 min, 178000*g*, Beckman Airfuge, room temperature) before use to remove any actin oligomers formed during the dialysis. The concentration of actin was measured spectrophotometrically at 290 nm by using an extinction coefficient of 0.63 $L \cdot g^{-1}$ (Houk & Ue, 1974) and a molecular weight of 42 300 (Elzinga et al., 1973).

Platelet actin was initially purified according to the procedure of Zechel (1980), followed by a cycle of actin polymerization and depolymerization to select for polymerization-competent actin monomer. This platelet actin was then dialyzed against A buffer as described above for skeletal muscle actin. The concentration of platelet actin was determined spectrophotometrically by using the same extinction coefficient as for skeletal muscle actin. The actin concentrations that were used in these studies were below the critical actin concentration for actin filament formation as assessed by the highly sensitive pyrene–actin fluorescence enhancement that occurs upon incorporation of this labeled actin into actin polymer (Tellam & Frieden, 1982; Tellam, 1985). Thus, the following results apply to actin monomer. (One exception is the measurement of the amount of P_i released from 9 μM actin in the presence of 0.5 mM Mg^{2+}. The critical actin concentration under these conditions is 5.7 μM. However, this measurement of P_i released was performed only 30 min after the addition of the

[1] Abbreviations: G buffer, 2 mM Tris-HCl, 200 μM ATP, 200 μM $CaCl_2$, and 1.5 mM NaN_3, pH 8.0; A buffer, 2 mM Tris-HCl, 50 μM $CaCl_2$, 50 μM ATP, and 1.5 mM NaN_3, pH 8.0; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; binary complex, actin–gelsolin; ternary complex, $actin_2$–gelsolin.

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Mg^{2+} to the actin when less than 2% of the total actin was polymeric.)

Platelet gelsolin was isolated and purified according to the procedure described by Tellam and Frieden (1982). After separation of the gelsolin from platelet actin by DE52 (Whatman) ion-exchange chromatography in the presence of 6 M urea, the gelsolin was concentrated in a Centricon (Amicon) cell (30000 molecular weight cutoff) and dialyzed against 2 mM Tris, pH 8.0, 0.1 mM PMSF, and 20 μM leupeptin at 4 °C. The final purity of this gelsolin was greater than 95% as judged by densitometry of a sample subjected to SDS–polyacrylamide gel electrophoresis. The concentration of platelet gelsolin was measured spectrophotometrically at 280 nm by using an extinction coefficient of 1.63 $L.g^{-1}$ (Kilhoffer & Gérard, 1985) and a molecular weight of 90000 (Wang & Bryan, 1981). This extinction coefficient was determined for brevin, a gelsolinlike protein present in serum, which has amino acid sequence homology with macrophage gelsolin and a similar amino acid composition (Yin et al., 1984). In addition, the concentration of platelet gelsolin determined in this manner agreed well with that obtained by the method of Lowry et al. (1951) with bovine serum albumin as a standard. A stable binary complex of platelet gelsolin and skeletal muscle actin can be isolated by incubation of the two proteins in A buffer (which contains 50 μM Ca^{2+}), followed by Sephadex G-150 chromatography in A buffer plus 5 mM EGTA. Densitometry of samples from the major chromatographic peak, which were subjected to SDS–polyacrylamide gel electrophoresis, showed a stoichiometry of 0.95 ± 0.07 (n = 4) (actin/gelsolin). In addition, a stable binary complex of platelet actin and platelet gelsolin can be isolated during the purification of platelet gelsolin by the procedure of Tellam and Frieden (1982). This platelet binary complex was then separated from free actin by Sephadex G-150 chromatography as described above. A ternary complex consisting of two actins and one gelsolin can be isolated (only in the presence of >1 μM Ca^{2+}) by the following procedure. A 2:1 molar ratio of skeletal muscle actin to gelsolin, respectively, was incubated for 30 min in A buffer. This sample was then subjected to Sephadex G-150 chromatography (equilibrated with A buffer), which showed that 88% of the protein was in a peak that had an actin:gelsolin molar ratio of 2.2 ± 0.3 (n = 5). This ratio was determined by densitometry of SDS–polyacrylamide gels of protein samples from this major peak. The concentrations of the binary and ternary complexes were determined by the method of Lowry et al. (1951).

