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Immunochemical Probing of the N-Terminal Segment on Actin: The Polymerization Reaction†

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Received August 23, 1989; Revised Manuscript Received November 27, 1989

ABSTRACT: The N-terminal segment of actin contains a cluster of acidic residues which are implicated in macromolecular interactions of this protein. In this work, the interrelationship between the N-terminal segment and the polymerization of actin was studied by using affinity-purified antibodies directed against the first seven N-terminal residues on α-skeletal actin (SαN). The F_{ab} fragments of these antibodies showed equal affinities for G- and F-actin while the bivalent IgG bound preferentially to the polymerized actin. As monitored by pyrene fluorescence measurements, the binding of F_{ab} to G-actin did not alter the kinetics of the $MgCl_2$-induced polymerization; IgG accelerated this reaction considerably. Consistent with these observations, the binding of F_{ab} to F-actin did not change its morphological appearance in electron micrographs and had no effect on the stability and the rate of dissociation of actin filaments. These results are discussed in terms of their implications to the spatial relationship between the N-terminal segment and the rest of the molecule and in the context of the polymerization reaction of actin in vitro and in vivo.

The regulation of actin polymerization and depolymerization through its interactions with a large number of proteins (Stossel et al., 1985; Pollard & Cooper, 1986) appears central to the function of actin in nonmuscle cell motility. The interest in the molecular mechanism of these interactions has stimulated numerous studies which attempt to map the binding sites between actin and actin-binding proteins (Tellam et al., 1989). A frequent approach in these investigations, initiated by Mornet et al. (1981), was to detect the protein–protein contact sites by cross-linking the protein complexes with carbodiimide. In most cases, such experiments implicated the acidic N-terminal residues on actin in the interactions with actin-binding proteins (Sutoh, 1982; Muneyuki et al., 1985; Sutoh & Mabuchi, 1986; Sutoh & Hatano, 1986; Hambley et al., 1986; Doi et al., 1987). The profound effect that many of these proteins have on the polymerization and depolymerization of actin filaments raises the possibility that the conformation or the net charge of the N-terminal segment influences the formation and stability of F-actin.

A convenient tool for testing the relationship between actin's N-terminus and the polymerization of this protein has become available with the preparation of site-specific antibodies (SαN)[1] directed against the N-terminal segment on actin (Bulinski et al., 1983; Mejean et al., 1986, 1987a; Miller et al., 1987). The SαN antibodies raised against a synthetic peptide corresponding to the first seven N-terminal residues on skeletal α-actin show specific interaction with the N-terminal segment in both monomeric and assembled actin and do not bind to other sites on this protein (Miller et al., 1987). The antibodies completely inhibit actomyosin interactions in the presence of MgATP (DasGupta & Reisler, 1989) but do

† This work was supported by Grant AR22031 from the National Institutes of Health, Grants DMB 85-0857 and DCB 89-40564 from the National Science Foundation, and Grant 877IG2 from the American Heart Association, Greater Los Angeles Affiliate.

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[1] Abbreviations: pyrene-labeled actin, actin modified at Cys-374 with *N*-(1-pyrenyl)iodoacetamide; S-1, myosin subfragment 1; SαN antibodies, peptide antibodies raised against the first seven N-terminal residues of α-skeletal actin; ELISA, enzyme-linked immunosorbent assay; phosphate-buffered saline, phosphate buffer containing 171 mM NaCl, 3.35 mM KCl, 10.1 mM Na_2HPO_4, and 1.84 mM KH_2PO_4, pH 7.4, and, when indicated, 0.05% Tween 20 and 0.1% BSA; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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not block them in the absence of the nucleotide (Mejean et al., 1987a; Miller et al., 1987). In this work, we report that the F_{ab} fragments of these antibodies bind equally well to G- and F-actin, do not affect the stability and appearance of actin filaments, and do not have any significant effect on the Mg^{2+}-induced polymerization of actin. These results confirm the surface location of actin's N-terminus and suggest that it is distinct from the inter-actin interface in the filament. Not surprisingly then, it appears also that interactions with the N-terminus may be a necessary but not a sufficient condition for the regulation of actin polymerization by various effectors.

