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Two opposite effects of cofilin on the thermal unfolding of F-actin: a differential scanning calorimetric study

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Abstract

Differential scanning calorimetry was used to examine the effects of cofilin on the thermal unfolding of actin. Stoichiometric binding increases the thermal stability of both G- and F-actin but at sub-saturating concentrations cofilin destabilizes F-actin. At actin:cofilin molar ratios of 1.5–6 the peaks corresponding to stabilized (66–67 °C) and destabilized (56–57 °C) F-actin are observed simultaneously in the same thermogram. Destabilizing effects of sub-saturating cofilin are highly cooperative and are observed at actin:cofilin molar ratios as low as 100:1. These effects are abolished by the addition of phalloidin or aluminum fluoride. Conversely, at saturating concentrations, cofilin prevents the stabilizing effects of phalloidin and aluminum fluoride on the F-actin thermal unfolding. These results suggest that cofilin stabilizes those actin subunits to which it directly binds, but destabilizes F-actin with a high cooperativity in neighboring cofilin-free regions.

Keywords: Cofilin; Actin; Thermal unfolding; Differential scanning calorimetry

1. Introduction

Actin filaments and their dynamics play essential roles in the development and function of eukaryotic organisms. A large number of actin-binding proteins can control assembly, disassembly, and rearrangement of actin filaments [1]. Small proteins that belong to the actin-depolymerizing factor (ADF)/cofilin family play a central role in the regulation of actin dynamics in cells. These proteins dramatically alter actin microfilament assembly, probably by accelerating the off-rate for actin subunits from the pointed ends of the filaments [2–4]. Cofilin can also depolymerize and sever actin filaments [5]. Cofilin binds stoichiometrically to both monomeric (G-) and polymeric (F-) actin [6]. Binding of cofilin alters the conformation of the so-called small domain of actin, reducing the distance between probes attached to specific sites in subdomains 1 and 2 [7]. A model proposed for the decorated filaments [8] suggests that at least one binding site for cofilin

Abbreviations: ADF, actin depolymerizing factor; DSC, differential scanning calorimetry..

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lies between successive monomers along the long-pitch, making contacts with the lower subdomains of the upper monomer and upper subdomains of the lower subunit.

Cofilin changes the twist of F-actin by decreasing the rotation of one monomer with respect to its neighbors. This decrease (approx. 5°) results in a shorter actin long-pitch helix and a slightly increased diameter. These changes produce a loss of the phalloidin-binding site [8], which in undecorated filaments is located between three adjacent monomers. It is suggested that cofilin can preferentially stabilize one of the existing helical variations in F-actin [9]. Binding of cofilin is associated with weakening of the longitudinal contacts in F-actin, a conclusion that is confirmed by studies on the longitudinal interactions between F-actin subunits in solution [10]. Electron microscopic observations showed that cofilin also disrupts lateral contacts within actin filaments [11].

Differential scanning calorimetry (DSC) is the most effective and commonly employed method to study the thermal unfolding of proteins [12,13]. Previously, this method was successfully used to investigate domain structure of G-actin [14] and to probe changes in the thermal unfolding of actin caused by polymerization [15,16] and its interactions with phalloidin [16,17], membrane lipids [18], nucleotides [19], and phosphate analogs [16,20]. DSC is also useful for structural characterization of protein–protein interactions as a direct method to measure the thermal unfolding of proteins interacting with each other [21]. This approach reveals structural changes, which occur in proteins due to their interaction. Previously, we used the DSC approach to studies on the interaction of F-actin with myosin heads [22–26] and with tropomyosin [27,28]. While these interactions significantly affected the thermal unfolding of myosin and tropomyosin, they had no appreciable influence on the thermal denaturation of F-actin.

In the present study, we apply the DSC approach to determine the effects of cofilin on the thermal unfolding of actin. Our results show that cofilin binding increases the thermal stability of G-actin but different effects are observed on F-actin, depending on a degree of saturation with cofilin. At saturating concentrations, cofilin stabilizes F-actin, but at sub-saturating concentrations it causes a strong decrease in F-actin thermal stability. These results suggest that cofilin stabilizes those actin subunits to which it directly binds, but destabilizes filaments with a high cooperativity in regions of the actin filament that are free of cofilin.

