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Probing the Dynamic Equilibrium of Actin Polymerization by Fluorescence Energy Transfer

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Summary

Fluorescence resonance energy transfer was used to follow the dynamic equilibrium of actin polymerization. We prepared fluorescent analogs of actin by labeling actin covalently with fluorescein (as the donor) and with eosin (as the acceptor). The copolymer of donor- and acceptor-labeled actin exhibits a 60%–70% efficiency of energy transfer. We followed the subunit exchange among filaments both by mixing the donor–acceptor copolymer with unlabeled F actin, and by mixing donor-labeled F actin with acceptor-labeled F actin. The extent of subunit exchange is dependent on ionic conditions. In addition, different kinetics are observed in the two approaches in the presence of excess magnesium ions. The effects of cytochalasin B as a model actin-binding factor were also investigated. One micromolar cytochalasin B reduces the rates of subunit exchange, monomer incorporation and filament depolymerization. At 10 μM cytochalasin B, we detected destabilization of filaments using a morphological assay. The results are discussed in relation to existing models of actin subunit exchange, and to proposed mechanisms of actin–cytochalasin interactions.

Introduction

It has been proposed that actin in nonmuscle cells undergoes reversible cycles of assembly during various cell functions (for review see Clark and Spudich, 1977; Taylor and Condeelis, 1979). The rates and sites of the reversible assembly process could be controlled by cellular metabolism and various cytoplasmic messengers, resulting in a highly dynamic cytoskeletal structure. To understand the mechanism of nonmuscle cell motility, we must first understand the detailed mechanism of actin assembly–disassembly in vitro.

It is known that continuous assembly–disassembly of actin subunits persists in F-actin solutions at steady state (Oosawa and Kasai, 1971). The detailed mechanism of the process, however, remains unclear. Earlier investigators assumed that the dynamic equilibrium is more or less limited to filament ends (Kasai and Oosawa, 1969). Wegner (1976) proposed the model of head-to-tail polymerization, or "treadmilling," of actin subunits. In addition, filament breakage and reannealing have also been observed (Kondo and Ishiwata, 1976). These processes would cause actin subunits to exchange constantly and extensively among filaments. Two recent reports support the view that extensive subunit exchange occurs at steady state. Significant differences in the rates of subunit association and dissociation between the two filament ends have been observed with electron microscopy (Pollard and Mooseker, 1981). The results are consistent with the treadmill model. In addition, extensive exchange of subunits is indicated by the exchange of a fluorescent ATP analog with subunit-associated nucleotides (Wang and Taylor, 1981).

We used the technique of fluorescence resonance energy transfer to examine directly the association–dissociation of actin subunits. Resonance energy transfer has been used widely as a tool for the study of macromolecular structures (Fairclough and Cantor, 1978; Stryer, 1978). The efficiency of energy transfer is highly dependent on the distance between the donor and the acceptor fluorophores, with an optimal application range between 10 and 70 Å. As a result, the technique is sensitive to small changes in donor–acceptor distances and is relatively site-specific. Furthermore, dynamic processes can be followed in real time with minimal mechanical perturbations to the experimental system. Fluorescence energy transfer has been recently applied to the detection of the polymerization of actin by measurement of the increase in energy transfer between donor- and acceptor-labeled subunits (Taylor et al., 1981).

We have used energy transfer to determine if there is an extensive exchange of actin subunits under specific ionic conditions. We have investigated the process by measuring the changes in energy transfer, both when donor–acceptor copolymers are mixed with unlabeled filaments, and when donor-labeled filaments are mixed with acceptor-labeled filaments. In addition, we used the technique to study the effects of actin-binding factors on the assembly–disassembly processes, using cytochalasin B as a model factor.

Results

Resonance Energy Transfer in Donor–Acceptor Copolymers

Fluorescein and eosin were chosen as the energy donor and acceptor, respectively. Resonance energy transfer was qualitatively demonstrated by measurement of the emission spectra of F-actin samples that contained a tenfold excess of acceptor-labeled subunits over donor-labeled subunits (see Experimental Procedures). The samples were irradiated at a wavelength (450 nm) that preferentially excites the donor fluorophore. When donor- and acceptor-labeled subunits were located on separate filaments, an emission peak was observed at ~555 nm and a prominent shoulder was observed at ~520 nm, corresponding to the peak emission wavelengths of the acceptor and

the donor, respectively. When donor- and acceptor-labeled subunits were copolymerized, a significant decrease (~70%) in donor fluorescence and a significant increase (~30%) in acceptor fluorescence were observed (Figure 1). The results indicate that there is an extensive intrafilament energy transfer among neighboring labeled subunits.