Materials and Methods

Reagents. Distilled and Millipore-filtered water and analytical-grade reagents were used in all experiments. Papain, alkaline phosphatase conjugated goat anti-rabbit IgG, cyanogen bromide activated Sepharose, alkaline phosphatase substrate, Freund's adjuvent, TLCK-treated α-chymotrypsin, and ATP were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-(1-Pyrenyl)iodoacetamide was obtained from Molecular Probes (Junction City, OR). The synthetic peptide Ac-Asp-Glu-Asp-Glu-Thr-Thr-Ala-Tyr used for immunization of rabbits was purchased from the custom peptide synthesis facility at the University of California, San Diego. The specific activity of the ^{14}C-labeled peptide was 35 cpm/nmol. Keyhole limpet hemocyanin was from Calbiochem (La Jolla, CA). Bradford protein assay solution and protein A–agarose were from Bio-Rad (Richmond, CA). ELISA plates (Dynatech Immulon I) were purchased from Fisher Scientific Co.

Preparation of Proteins. Skeletal muscle actin was prepared in G-actin buffer (0.5 mM β-mercaptoethanol, 0.2 mM ATP, 0.2 mM $CaCl_2$, and 5 mM Tris, pH 7.6) by the procedure of Spudich and Watt (1971). Gel-filtered actin was obtained as described by MacLean-Fletcher and Pollard (1980). Myosin was prepared as described by Godfrey and Harrington (1970). Subfragment 1 (S-1) was prepared by chymotryptic digestion of myosin according to the method of Weeds and Pope (1977).

Preparation of Actin Oligomers. The covalently cross-linked actin oligomers were prepared by reacting F-actin with *p*-phenylenebis(maleimide) according to Tobacman and Korn (1983). The cross-linked actin was used without any fractionation. SDS–PAGE analysis (Laemmli, 1970) of these samples revealed in decreasing abundance the presence in solution of actin monomers, dimers, trimers, and higher order species. The protein concentration of solutions of actin oligomers was measured by the Bradford technique (1976).

Preparation of Peptide Antibodies. Polyclonal antibodies against the synthetic peptide Ac-Asp-Glu-Asp-Glu-Thr-Thr-Ala-Tyr, corresponding to the first seven N-terminal amino acid residues of skeletal muscle actin (Vandekerchove & Weber, 1978a–c), were raised in rabbits as described previously (Miller et al., 1987). The actin-specific IgG and F_{ab} (SαN IgG and SαN F_{ab}) were purified by chromatography through a series of affinity columns as described previously by Miller et al. (1987), quick-frozen with dry ice–acetone, and stored at −70 °C. The titer of these antibodies was checked in ELISA.

Immunochemical Assays. Enzyme-linked immunosorbent assays (ELISA) were performed according to the method of Atherton and Hynes (1981). For competitive immunoassays, the ELISA plates were coated with skeletal muscle G- or F-actin and incubated overnight at 4 °C. Different amounts of the competing antigens, either G- or F-actin, were added to triplicate wells along with affinity-purified F_{ab} or IgG (0.4 μg/well each) and incubated for 2 h at 37 °C. To eliminate nonspecific binding of antibodies to the wells, the microtitration plates were incubated with 5% BSA in phosphate-buffered saline and 0.05% Tween 20 prior to the addition of antibodies. The plates were then washed several times and incubated with alkaline phosphatase conjugated goat anti-rabbit IgG (1:1000 dilution in PTB) for 2 h at 37 °C. After a final washing, the alkaline phosphatase substrate (1 mg/mL in 1 M diethanolamine) was added to the wells, and the developed color was measured at 410 nm in a Dynatech MR600 microplate reader.

Pyrene Labeling of Actin. Pyrene-labeled actin was prepared by the method of Cooper et al. (1983). The extent of labeling was measured by using a molar extinction coefficient of $\epsilon_{344} = 2.2 \times 10^4\ M^{-1}\ cm^{-1}$ for the protein–dye complex (Kouyama & Mihashi, 1981). The concentration of pyrene-labeled actin was measured in Bradford assays (1976).