ATP Hydrolysis. The hydrolysis of [γ-^{32}P]ATP was measured by determining the release of [^{32}P]P_i according to an adaption of the method described by Pollard and Korn (1973). [γ-^{32}P]ATP was purchased from Biotechnology Research Enterprises of Australia. The differing incubation times for the measurement of P_i released from actin, the binary complex, and the ternary complex in the absence (10 h) and presence (30 min) of 0.5 mM Mg^{2+} were required because of the very low ATPase activities of these species in the absence of Mg^{2+} and the desire to minimize free actin polymerization in the presence of 0.5 mM Mg^{2+}.

The nucleotide contents of the skeletal muscle muscle actin–gelsolin binary and ternary complexes as well as actin monomer were determined by the following method. The free ATP of protein solutions was removed by three treatments with Dowex I and then protein-bound nucleotide was released by 7% $HClO_4$ (Brenner & Korn, 1980). After centrifugation and adjustment of these protein-free solutions to pH 7.0 with a concentrated stock solution of Tris, the absorbance at 259 nm was measured against a suitable blank solution. The concentration of nucleotide was determined by using a molar extinction coefficient of 15400 M^{-1} cm^{-1}.

Densitometry. Protein samples were subjected to SDS–polyacrylamide gel electrophoresis (Laemmli, 1970) and then stained with Coomassie brilliant blue. The stained gel was then scanned by a spectrodensitometer (Schoeffel Instrument Corp.) at 550 nm. Individual peaks were cut out and weighed for comparisons of areas. The concentrations of actin and gelsolin applied to the gel were always carefully chosen so as to be in the linear response range. The ratio of peak areas for the same mass of gelsolin and actin was 1.08 (G:A), indicating that the dye bound equally well to both proteins.

Nucleotide Exchange. The fluorescence of 1,N^6-ethenoadenosine 5′-triphosphate (ϵ-ATP) is substantially enhanced when bound to the nucleotide binding site on actin (Waechter & Engel, 1975). Several studies have utilized this fluorescence change to measure the association and dissociation rates for ATP binding to monomeric actin [e.g., Waechter and Engel (1975, 1977)] and the influence of actin-binding proteins on nucleotide exchangeability (Mannherz et al., 1980; Nishida, 1985). The exchange of actin-bound ATP is a unimolecular reaction (Kuehl & Gergely, 1969; Waechter & Engel, 1975), and thus in the presence of a large excess of ϵ-ATP over ATP, the rate constant for the fluorescence increase, as ϵ-ATP is incorporated into actin, is a measure of the dissociation of ATP from actin monomer [see Nishida (1985)]. Fluorescence measurements were made in a Perkin-Elmer Model LS-5 luminescence spectrometer with a cell holder thermostated to 20 °C. The excitation wavelenth was set at 360 nm (slit width 5 nm) and the emission wavelength set at 410 nm (slit width 10 nm). There was no significant bleaching of the ϵ-ATP fluorescence during the time course of these experiments. The free ATP and free Ca^{2+} associated with actin monomer solutions in A buffer (50 μM each) were reduced by dilution of this actin sample into a buffer containing 2 mM Tris and 1.5 mM NaN_3, pH 8.0. Sufficient free Ca^{2+} and ATP (2 μM each) were still present after this dilution to prevent the irreversible denaturation of the actin monomer.

RESULTS

State of the Nucleotide on Actin in the Presence of Gelsolin. Incubation of actin monomer with platelet gelsolin, in the presence of Ca^{2+}, leads to the formation of a ternary complex (two actins and one gelsolin) that can be isolated by gel chromatography (also in the presence of Ca^{2+}). However, when the gel chromatography is performed in the absence of Ca^{2+} (i.e., <1 μM), a highly stable binary complex of gelsolin and actin is isolated (Wang & Bryan, 1981; Bryan & Kurth, 1984; Kurth & Bryan, 1984; my unpublished observations). Low concentrations of Mg^{2+} (<0.5 mM) have no effect on the formation of either of these complexes (unpublished results). Since the hydrolysis of actin-bound ATP is associated with actin polymerization (although this ATP hydrolysis is not an absolute requirement for polymerization as ADP–actin monomer can also polymerize; Korn, 1982), then hydrolysis of actin-bound ATP may also be occurring in association with the formation of the binary and ternary complexes of actin monomer and gelsolin. To test this possibility, the ATPase activity of skeletal muscle actin monomer solutions has been measured in the presence and absence of gelsolin (Table I).