2. Materials and methods

2.1. Protein preparations

Cofilin was prepared as a recombinant protein using a cDNA sequence derived from chick embryo and kindly supplied by Dr Takashi Obinata (Chiba, Japan). The protein was expressed in *Escherichia coli* using a *pGEX* plasmid and isolated by affinity chromatography. The resulting cofilin was obtained with a purity of >95%. Its concentration was determined spectrophotometrically using the absorption coefficient $E_{1\%,\ 1\ cm,\ 280\ nm}$=9.8. Actin was prepared from rabbit skeletal muscle acetone powder [29]. G-actin in G buffer (2 mM Tris–HCl, pH 8.0, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, and 1 mM NaN_3) was used within 1 week. Actin concentration was determined spectrophotometrically using the absorption coefficient $E_{1\%,\ 1\ cm,\ 290\ nm}$=6.3. G-actin was polymerized by the addition of 4 mM $MgCl_2$. Prior to experiments, F-actin was diluted to a final concentration of 25 μM with 30 mM Hepes, pH 7.3, containing 2 mM $MgCl_2$ and 0.2 mM ADP. F-actin was stabilized by the addition of 1.5-fold molar excess of phalloidin (Sigma), or by forming a complex with aluminum fluoride (AlF_4^-). To obtain the latter, 0.5 mM $AlCl_3$ was added to F-actin in the presence of 0.2 mM ADP and 5 mM NaF [20]. Prior to formation of the actin–cofilin complexes, cofilin was dialyzed against an appropriate buffer (either G buffer or 30 mM Hepes, pH 7.3, 2 mM $MgCl_2$ and 0.2 mM ADP).

2.2. Co-sedimentation assay

Samples containing F-actin (or G-actin) and cofilin were sedimented (100 000 *g*, 90 min, 15 °C) and the protein composition of supernatants and pellets was analyzed by SDS polyacrylamide gel electrophoresis [30]. This approach was used to examine the ability of cofilin to polymerize G-actin and to depolymerize F-actin.

2.3. Differential scanning calorimetry (DSC)

DSC experiments were performed on a DASM-4M differential scanning microcalorimeter (Institute for Biological Instrumentation, Pushchino, Russia) as described earlier [16,20,22–28]. All measurements were carried out at a scanning rate of 1 K/min. The reversibility of the thermal transitions was tested by checking the reproducibility of the calorimetric trace in a second heating of the sample immediately after cooling. The thermal denaturation of all protein samples was fully irreversible. Calorimetric traces were corrected for instrumental background and for possible aggregation artifacts by subtracting the scans obtained from the second heating of the samples. The temperature dependence of the excess heat capacity was further analyzed graphically using Origin software (Microcal Inc.). Transition temperatures (T_m) were determined from the maximum of thermal transition.

3. Results

3.1. Stabilization of actin by cofilin

Fig. 1a illustrates the thermally induced unfolding of G-actin, cofilin, and their binary complex where the molar ratio of actin:cofilin was 1:1.2. Separate proteins have thermal transitions with single maxima of 60.3 °C for G-actin and 56.5 °C for cofilin. In the complex, both proteins are mutually stabilized and denature as a unit resulting in a new sharp thermal transition with maximum at 66.4 °C (solid line in Fig. 1a). The width at the half-height of this new transition, $\Delta T_{0.5}$, is equal to 4.4 °C for the G-actin/cofilin complex compared to 7.5 °C for G-actin and 8.5 °C for cofilin. The substantial change in the thermal unfolding of G-actin is a result of binding to cofilin and cannot be due to actin polymerization because co-sedimentation results demonstrate there is no sign of actin polymerization in the presence of saturating concentrations of cofilin (data not shown). Thus, binding of cofilin to G-actin strongly increases the thermal stability of both proteins (Fig. 1a).

A very similar effect is observed for F-actin in the presence of saturating cofilin concentrations (Fig. 1b).