Fluorescence and Light-Scattering Measurements. Fluorescence intensities were measured in the Spex Fluorolog spectrophotometer (Spex Industries, Inc., Edison, NJ) as previously described (Miller et al., 1988). The excitation monochromator was set at 368 nm and the emission monochromator at 407 nm. Enhancement of pyrene fluorescence was monitored as an indicator of actin polymerization (Kouyama & Mihashi, 1981). A small amount of pyrene-labeled actin (between 0.3 and 0.46 μM) was combined with G-actin and affinity-purified SαN IgG or SαN F_{ab} in G-actin buffer. Polymerization of actin was induced by the addition of 2 mM $MgCl_2$ to the cuvette and was monitored by pyrene fluorescence measurements in a ratio mode. Prior to the fluorescence measurements, both F_{ab} and IgG were dialyzed against G-actin buffer and centrifuged for 10 min in an Eppendorf 5414 table-top centrifuge.

Light-scattering measurements at 90° were conducted in the Spex Fluorolog spectrophotometer at 310 and 660 nm at 25 °C.

Fluorescence intensity and light-scattering data are presented in arbitrary units and can be compared mainly for experiments shown in the same figure.

Concentration Determinations. Protein concentrations were determined spectrophotometrically by using the following extinction coefficients at 280 nm: myosin, $E^{1\%} = 5.55\ cm^{-1}$; actin, $E^{1\%} = 11.0\ cm^{-1}$; S-1, $E^{1\%} = 7.50\ cm^{-1}$; IgG, $E^{1\%} = 15.0\ cm^{-1}$; F_{ab}, $E^{1\%}$ in $16.0\ cm^{-1}$.

Electron Microscopy. G-Actin solutions (12 μM) were polymerized by either 2 mM $MgCl_2$ or 2 mM $MgCl_2$ and 0.1 M KCl in the absence and presence of stoichiometric amounts of SαN F_{ab} (12 μM) for 30 min at room temperature. Higher concentrations of F_{ab} were avoided in these experiments because of the resulting preponderant presence of unbound antibodies on the grids. Cross-linking of acto–F_{ab} complexes with carbodiimide (20 mM, up to 60 min at room temperature) produced only small amounts of cross-linked products as assayed by SDS–PAGE (not shown). All actin samples were diluted with Millipore-filtered solvent containing 10 mM imidazole pH 7.0, 10 mM KCl, and 2 mM $MgCl_2$ to a final concentration of 3 μM immediately prior to grid preparation. Electron microscope grids were prepared and examined as described previously (Miller et al., 1988).

Results

Binding of SαN Antibodies to G- and F-Actin. Earlier work has established that antibodies prepared against the N-terminal peptide of skeletal actin (SαN) have high reactivity toward actin and are specific for its N-terminal segment (Bulinski et al., 1983; Mejean et al., 1987a; Miller et al., 1987). As verified by dot blot experiments (Miller et al., 1987), the SαN antibodies could bind to G- and F-actin and denatured forms of

