Biophysical Chemistry 11 (1980) 443–446

THE FLEXIBILITY OF F-ACTIN

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1. Introduction

This is a brief summary of our study on the flexibility of F-actin in vivo and in vitro. Actin was first discovered as one of the major proteins in muscle and has been found to be widely distributed in a variety of cells of animals and plants. It is in a state of dispersed monomers, G-actin, in a salt-free solvent and forms a double stranded helical polymer, F-actin, with the addition of neutral salts [1] (fig. 1). The molecular weight of actin is about 41 k. The cross-over repeat of the double stranded helix of F-actin is about 38 nm in which about 14 monomers are contained (fig. 2). The length of F-actin attains several microns.

As a polymer made by the secondary bonding between globular protein molecules, F-actin should not be completely rigid, but flexible.

2. Flexibility in vitro

The flexibility of F-actin was first estimated by Fujime by the measurement of intensity fluctuation of scattered light from F-actin in solution [2]. The relaxation time of the thermal bending motion of F-actin, the average length of which was about 2 μm, was found to be about 10 ms and the flexural rigidity was calculated to be about 2×10^{-17} dyn cm^2. Since then, various experimental methods have been employed to estimate the flexibility of F-actin. Among them, the electronmicroscopic analysis of the average shape of a large number of negatively stained F-actin gave a value of the rigidity in a good agreement with the previous result of light scattering [3].

Last year, it was found by Nagashima that if decorated with myosin or heavy meromyosin, single filaments of F-actin can be directly observed under a dark field optical microscope [4]. The filaments showed thermal bending motion and the relaxation time of the motion was of the order of seconds for F-actin of a length of about 10 μm. This is reasonable because the relaxation time must be proportional to the fourth power of the length. From the statistical analysis of the shape of moving filaments, the flexural rigidity was estimated to be about 7×10^{-17} dyn cm^2, which is larger than, but not very different from the value obtained previously.

Thus, there is no doubt about that F-actin is semi-flexible and its flexural rigidity is in a range of $2 \sim 7 \times 10^{-17}$ dyn cm^2.

Fig. 1. An electronmicrograph of negatively stained F-actin filaments, showing flexibility. (Taken by S. Higashi–Fujime).

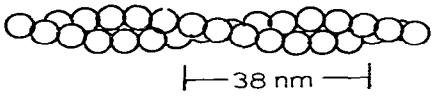

Fig. 2. A model for the structure of F-actin.

Table 5
The flexibility of F-actin or thin filament

Methods	Flexibility		
	flexural rigidity (dyn cm^2)	persistence length (μm)	Ref.
in vitro (in solution)			
light scattering	1.7×10^{-17}	6.0	[2]
electronmicroscopy	1.7	6.0	[3]
optical microscopy a)	7.2	25	[4]
in vivo (in fiber)			
electro-optic effect b)	3.1	11	[6]
polarized fluorescence	5.3	17	[7]

a) F-actin with heavy meromyosin.
b) F-actin with tropomyosin and troponin.

3. Flexibility in vivo

In living cells, actin is mostly in a state of polymer. The thin filament in the striated muscle consists of F-actin having bound tropomyosin and troponin. Does F-actin or the thin filament in vivo have a flexibility similar to that in vitro? It was found by Umazume and Fujime that an electric field applied along a muscle fiber induced a better parallel orientation of thin filaments in the fiber and the disorientation of the filaments after cutting off the field gave information about the flexibility of the filaments [5]. The flexural rigidity of thin filaments was obtained by Yoshino from the phase difference between the applied alternating field and the oscillation of filament orientation [6].

The flexibility of thin filaments in muscle was also estimated by Yanagida from the average shape determined by the polarized fluorescence of a fluorescent analogue of ADP tightly bound to F-actin [7]. The estimation from the movement and the shape of thin filaments in vivo gave similar values of the flexural rigidity as in vitro, as summarized in table 1.

It must be emphasized again that the results obtained by different dynamic and static methods all showed good agreement. Taking into consideration differences in experimental conditions, the agreement is satisfactory.

4. Bond elasticity

To calculate the flexural rigidity from the relaxation time of bending motion or from the average shape, F-actin or the thin filament was regarded to be a uniform semiflexible filament [8]. The persistence length of the same filament can be calculated from the flexural rigidity. The value of the persistence length is also listed in table 1. It is in a range of 6–20 μm, which is much longer than the pitch of the double stranded helix of F-actin, but not too long. F-actin longer than 10 μm may assume a curved or coiled form rather often. Actually, in some kinds of non-muscle cells, long F-actin or its bundle has been found in a strongly curved form.

Assuming a uniform cylindrical filament, the elastic modulus for stretching, Young's modulus, can be calculated from the flexural rigidity. Young's modulus is related to the microscopic elastic constant for stretching of the monomer-monomer bond. The above value of the flexural rigidity of F-actin gives Young's modulus of the order of 10^{11} dyn cm^{-2} and the microscopic elastic constant of the bond of about 2×10^4–10^5 dyn cm^{-1}, although these values depend on the effective radius of the cross section of F-actin [8].