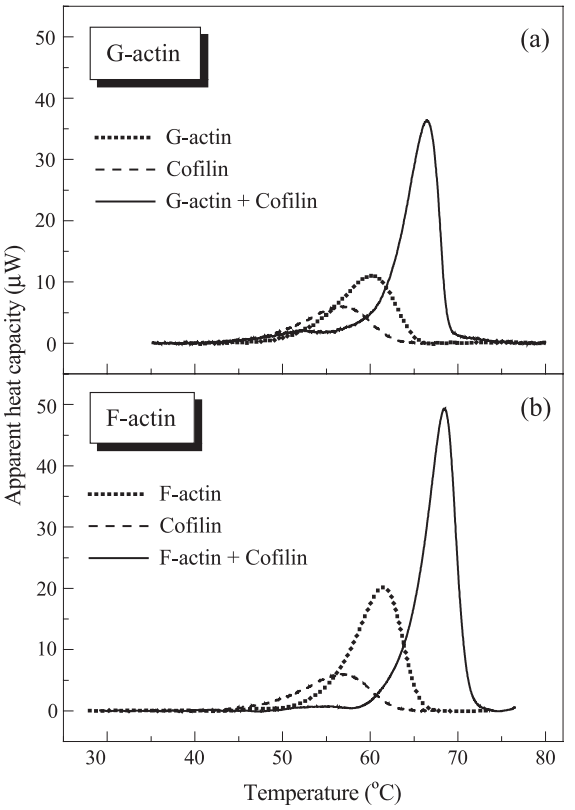

Fig. 1. DSC scans of G-actin (a) and F-actin (b) alone (dotted line curves) and complexed with cofilin (solid curves). The thermal unfolding of cofilin alone is shown by dashed curve. Conditions: 25 μM actin, 30 μM cofilin, G buffer. In the case of F-actin (b) the medium also contained 2 mM $MgCl_2$. Heating rate 1 K/min.

Mg^{2+}-induced polymerization results in changes in the thermal unfolding of actin (dotted line curves, Fig. 1a,b). Although polymerization does not significantly affect the maximum temperature of the transition (T_m increases from 60.3 °C for G-actin to 61.5 °C for F-actin), it produces a pronounced change in the thermal unfolding of actin ($\Delta T_{0.5}$ decreases from 7.5 °C for G-actin to 6.2 °C for F-actin, and ΔH_{cal} increases from 500 to 750 kJ/mol). Saturation of F-actin with cofilin (actin:cofilin=1:1.2) causes a new transition to appear at higher temperature (T_m=68.5 °C; $\Delta T_{0.5}$=4.0 °C). Like G-actin/cofilin (Fig. 1a), the appearance of this new transition is accompanied by the disappearance of the transitions of the separate proteins (Fig. 1b).

Thus, under saturating conditions, the binding of cofilin strongly increases the thermal stability of F-