Quantitation of the energy transfer of the donor–acceptor copolymer was carried out by measurement of the donor fluorescence intensities in the presence and absence of neighboring acceptors (Table 1). Efficiencies between 60% and 70% were consistently observed. We obtained similar values by measuring sensitized fluorescence emitted by the acceptor.

Subunit Exchange in F-Actin Solutions

We followed subunit exchange among steady-state F-actin filaments using two different procedures (Figure 2). In the mixed-polymer experiment, donor-labeled filaments were mixed with a large excess of acceptor-labeled filaments. If exchange occurred, then an increasing number of donor-labeled subunits would become surrounded by acceptor-labeled subunits, resulting in an increase in the efficiency of energy transfer. In the copolymer experiment, donor–acceptor copolymers were mixed with a large excess of unlabeled filaments. If exchange occurred, then an increasing number of donor-labeled subunits would become dissociated from neighboring acceptor-labeled subunits, resulting in a decrease in the efficiency of energy transfer.

Figure 3 shows the results of the two experimental procedures performed in a polymerization buffer containing 10 mM PIPES, 2.5 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol (pH 7.0). A relatively rapid change of energy transfer was observed during the first 2 hr with both procedures, followed by a slower phase of change in transfer efficiency. The kinetics observed in the two procedures are, however, significantly different. In the copolymer experiment, the initial rate was much slower and the subsequent rate was significantly higher as compared with those of the mixed-polymer experiment. Neither pretreating the unlabeled actin with N-ethyl maleimide, nor shearing the unlabeled filaments before mixing with the copolymer, changes the rates of the copolymer experiment to values comparable with those of the mixed-polymer experiment.

It is possible that in the mixed-polymer experiment, a significant part of the initial increase in energy transfer is due to filament–filament (interfilament) interactions. Two experiments were performed to test this possibility. In the first experiment, the mixed-polymer procedure was carried out in the presence of 5 μM phalloidin, which inhibits the dynamic equilibrium of actin polymerization but does not affect interfilament interactions as measured by low-shear viscometry (Wieland, 1977; Estes et al., 1981; our unpublished observation). As shown in Figure 4, the increase in energy transfer was almost completely inhibited by phalloidin. When the magnesium ion concentration was increased to 20 mM to induce paracrystal formation, however, a transfer efficiency of 65%–80% could be seen (data not shown). These results suggest that the increase in energy transfer in the mixed-polymer experiment is not caused by interfilament interactions. We performed a second experiment by incubating the mixed-polymer system for 1 hr, followed by a 1:10 dilution into the polymerization buffer with 5 μM phalloidin (final polymer concentration, 20

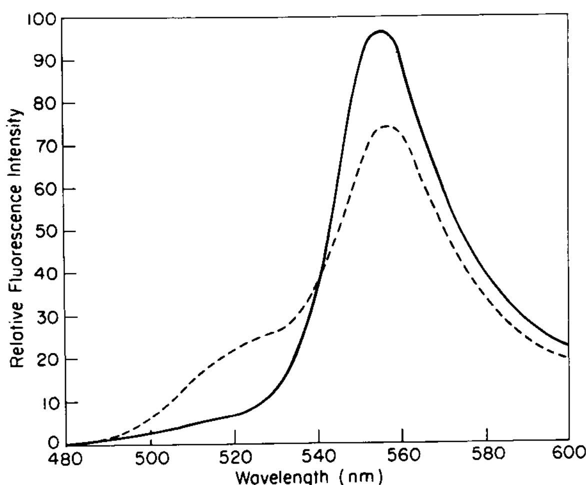

Figure 1. Fluorescence Emission Spectra of Labeled Actin Filaments
Samples contained 20 μg/ml donor-labeled subunits and 200 μg/ml acceptor-labeled subunits, with the two species of subunits copolymerized (——) or polymerized separately (- - -). Phalloidin (5 μM) was added to the polymerization buffer to stabilize the configuration of the filaments. Samples were excited at 450 nm with 10 nm bandpass. The bandpass of the emission monochromator was set at 2 nm. A standard microcuvette (4 mm path length) was used.

