Intermolecular Coupling Between Loop 38–52 and the C-Terminus in Actin Filaments

Eldar Kim and Emil Reisler
Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90095 USA

ABSTRACT The recently reported structural connectivity in F-actin between the DNase I binding loop on actin (residues 38–52) and the C-terminus region was investigated by fluorescence and proteolytic digestion methods. The binding of copper to Cys-374 on F- but not G-actin quenched the fluorescence of dansyl ethylenediamine (DED) attached to Gln-41 by more than 50%. The blocking of copper binding to DED-actin by *N*-ethylmaleimide labeling of Cys-374 on actin abolished the fluorescence quenching. The quenching of DED-actin fluorescence was restored in copolymers (1:9) of *N*-ethylmaleimide-DED-actin with unlabeled actin. The quenching of DED-actin fluorescence by copper was also abolished in copolymers (1:4) of DED-actin and *N*-ethylmaleimide-actin. These results show intermolecular coupling between loop 38–52 and the C-terminus in F-actin. Consistent with this, the rate of subtilisin cleavage of actin at loop 38–52 was increased by the bound copper by more than 10-fold in F-actin but not in G-actin. Neither acto-myosin subfragment-1 (S1) ATPase activity nor the tryptic digestion of G-actin and F-actin at the Lys-61 and Lys-69 sites were affected by the bound copper. These observations suggest that copper binding to Cys-374 does not induce extensive changes in actin structure and that the perturbation of loop 38–52 environment results from changes in the intermolecular contacts in F-actin.

INTRODUCTION

It has been recognized recently that actin filaments may exist in different conformational states depending on the divalent cation, nucleotides, and proteins bound to actin (Orlova and Egelman, 1995; Orlova et al., 1995; Egelman and Orlova, 1995). The observations of these authors linked the structural differences between the Ca^{2+}- and Mg^{2+}-polymerized actins (Orlova and Egelman, 1993), as well as between standard F-actin and the filaments stabilized by the P_i analog BeF_x (Orlova and Egelman, 1992), to conformational differences in subdomain 2 of actin, and the changes in the radial position of the DNase I binding loop 38–52 in that subdomain. These findings are of considerable interest because of the proposed involvement of loop 38–52 in intermolecular contacts in F-actin (Lorenz et al., 1993; Tirion et al., 1995) and the functional implications of filament structure modulation via this site (Egelman and Orlova, 1995).

Additional insight into the structural determinants of the different F-actin states was obtained by Orlova and Egelman (1995) through image reconstruction of electron micrographs of several forms of actin filaments. These authors identified a bridge of density between the two strands of actin in the Ca^{2+}- F- but not Mg^{2+}-F-actin, which they attributed to the C-terminus of actin. Tryptic cleavage of the last two C-terminal residues on actin abolished the connectivity between the two strands and, strikingly, resulted in an Mg^{2+}-like state of subdomain 2 in Ca^{2+}-F-actin (Orlova and Egelman, 1995). The conformational coupling between the C-terminus and the 38–52 loop in actin, both of which are perturbed by myosin binding (Kasprzak et al., 1988; Kim et al., 1996 Fievez and Carlier, 1993; Duong and Reisler, 1994; Kouyama and Mihashi, 1981; Egelman and Orlova, 1995) may be of intramolecular or intermolecular nature. In G-actin such coupling has been demonstrated via the exposure of a new, C-terminal proteolytic site upon DNase I binding to actin (Crosbie et al., 1994). The intermolecular coupling in F-actin would be consistent with structural models of F-actin in which three regions from three neighboring monomers, the C-terminus, the hydrophobic plug 262–274, and the 38–52 loop are in close proximity to each other (Tirion, 1995; Lorenz et al., 1993; Owen and DeRosier, 1993). These sites would be a part of the intermolecular interface in F-actin and thus, changes at one site could easily affect the other two sites.

Because of the functional importance of loop 38–52 and the C-terminus on actin, as demonstrated by significant changes in the in vitro motility of actin filaments after loop 38–52 cleavage (Schwyter et al., 1990) and Cys-374 modification (Crosbie et al., 1994), the connection between these two sites in F-actin was further explored in this study. Proteolytic digestions and spectroscopic evidence based on the conformational sensitivity of the dansyl ethylenediamine (DED) probe attached to Gln-41 and on the specific binding of copper to Cys-374 (Lehrer et al., 1972) provide support for the existence of intermolecular coupling between loop 38–52 and the C-terminus in F-actin.

Received for publication 28 March 1996 and in final form 27 June 1996.

Address reprint requests to Dr. Emil Reisler, Department of Chemistry and Biochemistry, University of California, 405 Hilgard Avenue, Los Angeles, CA 90024-1569. Tel.: 310-825-2668; Fax: 310-206-7286; E-mail: reisler@ewald.mbi.ucla.edu.