The ATPase activities of monomeric actin, the binary complex of skeletal muscle actin and gelsolin, and the ternary complex of gelsolin and two skeletal muscle actin monomers were virtually negligible in A buffer. In the presence of 0.5 mM $MgCl_2$, there was a significant ATPase activity of

Table I: ATPase Activity of Skeletal Muscle Actin Monomer, Binary Complex, and Ternary Complex

	mol of P_i·(mol of actin)$^{-1}$·h^{-1} [a]	
	A buffer	A buffer + 0.5 mM $MgCl_2$
actin	0.01	0.25
binary complex (actin–gelsolin)	<0.01	<0.01
ternary complex (actin$_2$–gelsolin)	0.01	0.16

[a] Protein samples (each 9 μM) were incubated with A buffer or with A buffer plus 0.5 mM $MgCl_2$ for 10 or 0.5 h, respectively, at 20 °C in a total reaction volume of 1 mL. A buffer contained 50 μM [γ-^{32}P]-ATP (specific activity = 36 mCi/mmol). At the end of these incubations the amount of [^{32}P]P_i was determined and corrected for spontaneous ATP hydrolysis by a control experiment in which no protein was added. The amount of [^{32}P]P_i released was corrected for the actin concentration (9 μM for actin and the binary complex and 18 μM for the ternary complex) and for the differing incubation times in the absence and presence of 0.5 mM $MgCl_2$. Less than 2% of the free actin sample was polymeric after 30 min of incubation with 0.5 mM $MgCl_2$.

"monomeric" actin (greater than 98% monomer after 30 min of incubation with 0.5 mM Mg^{2+}) and of the ternary complex but little or no activity of the binary complex. The ATPase activity of the ternary complex could be due to one or both of the actin monomers in the complex. Since there is little ATPase activity associated with the binary complex, then the activity of the ternary complex is probably due to only one of its two actin monomers. In this case, the specific activity of that actin monomer is in reality 0.32 mol of P_i·(mol of actin)$^{-1}$·h^{-1} as a consequence of the actin stoichiometry within the ternary complex. This ATPase activity is slightly higher than that found for free actin "monomer" [0.25 mol of P_i·(mol of actin)$^{-1}$·h^{-1}] in the presence of 0.5 mM Mg^{2+}.

The nucleotide contents of the actin monomer, the binary complex, and the ternary complex were 0.93, 0.95, and 0.90 mol of nucleotide·(mol of actin)$^{-1}$, respectively. The method used for the determination of nucleotide in these samples does not distinguish between ADP and ATP. However, comparable experiments performed with [γ-^{32}P]ATP have demonstrated that the bound nucleotide consists of greater than 90% ATP and has a similar stoichiometry [~0.9 mol of ATP·(mol of actin)$^{-1}$] for all three protein samples (results not shown). This finding is consistent with the results shown in Table I, which demonstrate the virtually absent ATPase activity of the actin monomer and of the binary and ternary complexes in A buffer. Gelsolin itself did not bind ATP nor cause the hydrolysis of ATP (results not shown).

Effect of Gelsolin on the Nucleotide Exchange of Actin Monomer. The exchange of ε-ATP into the skeletal muscle actin monomer and into the stable binary and ternary complexes of skeletal muscle actin and gelsolin has been measured by following the fluorescence enhancement of ε-ATP that occurs when this molecule binds to actin. These nucleotide exchange experiments were all performed in the absence of Mg^{2+} so as to eliminate complications that arise from ATP hydrolysis. Figure 1 shows the kinetics of the fluorescence enhancement of ε-ATP in the presence of actin and the binary and ternary complexes. The large excess of ε-ATP, relative to ATP, which was used in these experiments, ensured that near complete exchange of nucleotide was achieved with the actin monomer. There was only a very slow and small fluorescence enhancement associated with the binary complex. Indeed, the final extent and first-order rate constant for the fluorescence change of the binary complex were only 6% and 2%, respectively, of the values for the same concentration of skeletal muscle actin. ATP is still bound to the binary complex