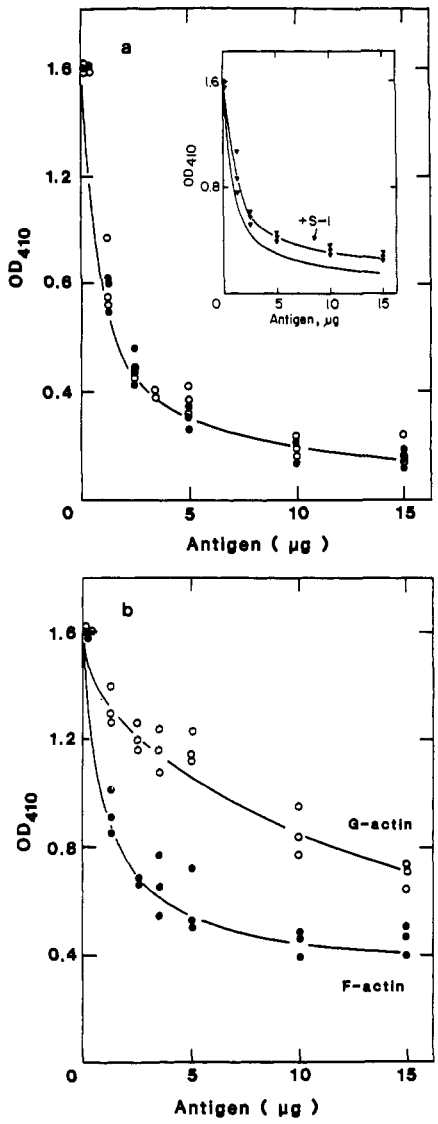

FIGURE 1: (a) Competition ELISA of the binding of SαN F_{ab} to an adsorbed actin in the presence of G- and F-actin. The experimental details of immunoassays are given under Materials and Methods. Affinity-purified F_{ab} (0.4 μg/well) was added in the presence of G-actin (●) and F-actin (○), in their respective solvents, to microtitration plates coated with F-actin. G-Actin solvent contained 5 mM Tris, pH 7.6, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, and 0.2 mM ATP. F-Actin solvent contained in addition to that 2 mM $MgCl_2$. OD readings at 410 nm monitor the amounts of SαN bound to actin adsorbed to the microtitration wells. The inset shows the comparative competitions of F-actin and acto-S-1 for F_{ab} (against the adsorbed actin). (b) Competition ELISA of the binding of SαN IgG to an adsorbed actin in the presence of G- and F-actin. Affinity-purified IgG (0.4 μg/well) was allowed to bind to actin immobilized in the wells in the presence of competing G-actin (○) or F-actin (●).

actin adsorbed to nitrocellulose.

The solution interactions between SαN F_{ab} and G- and F-actins were compared by ELISA titrations. G- and F-actin competed equally well against adsorbed F-actin for the SαN antibodies (Figure 1a). This result was independent of the F_{ab} concentration employed in the competitive ELISA titrations and suggested similar binding affinities of SαN F_{ab} for G- and F-actins.

The sensitivity and validity of competitive ELISA were tested in two types of measurements. The first one, presented in the inset to Figure 1a, reveals a rather small effect of S-1 on the competition for F_{ab} between F-actin in solution and that adsorbed to the wells. This result agrees well with the findings of previous direct immunoassays (Mejean et al., 1987a). The second experiment (Figure 1b) demonstrates that competitive ELISAs readily detect differences in antibody–antigen affinities. As expected, the bifunctional SαN IgG binds better to F-actin than to G-actin. A similar distinction between G- and F-actin was also observed in competitive ELISA titrations utilizing the unfractionated antiserum.

The binding of F_{ab} to actin did not affect significantly the stability and structure of thin filaments. The addition of saturating amounts of F_{ab} (12 μM) to F-actin (3 μM) (Miller et al., 1987) containing small amounts of pyrene-labeled protein (at Cys-374) did not produce any changes in pyrene fluorescence. Under the same conditions, the binding of F_{ab} to actin increased the 90° light scattering from actin solutions by a factor of 2.5. This change occurred within the mixing time of the samples, and the resulting readings were stable over the standard observation times (30 min). Thus, the observed increase in light scattering could be ascribed to changes in the molecular weight of actin filaments upon formation of F_{ab}–actin complexes.

Electron micrographs of F_{ab}–actin complexes (Figure 2) showed normal actin filament structures and did not reveal any cross-linking, bundling, or fragmentation of F-actin by F_{ab}. The "decoration" of actin filaments by F_{ab}, although infrequent, could easily be detected and did not show any preferred orientation of antibodies with respect to the thin filaments. The low degree of decoration was probably caused by grid preparation procedures, the affinities of F_{ab} for actin, and the inefficient cross-linking of F_{ab} to actin by carbodiimide. Yet even at low densities of decoration, the F_{ab} tended to cluster on actin filaments (Figure 2). In contrast to F_{ab}, IgG produced bundling of actin filaments on EM grids. The IgG caused also a continuous and large increase in the light scattering of F-actin solutions, indicative of filament cross-linking by the antibodies.

