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The effect of ADF/cofilin and profilin on the dynamics of monomeric actin

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ABSTRACT

The main goal of the work was to uncover the dynamical changes in actin induced by the binding of cofilin and profilin. The change in the structure and flexibility of the small domain and its function in the thermodynamic stability of the actin monomer were examined with fluorescence spectroscopy and differential scanning calorimetry (DSC). The structure around the C-terminus of actin is slightly affected by the presence of cofilin and profilin. Temperature dependent fluorescence resonance energy transfer measurements indicated that both actin binding proteins decreased the flexibility of the protein matrix between the subdomains 1 and 2. Time resolved anisotropy decay measurements supported the idea that cofilin and profilin changed similarly the dynamics around the fluorescently labeled Cys-374 and Lys-61 residues in subdomains 1 and 2, respectively. DSC experiments indicated that the thermodynamic stability of actin increased by cofilin and decreased in the presence of profilin. Based on the information obtained it is possible to conclude that while the small domain of actin acts uniformly in the presence of cofilin and profilin the overall stability of actin changes differently in the presence of the studied actin binding proteins. The results support the idea that the small domain of actin behaves as a rigid unit during the opening and closing of the nucleotide binding pocket in the presence of profilin and cofilin as well. The structural arrangement of the nucleotide binding cleft mainly influences the global stability of actin while the dynamics of the different segments can change autonomously.

1. Introduction

It is widely accepted that the function of proteins depends on their conformational and dynamic properties in a certain physico-chemical environment. The conformational heterogeneity of proteins is directly correlated with their flexibility. The concept of flexibility involves intra-molecular motions from picosecond-scale thermal fluctuations to millisecond-scale (or slower) conformational transitions [1]. There is a strong coupling between the flexibility, stability and function of the proteins. The higher flexibility is generally the consequence of a less stable protein structure that can be often related to increased biological activity [2].

The dramatic change in the actin dynamics can be recognized when actin is under the influence of intracellular effectors such as actin binding proteins [3–11]. Based on structural information the actin monomers are composed of a small and a large domain with a bound nucleotide and a divalent cation inserted between them [12]. The small domain contains subdomains 1 and 2 while the large domain is built up from subdomains 3 and 4. Comparing different actin structures revealed that actin is composed of rigid cores and variable conformational regions [13]. The rigid core of the large domain is shared by subdomains 3 and 4 while the rigid core of the small domain is restricted exclusively to subdomain 1. This arrangement of actin suggests that the rigid cores of the two main domains can move relative to each other through a shear region while the subdomain 2 can rotate independently [13]. Based on studies of intramolecular dynamics the limited dynamical heterogeneity of the core regions is very similar in the different states (G- and F-actin) and origin (i.e. yeast and skeletal) of actin while the variability of the flexible regions is highly pronounced [14,15]. The flexible regions involve segments participating in the interaction of actin protomers and in the docking area of actin binding proteins [14–16].

The intracellular organization of the actin cytoskeleton is regulated by a large number of actin binding proteins (ABP). The functions of the ADF/cofilin family members are highly versatile and complex, which can emphasize their strategic position in organizing intracellular processes. ADF/cofilin enhances the disintegration of the actin filament meshwork via severing the filaments, promoting the dissociation of

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actin subunits at the pointed end and decreasing the rate of nucleotide exchange on actin monomers [17–19]. The members of ADF/cofilin family act on actin in a concentration dependent manner as their function of severing and filament nucleation is emphasized at different ADF/cofilin levels [20]. ADF/cofilin severing activity is more pronounced at the boundaries between decorated and non-decorated sections of actin filaments [21,22] and their binding to F-actin can accelerate the release of inorganic phosphate from the filament [23]. ADF/cofilins can induce a steady regime in the filament length fluctuation and can maintain the actin filaments in a very dynamical state [24]. Members of the profilin family facilitate the nucleotide exchange on monomeric actin and are capable to maintain a large pool of polymerization competent actin subunits intracellularly [25,26]. Profilin inhibits the formation of new actin seeds while assists in the incorporation of actin monomers at the barbed end in the presence of formins [27–29].

It was confirmed that ADF/cofilin binds to G-actin in a similar fashion like twinfilin-1 [30]. The ADF homology domain interacts with the hydrophobic cleft between the subdomains 1 and 2 on G-actin [31,32]. The nucleotide binding cleft is closed in the complex which can explain the decreased nucleotide exchange rate on actin in the presence of the ADF homology domain [31,33].

Two types of actin–profilin complex (tight and open) were identified based on X-ray crystallography measurements [34,35]. In both crystal structures profilin binds to the barbed end of the actin at the groove between the subdomains 1 and 3. In the open structure the nucleotide binding cleft is more exposed to the solvent which results in a high nucleotide exchange rate on actin. The open form of the complex can be transformed into the tight form via the closure of the nucleotide binding pocket by the rotation of subdomain 2 [34,35]. Molecular dynamics simulation showed that the open structure is thermodynamically unstable in the absence of profilin and removing profilin from the complex results in the closure of the nucleotide binding cleft within 200 ps [36].