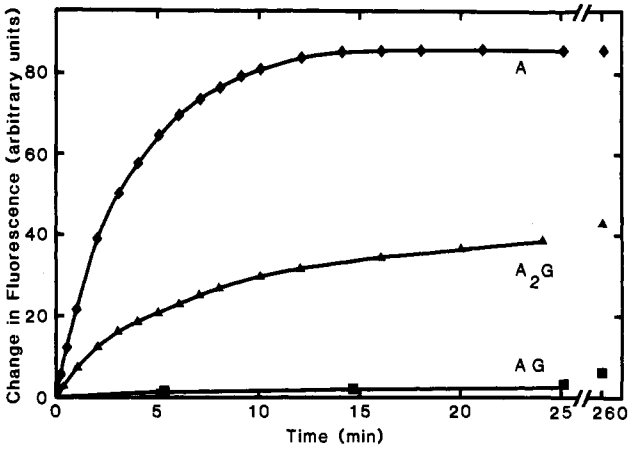

FIGURE 1: Time courses of nucleotide exchange for actin monomer, binary complex, and ternary complex. The nucleotide exchange was initiated by the addition of 50 μM ε-ATP to 600 nM skeletal muscle actin monomer (A, ♦), 600 nM binary complex (AG, ■), and 300 nM ternary complex (A_2G, ▲). Thus, in this experiment, a constant actin concentration was maintained for all samples. Each protein species was initially in A buffer and then diluted into 2 mM Tris and 1.5 mM NaN_3, pH 8.0. Sufficient CaATP was added to this buffer to supplement the "carryover" CaATP to a total concentration of 2 μM. The change in the fluorescence of ε-ATP as it binds to actin was monitored as described under Experimental Procedures. The final extent of the fluorescence changes is also shown.

even after several days of dialysis followed by chromatography (Sephadex G-150) of the complex in an ATP-free buffer (2 mM Tris, 50 μM $CaCl_2$, 1.5 mM NaN_3, 0.1 mM PMSF, 20 μM leupeptin, pH 8.0; results not shown). This observation suggests that the nucleotide bound to the actin in the binary complex is incapable of exchange, as further evidenced by the very low fluorescence enhancement of ε-ATP in the presence of this complex. This small but finite fluorescence enhancement may be due to a very low concentration of free actin present with the binary complex.

The final extent of the fluorescence enhancement for the ternary complex is 49% of that for the actin monomer alone. This result must be considered in relation to the presence of two actin monomers within the ternary complex and the lower molar concentration of the ternary complex (300 nM) compared with the binary complex and actin monomer (each 600 nM) used in this experiment. Thus, a simple explanation for this result is that only one of the two actins in the ternary complex has an exchangeable nucleotide. This result is consistent with the lack of nucleotide exchange into the binary complex. Alternatively, the final fluorescence enhancement of ε-ATP upon binding to both actins in the ternary complex may be altered to a value fortuitously similar to that for a single free actin monomer. However, studies with [γ-^{32}P]ATP have also demonstrated that only one of the two nucleotides bound to the ternary complex is exchangeable (result not shown). First-order plots of these time courses were linear (results not shown) and the observed rate constant for nucleotide exchange from the ternary complex was 0.12 min^{-1} compared with a value of 0.27 min^{-1} for the actin monomer.

Figure 2a shows the effect of increasing concentrations of gelsolin on the nucleotide exchange from a fixed concentration skeletal muscle actin monomer. Gelsolin, in a concentration-dependent manner, decreases the final extent of the fluorescence change. Figure 2b shows representative first-order plots of two of these time courses. These plots were always linear and thus were consistent with earlier reports (Kuehl & Gergely, 1969; Waechter & Engel, 1975) of a unimolecular dissociation of ATP from actin monomer. Figure 3 shows the