Table 1. Measurement of the Efficiency of Energy Transfer Based on Donor Quenching

Components (Final Concentration)[a]		Relative Fluorescence Intensity
20 μg/ml	200 μg/ml	
D	U	796 (I_1)
D	A	324 (I_2)
U	A	68 (I_3)
U	U	15 (I_4)

$$E = 1 - \frac{I_2 - I_3}{I_1 - I_4} = 67\%$$

Subunits in buffer A were mixed and diluted into 4 volumes of the polymerization buffer (for description of buffers, see Experimental Procedures). Intensity measurements were carried out after 2 hr of incubation. Samples were excited at 450 nm and emission was measured at 520 nm. Microcapillary cuvettes were used.

[a] D: donor-labeled subunits. U: unlabeled subunits. A: acceptor-labeled subunits. E: efficiency of energy transfer.

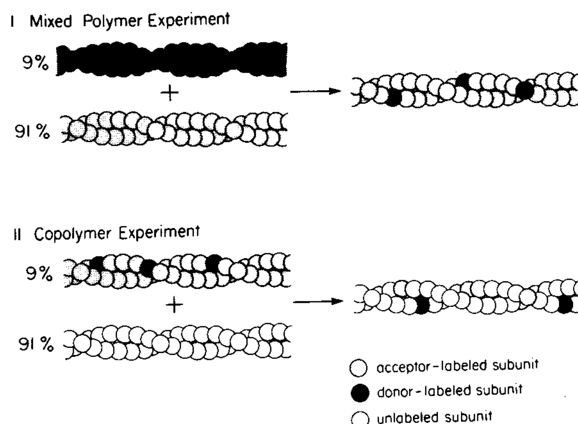

Figure 2. Diagrammatic Representation of the Approaches Used to Follow the Exchange of Subunits among Actin Filaments

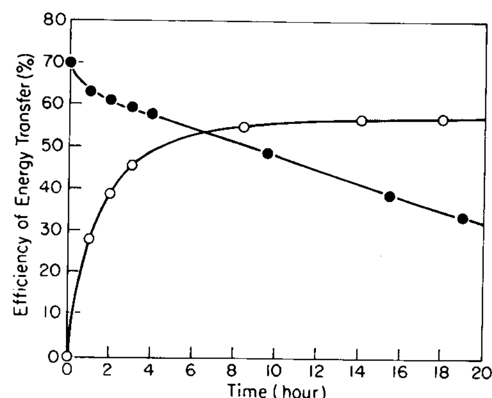

Figure 3. Exchange of Subunits among Actin Filaments in the Polymerization Buffer Containing 2.5 mM $MgCl_2$

(○——○) Mixed-polymer experiment; (●——●) copolymer experiment. The sample for the mixed-polymer experiment contained 200 μg/ml acceptor-labeled filaments and 20 μg/ml donor-labeled filaments. The sample for the copolymer experiment contained 200 μg/ml unlabeled filaments and 20 μg/ml donor–acceptor copolymers (with a tenfold excess of acceptor-labeled subunits over donor-labeled subunits). The excitation wavelength was 450 nm for the mixed-polymer experiment and 490 nm for the copolymer experiment. Emission was measured at 520 nm. Measurements were carried out in the microcapillary cuvettes.

μg/ml). If the increase in energy transfer during the first hour is caused by interfilament interactions, the efficiency should decrease upon dilution. Intrafilament energy transfer, however, should not be affected by dilution into a phalloidin-containing buffer. The result indicates that no change in the efficiency of energy transfer (~37%) was induced upon dilution, consistent with intrafilament energy transfer.

To determine if the exchange is affected by ionic conditions, we also carried out the mixed-polymer experiment in a buffer of low free magnesium ion concentration (containing 10 mM PIPES, 1 mM ATP, 1 mM $MgCl_2$, 100 mM KCl, 0.5 mM dithiothreitol [pH 7.0]). As shown in Figure 5, a significantly lower rate of exchange compared with that in our standard polymerization buffer was observed. Interestingly, increasing the concentration of $MgCl_2$ in this buffer to 3 mM restores the rate of exchange to a level similar to that seen with the standard polymerization buffer, suggesting that the exchange of subunits is stimulated by excess free magnesium ions.

Effects of Cytochalasin B

The energy transfer technique can also be used to characterize the action of various actin-binding factors. In the present study, cytochalasin B was chosen as a model actin-binding factor. The transfer efficiency of the donor–acceptor copolymer was not significantly affected by cytochalasin B. As shown in Table 2, 10 μM cytochalasin B caused only a ~10% decrease in the efficiency of energy transfer, indicating that there is no extensive depolymerization of filaments. The exchange of subunits as indicated by the mixed-polymer experiment, however, was markedly affected (Figure 4). At 1 μM cytochalasin B, the rate of the increase in energy transfer was reduced by more than 50%.

