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Dynamic structure of F-actin and its Ca -dependent regulation under the influence of tropomysoin-troponin system

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ABSTRACT

Regulated F-actin was reconstituted in vitro from eosin-labeled F-actin, tropomyosin and troponin. After excitation of the labeled eosin probe with a polarised, pulsed laser light, the time-resolved optical anisotropy decay was measured in a range between microsecond and millisecond. The results showed that the torsional rigidity of the regulated eosin-F-actin did not change significantly with the concentration of calcium ion in the solution, while the flexural rigidity of the regulated F-actin is known to increase with the lowering calcium concentration. Therefore it is suggested that the anisotropy of the microscopic elasticity of F-actin (i.e., the apparent difference in the flexural rigidity and the torsional rigidity) is modulated in the regulated F-actin depending on the Ca-receptive state of troponin. Corresponding to this, we found that the subunit exchange at the P-end of regulated F-actin is suppressed by the removal of calcium from troponin more significantly than the subunit exchange at the B-end. These results of two different types of experiment are interpreted in terms of the Ca-dependent regulation of the dynamic polarity of F-actin.

INTRODUCTION

F-actin is a two-stranded helical polymer of protomers (G-actin) whose molecular weight is approximately 42,500 (Oosawa, 1983). Each actin protomer in F-actin interacts with four neighbouring protomers through a group of non-covalent bonds. The bonding pattern in F-actin is illustrated in Fig. 1. Bonds are classified into two groups, one connecting protomers along the genetic helix (P-B bond) and the other connecting protomers in the same strand (p-b bond). Because of the nature of non-covalent bonds, the bonds are not rigid and the coupling of protomers fluctuates. If the amplitude of the fluctuation is large enough, in some occasion bonds may break spontaneously. Under thermal equilibrium condition, formation and breaking of bonds continue to exist, and protomers in F-actin have a limited freedom of rotational motion around the equilibrium positions. This fluctuation of inter-protomer bonds underlies the macroscopic flexibility of F-actin on the one hand, and is the basis of the protomer exchange at the two terminals of F-actin.

The flexibility of F-actin is characterised by the flexural rigidity (Y) and the torsional rigidity (C). The experimental values obtained in previous works are given in Table 1 (Fujime & Ishiwata, 1971; Oosawa 1983; Yoshimura et al., 1984). The classical theory of elasticity predicts that the torsional rigidity and the flexural rigidity of a thin flexible rod composed of homogenious isotropic material are related each other in the form (Barkley & Zimm, 1979):

$$Y = C(1 + \sigma)$$

where σ (Poisson's ratio) is about 0.5 (Landau & Lifshitz, 1970). The previous results (Table 1) indicates that Poisson's ratio of F-actin must be at least eleven times larger than the value for a uniform rod. Therefore F-actin is not isotropically flexible, but is more flexible in twisting than in bending (Mihashi et al., 1983). This is reasonable because of the helical arrangement of protomers in F-actin (Fig. 1). The nature of the change in inter-protomer coupling required for twisting will be different from the change required for bending. Macroscopic flexibilities (torsional and flexural rigidities) are determined by the helical distribution of these microscopic elasticities.

On the other hand, if the deformation of bonds occur at both B-P bond and b-p bond simultaneously of the terminal protomer, then the terminal protomer will dissociate from the filament (Fig. 1). The rate of dissociation of the protomer is promoted thermally. Inversely, the rate of association of a new protomer to the terminal of F-actin will be also promoted thermally. It has been demonstrated (Pollard & Mooseker, 1981) that the rate of both dissociation and association of protomers at the B-end are at least several times larger than those of the P-end, and by this reason the B-end is sometimes called as the "active-end" and the P-end as the "inactive-end." The conformation change which will occur in the course of dissociation (or association) will be different between the B-end and the P-end. It

is to be noted that in the middle of F-actin the dissociation of actin protomer is a less frequent event than at the terminal, since dissociation at the terminal requires breaking of B-P and b-p bonds simultaneously.