0006-3495/96/10/1914/06 $2.00

MATERIALS AND METHODS

Reagents

ATP, ADP, α-chymotrypsin, trypsin and subtilisin Carlsberg were obtained from Sigma Chemical Co. (St. Louis, MO). Bradford reagent was from

Bio-Rad (Richmond, CA). DED (dansyl ethylenediamine) was purchased from Molecular Probes (Eugene, OR).

Preparation of proteins

Actin and myosin were prepared according to Spudich and Watt (1971) and Godfrey and Harrington (1970), respectively. S1 was prepared as described by Weeds and Pope (1977). Microbial transglutaminase was a generous gift from Drs. M. Motoki and K. Seguro.

DED-labeled actin

The labeling of Gln-41 on G-CaATP-actin (50 μM) with DED (100 μM) was carried out in the presence of 0.4 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 5.0 mM Tris-HCl at pH 7.7, and 0.5 unit/ml of microbial transglutaminase for 2 h at 23°C (Kim et al., 1995). The labeled actin was centrifuged through spin columns to remove free DED and β-mercaptoethanol. The labeled actin was polymerized with 2.0 mM $MgCl_2$ after replacing the bound Ca^{2+} in G-actin with Mg^{2+} (Kim et al., 1995). The amount of DED bound to actin and the concentration of the modified actin were determined as before (Kim et al., 1995). The stoichiometry of G-actin labeling with DED ranged between 0.9 and 1.0 label per actin. Cu-F-actin and Cu-G-actin were prepared by adding Cu^{2+} to F- or G-actin free of β-mercaptoethanol.

NEM modification of actin

G-actin and DED-G-actin (50 μM), free of β-mercaptoethanol, were modified at Cys-374 with 500 μM NEM at 4°C for 3 h. The reaction was terminated with β-mercaptoethanol and the modified proteins were dialized against 0.2 mM CaCl, 0.2 mM ATP, 0.5 mM β-mercaptoethanol, and 5.0 mM Tris-HCl at pH 7.7. After the dialysis the modified G-actins were centrifuged for 2 h at 40,000 rpm, at 4°C, in a Beckman ultracentrifuge and the supernatant was used in subsequent experiments. The extent of Cys-374 labeling on actin by NEM was determined by monitoring the Cu^{2+}-induced quenching of tryptophan fluorescence of actin (Lehrer et al., 1972). NEM labeling of actin resulted in between 95 and 100% loss of Cu^{2+}-induced quenching of tryptophan fluorescence. These preparations were assumed to contain between 0.95 and 1.0 NEM/actin.

Proteolytic digestions

G- and F-actin (1.0 mg/ml) were digested with subtilisin at 25°C at 500:1 and 66:1 ratios (w/w) of actin to protease, respectively. The reaction was stopped with 1.0 mM PMSF. Tryptic digestions of G- and F-actin (1.0 mg/ml) were done at 25°C, at 25:1 and 5:1 ratios of (w/w) of actin to trypsin, respectively. The digestions were stopped with a 2.5-fold molar excess of soybean trypsin inhibitor over trypsin. For both proteases G-actin was equilibrated with 0.2 mM $CaCl_2$, 0.2 mM ATP, and 5.0 mM Tris-HCl at pH 7.7. F-actin contained 2.0 mM $MgCl_2$, 0.2 mM ATP, and 5.0 mM Tris-HCl at pH 7.7. When used, copper was added at stoichiometric ratios to actin and incubated with actin for 10 min before the addition of proteases. The rates of actin cleavage were determined as before (Muhlrad et al., 1994), by densitometric analysis of reaction aliquots displayed on SDS-PAGE. The disappearance of the 42-kDa actin band on SDS gels (due to actin cleavage) followed a first order process and yielded a rate constant for the digestion reaction.

Fluorescence measurements

All fluorescence measurements were carried out in a Spex Fluorolog spectrofluorometer (Spex Industries, Inc., Edison, NJ) at 25°C. The fluorescence of DED F-actin and the copolymers of DED-actin and NEM-actin or unlabeled actin was monitored after excitation at λ = 334 nm.

Copper binding assay

The binding of copper to DED labeled F-actin was monitored via changes in the absorption at 348 nm (Lehrer et al., 1972) or the changes in DED fluorescence at λ = 525 nm. The absorption measurements were done at 10 μM F-actin; the fluorescence was monitored at 3.0 μM F-actin.

Acto-S1 ATPase activity measurements

Actin-activated MgATPase activity of S1 was determined under steady-state conditions, over a time interval between 30 and 90 s, at 25°C by colorimetric assays. The retention of bound copper by actin over this time scale under ATPase assay conditions was confirmed by DED-fluorescence quenching measurements. The solutions contained 10 μM F-actin or Cu-F-actin and 3.0 mM ATP, 3.0 mM $MgCl_2$, 5 mM KCl, 10 mM Imidazole at pH 7.0, and S1 at concentrations ranging between 5 and 30 μM. ATPase activities were calculated as micromoles of P_i released per micromole of S1/s and were corrected for the ATPase of S1 alone.