Since the inhibition of exchange could be caused by inhibition of subunit dissociation and/or incorporation, the effects of cytochalasin B on the two processes were examined separately. We followed subunit dissociation by diluting the copolymer to a final concentration close to the critical concentration, while maintaining the same buffer condition. As donor-labeled subunits became separated from acceptor-labeled subunits, the donor fluorescence intensity would increase as a result of the decrease in energy transfer. In the absence of cytochalasin B, the increase in donor intensity followed a quasi-first-order kinetics, with a half-life of <3 min. The rate constant was reduced by ~40% in the presence of 1 μM cytochalasin B and by ~60% in the presence of 10 μM cytochalasin B (Figure 6).

We measured the incorporation of subunits into filaments by adding 2 μg/ml donor-labeled monomers to a steady-state solution of acceptor-labeled filaments. The concentration of donor-labeled subunits was not high enough to self-polymerize or to cause significant perturbation to the equilibrium of the acceptor-labeled filaments. The incorporation of donor-labeled subunits into acceptor-labeled filaments was indicated by an increase in energy transfer, measured as a decrease in donor fluorescence intensity (Figure 7). In the presence of 1 μM cytochalasin B, the rate of decrease in donor fluorescence was reduced by ~60% as compared with that of the control (Figure 7). The two experiments indicate that both subunit assembly and disassembly are affected by the binding of cytochalasin B.

To investigate further the effect of cytochalasin B on the integrity of actin filaments, we used a morphological approach to visualize the filaments directly. It has been shown that polylysine-coated polystyrene

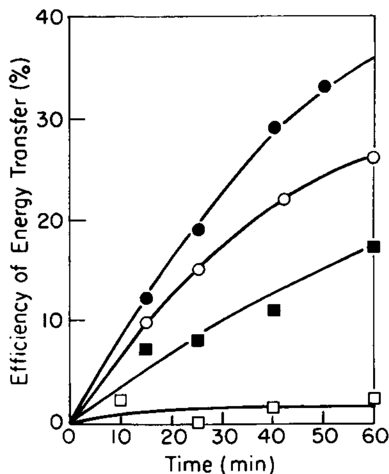

Figure 4. Effects of Cytochalasin B and Phalloidin on Subunit Exchange

Conditions were the same as those for the mixed-polymer experiment. Acceptor-labeled filaments were incubated with no cytochalasin B (0.1% DMSO) (●——●), 0.1 μM cytochalasin B (○——○) or 1 μM cytochalasin B (■——■) for 1 hr before mixing with donor-labeled filaments. The curve for 10 μM cytochalasin B was similar to that for 1 μM cytochalasin B. In the phalloidin experiment (□——□), donor-labeled filaments and acceptor-labeled filaments were assembled in the polymerization buffer with 5 μM phalloidin.

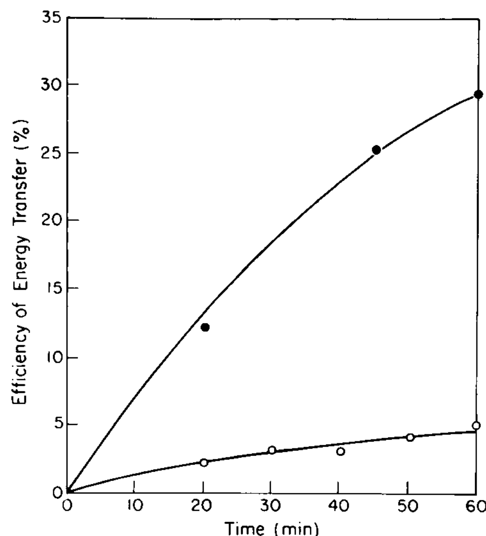

Figure 5. Effects of Buffer Conditions on Subunit Exchange

Conditions were the same as those for the mixed-polymer experiment, except that the polymerization buffer was replaced by a buffer of 10 mM PIPES, 1 mM ATP, 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 100 mM KCl (pH 7.0) (○——○). (●——●) Results with the polymerization buffer, shown for comparison.

beads stimulate the nucleation of actin polymerization. The resulting filaments remain associated with the beads and have an uniform polarity, as indicated by decoration with heavy meromyosin (with the polarity pointing towards the bead; Brown and Spudich, 1979a). Using fluorescein-labeled actin, we observed arrays of fluorescent filaments in association with the beads under the fluorescence microscope (Figure 8A). When beads with bound fluorescein-labeled filaments were incubated with 10 μM cytochalasin B for 30 min, a dramatic reduction in the associated filaments was observed (Figure 8B). No apparent effect was detected, however, with 1 μM cytochalasin B or in the DMSO control after the same period of incubation.