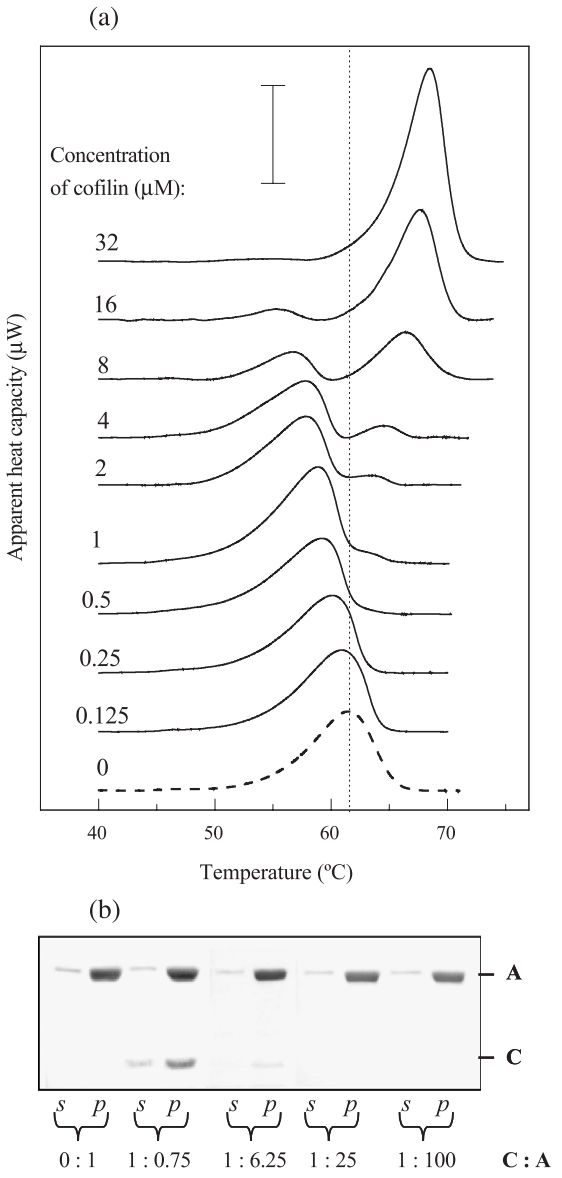

Fig. 2. (a) DSC curves of F-actin-cofilin complexes obtained at various cofilin:actin molar ratios. F-actin concentration (25 μM) was held constant. Concentrations of cofilin are indicated for each curve. Other conditions: 30 mM Hepes, pH 7.3, 2 mM $MgCl_2$, 0.2 mM ADP. A dotted vertical line indicates the maximum of the thermal transition for cofilin-free F-actin (T_m=61.5 °C). The vertical bar corresponds to 25 μW. (b) Prior to DSC scans, polymerization was confirmed by centrifugation of the F-actin-cofilin samples and supernatants (*s*) and pellets (*p*) were run on a 15% SDS PAGE gel. The molar ratios of cofilin:actin (C:A) are illustrated below each lane.

actin, an effect very similar to that observed with G-actin in the presence of cofilin. This stabilizing effect does not depend on buffer conditions because it is observed both in G buffer (pH 8.0) containing 2 mM $MgCl_2$ and 0.2 mM ATP (Fig. 1b), and in 30 mM Hepes, pH 7.3, containing 2 mM $MgCl_2$, and 0.2 mM ADP (Fig. 2a).

3.2. Destabilization of F-actin in the presence of sub-saturating concentrations of cofilin

Contrary to the stabilizing effect of saturating cofilin on the F-actin thermal denaturation (Fig. 1b), sub-saturating concentrations of cofilin destabilize F-actin (Fig. 2a). We observed a substantial decrease in the thermal stability of F-actin with T_m shifted to lower temperatures by 1–6 °C, depending on the molar ratio of cofilin. The most pronounced effect is observed at cofilin concentrations 4–16 μM (i.e. at actin:cofilin molar ratios from 6.25 to 1.56). Under these conditions, two peaks are observed: a cofilin-stabilized F-actin peak at 65–67 °C, and a cofilin-destabilized peak at 56–58 °C (Fig. 2a). On decreasing the molar ratio of cofilin, the cofilin-stabilized peak decreased, whereas the cofilin-destabilized peak increased. At low concentrations of cofilin (0.125–1.0 μM) only the destabilized peak is observed (Fig. 2a) and its maximum shifts to higher temperatures as the molar ratio of cofilin decreases (Table 1). However, the maximum of the

Table 1
The values of the maximum temperature (T_m) for the thermal transitions of F-actin destabilized by sub-saturating concentrations of cofilin (T_{m1}), and for those of F-actin stabilized by cofilin (T_{m2})

Cofilin concentration (μM)	Molar ratio (actin:cofilin)	T_{m1} (°C)	T_{m2} (°C)
0	–	61.5	–
0.125	200	60.8	–
0.25	100	60.1	–
0.5	50	59.2	–
1	25	58.8	63.1
2	12.5	57.85	63.4
4	6.25	57.8	64.5
8	3.13	56.7	66.4
16	1.56	55.4	67.7
32	0.78	–	68.5

The absolute error of the given T_m values did not exceed ±0.2 °C. Concentration of F-actin was 25 μM. The data were extracted from Fig. 2a.