RESULTS

Spectroscopic experiments

Previous work documented the sensitivity of DED attached to Gln-41 on actin to conformational changes in loop 38–52 in G- and F-actin (Kim et al., 1995, 1996). The environmental sensitivity of DED suggested that this probe might be useful in testing the structural connectivity between loop 38–52 and the C-terminus of actin. Fig. 1 shows the emission spectra of DED-F-actin and DED-F-actin with a stoichiometric amount of Cu^{2+} bound to Cys-374. Strikingly, the binding of copper to actin quenches the fluorescence of the probe by more than 50%. Previous observations contribute to the interest in this effect: 1) The binding of Cu^{2+} to F-actin (and G-actin) is highly specific to Cys-374

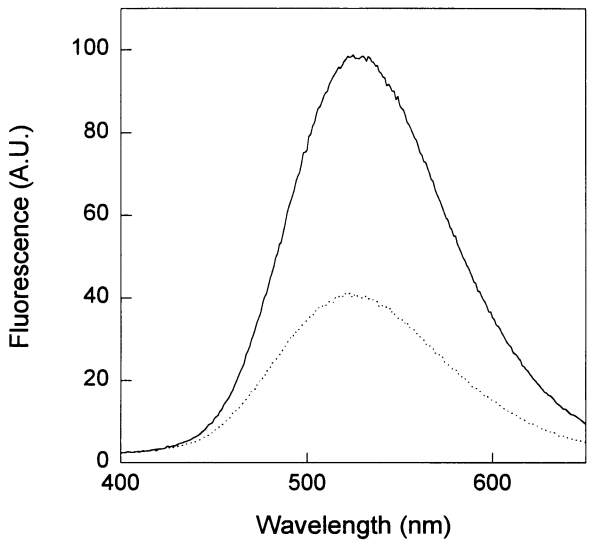

FIGURE 1 Emission spectra of DED-F-actin. The spectra were recorded at a concentration of 3.0 μM DED-F-actin in the absence (solid trace) and presence (dotted trace) of a stoichiometric amount of copper after excitation at λ = 334 nm. The solvent contained 0.2 mM ATP, 2.0 mM $MgCl_2$, and 5.0 mM Tris-HCl at pH 7.7. Fluorescence intensities are reported in arbitrary units (A.U.)

(Lehrer et al., 1972). Because of copper's extremely high affinity for actin, virtually no free metal ion is present in solutions containing Cu^{2+} at substoichiometric or stoichiometric molar ratios to actin. 2) In the absence of spectral overlap copper can not serve as an acceptor for energy transfer from DED (Kim et al., 1995). 3) The binding of Cu^{2+} to Cys 374 has no effect on DED fluorescence in G-(MgATP)-actin (Kim et al., 1995). These observations show that the binding of Cu^{2+} to the C-terminus region on G-actin does not perturb the environment of loop 38–52 and suggest, therefore, that the copper effect on DED fluorescence in F-actin results from intermolecular interactions in actin filaments.

A titration of DED-F-actin with Cu^{2+} shows a linear dependence of fluorescence quenching on the saturation of actin with copper (Fig. 2) and does not indicate any cooperative transitions in the environment of loop 38–52. The time dependence of DED quenching by copper (Fig. 3) reveals that this process is relatively slow, and at the low protein and Cu^{2+} concentrations used here takes ~100 s for completion. To test whether the quenching of DED fluorescence is rate-limited by the binding of Cu^{2+} to Cys 374 on F-actin or, perhaps, by a subsequent isomerization process, the binding step has been monitored via absorption measurements at 348 nm (Fig. 3) (Lehrer et al., 1972). Although for practical reasons the fluorescence and absorption changes are followed at different protein concentrations, it is clear that both changes occur on the same time scale (Fig. 3). Thus, it appears that the slow quenching of DED-F-actin fluorescence by Cu^{2+} is rate limited by its binding to actin. Notably, similar measurements with G-actin revealed that the absorption at 348 nm changed within the mixing time of G-actin and copper (not shown).