Taken together, the light scattering, pyrene fluorescence, and electron microscopy experiments showed that F_{ab} did not perturb in any significant way the polymerization equilibrium of actin. The actual binding of F_{ab} to F-actin in these experiments was verified by the copelleting of these proteins in airfuge centrifugations. As was found earlier (Miller et al., 1987), F-actin was saturated by F_{ab} under the conditions employed in the present study.

Polymerization of Actin in the Presence of SαN Antibodies. The rates of polymerization of actin by 2 mM $MgCl_2$ in the presence and absence of antibodies were followed by the increase in the fluorescence of pyrene-labeled actin. The validity of this approach is confirmed in Figure 3a, which documents the effect of SαN IgG on the polymerization of actin by $MgCl_2$. Because of the bivalent nature of IgG and its consequent higher affinity for F-actin than G-actin (Figure 1b), it was expected that these antibodies would accelerate the rates of actin polymerization. Figure 3a shows that IgG indeed has a striking effect on the Mg^{2+}-induced polymerization of actin; it shortens the initial lag phase which is normally associated with the nucleation step of the assembly reaction. Since the antibodies did not alter the fluorescence of pyrene–F-actin, the IgG-induced changes in the fluorescence of the monomeric protein could be ascribed to an accelerated polymerization of actin.

In contrast to the bivalent IgG, F_{ab} had no effect on the polymerization of filtered and unfiltered actin. Essentially the same rates of assembly were observed for the free G-actin and in the presence of up to saturating amounts of SαN F_{ab} (Figure 3b), i.e., at a 4:1 molar ratio of F_{ab} added to actin (which yields a binding stoichiometry of 0.9 F_{ab}/actin; Miller et al., 1987).