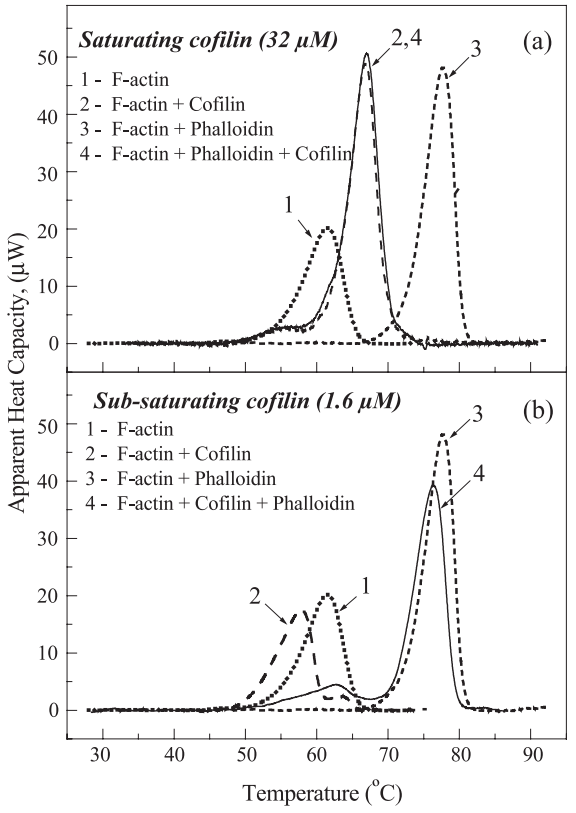

Fig. 3. Effects of saturating (a) and sub-saturating (b) concentrations of cofilin on the thermal stability of F-actin in the presence and absence of phalloidin. DSC curves of F-actin were obtained in the absence (curves 1 and 2) and in the presence of phalloidin (curves 3 and 4). Curves 2 and 4 were obtained in the presence of cofilin, without (curve 2) and with (curve 4) phalloidin. Concentrations of cofilin were 32 μM (a) or 1.6 μM (b). Concentrations of F-actin and phalloidin were 25 μM and 38 μM respectively. Other conditions: 30 mM Hepes, pH 7.3, 2 mM $MgCl_2$, 0.2 mM ADP.

stabilized peak shifts to lower temperature with decreasing the cofilin/actin ratio (Fig. 2a, Table 1). The destabilizing effect of cofilin is highly cooperative, and is observed even at molar ratios as small as 1 cofilin per 100–200 actin monomers.

Co-sedimentation experiments indicate that at sub-saturating molar ratios of cofilin actin is found only in pellets (Fig. 2b). Therefore, the thermal destabilization of F-actin by sub-saturating cofilin is not caused by actin depolymerization but appears to be associated with structural reorganization of the actin filaments.

3.3. Effects of cofilin on the thermal stability of F-actin in the presence of phalloidin or aluminum fluoride (AlF_4^-)

The following experiments were designed to investigate the effects of two F-actin stabilizers, phalloidin and AlF_4^-, on the cofilin-induced thermal stabilization or destabilization of F-actin. In good agreement with previous reports [16,17], the binding of phalloidin significantly increases the thermal stability of F-actin (Fig. 3). Phalloidin shifts the maximum of the F-actin thermal transition from 61.5 to 78 °C (curves 1 and 3 in Fig. 3a,b). When saturating