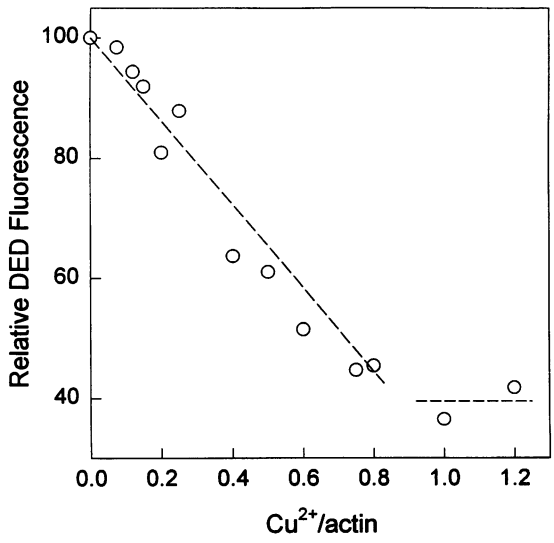

FIGURE 2 Quenching of DED-F-actin fluorescence as a function of a molar ratio of copper to actin. DED-F-actin (3.0 μM) was titrated with copper in the presence of 0.2 mM ATP, 2 mM $MgCl_2$, and 5.0 mM Tris-HCl, pH 7.7. DED-F-actin emission was monitored at λ = 525 nm after excitation at λ = 334 nm.

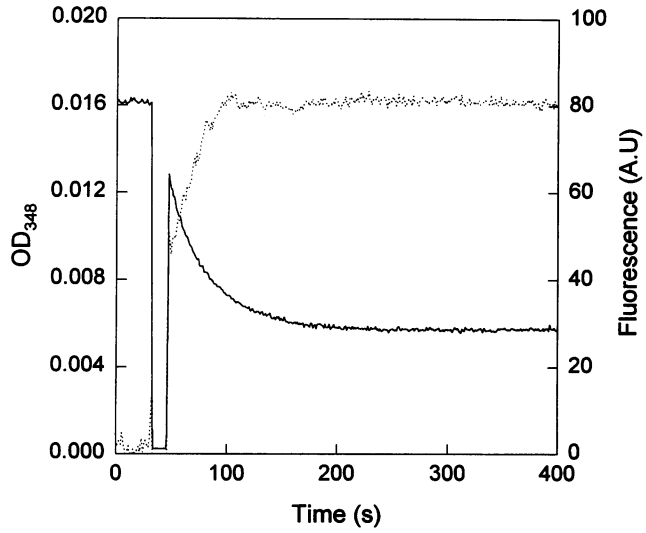

FIGURE 3 Time dependence of Cu^{2+}-induced changes in DED-F-actin. Absorption changes (*dotted trace*) at 348 nm were monitored in an HP spectrophotometer at 10 μM DED-F-actin after the addition of 10 μM Cu^{2+}. The fluorescence change by copper (quenching of DED-F-actin fluorescence) was monitored at 3.0 μM concentrations of F-actin and copper (*solid trace*). Solvent conditions and excitation and emission settings were the same as in the legend to Fig. 2.

Proteolytic digestions of actin

Additional information on Cu^{2+}-induced changes in loop 38–52 on actin was obtained from proteolytic digestion experiments. Subtilisin cleavage of F-actin in the 38–52 loop (Schwyter et al., 1989) occurred at a rate that was more than 10-fold faster in the presence of stoichiometric amount of copper ($k = 0.42 \pm 0.03$ min^{-1}) than in its absence ($k = 0.032 \pm 0.003$ $min.^{-1}$) (Table 1). This large difference in the proteolytic susceptibility of F-actin to subtilisin, but not in the reaction products, suggests a Cu^{2+}-induced exposure of loop 38–52 to the protease. Similar digestions of F-actin by subtilisin carried out at different substoichiometric molar ratios of copper to actin showed a linear increase in the digestion rate of F-actin with its saturation by Cu^{2+}. Significantly, and in contrast to F-actin, the digestion rates of G-actin by subtilisin were identical in presence and absence of copper (Table 1). These observations are consistent with the results of fluorescence experiments: the Cu^{2+}-induced

TABLE 1 Rate constants for proteolytic cleavage of actin

	Rate constants (min^{-1})	
Actin	Subtilisin	Trypsin
F-actin	0.032 ± 0.003 (66:1)	0.13 ± 0.01 (5:1)
Cu-F-actin	0.42 ± 0.03 (66:1)	0.16 ± 0.01 (5:1)
G-actin	0.33 ± 0.06 (500:1)	0.19 ± 0.04 (25:1)
Cu-G-actin	0.35 ± 0.05 (500:1)	0.21 ± 0.03 (25:1)

First order rate constants for tryptic and subtilisin digestions of G-actin and F-actin (1.0 mg/ml) at 25°C were determined as described in Materials and Methods. Actin to protease ratios (w/w) at which the rates have been determined are given in parentheses.

quenching of DED emission on F-actin (Fig. 1) but not on G-actin (Kim et al., 1995), and the linear dependence of the quenching of DED-F-actin fluorescence on the binding of Cu^{2+} (Fig. 2).