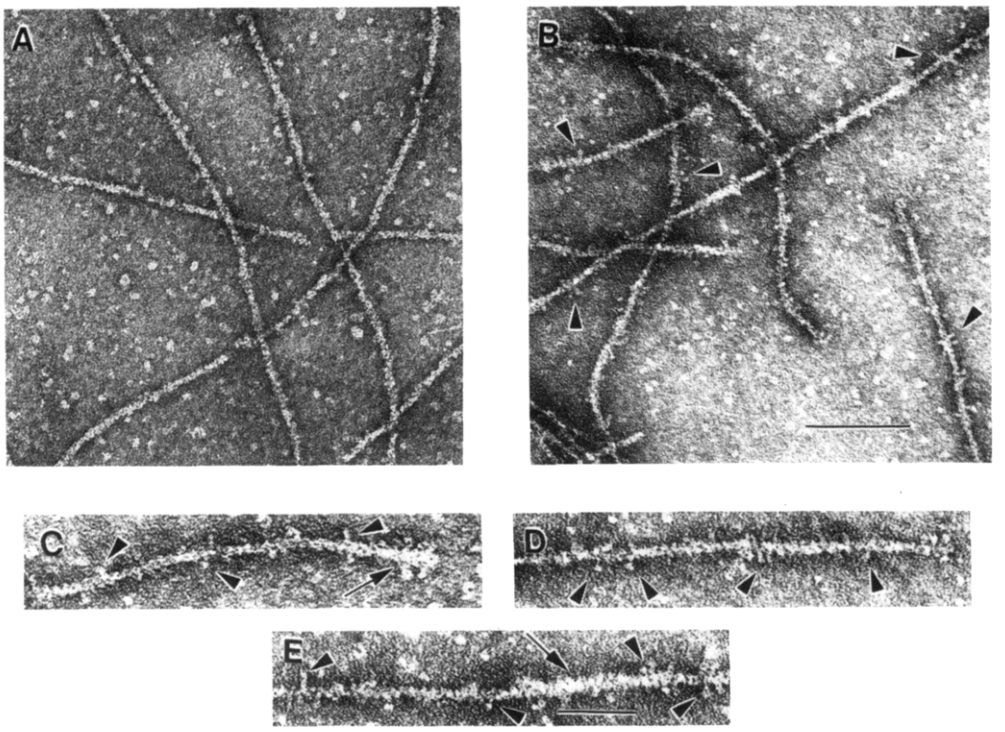

FIGURE 2: Electron microscopy of F-actin and F-actin decorated with SαN F_{ab}. Samples were prepared as described under Materials and Methods and diluted to a final actin concentration of 3 μM just before grid preparation. (A) F-Actin control; (B) F-actin + F_{ab} cross-linked with 20 mM carbodiimide for 60 min. The arrowheads indicate some of the bound F_{ab}. The bar represents 1000 Å. (C–E) Higher magnification pictures showing the bound F_{ab} more clearly. Arrowheads indicate some of the bound F_{ab}. The arrows indicate regions of the actin filament where the stain becomes more diffuse, possibly because F_{ab} is protruding out of the plane of the electron microscope grid. The bar represents 500 Å.

In order to check more directly whether the elongation step in the formation of F-actin was affected by F_{ab}, the polymerization experiments were done also in the presence of cross-linked oligomers of actin. When added in small amounts (>5% w/w) to G-actin, the *p*-phenylenebis(maleimide)-cross-linked dimers and trimers result in "seeded" elongation of actin filaments and the elimination or great shortening of the nucleation step in the assembly reaction (Tobacman & Korn, 1983). This is demonstrated by the comparison between curves A and C in Figure 4. As shown in the same figure (curves A and B), the "seeded" elongation was not affected by the binding of F_{ab} to actin and proceeded at the same rates in the presence and absence of antibodies.

Light-scattering measurements of actin polymerization in the presence and absence of F_{ab} provide somewhat less direct comparisons of these reactions than pyrene fluorescence data. However, when normalized for the different scattering intensities of F-actin and F-actin–F_{ab} complexes, the light scattering confirmed (not shown) the fluorescence measurements and did not reveal any effect of F_{ab} on the polymerization of actin. Consistent with this conclusion, and as monitored by pyrene fluorescence measurements over a period of 30 min, the depolymerization of F-actin upon its dilution to G-actin buffer was insensitive to the binding of F_{ab} (data not shown).

Discussion

Several lines of evidence indicate that the N-terminal segment of actin and the polymerization of this protein may be interrelated. The interactions between actin-binding proteins, which affect the monomer–polymer equilibrium in actin, and the acidic N-terminal residues have been mentioned in the preceding sections. By analogy to tubulin, in which the C-terminal acidic fragment appears to modulate the polymerization of tubulin into microtubules (Serrano et al., 1984; Maccioni et al., 1986), a similar role in the assembly reaction may be envisaged for the N-terminal segment of actin. The general role of acidic residues in the polymerization of actin is evident from the polycation-induced assembly reactions (Brown & Spudich, 1979; Magri et al., 1978).

The availability of the N-terminal residues in F-actin for chemical cross-linking reactions and to antibodies in solid-phase (direct) and solution (competitive ELISA) (Mejean et al., 1986, 1987a,b) suggests an unconstrained location of the N-terminal segment, most likely away from the rest of the molecule (Benyamin et al., 1986). Although the binding of the SαN antibodies to F-actin has been demonstrated also in solution (Miller et al., 1987), no attempt has been made yet to compare the conformational properties of the N-terminal segment in G- and F-actin and to examine them in the context of actin's polymerization.

In this work, we have monitored the effect of F_{ab} on the polymerization of actin and the stability of its filaments, and compared the binding of antibodies to G- and F-actins. The results of Figure 1, which show similar affinities of F_{ab} for G- and F-actin, are consistent with the earlier conclusions on the accessibility, solvent exposure, and surface location of the N-terminal segment on actin (Benyamin et al., 1986; Barden & Dos Remedios, 1983). The fact that we have not detected polymerization-induced changes in actin–F_{ab} interaction in the competitive ELISA titrations cannot be attributed to experimental difficulties or lack of sensitivity. Similar ELISA measurements (inset to Figure 1a) readily detect the small effect of S-1 on the binding of F_{ab} to actin (Mejean et al., 1987) and the higher affinity of IgG for F-actin than for G-actin (Figure 1b).