Discussion

Subunit Exchange

Significant changes in the efficiency of energy transfer were observed in the exchange experiments performed in the presence of excess free magnesium ions, indicating that at least under some ionic conditions, actin subunits can exchange extensively among filaments. The phenomenon cannot be explained if subunit exchange under these conditions is limited to filament ends. The results are consistent either with extensive breakage–reannealing coupled to end-on subunit exchange or with head-to-tail polymerization.

The results of the mixed-polymer experiment in different buffers indicate that the exchange of subunits is highly dependent on ionic conditions. The present observation could be explained if the exchange is stimulated by excess free magnesium ions. The change in the rate of exchange could be caused either by a shifting in the filament-length distribution or by a change in the kinetic constants. It is possible that the exchange process is also affected by other factors, such as actin concentration, free calcium ion concentration and pH. The effects of ionic conditions have important implications on the regulation of the actin-containing cytoskeleton in living cells, and warrant further study.

We followed subunit exchange in the presence of millimolar quantities of free magnesium ions using two different approaches. In the mixed-polymer experiment, the rate of change in energy transfer was affected directly by the rate of subunit incorporation. In the copolymer experiment, the rate of change in energy transfer was affected directly by the rate of subunit dissociation. Based on the simplest model of treadmill, with monomers as the basic unit of exchange (Wegner, 1976; Kirschner, 1980), the rate of incorporation should equal the rate of dissociation at steady state. In addition, each subunit should undergo a complete replacement of neighboring subunits as it becomes dissociated from the original filament and reassociated with a new filament. As a result, the rate of change in energy transfer in the copolymer experiment should match that in the mixed-polymer experiment.

The difference in kinetics observed in the mixed-polymer experiment and in the copolymer experiment can be explained in several ways. It is possible that

Table 2. Effect of Cytochalasin B on the Efficiency of Energy Transfer

Cytochalasin B Concentration (μM)	Transfer Efficiency	Percentage of Control
0 (0.1% DMSO)	64	100
0.1	63	98
1	62	97
10	58	90

A set of four samples was prepared as described in Table 1 for each cytochalasin B concentration. Actin was allowed to polymerize for 2 hr before addition of cytochalasin B. Fluorescence intensity was measured after >30 min of incubation.

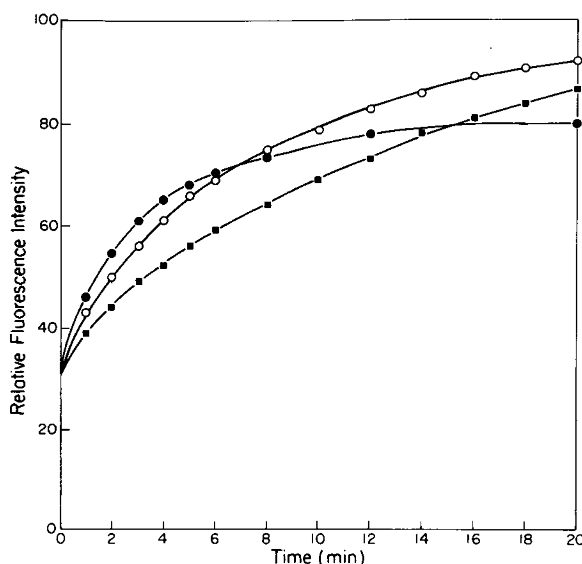

Figure 6. Effects of Cytochalasin B on Filament Depolymerization

Donor-labeled subunits (40 μg/ml) were copolymerized with 400 μg/ml acceptor-labeled subunits. At time zero, the copolymer was diluted into 19 volumes of the polymerization buffer containing no cytochalasin B (●——●), 1 μM cytochalasin B (○——○) or 10 μM cytochalasin B (■——■). The curve for 0.1 μM cytochalasin B was very close to that for no cytochalasin B. Samples were excited at 490 nm and emission was measured at 520 nm. A standard microcuvette (4 mm path length) was used.

fluorescent labeling changes the rates of subunit association–dissociation. However, pretreatment of the unlabeled actin with N-ethyl maleimide did not alter the result of the copolymer experiment. In addition, no significant change in critical concentration could be detected upon fluorescent labeling (Wang and Taylor, 1980; see Experimental Procedures). It is also possible that fluorescent labeling dramatically alters the length distribution of actin filaments. However, shearing of unlabeled filaments in the copolymer experiment did not eliminate the difference in kinetics. Furthermore, no significant difference could be detected between labeled and unlabeled filaments by either high-shear viscometry or electron microscopy. An alternative explanation is that during each cycle of