To test whether the perturbation of loop 38–52 by Cys-374 bound Cu^{2+} in F-actin extends to other regions of subdomain 2, the rates of tryptic digestions of actin were determined in the presence and absence of copper. These digestions, which occur at Lys-61 and Lys-68 in subdomain 2 of actin (Jacobson and Rosenbusch, 1976) yielded the same products and at similar rates irrespective of the presence or absence of bound Cu^{2+}, both in G-actin and in F-actin (Table 1). Thus, the structural perturbation of subdomain 2 by the Cys-374 bound copper appears to be limited to loop 38–52. Additional support for the conclusion on rather limited changes in F-actin by Cu^{2+} comes from acto-S1 ATPase measurements. These assays did not reveal any significant effect of the bound copper on the activation of S1 ATPase by actin (Table 2).

Fluorescence quenching in copolymers of DED-actin

Neither the quenching of DED fluorescence (Fig. 1) nor the acceleration of subtilisin cleavage (Table 1) by Cu^{2+} in F-actin but not in G-actin could eliminate completely the possibility that actin monomers in the F-actin might show intramolecular coupling between loop 38–52 and the C-terminus. In a more stringent test of such a possibility DED fluorescence was measured on several copolymers of actin assembled from DED-actin, NEM-blocked DED-actin, G-actin, and NEM-blocked actin. The rationale for blocking Cys-374 on actin by NEM is that such a modification prevents the binding of Cu^{2+} to actin (Lehrer et al., 1972). As judged by the loss of tryptophan fluorescence quenching in G-actin by Cu^{2+}, all preparations contained between 0.95 and 1.00 NEM/actin.

As shown in Fig. 4, emission spectra of a copolymer of 1.0 μM NEM-DED-actin and 9.0 μM unlabeled actin (*solid trace*) and that of 1.0 μM NEM-DED-F-actin (stabilized by 1.0 μM phalloidin) in the presence of Cu^{2+} (*dotted trace*) were virtually identical. Equimolar amounts of Cu^{2+} also had no effect on NEM-DED-F-actin fluorescence at higher actin concentrations (5.0 μM) in the absence of phalloidin. These spectra revealed that the copolymerization of DED-actin with unlabeled actin did not alter DED fluorescence and, as expected, that the NEM labeling of DED-actin completely blocked the quenching of its fluorescence by Cu^{2+}. However, normal DED fluorescence quenching (i.e., >50%) was observed upon additions of 10 μM Cu^{2+} to copolymers of 1.0 μM NEM-DED-actin and 9.0 μM unlabeled actin (Fig. 4, *dashed trace*). Clearly, because the fluorescence of NEM-DED-F-actin alone was not quenched by Cu^{2+}, the quenching observed in the copolymers with unlabeled actin (*dashed trace*) demonstrated intermolecular coupling between the C-terminus of unlabeled actin and the 38–52 loop of DED-actin. Moreover, the approximately similar level of DED fluorescence quenching by Cu^{2+} in DED-F-actin alone (Fig. 1) and in the copolymers of NEM-DED-actin and unlabeled actin (Fig. 4) suggests that most of the quenching, if not all, is due to such an intermolecular coupling.

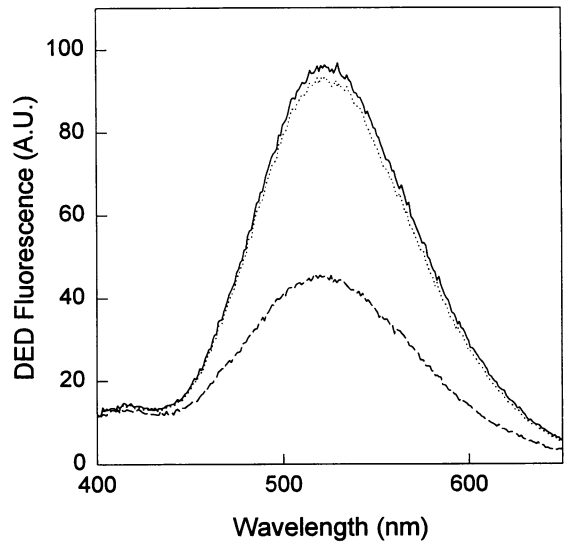

FIGURE 4 Emission spectra of DED-actin copolymers. The spectra were recorded for copolymers of 1.0 μM actin labeled with N-ethylmaleimide at Cys-374 and with DED at Gln-41 and 9.0 μM unlabeled actin in the absence (*solid trace*) and presence (*dashed trace*) of 10 μM copper and for 1.0 μm *N*-ethylmaleimide-DED-actin (stabilized by 1.0 μm phalloidin) in the presence of 1.0 μM copper (*dotted trace*). Solvent conditions and excitation wavelength were the same as in legend to Fig. 1. The copolymers of *N*-ethylmaleimide DED-actin and unlabeled actin were formed by polymerizing their mixtures with 2.0 mM $MgCl_2$.

