Ph.D. thesis

Toxin Induced Conformational and Dinamical Changes in Actin Filaments

Balázs Visegrády

Program: Biochemistry and molecular biology

Program leader: Prof. Dr. Balázs Sümegi

Subprogram: B-130: Functional protein dynamic studies by biophysical methods

Subprogram leader: Prof. Dr. Béla Somogyi

Tutors: Dr. Nyitrai Mklós

Dr. Lőrinczy Dénes

University of Pécs, Faculty of Medicine, Institute of Biophysics

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Introduction

Phalloidin, a cyclic peptide from *Amanita phalloides* can tightly bind to actin filaments and stabilizes their structure (Faulstich et al., 1977; Miyamoto et al., 1986). Phalloidin stabilized actin filaments were extensively used in *in vitro* studies. Fluorescent derivatives of phalloidin (e.g.: rhodamine-phalloidin, fluorescein-phalloidin, eosin-phalloidin) were also applied to visualize the architecture of the actin cytoskeleton by fluorescence microscopic methods in intracellular studies.

The effect of another cyclic peptide, jasplakinolide from a marine sponge (*Jaspis johnstoni*) on actin filaments is understood to a much lesser extent than that of phalloidin. It is known that jasplakinolide binds to F-actin, and stabilize it's structure *in vitro* (Bubb et al., 1994). Jasplakinolide accelerates actin polymerization (Bubb et al., 1994), promotes actin polymerization under nonpolymerizing conditions. Although phalloidin can stabilize actin oligomers, similar effect from jasplakinolide was not observed (Spector et al., 1999). Jasplakinolide and phalloidin competitively bind to actin filament, which suggest that the binding sites of the two drugs are identical or similar in high extent (Senderowicz et al., 1995). Another important difference between the two drugs is that in contrast to phalloidin, jasplakinolide readily enters cells. It appears that the effect of jasplakinolide is similar to that of phalloidin in some aspects, while other effects are different for the two toxins. In this study we compared the effect of phalloidin and jasplakinolide on the dynamic properties and thermal stability of actin filaments.

Aims

1. Study the differences between the effect of phalloidin or jasplakinolide on actin filament using fluorescence spectroscopy.

2. Describe and compare the thermodinamical parameters of actin filament in the presence or absence of toxins.

3. Characterization of the actin filament under substoichiometric toxin concentrations.

4. Describe the allosteric effect of the toxins with a quantitative model.

Methods

Steady-state fluorescence experiments

The steady-state fluorescence measurements were performed with a Perkin-Elmer LS50B Luminescence Spectrometer equipped with a thermostatic sample holder flushed with dry air against the water condensation. The experiments were carried out at pH 8.0 in a buffer containing 2 mM Tris/HCl, 0.2 mM ATP, 0.005 % NaN_3, 0.5 mM 2-mercaptoethanol, 100 mM KCl and 2 mM $CaCl_2$ (buffer A).

The efficiency of FRET (E) can be determined from the donor intensities by using the following equation:

$$E = 1 - (F_{DA}/F_D) \quad (1)$$

where F_{DA} and F_D are the fluorescence intensities of the donor in the presence and absence of the acceptor, respectively.

The normalized FRET efficiency, f ', was defined as the ratio of the FRET efficiency to the fluorescence intensity of the donor measured in the presence of the acceptor (Somogyi et al., 1984):

$$f' = E/F_{DA} \quad (2)$$

An experimental strategy has been developed to obtain information regarding the flexibility of the different forms of a protein by temperature dependent FRET measurements. The temperature profile of f ' (Eq. 2.) is characteristic for the flexibility of the protein matrix between the applied donor and acceptor (Somogyi et al., 1984).

Differential scanning calorimetry (DSC)

The thermal denaturation of actin filaments was monitored between 0 °C and 100 °C with a SETARAM Micro DSC-II calorimeter. Calorimetric enthalpy change (ΔH) of endothermic transitions were calculated from the area under the heat absorption curve using two-point setting SETARAM peak integration. Transition entropy change (ΔS) was calculated for the peak transition temperature (T_m) from the following equation:

$$\Delta S = \Delta H/T_m. \quad (3)$$

The Gibbs free enthalpy change was calculated from the following equation:

$$\Delta G = \Delta H - T\Delta S. \quad (4)$$

Results

Study the flexibility and thermal stability of Ca-actin filaments

In this work our aim was to compare the effect of phalloidin and jasplakinolide on the dynamics of actin filaments. The filament flexibility showed little dependence on the presence of phalloidin in the case of Mg-F-actin (Nyitrai et al., 2000), while for Ca-F-actin the filament flexibility was greatly reduced after phalloidin binding. Therefore, we have chosen Ca-F-actin as a model system in this study. The actin filaments were treated with toxins in 1 : 1 molar ratio in all cases befor the experiments.