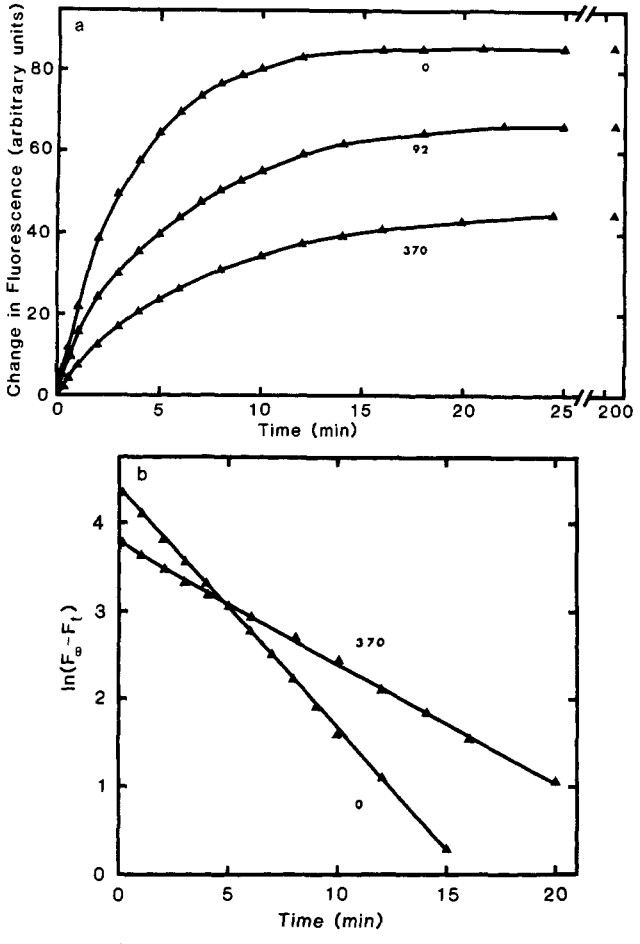

FIGURE 2: Effect of gelsolin on nucleotide exchange of skeletal muscle actin monomer. The conditions for these experiments are identical with those described in the caption to Figure 1. Actin monomer (600 nM) was preincubated with varying concentrations of gelsolin, for 5 min, before the addition of 50 μM ϵ-ATP. (a) Representative time courses of ϵ-ATP fluorescence changes for actin solutions containing 0, 92, and 370 nM gelsolin. (b) First-order plots of the time courses for 0 and 370 nM gelsolin. F_t is the change in fluorescence at time t, and F_∞ is the final change in fluorescence at 200 min.

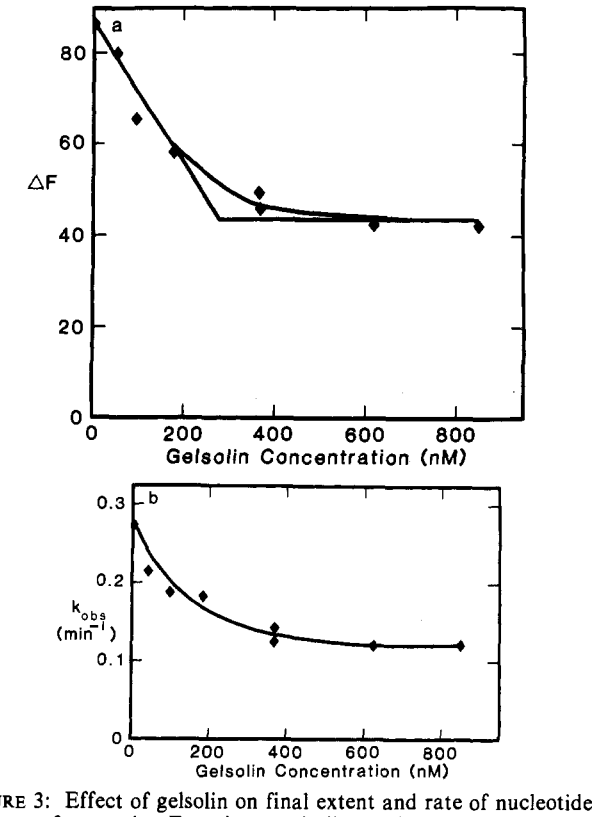

FIGURE 3: Effect of gelsolin on final extent and rate of nucleotide exchange from actin. Experiments similar to those described in the caption to Figure 2 were performed over a range of gelsolin concentrations (0–850 nM) at a fixed actin concentration (600 nM). (a) The final extent of the fluorescence change (ΔF) plotted aginst the gelsolin concentration. The lines that have been drawn represent the initial and final slopes. (b) The observed rate constant, k_{obsd} was plotted against the gelsolin concentration. The magnitudes of k_{obsd} were determined from the slopes of first-order plots similar to those shown in Figure 2b.

gelsolin concentration dependence of the final extent (ΔF) and of the apparent rate constant for nucleotide exchange (k_{obsd}), the latter being determined from the slopes of first-order plots such as those shown in Figure 2b. The gelsolin concentration dependence of ΔF is apparently biphasic, suggesting that it represents more a titration curve rather than a binding curve. Lines representing the initial and final slopes intersect at a gelsolin to actin stoichiometry of 0.48. This result demonstrates first that the binding interaction is very strong and second that about 50% of the actin in the presence of an excess of gelsolin is unable to exchange its nucleotide. Further, the apparent rate constant for nucleotide exchange is decreased by about 60% at saturation (Figure 3b). These results are very similar to those for the purified ternary complex, suggesting a strong predominance of this species in these actin–gelsolin mixtures.