TABLE 2 S1 ATPase activities activated by F-actin and Cu-F-actin

	ATPase activities (s^{-1})	
S1 (μM)	F-actin	Cu-F-actin
5.0	1.80 ± 0.15	1.76 ± 0.02
10.0	3.40 ± 0.10	3.15 ± 0.05
20.0	5.00 ± 0.25	4.65 ± 0.10

Acto-S1 ATPase activities were determined at a constant actin concentration (10 μM) at 25°C as described in Materials and Methods. The activities are corrected for the ATPase of S1 alone.

Additional test of the quenching mechanism was provided by monitoring the extent of DED fluorescence quenching by Cu^{2+} as a function of NEM-actin concentration in the copolymers of 1.0 μM DED-actin and NEM-actin. Fig. 5 shows that the quenching by Cu^{2+} is abolished completely at molar ratios of NEM-actin to DED-actin ≥ 4.0. In these copolymers DED-actin should still bind Cu^{2+} and thus, the lack of quenching of its fluorescence must be associated with the decreased frequency of DED-actin pairs in F-actin (NEM-actin does not bind Cu^{2+}). This result shows clearly that intermolecular interactions between loop 38–52 and the Cu^{2+} bound to actin's C-terminus are necessary for the quenching of DED fluorescence.

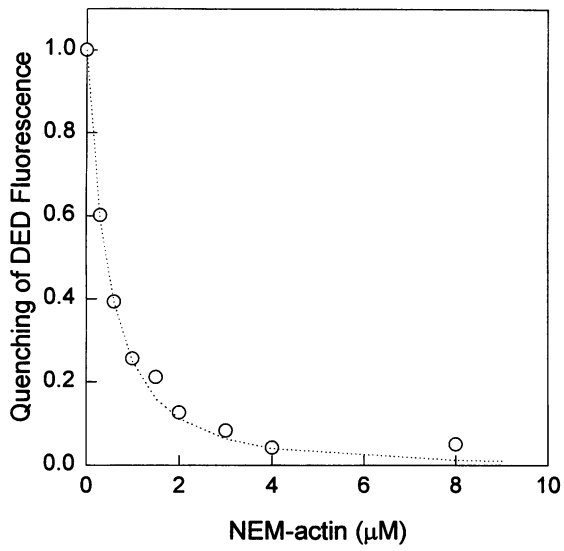

FIGURE 5 Quenching of DED-actin fluorescence by copper in copolymers with actin labeled with *N*-ethylmaleimide at Cys-374. The copolymers of DED-actin and actin labeled with *N*-ethylmaleimide at Cys-374 were obtained by polymerizing the mixtures of 1.0 μM DED-G-actin and *N*-ethylmaleimide-G-actin (between 0.25 and 8.0 μm) with 2.0 mM $MgCl_2$ in the presence of equimolar amounts of phalloidin. Fluorescence intensities were measured with the same emission and excitation settings as in legend to Fig. 2. All solutions contained 9.0 μM copper. Full quenching of DED fluorescence by Cu^{2+} (1.0 on the quenching scale) corresponds to the quenching observed for a solution of 1.0 μM DED-F-actin.

DISCUSSION

The connectivity between distant structural elements on G-actin has been demonstrated and discussed in several recent publications (Strzelecka-Golaszewska et al., 1993; Khaitlina et al., 1993; Muhlrad et al., 1994; Drummond et al., 1992; Adams and Reisler, 1994; Crosbie et al., 1994). It can be rationalized on the basis of the dynamic structure of G-actin and the internal motions predicted by normal mode analysis (Tirion and ben Avraham, 1993). Equally strong evidence exists for the dynamic rearrangements in F-actin, and in particular for changes in subdomain 2 and the C-terminus region of actin (Orlova and Egelman, 1995; Orlova et al., 1995; Muhlrad et al., 1994; Crosbie et al., 1994). Electron microscopy studies demonstrated that both subdomain 2 and the C-terminus can exist in different conformations (Orlova and Egelman, 1995; Orlova and Egelman, 1992; 1993) and showed the connectivity in F-actin between these regions of the protein.

The main goal of this study has been to test whether the steric proximity of loop 38–52 and the C-terminus on an adjacent actin in F-actin (Tirion et al., 1995; Owen and DeRosier, 1993; Lorenz et al., 1993) can lead to the coupling between these sites. The use for that purpose of DED attached to Gln-41 on actin, (Kim et al., 1995) and copper chelated to Cys-374 and the adjacent residues (Lehrer et al., 1972), is particularly attractive. The DED probe on Gln-41 offers the combined advantages of high environmental sensitivity and a minimal or limited structural perturbation of F-actin, as evidenced by the unchanged in vitro motility and acto-S1 ATPase activity (V_{max} and K_m) of the labeled actin (Kim et al., 1996). Similarly, as shown in this study, Cu^{2+} binding to Cys-374 on F-actin does not alter the acto-S1 ATPase activity and thus, probably impacts the structure of F-actin to a smaller extent that other popular Cys-374 probes (Crosbie et al., 1994).