We wish to propose here to call the above-mentioned picture of F-actin in terms of "dynamic polarity" in contrast to the static polarity already used in previous works (Huxley, 1963). In the present study, we examined the effect of regulatory protein on the "dynamic polarity" of F-actin by measuring the torsional motion of regulated F-actin on one hand and by measuring the rate of association of actin protomer to the terminals of regulated F-actin. We have focused our attention to the Ca-dependent regulation. Preliminary results of the studies were reported in Mihashi et al. (1983) and Mihashi (1984).

MATERIALS AND METHODS

2.1. Optical Anisotropy Decay Measurements for Detection of Twisting of F-actin.

The basic principle of the optical anisotropy decay measurements has been reviewed elsewhere (Kinosita et al., 1984), and a brief description will be given below. F-actin labeled with an optical probe, eosin-iodoacetamide (EIA), at the site of Cys-374 of actin peptide was prepared in the previous way (Yoshimura et al., 1984). The labeled F-actin was dialysed in low ionic strength to give EIA-G-actin. EIA-G-actin was polymerised together with tropomyosin (TM) and troponin (TN) in a molar ratio of TM:TN:G-actin equal to 1:1:5.5 to give the labeled regulated F-actin. The solution was dialysed in ATP solution containing 0.2 mM ATP 60 mM KCl, 2 mM $MgCl_2$, 10 mM Tris-acetate (pH 7.6), 1 mM 2-mercaptoethanol, 1 mM NaN_3. The solution of regulated EIA-F-actin thus obtained was excited with a polarised, pulsed light at the wave length 540 nm. This results in an anisotropic excitation of the probe, since those probes that are oriented parallel to the polarization axis are excited preferentially. Optical signals from the probe, such as phosphorescence, or transient absorption change, are therefore anisotropic. The anisotropy of the optical signal is generally giving the maximum value at the time of excitation and decays with time as a result of the Brownian rotational motion of the labeled molecules. The signal becomes isotropic when the excited probe have rotated into completely random orientations. The anisotropy of the optical signal as a function of time thus reflects directly the rotational processes. For instance, if the probe is rigidly attached to the F-actin, the measured anisotropy decay tells about the rotational motion of F-actin. Measurement was done in a time range of μs to ms.

In the optical anisotropy decay measurement, we learn mainly two things, the rate and range of the Brownian rotational motion. The rate of rotation is assessed from the rate of anisotropy decay. The range of rotation is unlimited in solution. However, when the probe is incorporated in a highly organised structure, the surrounding structure generally restricts the rotation range. In this case, the optical anisotropy does not decay to zero but levels off at a finite value. The residual anisotropy tells us the range of motion. In the case of filamentous structure like F-actin, which may be regarded as a linear array of protomers

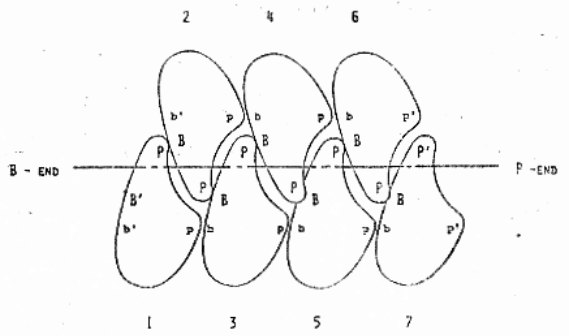

Fig. 1(a) The Mode of Protomer-Protomer Coupling in F-actin. Actin protomer consists of two domains (large/small). Around the helical axis, the small domains are connected through B-P bond, and the large domains are in contact with each other through b-p bonds in the outer region. When the B-end terminal protomer (# 1 in the figure) dissociates, some structural changes will occur at least at the sites of P and p of the protomer. On the other hand, when the P-end terminal protomer (# 7) dissociates, some structural changes at the sites of B and b will be necessary to occur. This kind of assymmetry of dynamic structure is the basis of "dynamic polarity" of F-actin.