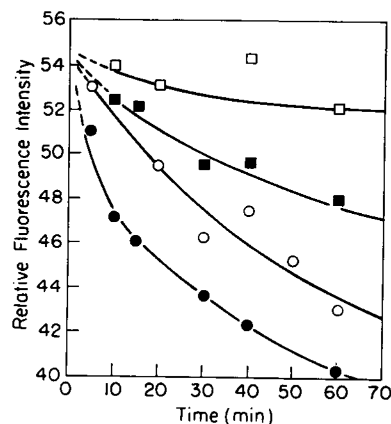

Figure 7. Incorporation of Donor-Labeled Subunits into Acceptor-Labeled Filaments

Donor-labeled monomers (2 μg/ml) were added to 200 μg/ml acceptor-labeled filaments in the presence of no cytochalasin B (●——●), 0.1 μM cytochalasin B (○——○), 1 μM cytochalasin B (■——■) or 10 μM cytochalasin B (□——□). Samples were excited at 490 nm and emission was measured at 520 nm. Microcapillary cuvettes were used.

subunit exchange, there is an incomplete replacement of neighboring subunits. Assuming that each donor-labeled subunit in the copolymer interacts with two neighboring acceptor-labeled subunits at equal distances to generate an efficiency of 70%, we can calculate that when the donor-labeled subunit has only one interacting acceptor-labeled subunit, the transfer efficiency should be ~54%. A large increase in energy transfer should therefore be observed when a donor-labeled subunit with no neighboring acceptor becomes associated with the first acceptor fluorophore (that is, during the early phase of the mixed-polymer experiment). A small decrease in energy transfer should be observed when a donor-labeled subunit with multiple neighboring acceptors loses one associated acceptor fluorophore (that is, during the early phase of the copolymer experiment). This explanation is consistent with either treadmilling of oligomers or extensive breakage and reannealing of filaments.

Effects of Cytochalasin B

The small effect of cytochalasin B on the efficiency of energy transfer of the donor–acceptor copolymer indicates that cytochalasin B does not cause extensive depolymerization of actin filaments. The slight decrease in energy transfer could be caused either by a slight increase in monomer concentration or by an increase in oligomer concentration. Consistent observations have been made with other techniques (Brown and Spudich, 1979b; MacLean-Fletcher and Pollard, 1980).

Previous experiments indicate that there are two classes of cytochalasin-binding sites on actin filaments (Brown and Spudich, 1979b; Hartwig and Stos-

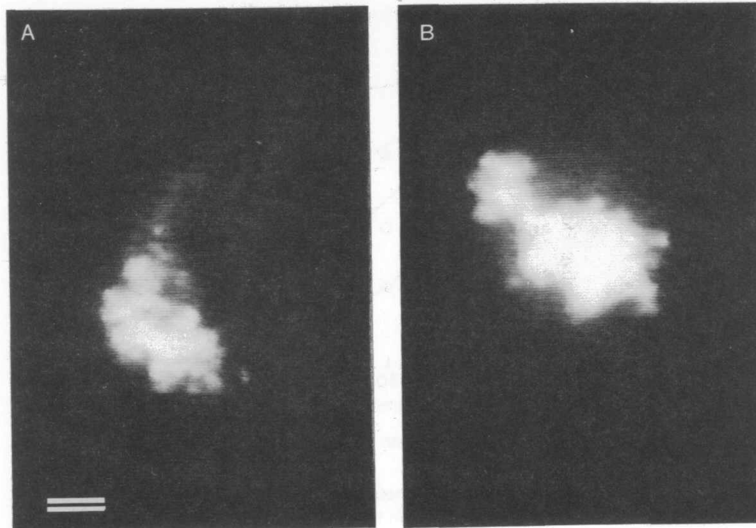

Figure 8. Effects of Cytochalasin B on Bead-Associated Filaments

Fluorescein-labeled G actin (0.2 mg/ml) was mixed with 0.5 mg/ml polylysine-coated polystyrene beads in a buffer containing 10 mM PIPES, 2.5 mM $MgCl_2$, 120 mM KCl, 0.5 mM ATP, 0.5 mM dithiothreitol (pH 7.0). The suspension was incubated for 30 min at room temperature to allow complete polymerization. We subsequently added 0.1% DMSO (A) or 10 μM cytochalasin B (B), followed by a 30 min incubation at room temperature. Arrays of long filaments extending from an aggregate of beads are readily detectable in the DMSO control, but not in the presence of 10 μM cytochalasin B. Bar = 10 μm.