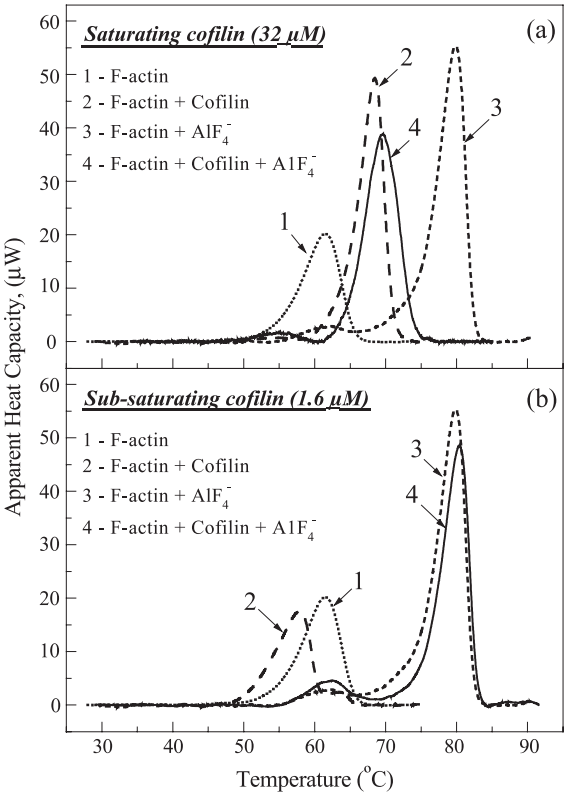

Fig. 4. Effects of saturating (a) and sub-saturating (b) concentrations of cofilin on the thermal stability of F-actin in the presence and absence of aluminum fluoride (AlF_4^-). DSC curves of F-actin were obtained in the absence (curves 1 and 2) and in the presence (curves 3 and 4) of AlF_4^- (5 mM NaF and 0.5 mM $AlCl_3$). Curves 2 and 4 were obtained in the presence of cofilin, in the absence (curve 2) and in the presence of AlF_4^- (curve 4). Concentrations of cofilin were 32 μM (a) or 1.6 μM (b); concentration of F-actin was 25 μM. Other conditions were the same as in Fig. 3.

concentrations of cofilin are added to F-actin/phalloidin, the shape of the DSC trace (curve 4 in Fig. 3a) is essentially the same as it is for the F-actin–cofilin complex in the absence of phalloidin (curve 2 in Fig. 3a). Thus, cofilin abolishes the stabilizing effect of phalloidin. However, sub-saturating concentrations of cofilin had almost no effect on the thermal stability of F-actin stabilized by phalloidin (curves 3 and 4 in Fig. 3b). In this case, addition of phalloidin completely prevented the destabilizing effects of cofilin (Fig. 3b).

Similar effects are observed when cofilin is added in the presence of AlF_4^- (Fig. 4). This anion binds to F-ADP-actin with a very high affinity [31] and strongly increases the thermal stability of F-actin [16,20]. Similar to the effects of phalloidin, addition of AlF_4^- to F-actin shifts the thermal transition of F-actin by 18.5 °C, from 61.5 to 80 °C (curves 1 and 3 in Fig. 4). This effect is almost completely abolished in the presence of saturating cofilin (curve 4 in Fig. 4a). However, addition of AlF_4^- prevents the destabilizing effects of sub-saturating cofilin (Fig. 4b). Thus, both phalloidin and AlF_4^- can overcome the destabilizing effects of sub-saturating concentrations of cofilin whereas saturating concentrations of cofilin abolish their stabilizing effects.

4. Discussion

The data presented here show that cofilin binding can have opposite effects on the thermal stability of F-actin, depending on the molar ratio of cofilin. At saturation, cofilin stabilizes the F-actin structure as seen by the increase in the temperature of thermal transition. However, sub-saturating cofilin strongly destabilizes F-actin, i.e. decreases the thermal stability of F-actin.

The stabilizing effect of saturating cofilin on F-actin (Fig. 1b) is very similar to that observed with G-actin (Fig. 1a). This suggests that cofilin may induce similar structural changes in actin monomers and in those subunits of F-actin to which it binds directly. Elsewhere, we have shown that cofilin decreases the distance between fluorescent probes attached to Gln-41 in subdomain 2 and Cys-374 in subdomain 1 of the small domain of actin [7]. Blondin et al. also have proposed a cofilin-mediated rotation of subdomain 2 accompanied by changes in a FRET distance between Cys-374 and Lys-61 [32]. In F-actin cofilin bridges two longitudinally associated actin subunits, inducing a change in the filament twist [8], altering the interface between subdomains 1 and 2 in F-actin [10] and stabilising lateral interactions in the filament [33]. A changed tilt of F-actin subunits was proposed to stabilise the F-actin structure [9].

Stabilization of F-actin by cofilin completely prevents the effect of phalloidin (Fig. 3a), as though cofilin displaces the phalloidin from F-actin. This is consistent with a recent observation that cofilin can displace rhodamine-phalloidin from F-actin under some specific conditions [34]. Cofilin/actin rods that are induced in cultured cells by heat shock and other stresses to the cell fail to bind phalloidin [35], probably due to changes in the filament twist [8]. However, the affinity of cofilin to F-actin is known to be much weaker than that of phalloidin, and therefore phalloidin usually inhibits the binding of cofilin to F-actin in the in vitro experiments [2,33,36,37]. It is possible, however, that the affinity of cofilin to F-actin may strongly increase upon the heating during DSC experiments. This may result in cofilin binding to F-actin in the presence of phalloidin, therefore, completely preventing the effect of phalloidin on the thermal unfolding of F-actin (Fig. 3a).