Fluorescence resonancy energy transfer (FRET)

In one set of experiments, the separately labeled IAEDANS-actin and IAF-actin monomers were mixed before the polymerization. These experiments therefore reported on the intermonomer flexibility of actin filaments. In another set of experiments the double labeled IAEDANS-FC-actin monomers were incorporated into actin filaments, and the intramonomer flexibility of individual actin protomers within the filaments was characterized.

The smaller temperature induced increase of *f* ' in the presence of toxins (phalloidin or jasplakinolide) indicated that the monomer-monomer interactions along the actin filament became stiffer and the flexibility of the actin filaments, either intermonomer or intramonomer cases. The effect of jasplakinolide was slightly greater than that of the phalloidin in all cases.

Differential scanning calorimetry (DSC)

The calorimetric curves were obtained between 0 °C and 100 °C for Ca-F-actin in the absence or presence of toxins in 1 : 1 molar ratio. We found one large transition - corresponding to the filament heat denaturation - at each cases (> 60 °C), which we attribute

to the thermal denaturation of the filaments. The melting temperature (T_m) from this transition was 67.3 °C in the absence of toxins, and shifted to 79.3 °C and 87.7 °C in the presence of phalloidin or jasplakinolide, respectively. The larger T_m is attributed to the greater resistance to heat denaturation, and thus the variation of the T_m values indicated that the thermal stability of Ca-F-actin was increased after the binding of either phalloidin or jasplakinolide. In agreement with the fluorescence data the stabilizing effect of jasplakinolide was greater on Ca-actin filaments than that of phalloidin.

The cooperative effect of the toxins on Mg-actin filaments

A number of ligands can induce conformational changes in actin filaments, which propagate to distant protomers from the binding site (Drewes and Faulstich, 1993; Orlova and Egelman, 1995; Steinmetz et al., 1997). One of these ligands is phalloidin (Drewes and Faulstich, 1993; Orlova and Egelman, 1995). We used here the phalloidin-actin complex as a reference model. We tested whether the effect of the binding of jasplakinolide was cooperative as well.

The conformations of actin in the presence of phalloidin or jasplakinolide

DSC experiments were carried out at a series of drug : actin protomer ratios ranging from 1 : 500 to 1 : 1. At substoichiometric drug concentrations the DSC curves usually showed three transitions with three characteristic melting points. This complex nature of the DSC curves suggested that the actin filaments were present in multiple conformational states in these samples. The processing of the DSC data was carried out by determining the T_m values for each melting components and then the integrals attributed to the heat absorption curves at each T_m. The latter analyses involved deconvolution and assumed that the individual melting curves were Gaussian. We used the enthalpies attributed to the three T_m values as the measure of the amount of actin denatured at the corresponding T_m value. T_m^a, T_m^b and T_m^c are the T_m values at the lowest, intermediate and the highest temperatures, respectively (Figure 1.a,b). At each drug : actin protomer ratio T_m^a was identical to the T_m value measured in the absence of drugs and corresponded to the actin conformation unaffected by drug binding. On the applied temperature scale another thermal transition peak (T_m^c) appeared at ~ 80 ^{o}C and ~ 90 ^{o}C for phalloidin and jasplakinolide, respectively and were similar to that measured at a 1 : 1 drug : actin protomer ratio. The higher T_m^c value for jasplakinolide indicates that the stabilisation effect of this drug is greater on Mg-F-actin than that of phalloidin. The integral attributed to the heat absorption curve corresponding to T_m^c depended linearly on the drug concentrations (Figure 1.a,b).

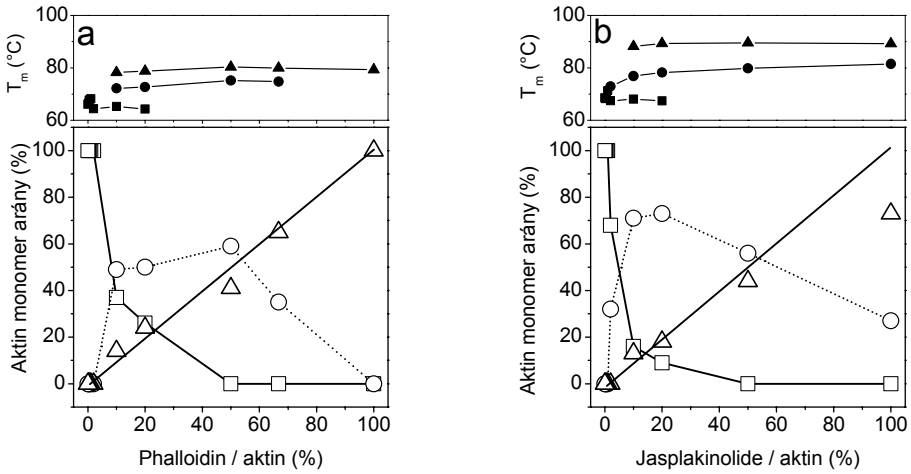


Figure 1.