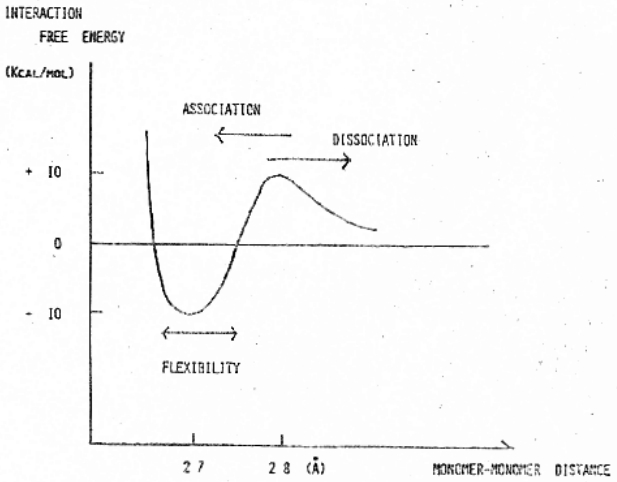

Fig. 1(b) The Potential Profile of Protomer-Protomer Coupling in F-actin. Interaction free energy between actin protomers calculated from thermodynamical data is biven as a function of the protomer-protomer distance along the helical axis (Oosawa, 1983). This is simplified description of the potential profile, because the actual shape of the potential will be different depending on the position of the protomer along the filament axis.

connected with elastic springs, the rate and range of rotation interplay in the anisotropy decay (Barkley & Zimm, 1979; Allison & Schurr, 1979). Initially the protomer carrying the optical probe rotates independent of the rest of the chain with a rate characteristic to the size of the protomer. However, the range of this independent rotation is very limited owing to the spring action. Next follows the rotation of three consecutive protomers with the probe at the middle. The combined rotation of this unit is much slower than the first one and again the range is limited. Then

follows the rotation of five consequtive protomers, which is still slower. In this way emerges a characteristic anisotropy decay, in which the decay rate decreases continuously with time. Actually this was the case of EIA-F-actin (Fig. 2). It is important to note that, of the two modes of internal motion of F-actin, the twisting motion dominates the anisotropy decay characteristics, since the bending motion is much slower owing to a larger hydrodynamic friction. Thus, from an anisotropy decay curve, we can estimate the torsional rigidity of F-actin, or the rigidity of the springs that act against twisting.

2.2. Association of the Pyrenyl-G-actin to the Terminal of Regulated F-actin.

Pyrenyl-F-actin (PIA-F-actin) was prepared according to the previous method (Kouyama & Mihashi, 1981). Pyrenyl-G-actin was then obtained by dialysis of pyrenyl-F-actin against an ATP solution containing 0.2 mM ATP, 0.05 mM $CaCl_2$, and bicarbonate 1 mM at 0°C. Regulated F-actin was prepared by mixing TM, TN and G-actin in a molar ratio of 1:1:5.5 and then dialysed against a large volume of a solution containing 90 mM KCl, 2 mM MgCl2, and 15 mM Tris-acetate (pH 7.6) at 4°C. Finally the solution was dialysed against a large volume of ATP solution containing 0.2 mM ATP, 20 mM KCl, 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, 1 mM NaN_3, 1 mM 2-mercaptoethanol and 10 mM MOPS (pH 7.2).

RESULTS AND DISCUSSION

3.1. Flexibility of F-actin.

With a triplet probe, eosin-5-iodoacetamide (EIA), the long life time of the excited triplet state of the probe (a few millisecond at room temperature in deoxgenated samples), allowed observation of the anisotropy decay over a wide range of time up to several milliseconds.

3.1.1. Torsional Flexibility of Pure F-actin

Fig. 2 shows typical anisotropy decays of EIA-F-actin at 20°C and 5°C (Yoshimura et al., 1984). The decay slows down markedly with time, in accord with prediction for an internal motion of an elastic filament of F-actin. The observed decay in the millisecond time range is too slow to be accounted for by an independent rotation of each actin protomer which is in a several tens nanosecond range (Mihashi & Wahl, 1974). On the other hand, if F-actin were completely rigid, we would observe the rotation of F-actin as a whole which gives much slower anisotropy decay. Therefore the observed decays appear to arise from an internal motion of F-actin. Thus F-actin is flexible.