Excess cofilin also prevents to a great extent, the stabilizing effect of AlF_4^- (Fig. 4a). These data suggest that structural changes induced by cofilin in the small domain of actin [7] may inhibit structural changes induced by AlF_4^- [20].

An intriguing result of the present work is a destabilization of F-actin by sub-saturating concentrations of cofilin (Fig. 2a). This effect could be explained by either depolymerisation or alteration of an existing F-actin structure, making it less thermostable. Depolymerisation is excluded by DSC data that demonstrate that the addition of phalloidin to cofilin-destabilized F-actin ‘repairs’ the destabilization (Fig. 3b). Pelleting assays (Fig. 2b) confirmed that actin was in the filamentous form. It is important to note that the peaks of stabilized and destabilized F-actin may co-exist on the same thermogram (Fig. 2a). These data suggest that cofilin possibly stabilizes only those actin subunits to which it directly binds, but it destabilizes with a high cooperativity neighboring regions of the actin filament that are free of cofilin. This conclusion is consistent with the electron micro-

scope observations showing that actin subunits within cofilin-free regions of cofilin-decorated actin filaments differ in their orientation from those in undecorated F-actin [9].

The cofilin-induced destabilization of F-actin is prevented by the addition of phalloidin or AlF_4^- indicating that actin subunits destabilized by cofilin retained their ability to bind other stabilizers of F-actin. Thus, binding sites may be still available for the binding of phalloidin in unsaturated filament areas.

These DSC data also suggest that cofilin-induced changes in the conformation of F-actin can be propagated along the filament from the monomers with cofilin bound to cofilin-free actin subunits. This explanation implies the co-existence of at least two different conformations in partially decorated filaments, namely thermostable cofilin-decorated F-actin and thermally unstable F-actin in the undecorated parts of the same filament. The destabilizing effect of cofilin is highly cooperative as it is observed even at very low concentrations of cofilin, as small as 1 cofilin per 100–200 actin subunits (Fig. 2a). The cooperativity of cofilin-induced structural destabilization of actin filaments is much higher than the cooperativity of cofilin binding to F-actin [8]. Therefore, only few cofilin molecules bound to the actin filament are enough to make structural changes in hundreds of actin subunits.

Current models of F-actin acknowledge the possible existence of several conformations of the filament with allosteric interactions within actin subunits and long-range cooperative effects that occur between subunits [38–43]. For example, Orlova et al. observed a large shift in the actin C-terminus that was cooperatively propagated from the gelsolin nucleation point along the filament [44]. A similar high cooperativity of sub-saturating concentrations of AlF_4^- on the F-actin thermal stability has been previously demonstrated by DSC [20].

The destabilizing effect of cofilin demonstrated by this work may be important to provide a possible molecular mechanism for the actin-severing activity of cofilin [5]. Structural destabilization of cofilin-free regions may lead to ‘brittleness’ at the points where the decorated and undecorated regions meet, and actin filaments may be prone to breakage or severing at these ‘weak’ points. The breakage may lead, in turn, to appearance of new pointed and barbed ends, thus leading to the increase in treadmilling. It seems, however, very unlikely that thermal destabilization of F-actin can be the direct result of severing, because the thermal stability of F-actin destabilized by sub-saturating concentrations of cofilin (Fig. 2a) is much less than that of monomeric G-actin (Fig. 1a). This suggests that cofilin-induced destabilization of F-actin includes some serious rearrangement of actin subunits in the destabilized regions of the filament, as well as significant conformational changes in these subunits.

The thermal unfolding of F-actin is a very complicated process, and therefore the effects of cofilin presented here cannot be explained by any existing models that describe the thermal unfolding of proteins interacting with each other. As to stabilizing effect of cofilin, it may be described, although very approximately, by simulations for tight protein–protein interactions [21] or very tight binding of ligands stabilizing the folded state of the protein [13]. However, there were no models whatever that could explain the cooperative effect of F-actin destabilization by sub-saturating concentrations of cofilin. A new model for the thermal unfolding of F-actin filaments has been recently proposed from the DSC data [45]. An important feature of this model is that the irreversible thermal unfolding of F-actin is preceded by at least two stages; one of them is dissociation of actin monomers (or short oligomers) from the pointed end of the filament. In the light of this model, the effects of cofilin on the thermal unfolding of F-actin can be explained as follows.