The main conclusion of this work is that the connectivity between the conformational states of loop 38–52 and the C-terminus on actin can result from the intermolecular coupling between these sites. This conclusion is based on the quenching of DED fluorescence by copper in F-actin but not in G-actin, and by the 10-fold acceleration of subtilisin cleavage in F-actin but not in G-actin by the bound copper. The intermolecular coupling is shown even more convincingly by DED quenching (by Cu^{2+}) in copolymers of NEM-DED-actin and unlabeled actin, and by the loss of quenching in copolymers of DED-actin and NEM-actin. The fact that copper has no effect on the acto-S1 ATPase activity and the tryptic digestion of F-actin, as well as on the properties of G-actin, indicates that this metal ion does not alter significantly the actin structure. Furthermore, because subtilisin digestion of F-actin is accelerated by copper for the unlabeled protein, the spectroscopically detected coupling between loop 38–52 and the C-terminus is not an artifact of a dual labeling of actin at Gln-41 and Cys-374. Obviously, however, in those cases in which the effect of G-actin modification is transmitted between loop 38–52 and the C-terminus (Khaitlina et al., 1993), the intramolecular coupling between these sites may be also preserved in F-actin (Kuznetsova et al., 1996).

The relatively slow binding of Cu^{2+} to F-actin, as well as the frequently noted slower modification of Cys-374 in F-actin than in G-actin, may reflect the partial burial of the C-terminus in F-actin structure where in some cases it appears to make contact with loop 38–52 and helix 223–230 (Owen and DeRosier, 1993). In view of the dynamic changes in loop 38–52 and the C-terminus, it is likely that the chelation of Cu^{2+} occurs primarily in the "open" and solvent exposed conformation of the C-terminus. The binding of Cu^{2+} to the C-terminus of actin changes, in turn, the conformation of that region as shown by its decreased susceptibility to carboxypeptidase A (Drabikowski et al. 1977). Both the quenching of DED-F-actin fluorescence by Cu^{2+} and the Cu^{2+}-accelerated subtilisin cleavage of F-actin are consistent with the exposure of loop 38–52 to solvent. This may occur through a rotation of the 38–52 loop to a greater radial distance from the filament axis (Orlova and Egelman, 1995). Such a decreased involvement of loop 38–52 in the intermolecular contacts in F-actin would be a direct consequence of the Cu^{2+}-induced change in the C-terminus of an adjacent actin monomer in F-actin.

It is interesting that the changes in loop 38–52 environment due to Cu^{2+} binding to Cys-374 are noncooperative. This implies that Cu^{2+}-induced changes in the intermolecular contacts in F-actin are less extensive than those produced by filament stabilizing factors, phalloidin and BeFx,

which evoke cooperative transitions in F-actin (Drewes and Faulstich, 1993; Muhlrad et al., 1994). Thus, it appears that cooperative transitions in F-actin may occur in response to more significant changes in intermolecular contacts than those induced by copper binding to Cys-374.

This work was supported by National Science Foundation grant MCB 9206739.

REFERENCES

Adams, S. B., and E. Reisler. 1994. Sequence 18–29 on actin: antibody and spectroscopic probing of conformational changes. *Biochemistry*. 33: 14426–14433.

Crosbie, R. H., C. Miller, P. Cheung, T. Goodnight, A. Muhlrad, and E. Reisler. 1994. Structural connectivity in actin: effect of C-terminal modifications on the properties of actin. *Biophys. J.* 67:1957–1964.

Drabikowski, W., S. Lehrer, B. Nagy, and J. Gergely. 1977. Loss of Cu^{2+} binding to actin upon removal of the C-terminal phenylalanine by carboxypeptidase A. *Arch. Biochem. Biophys.* 181:359–361.

Drewes, G., and H. Faulstich. 1993. Cooperative effects on filament stability in actin modified at the C-terminus by substitution or truncation. *Eur. J. Biochem.* 212:247- 253.

Drummond, D. R., E. S. Hennessey, and J. C. Sparrow. 1992. The binding of mutant actins to profilin, ATP and DNase I. *Eur. J. Biochem.* 209:171–179.

Duong, A. M., and E. Reisler. 1994. Actin: Biophysics, Biochemistry, and Cell Biology. P. J. Higgins and J. E. Estes, editors. Plenum Publishing Corp., New York. 59–70.

Egelman, E. H., and A. Orlova. 1995. New insights into actin filament dynamics. *Curr. Opin. Struct. Biol.* 5:172–180.

Fievez, S., and M. Carlier. 1993. Conformational changes in subdomain-2 of G-actin upon polymerization into F-actin and upon binding myosin subfragment-1. *FEBS Lett.* 316:186–190.