The equal affinities of F_{ab} for monomeric and polymeric actin agree also with the unperturbed polymerization of actin in the presence of F_{ab}. Neither pyrene fluorescence nor light-scattering measurements could detect any alteration in

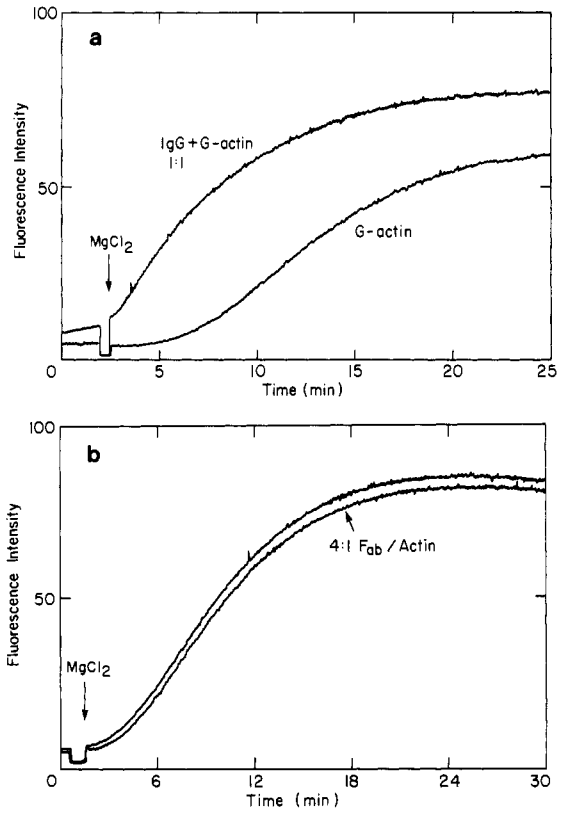

FIGURE 3: (a) Time course of actin polymerization by $MgCl_2$. The polymerization was monitored by the increase in fluorescence of pyrene-labeled actin, in the presence and absence of SαN IgG at 25 °C. The polymerization process was induced by the addition of 2 mM $MgCl_2$ to G-actin (3 μM) containing small amounts of pyrene-labeled actin. (b) Time course of actin polymerization by $MgCl_2$ in G-actin buffer in the presence and absence of SαN F_{ab}. The polymerization was monitored by the increase in the fluoresence of pyrene-labeled actin. Small amounts of pyrene-labeled actin (0.4 μM) were mixed with unlabeled G-actin (3 μM), and the polymerization process was induced by the addition of 2 mM $MgCl_2$ at 25 °C. Whenever used, filtered actin was present at somewhat higher concentrations in these experiments (4 or 5 μM) in order to shorten the nucleation time (not shown). F_{ab} and G-actin (at 4:1 molar ratio) were preincubated for up to 20 min prior to the addition of $MgCl_2$. The addition of F_{ab} to G-actin did not result in any changes in pyrene fluorescence readings.

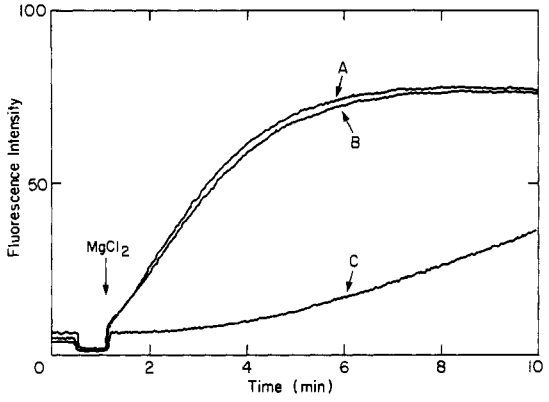

FIGURE 4: Time course of F-actin elongation. The elongation was monitored by the increase in the fluorescence of pyrene-labeled actin in the presence and absence of SαN F_{ab}. 5% *p*-phenylenebis(maleimide)-cross-linked actin oligomers were added to an unlabeled G-actin solution (3 μM) containing small amounts of pyrene-labeled actin (0.4 μM) to monitor the elongation process. The polymerization was induced by the addition of 2 mM $MgCl_2$ at 25 °C. (A) Polymerization of G-actin in the presence of cross-linked oligomers; (B) polymerization of actin in the presence of cross-linked oligomers and SαN F_{ab} (4:1 F_{ab}/actin); (C) polymerization of G-actin in the absence of cross-linked oligomers.