sel, 1979; Flanagan and Lin, 1980). At a concentration of 1 μM, cytochalasin B occupies predominantly the high-affinity binding sites; at 10 μM cytochalasin B, the low-affinity binding sites also become occupied. The effects of cytochalasin B on subunit exchange increase significantly at concentrations between 0.1 μM and 1 μM, suggesting that they are caused by binding to the high-affinity sites. This effect cannot be explained by filament "cutting" alone, since the cutting hypothesis predicts an increase instead of a decrease in both the rate of monomer incorporation and the rate of filament disassembly in the presence of cytochalasin B. The results are consistent, however, with the now common hypothesis that cytochalasin blocks the fast-growing end of the filament (Brenner and Korn, 1979; Lin et al., 1980; MacLean-Fletcher and Pollard, 1980; Maruyama et al., 1980; Brown and Spudich, 1981). It is interesting to note that if oligomers are the major unit for exchange, then blocking actin oligomers could also cause significant effects on the assembly-disassembly processes.

We observed previously that 0.1–10 μM cytochalasin B does not significantly affect the exchange of actin-bound nucleotides (Wang and Taylor, 1981). Normally, the exchange of nucleotides is coupled to the exchange of subunits. The different effects of cytochalasin B on subunit exchange and on nucleotide exchange suggest that cytochalasin B at least partially uncouples the two exchange processes.

The effect of cytochalasin B on bead-associated filaments is readily observable only at relatively high concentrations, suggesting that it is caused by binding to the low-affinity binding sites. Since 10 μM cytochalasin B does not cause significant depolymerization of filaments, as indicated by the efficiency of energy transfer (Table 2), the dramatic reduction of bead-associated filaments is most likely caused by destabilization of the filaments. Cytochalasin-induced filament "breaking" has been previously suggested (Hartwig and Stossel, 1979; Selden et al., 1980; Maruyama et al., 1980).

The present study represents the first step in the use of the energy transfer technique to investigate the properties of actin polymerization at the molecular level. Careful applications of this technique should yield new information not accessible by other assays. In addition, experiments similar to those performed with cytochalasin B can be carried out to characterize the effects of various cytoplasmic actin-binding factors. This information is crucial to the ultimate understanding of nonmuscle cell motility.

Experimental Procedures

Materials

5-Iodoacetamidofluorescein and eosin-maleimide were obtained from Molecular Probes. ATP and phalloidin were purchased from Boehringer Mannheim. Phalloidin was stored as a 1 mM stock solution in 2% dimethylsulfoxide (DMSO). Poly-L-lysine, cytochalasin B, DMSO and N-ethyl maleimide were obtained from Sigma. Cytochalasin B was stored as a 10 mM stock solution in DMSO. Polystyrene beads (1.1 μm diameter) were obtained from Dow Chemical Company.

Actin was prepared from rabbit leg and back muscles according to the method of Spudich and Watt (1971), and was further purified by gel filtration chromatography on Sephadex G-150.

Actin Labeling and Characterization

Actin was labeled with 5-iodoacetamidofluorescein as described previously (Wang and Taylor, 1980) with slight modifications. The reaction was stopped by dialysis of the dye-actin solution overnight against 500 volumes of a buffer containing 2 mM PIPES, 0.2 mM $MgCl_2$, 100 mM KCl, 0.5 mM ATP, 1 mM dithiothreitol and 0.02% NaN_3 (pH 7.0). The final fluorescein to actin molar ratio of fluorescein-labeled actin was typically between 0.70 and 0.75.

Eosin-labeled actin was prepared by reaction of F actin with a 15-fold molar excess of eosin-maleimide in a buffer of 25 mM PIPES, 0.2 mM $MgCl_2$, 100 mM KCl, 0.25 mM ATP (pH 7.2). Other labeling procedures were the same as those for fluorescein-labeled actin. We determined the concentration of bound eosin by measuring the optical density at 528 nm, using an extinction coefficient of 70,000 M^{-1}

cm^{-1} (Taylor et al., 1981). The final eosin to actin molar ratio was typically between 0.95 and 1.05.

Actin was stored in the G state by dialysis against 2 mM Tris–HCl, 0.5 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM dithiothreitol, 0.02% NaN_3 (pH 7.8) (buffer A), and was used within 4 days of preparation.