Cofilin-containing subunits of the filament dissociate together with bound cofilin and then unfold like monomeric G-actin stabilized by cofilin, thus producing a high-temperature thermal transition on the thermogram of F-actin. At sub-saturating concentrations of cofilin its binding to some subunits of the filament causes conformational changes in cofilin-free actin subunits. We propose that these subunits, when dissociate from the filament, can ‘memorize’ for some time the altered conformation, and due to this ‘conformational memory’ the cofilin-free subunits retain the conformation which promotes an increase in their denaturation rate. This is expressed in appearance of a low-temperature thermal transition on the thermogram (Fig. 2a). Thus, we propose that cofilin-free actin subunits of the filament destabilized by sub-saturating cofilin do not have time for relax-

ation to its normal conformation after dissociation from the filament, and therefore they rapidly denature in the altered conformation.

The mechanism proposed above suggests that thermally induced dissociation of subunits (or short oligomers) occurs at lower temperature than that of denaturation. It seems also quite possible that cofilin binding (particularly at sub-saturating concentrations of cofilin) lowers the temperature at which dissociation occurs (the ability of cofilin to accelerate the dissociation of actin subunits from the pointed ends of the filaments [2–4] is in favor of this assumption). If actin subunits dissociate at relatively low temperature (e.g. ~55 °C) from the filament partially decorated by cofilin, then the subunits with altered conformation destabilized by cofilin undergo to fast denaturation, whereas stabilized subunits with cofilin bound remain folded up to 63–65 °C. It is important to note that T_m of the stabilized peak shifts to higher temperature with increasing the cofilin/actin molar ratio (Fig. 2a, Table 1). This effect can be explained in part by the model describing stabilization of a protein by the binding of ligand (cofilin in this case) to the folded state of the protein [13]. Although this model is proposed for reversibly denaturing proteins, it can also be used for proteins, which unfold irreversibly. From this viewpoint, a ligand-induced desrease in denaturation rate may occur if the ligand binding to the folded state is stronger than its binding to the transition state. Actin monomers, when dissociate from the filament in the presence of cofilin, seems to meet these demands. This may explain why T_m of the stabilized peak increases with increasing the cofilin concentration.

The conformational changes spread for a long distance along the filament, from subunits stabilized by cofilin to subunits free of cofilin. Obviously, at low cofilin concentrations these changes become weaken with increasing the distance from subunits stabilized by direct binding of cofilin. This can explain why cofilin-induced destabilization is the most pronounced at rather high cofilin concentrations, from 2 to 16 μM (Fig. 2a, Table 1). Under these conditions, when many subunits in actin filament are directly stabilized by cofilin, the cofilin-free subunits are located not so far from subunits with cofilin bound, and therefore they can memorize a rather strong conformational changes when dissociate from the filament before denaturation.

Thus, the recently proposed ‘dissociative’ mechanism for the thermal denaturation of F-actin filaments [45] can explain, to some extent, the opposite effects of cofilin on the thermal unfolding of F-actin.

The stabilizing and destabilizing effects of cofilin may be important in living cells. Cofilin is localized near the cell membrane and is not so abundant towards the center of the cell [1]. Cellular concentrations of ADF/cofilin (approx. 20 μM) [46] are substantially lower than the concentration of actin (approx. 65 μM) [1]. Thus, actin microfilaments will be highly decorated just beneath the cell membrane and less so as the filaments extend into the cell. Our data suggest that in the absence of other factors, these filaments will become progressively unstable thereby generating actin monomers that can be reassembled at the cofilin-rich regions beneath the plasmalemma. This means that destabilization of actin filaments by cofilin demonstrated here may take place in living cells and play an important role in actin dynamics.

In conclusion, DSC studies on cofilin–F-actin complexes offer a new and promising approach for probing of the cofilin-induced structural changes in actin filaments. The DSC approach makes it possible to reveal two opposite effects of cofilin on the thermal stability of actin filaments, stabilization and highly cooperative destabilization of the filaments. This approach can be useful for further studies on the complexes of F-actin with other actin-binding proteins.

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