the rates of overall assembly or elongation of actin filaments upon F_{ab} binding. This somewhat surprising result, especially in view of the well-documented influence of many proteins on the assembly of actin, is supported by several observations. These include equal dissociation rates of actin filaments in the presence and absence of F_{ab}, the morphological similarity of free and F_{ab}-decorated filaments, and the absence of any changes in pyrene fluorescence of F-actin upon the binding of F_{ab}. Thus, while the results of carbodiimide cross-linking studies suggest that binding of proteins to the N-terminus of actin might modulate actin's polymerization, the experiments with F_{ab} show that this is not the case and that the antibodies bound to the N-terminus do not affect the assembly of actin.

Notably, and consistent with the results of ELISA titrations, IgG accelerated the Mg^{2+}-induced polymerization of actin. It is conceivable that same cellular factors might act in a similar way, i.e., accelerate the polymerization of actin by cross-linking its monomeric units.

The unperturbed polymerization of G-actin by $MgCl_2$ in the presence of F_{ab} and the equal affinities of F_{ab} for G-actin and F-actin have several implications with respect to actin. First, these results confirm the idea that the N-terminal segment of actin is on the surface or protrudes from the remainder of the molecule (Benyamin et al., 1986) and that it must be distant from the actin–actin interface. Most likely the N-terminus has considerable rotational freedom (Barden & Dos Remedios, 1983). The flexibility of this part of actin would explain its alternate cross-linking by carbodiimide to two different sites on the myosin head (Sutoh, 1983). Obviously, the flexibility of the N-terminal segment would also facilitate its electrostatic interactions with various actin-binding proteins. Second, our results indicate that the N-terminal segment does not play a direct role in the polymerization of actin, nor in the Mg^{2+}-triggered changes in the protein. The latter conclusion was reached also by Mejean et al. (1988), who did not detect any Mg^{2+} effect on ELISA titrations of actin with SαN antibodies. Yet, this conclusion is at variance with sequence homology considerations of Mornet and Ue (1984). The fact that KCl- and S-1- (but not Mg^{2+}-) induced assembly reactions are modified to different degrees by F_{ab} provides an example of the different modes of action and interactions with actin of various polymerization effectors (Wang & Taylor, 1981; Carlier et al., 1986). Finally, as shown by the polymerization experiments, the mere binding of proteins (or polycations) to the N-terminal segment and the resulting charge modification seem to be insufficient for inducing the polymerization of actin. The dominant effect of actin sites distinct from the N-terminus on the polymerization reaction is suggested also by the fact that both polymerizing (myosin; Sutoh, 1982) and depolymerizing proteins (fragmin; Sutoh & Hatano, 1986) interact with the N-terminal segment and that substitutions of Asp-3 and Asp-11 on actin have little impact on its assembly into filaments (Solomon, et al., 1988). In addition to this, thermodynamic studies on the polymerization of actin suggest an important role of hydrophobic interactions in the assembly process (Kasai, 1969; Swezey & Somero, 1985; Zimmerle & Frieden, 1986). It appears then that the bulk of evidence is consistent with an indirect relationship between the polymerization of actin and its N-terminal region. The binding of effectors to the N-terminal segment on actin may trigger in some cases intramolecular rearrangements or enable additional effector–actin contacts which in turn induce the polymerization reaction. The antibodies against the N-terminal segment and other sites on actin provide a sensitive tool for distinguishing between various effector sites on actin and determining the

role of these sites in the dynamic association properties of this protein.

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