Wen and colleagues raised the interesting idea that the subdomains 1 and 2 in actin evolved together to support the allosteric behavior of the small domain. They verified their idea as they found that the subdomains 1 and 2 of actin are forming an integrated unit in the presence of profilin [10]. In the present work the integrated function of the small domain of actin was tested in the presence of cofilin as well. Temperature dependent FRET experiments were applied to obtain information about the dynamics of the small domain in the presence of cofilin or profilin. This special type of FRET measurement was successfully applied before to test the flexibility of the protein matrix between the fluorescent donor–acceptor pairs attached to Lys-61 in subdomain 2 and Cys-374 in subdomain 1 in actin [37,38]. Time resolved anisotropy decay measurements were also performed to support the results of the temperature dependent FRET experiments. Further aim of the present work was to evaluate the influence of cofilin and profilin binding on the thermodynamic stability of actin monomers. The results revealed that subdomains 1 and 2 are forming a unified working unit under the influence of profilin and cofilin as well. The thermodynamic consequence of ABP binding to actin showed that cofilin can increase the global thermodynamic stability of actin while profilin induced an opposite effect.

2. Material and methods

2.1. Protein preparation

Acetone-dried muscle powder was obtained from rabbit skeletal muscle as described earlier by Feuer and colleagues [39]. The calcium bound G-actin was prepared according to the modified method of Spudich and Watt [40,41]. The concentration of the G-actin was determined spectrophotometrically with a Shimadzu UV-2100 spectrophotometer by using the absorption coefficient of 1.11 mg ml^{-1} cm^{-1} at 280 nm [42]. The G-actin was stored in G-buffer at pH 8.0 (4 mM MOPS, 0.2 mM ATP, 0.1 mM $CaCl_2$, 0.5 mM MEA, and 0.005% NaN_3).

The plasmid construction of GST-fusion mouse cofilin-1 (MW: 15.9 kDa) and His-tagged mouse profilin-1 (MW: 13.5 kDa) were expressed in *Escherichia coli* BL21 (DE3) strain and purified as described previously [33]. The concentration of mouse cofilin and profilin were estimated at 280 nm with a Shimadzu UV-2100 spectrophotometer by using a calculated absorption coefficient of 1.002 and 1.472 mg ml^{-1} cm^{-1}, respectively. The absorption coefficients were determined by ProtParam (http://us.expasy.org/tools) based on amino acid composition of the proteins. Proteins were stored in G-buffer at −80 °C and were used within 2 months.

2.2. Labeling of actin

The labeling of Cys-374 of actin with the donor fluorophore (IAEDANS) was performed as described earlier [43]. The concentration of IAEDANS labeled G-actin was determined spectrophotometrically taking into account the contribution of the label to the absorption at 290 nm [42]. The concentration of IAEDANS was calculated at 336 nm using the absorption coefficient of 6100 M^{-1} cm^{-1} [44]. The labeling ratio was in the range of 60–92%.

The acceptor (FITC) was attached to the Lys-61 on G-actin according to Miki and colleagues [43]. The concentration of double labeled IAEDANS–FITC-actin was determined by Bradford protein assay using unlabeled G-actin as standard [45]. The concentration of FITC was estimated spectrophotometrically with the absorption coefficient of 74,500 M^{-1} cm^{-1} at 493 nm [46]. The extent of FITC labeling was in the range of 95–99%.

2.3. Fluorescence quenching

The local environment of the donor (IAEDANS) fluorophore was investigated by quenching the fluorescence intensity with acrylamide in the presence and absence of cofilin and profilin. Fluorescence quenching measurements were carried out with a Perkin–Elmer LS50B spectrofluorometer at 20 °C. The concentration of the labeled actin was 2 μM in G-buffer and the final concentration of cofilin and profilin were adjusted to 10 and 25 μM, respectively. The emission spectrum of IAEDANS labeled actin was measured in the range of 365 and 600 nm with the excitation wavelength of 360 nm. The excitation and emission slits were set to 5 nm. The concentration of the acrylamide (Q) increased from 0 to 0.5 M and the area under the emission spectrums represented the fluorescence intensity in the calculations. The ratio of the fluorescence intensity in the absence (F_0) and presence (F) of the quencher molecule was plotted against the concentration of the acrylamide. The Stern–Volmer constant (K_{SV}) was obtained from the slope of the fitted straight line according to the Stern–Volmer equation [47]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q]. \quad (1)$$

2.4. Fluorescence anisotropy

Steady-state fluorescence anisotropy measurements were applied to determine the affinity of mouse cofilin and profilin to the IAEDANS labeled actin monomers. Fluorescence anisotropy experiments were performed at 20 °C with a Perkin–Elmer LS50B spectrofluorometer equipped with a thermostated cuvette holder. The excitation and emission wavelength were 360 nm and 475 nm, respectively. The excitation and emission slits were set to 5 nm. 2 μM IAEDANS G-actin was titrated with the different amount of cofilin and profilin during the measurements.

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