The results of similar experiments performed with platelet actin, platelet gelsolin, and the isolated platelet actin–gelsolin binary complex were essentially identical with those reported in Figures 1–3 for platelet gelsolin and skeletal muscle actin (results not shown).

Discussion

It has been demonstrated previously that gelsolin interacts with the actin monomer in the presence of Ca^{2+} to form a ternary complex consisting of two actins and one gelsolin. Exposure of this ternary complex to a Ca^{2+}-free environment causes the dissociation of one actin molecule and the formation of a highly stable binary complex (Yin et al., 1981b; Bryan & Kurth, 1984; Kurth & Bryan, 1984). In addition, a stable binary complex has been also isolated from a number of cell types (Markey et al., 1982; Kurth et al., 1983; Kanno et al., 1985), and Kurth and Bryan (1984) have demonstrated that platelet activation results in the formation of a stable gelsolin–actin complex. The present results demonstrate that the EGTA-stable binary complex contains one actin-bound nonexchangeable ATP. Bryan and Kurth (1984) have reported that one molecule of Ca^{2+} is "trapped" when the binary complex is formed and cannot be removed by EGTA. The nonexchangeability of both ATP and Ca^{2+} in this complex suggests an interrelationship between these two cofactors. Indeed, actin monomer has a single high affinity bound Ca^{2+} ion that influences the exchange of ATP from monomer (Waechter & Engel, 1975). However, gelsolin additionally has two calcium binding sites, $K_d \sim 1$ μM (Yin & Stossel, 1980; Bryan & Kurth, 1984), and therefore the position of the trapped Ca^{2+} ion (on actin or gelsolin) is not clear. Further, any Ca^{2+} dependence of the nucleotide exchange from actin monomer in the presence of gelsolin is difficult to interpret because of the combined effects of this bivalent cation on actin monomer nucleotide exchange and on formation of the ternary complex. The ternary complex has a single exchangeable actin-bound ATP molecule. Presumably, the nonexchangeable site reflects the properties of the nucleotide trapped within the binary complex. This observation and the release of only one of the two actins from the ternary complex

in the absence of Ca^{2+} demonstrate the nonequivalence of the actin monomers within the ternary complex.

In the presence of 0.5 mM Mg^{2+} there is a significant ATPase activity associated with the ternary complex and little or no activity associated with the binary complex. This result may indicate that the single exchangeable nucleotide bound to the ternary complex is also capable of hydrolysis. It is difficult to compare the specific ATPase activity of the ternary complex with that for the actin monomer as the latter may have a small amount of actin polymer associated with it in the presence of 0.5 mM Mg^{2+}. However, it is clear that the specific activity of the whole ternary complex [0.16 mol of P_i·(mol of actin)$^{-1}$·h^{-1}] is substantially less than that for the actin "monomer" [0.25 mol of P_i·(mol of actin)$^{-1}$·h^{-1}] under these conditions.

In the presence of Ca^{2+}, there is a strong preference for the formation of the ternary complex compared with the binary complex as evidenced by the results shown in Figures 1 and 2 and gel chromatography studies (results not shown) as well as in other studies (Bryan & Kurth, 1984; Lees et al., 1984; Harris, 1985a,b; Coue & Korn, 1986). This result is in apparent contradiction to the known stability of the isolated EGTA-stable binary complex. Thus the EGTA-stable binary complex may be a quite distinct species from the putative binary complex intermediate formed during ternary complex formation in the presence of Ca^{2+}. Indeed, Kurth and Bryan (1984) have established that Ca^{2+} is required for the initial formation of the isolatable binary complex, but subsequently this complex is stable in the presence of EGTA although it contains one trapped Ca^{2+} ion. Further, the formation and isolation of the EGTA-stable binary actin–gelsolin complex from a number of different cell types may be simply due to the exposure of endogenous actin–gelsolin ternary complexes to calcium-free buffers used during the isolation procedures.