Godfrey, J. E., and W. F. Harrington. 1970. Self-association in the myosin system at high ionic strength. I. Sensitivity of the interaction to pH and ionic environment. *Biochemistry*. 9:886–893.

Jacobson, G. R., and J. P. Rosenbusch. 1976. ATP binding to a protease-resistant core of actin. *Proc. Natl. Acad. Sci. USA*. 73:2742–2746.

Kasprzak, A. A., R. Takashi, and M. F. Morales. 1988. Orientation of the actin monomer in the F-actin filament: Radial coordinate of glutamine-41 and the effect of myosin subfragment-1 binding on the monomer orientation. *Biochemistry*. 27:4512–4522.

Khaitlina, S. Y., J. Moraczewska, and H. Strzelecka-Golaszewska. 1993. The actin/actin interactions involving the N-terminus of the DNase-I-binding loop are crucial for stabilization of the actin filament. *Eur. J. Biochem.* 218:911–920.

Kim, E., C. J. Miller, M. Motoki, K. Seguro, A. Muhlrad, and E. Reisler. 1996. Myosin-induced changes in F-actin: fluorescence probing of subdomain 2 by dansyl ethylenediamine attached to Gln-41. *Biophys. J.* 70:1439–1446.

Kim, E., M. Motoki, K. Seguro, A. Muhlrad, and E. Reisler. 1995. Conformational changes in subdomain 2 of G-actin: fluorescence probing by dansyl ethylenediamine attached to Gln-41. *Biophys. J.* 69: 2024–2032.

Kouyama, T., and K. Mikashi. 1981. Fluorimetry study of N-(1-pyrenyl) iodoacetamide-labeled F-actin. Local structural change of actin protomer both on polymerization and on binding of heavy meromyosin. *Eur. J. Biochem.* 113:33–38.

Kuznetsova, I., O. Antropova, K. Turoverov, and S. Khaitlina. 1996. Conformational changes in subdomain I of actin induced by proteolytic cleavage within the DNase I binding loop: energy transfer from tryptophanto AEDANS. *FEBS Lett.* 383:105–108.

Lehrer, S. S., B. Nagy, and J. Gergely. 1972. The binding of Cu^{2+} to actin without loss of polymerizability: the involvement of the rapidly reacting -SH group. *Arch. Biochem. Biophys.* 150:164–174.

Lorenz, M., D. Popp, and K. C. Holmes. 1993. Refinement of the F-actin model against x-ray fiber diffraction data by the use of a directed mutation alogarithm. *J. Mol. Biol.* 234:826–836.

Muhlrad, A., P. Cheung, B. C. Phan, C. Miller, and E. Reisler. 1994. Dynamic properties of actin: structural changes induced by beryllium fluoride. *J. Biol. Chem.* 269:11852- 11858.

Orlova, A., and E. H. Egelman. 1992. Structural basis for the destabilization of F-actin by phosphate release following ATP hydrolysis. *J. Mol. Biol.* 227:1043–1053.

Orlova, A., and E. H. Egelman. 1993. A conformational change in the actin subunit can change the flexibility of the actin filament. *J. Mol. Biol.* 232:334–341.

Orlova, A., and H. E. Egelman. 1995. Structural dynamics of F-actin. I. Changes in the C terminus. *J. Mol. Biol.* 245:582–597.

Orlova, A., E. Prochniewicz, and E. H. Egelman. 1995. Structural dynamics of F-actin: II. Cooperativity in structural transitions. *J. Mol. Biol.* 245:598–607.

Owen, C., and D. DeRosier. 1993. A 13 Å map of the actin-scruin filament from the Limulus acrosomal process. *J. Cell Biol.* 123:337–344.

Schwyter, D., M. Phillips, and E. Reisler. 1989. Subtilisin-cleaved actin: polymerization and interaction with myosin subfragment 1. *Biochemistry*. 28:5889–5895.

Schwyter, D. H., S. J. Kron, Y. Y. Toyoshima, J. A. Spudich, and E. Reisler. 1990. Subtilisin cleavage of actin inhibits in vitro sliding movement of actin filaments over myosin. *J. Cell. Biol.* 111:465–470.

Spudich, J. A., and S. Watt. 1971. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866–4871.

Strzelecka-Golaszewska, H., J. Moraczewska, S. Y. Khaitlina, and M. Mossakowska. 1993. Localization of the tightly bound divalent-cation-dependent and nucleotide-dependent conformation changes in G-actin using limited proteolytic digestion. *Eur. J. Biochem.* 221:731–742.

Tirion, M. M., D. Ben-Avraham, M. Lopez, and K. C. Holmes. 1995. Normal modes as refinement parameters for the F-actin model. *Biophys. J.* 68:5–12.

Weeds, A., and B. Pope. 1977. Studies of the chymotryptic digestion of myosin: Effects of divalent cations on proteolytic susceptibility. *J. Mol. Biol.* 111:129–157.