The critical concentration of eosin-labeled actin in 2 mM $MgCl_2$ was ~30 μg/ml (measured by the sedimentation assay). Electron microscopy of both polymerized fluorescein-labeled actin and polymerized eosin-labeled actin revealed filamentous structures typical of normal F actin. The high-shear viscosity of polymerized fluorescein-labeled actin or eosin-labeled actin was similar to that of unlabeled F actin (Wang and Taylor, 1980; Taylor et al., 1981). The low-shear viscosity of polymerized eosin-labeled actin, as measured by a rolling-ball viscometer (Griffith and Pollard, 1978), was about 45% of that of unlabeled actin. A similar decrease was observed when actin was modified with N-ethyl maleimide or with 5-iodoacetimidofluorescein. At least part of the decrease could be attributed to the "aging" of actin during the labeling procedure. However, the low-shear viscosity of labeled actin did maintain the sensitivity to cytochalasin B. At 1 μM cytochalasin B, the viscosity of 0.4 mg/ml eosin-labeled actin decreased by a factor of 2.3, as compared with a decrease by a factor of 3.3 for unlabeled actin. These data suggest that while there are some quantitative differences between labeled actin and unlabeled actin, the fluorescently labeled actins can be used as close analogs.

Microcapillary Cuvette

Most measurements of fluorescence intensity were carried out in glass capillary cuvettes (50 μl microcaps; Drummond Scientific Co.). The cuvettes were loaded by capillary action, and were subsequently inserted into the center hole of a brass cylinder, which in turn was mounted into a brass holder. Each sample was typically 10–15 μl in volume, while conventional microcuvettes require at least 200 μl of sample. The small diameter of the capillary also greatly alleviated the problem of inner-filter effect. Samples of up to 0.4 OD unit (measured in a standard 1 cm path length cuvette) could be reliably measured. The cuvette is transparent to the excitation light at wavelengths >400 nm, and emits little background fluorescence at wavelengths <580 nm.

To minimize the effect of photobleaching, we used each sample for no more than two measurements. Good agreement in the efficiency of energy transfer was obtained between the microcapillary cuvette and standard microcuvettes.

Fluorescence Measurements

Fluorescence intensities were measured with a Fluorolog (Spex Industries) in the E/R mode. Unless otherwise specified, the bandpass of both the excitation and the emission monochromators were set at 10 nm. The efficiency of energy transfer was calculated from donor quenching, as shown in Table 1. We detected sensitized fluorescence by exciting the sample at 470 nm and measuring the emission at 580 nm. Efficiencies based on sensitized fluorescence were calculated according to the method of Fairclough and Cantor (1978). In experiments involving G-F transformation of donor-labeled subunits, the results were expressed as relative fluorescence intensity, which is affected by both the energy transfer and the polymerization states of the donor-labeled subunits (Wang and Taylor, 1980).

Since donor quenching was measured in most experiments, it is preferable to have most donor-labeled subunits surrounded by acceptor-labeled subunits upon copolymerization. A tenfold excess of acceptor-labeled subunits over donor-labeled subunits was therefore used in most experiments. The optical densities of the samples at the excitation wavelength were maintained below 0.04.

We polymerized actin by diluting G actin in buffer A into 4 volumes of the polymerization buffer (10 mM PIPES, 2.5 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol [pH 7.00]), followed by a 2 hr incubation at room temperature. Filaments in the exchange experiments were assembled at a concentration of 400 μg/ml. The major species (those at 200 μg/ml final concentration; see Table 1) were first diluted 1:1.9 by the polymerization buffer. We subsequently added 1/20 volume of the minor species, followed by brief vortexing (<1 sec) to ensure proper mixing. All samples were prepared under dim red light illumination.

The transfer efficiency of the donor–acceptor copolymer was higher than that reported previously with fluorescein-labeled actin and actin labeled with eosin-5-iodoacetamide (Taylor et al., 1981). The exact explanation for the difference is unknown. It is possible that the bond between actin and eosin is more rigid when coupled by maleimide as compared with that coupled by iodoacetamide (Stone et al., 1970), resulting in a more favorable orientation between the donor and the acceptor for the resonance energy transfer to occur (Fairclough and Cantor, 1978). The question is presently under investigation.

Other Procedures

Modification of actin with N-ethyl maleimide was carried out as described previously by Wang and Taylor (1980). Experiments with polylysine-coated polystyrene beads were carried out as described by Brown and Spudich (1981). The fluorescence of the beads was visualized with a Zeiss Photomicroscope equipped with an epi-illuminator and a 25× Neofluar objective (numerical aperture = 0.6). Images were recorded with an RCA Silicon Intensified Target TV camera as described previously by Wang and Taylor (1980).

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