While this investigation was in progress, Harris (1985a) reported that plasma gelsolin, a protein similar to, but not identical with, platelet gelsolin, also forms a ternary complex with actin in the presence of Ca^{2+}. Further, in agreement with this study, it was shown that this complex contained two actin-bound ATP molecules, one of which was nonexchangeable. Even more recently, Coue and Korn (1986) reported that the ternary complex of plasma gelsolin and actin contains 1 mol/mol of actin-bound ATP. However, that study concluded that the ternary complex neither hydrolyzes the ATP (in the presence or absence of Mg^{2+}) nor exchanges it at a significant rate, a result differing from that reported herein and partially from that reported by Harris (1985a). Further studies will be required to determine whether these disparities result from the use of different "isoforms" of gelsolin or are perhaps due to differing purification procedures.

Gelsolin has the ability to accelerate the initial rate of Mg^{2+}-induced actin polymerization from actin monomer in the presence of Ca^{2+} (Tellam & Frieden, 1982). The ternary complex that is formed in the presence of Ca^{2+} is probably responsible for this effect by its ability to act as a nucleus for actin assembly (thereby circumventing or accelerating the normally rate-limiting formation of actin nuclei). However, elongation of the actin polymer would only occur in one direction due to the capping of the actin polymer by the presence of gelsolin on one end of the growing actin filament (Yin et al., 1981b; Harris & Weeds, 1984). In this regard the ternary actin$_2$–gelsolin complex may resemble one end of an actin filament. Indeed, it has been recently demonstrated that ATP is associated with actin protomers on the ends of actin filaments (Pantaloni et al., 1984, 1985), thereby suggesting a further similarity (in addition to nucleating activity and ATPase activity) between the ternary complex and a filament end. Indeed, the severing, nucleating, and capping activities of gelsolin may be regulated by the relative distributions of free gelsolin and the binary and ternary complexes within a cell. It is now recognized that, in the presence of Ca^{2+}, the EGTA-stable binary complex (Bryan & Coluccio, 1985; Janmey et al., 1985) and the ternary complex (Coue & Korn, 1986) are unable to sever actin filaments but are able to cap actin filaments at their fast-growing ends, whereas free gelsolin has both of these activities.

Activation of platelets by agents such as thrombin leads to an elevation of the intracellular free calcium ion concentration (Rink et al., 1982). Kurth and Bryan (1984) have suggested that this rise in calcium activates the formation of gelsolin–actin complexes, which may then initiate the rapid and site-specific actin polymerization accompanying platelet activation (Carlsson et al., 1979; Phillips et al., 1980).

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Depolymerization of Microtubules Alters Membrane Potential and Affects the Motional Freedom of Membrane Proteins

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ABSTRACT: Two independent lines of evidence were obtained indicating that microtubule depolymerization affects the functions and the physical state of membranes in intact Chinese hamster ovary cells. The first type of evidence was obtained by using the dye dihexyloxacarbocyanine iodide to measure membrane potential before and after treatment with several microtubule active agents. Microtubule depolymerization resulted in a decrease in cell fluorescence, whereas stabilization of microtubules with taxol resulted in an increase in cell fluorescence. These effects of the drugs were due to their interactions with microtubules and not to direct effects of the drugs on the plasma membranes for the following reasons: (1) effects were time dependent and required entry into the cells as indicated by the lack of fluorescence change in a multidrug-resistant mutant that does not accumulate antimicrotubule drugs and (2) a colcemid-resistant tubulin mutant did not show these effects on cell fluorescence. Evidence for altered motional freedom of membrane proteins in the plasma membrane was obtained by using electron spin resonance analysis of maleimide spin probe labeled cells. This study showed that depolymerization of microtubules results in increased motional freedom of maleimide-labeled sulfhydryl group containing proteins. Taken together, these data argue that microtubules function in mammalian cells to regulate the physical state of membranes and modulate membrane potential generated across cell membranes.

Several lines of evidence suggest that the microtubule network may interact with membrane components, modulating membrane potential (Vassilev et al., 1985), hormone responsiveness (Insel & Kennedy, 1978; Hagmann & Fishman, 1980), capping of receptors (Albertini & Clark, 1975; Malawista et al., 1978), and exocytosis (Wehland et al., 1982). Direct association of microtubules or tubulins with cell membrane components has been observed (Bhattacharya & Wolff, 1975; Bernier-Valentin et al., 1983; Pfeffer et al., 1983; Collot et al., 1984), and earlier studies have demonstrated that the microtubule system can directly influence plasma membrane dynamics such as microviscosity and fluidity (Berlin & Fera, 1977; Aszalos et al., 1985); however, the mechanism by which microtubules modulate different membrane functions is not yet fully understood.

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