Actually, F-actin is not a uniform cylinder but a helical polymer, along which the elasticity is not necessarily uniformly distributed. The above value of the elastic modulus is in the same range as the intramonomer elastic modulus of protein estimated theoretically and experimentally. In F-actin, it is likely that both monomer-monomer bonds and individual monomers have elasticities of a similar order of magnitude.

According to the above value of the microscopic elastic constant, the stretching of the monomer-monomer bond by 0.1 nm results in an increase of the free energy of about 6–30 kcal/mole which is of the same order as the free energy gain in the polymerization of actin [1].

5. Effects of temperature

The flexibility of F-actin or the thin filament has been found to increase with rising temperature [1,9]. The flexural rigidity decreases with rising temperature. This means that the force against bending or stretching of F-actin comes from the increase of enthalpy. On the other hand, it was previously found that the polymerization of actin is an endothermic process. The polymeri-

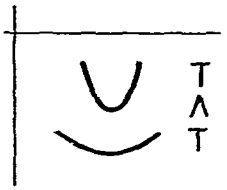

Fig. 3. Free energy profile of the actin-actin bond at two temperatures.

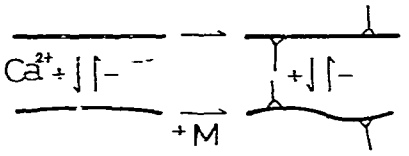

Fig. 4. Illustration of the flexibility change in the thin filament induced by Ca ion and myosin.

zation and depolymerization are associated, respectively, with increase and decrease of entropy. When an actin monomer approaches to an end of F-actin to form a bond, entropy increases. The rearrangement of water molecules around actin monomers may take part in this process. In the final stage of bonding, however, a short-range energetic force acts between monomers. The flexibility is determined by the short-range force.

As a function of the monomer-monomer distance, the the minimum of the momomer-monomer interaction free energy in F-actin is lowered and broadened with rising temperature. F-actin has a larger deformability at a higher temperature (fig. 3).

The polymerization of actin depends on the ionic condition. The optimum for polymerization is around 0.1 M of monovalent salts and neutral pH. It has been found, however, that the ionic condition changes the entropy for polymerization but does not change the enthalpy [9]. The flexibility of F-actin is not very much dependent on the ionic condition.

6. Flexibility of thin filament and Ca ion

In the striated muscle the interaction between myosin in thick filaments and actin in thin filaments coupled with the splitting of ATP generates the force for contraction. This interaction is initiated when Ca ion released from sarcoplasmic reticulum binds with troponin on the thin filament [10]. Since the thin filament is composed of F-actin, tropomyosin and troponin, the binding of Ca ion to troponin is expected to induce a conformational change in the thin filament which makes possible the interaction of actin with myosin.

The first finding was made by Ishiwata and Fujime in their light scattering experiment [11]. The thin filament reconstituted in vitro was less flexible in the absence of Ca ion than in its presence. The flexibility change occured at a micromolar concentration of free Ca ion, corresponding to the concentration required for muscle contraction.

This finding has been supported by the measurements of ultraviolet dichroism and polarized fluorescence due to ADP and its analogue bound to F-actin in the thin filament [12,13]. In this case also, all in vivo and in vitro, dynamic and static methods gave similar results. The flexibility of the thin filament in the presence of Ca ion is of the same order as or a little smaller than that of pure F-actin. The removal of Ca ion decreases the flexibility or increases the rigidity. More than ten times increase of the rigidity has been reported by Yoshino, based on the decrease of the relaxation time of orientation of the thin filament in a muscle fiber under alternating electric field [14].

The flexibility change must be associated with changes in intermonomer bonding and/or intramonomer conformation. The polarized fluorescence measurement is useful to obtain information about both the overall shape and the local conformation. Using this method, it was shown by Yanagida that the binding of Ca ion to troponin increases the bending fluctuation of the thin filament in a muscle fiber and also changes the direction of the adenine plane of bound ADP relative to the filament axis [13]. The small angle X-ray diffraction study by Maeda et al. showed that the transition of a muscle fiber from the resting state to the rigor state by the addition of Ca ion is associated with changes in the cross-over repeat and the helical symmetry of F-actin in the thin filament [15]. Similar changes were previously detected in electronmicrographs of reconstituted thin filaments [16]. Since the displacement of tropomyosin on F-actin also occured [17], it is likely that tropomyosin works as a mediator of conformational changes between troponin and F-actin.

Summarizing the results (fig. 4), the thin filament or F-actin is rigid in the resting state of muscle and is more flexible in the active state of muscle. The flexibil-

ity may be related to the motility of F-actin upon interaction with myosin and ATP. In fact, the binding of myosin or heavy meromyosin changes both the local conformation and the overall shape of F-actin only when it is in a flexible state [7,13,18]. In this condition, a cyclic reaction of F-actin with myosin coupled with the splitting of ATP may produce an oscillatory change in the conformation of F-actin.

The work on the flexibility of F-actin reported here has been developed by our colleagues in the Department of Biophysical Engineering of Osaka University, the Department of Physics and Institute of Molecular Biology of Nagoya University and the Mitsubishi-Kasei Institute of Life Sciences.

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