The upper panels show the drug concentration dependence of the determined T_m values with corresponding filled symbols. The lower panels show drug concentration dependence of the contribution of actin conformations corresponding to $T_m{}^a$ (empty squares), $T_m{}^b$ (empty circles) and $T_m{}^c$ (empty triangles) to the total enthalpy required for denaturation. The results are shown for phalloidin (a) and jasplakinolide (b).

These results suggest that there was a subpopulation of actin protomers which directly bound drug and melted at a temperature ($T_m{}^c$) characteristic for the most stable actin conformation. In both cases there was a third component of the thermal denaturation curves ($T_m{}^b$) at intermediate drug concentrations. We interpret these data as there was an actin population at intermediate drug : actin protomer ratios which did not bind the drugs directly, but was stabilised by the drug at locations distant from the binding site.

A model to describe cooperativity

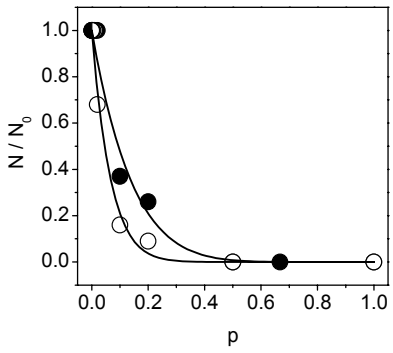
To quantitatively describe the cooperative interactions along the actin filaments we assumed that these drugs bind randomly to actin protomers. We attempted to determine the number of cooperative units (number of protomers), over which the effect of the binding of an individual drug molecule can propagate.

We determined the probability that a protomer is not affected by the drug binding (N/N_0) is given by:

$$N / N_0 = (1 - p)^{2k+1} \qquad (5)$$

where p is the probability that an actin protomer binds the drug, N_0 is the total number of monomers in a filament, and N the number of actin protomers in a filament, which are not affected by drug binding.

The N / N_0 ratio was experimentally determined from the relative contribution of the actin conformation which was characterised by T_m^a in Figure 1, while the actual value of p was given by the applied drug : actin protomer ratio. When Eq. 5. was fitted to the p *vs.* N / N_0 plots (Fig. 2.), the value of k was determined to be 3.0 ± 0.5 and 6.9 ± 1.1 for phalloidin and jasplakinolide, respectively.



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The ratio (N / N_0) of the concentration of Mg-actin monomers which were not affected by the binding of phalloidin (filled circles) or jasplakinolide (empty circles) as a function of the probability that an actin monomer binds a drug molecule.

Accordingly, the binding of one phalloidin molecule could change the conformation of $2k+1 = 7$ actin protomers, while one jasplakinolide could modify 15 actin protomers. These values showed that the range of allosteric interaction along the actin filament was longer for jasplakinolide than that for phalloidin.

Summary

In this work the effect of phalloidin and jasplakinolide on the dynamic properties and thermal stability of actin filaments was studied. The results showed that actin filaments became more rigid in the presence of phalloidin or jasplakinolide. The fluorescence and calorimetric measurements provided evidences that the extent of stabilization by jasplakinolide was greater than that by phalloidin.

We showed here that stabilisation effect of phalloidin and jasplakinolide on Mg-actin filaments was cooperative. To describe the experimental data we established a simple quantitative model, which revealed that the number of cooperative units was larger for jasplakinolide (15 actin protomers) than that for phalloidin (7 protomers).

Publications corresponding to the thesis

Visegrády B, Lőrinczy D, Hild G, Somogyi B, Nyitrai M. The Effect of Phalloidin and Jasplakinolide on the Fexibility and Thermal Stability of Actin Filaments. FEBS Lett. 2004, 565(1-3), 163-6.

Visegrády B, Lőrinczy D, Hild G, Somogyi B, Nyitrai M. A Simple Model for the Cooperative Stabilisation of Actin Filaments by Phalloidin and Jasplakinolide FEBS Lett. 2005, 579(1-3), 6-10.

Other publications

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