Bending motion of pure F-actin occurs in the millisecond or longer time range (Fujime & Ishiwata, 1971; Yanagida et al., 1984). Thus we describe the observed anisotropy decay here to the torsional motion of F-actin. In fact the data could be superimposed on theoretical curves for torsional motion of an elastic filament calculated by Barkley & Zimm (1979) and allison Schurr (1979); the broken lines in Fig. 2.

The torsional rigidity of F-actin estimated from the anisotropy decay was 0.2×10^{-17} dynx cm² at 20°C (Table 1). This value is an order of magnitude smaller than the literature values for bending rigidity. This means that twisting F-actin is much easier than bending it. Egelman et al. (1982) have found that F-actin is randomly twisted under the electron microscopic observation (Egelman et al., 1982).

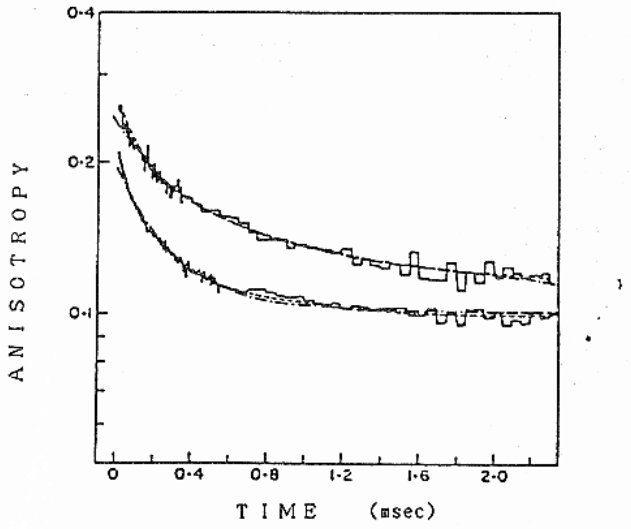

Fig. 2 Absorption Anisotropy Decays of EIA-F-actin at 20°C (lower curve) and at 5°C (upper curve). Solid lines, experimental; broken lines, the best fit theoretical curves for torsional anisotropy decay; chain lines, the best-fit double-exponential approximation. Actin concentration = 14 μM (Yoshimura et al., 1984)

The torsional flexibility of F-actin is best appreciated by calculating an average fluctuation angle. As is shown in Table 1, adjacent actin protomers in F-actin with a vertical distance of 5.5 nm in the long pitch (75 nm) helix are twisted over each other by as much as 6 degree. Thus at a radius of 3 nm, the twist results in a displacement of 0.3 nm. If therefore actin protomers are rigid, b-p bonds between adjacent protomers will be broken. It is worthwhile to note that the above values are of an average fluctuation corresponding to an average thermal energy. Instantaneous thermal fluctuations will ocassionally exceed the average amplitude. It could be infered that either actin protomers in F-actin are very flexible and/or the interaction between adjacent protomers is not tight but allows many twisted arrangements separated by low energy barriers.

Table 1. Flexibility Parameters of pure F-actin

mode	rigidity (10^{-17} dyn cm²)	fluctuation angle (per 5.5 nm)
bending	1.6 - 6.5	2.8 - 1.5 degree
twisting	0.2	6.0 degree

Twisting parameters for pure F-actin are from Yoshimura et al. (1984) and bending parameters for pure F-actin are from Fujime & Ishiwata (1979).

3.1.2. Torsional Flexibility of Regulated F-actin.

Addition of tropomyosin plus troponin apparently slowed down the anisotropy decay of F-actin. As is seen in Fig. 3, the anisotropy decay of the regulated F-actin was slower by a factor of 3 than its absence. Generally the reduction in the decay rate is expected when the hydrodynamic friction increases as a result of an increase in the radius of F-actin and/or when F-actin becomes torsionally stiffer. The decay rate is inversel proportional to the square of hydrodynamic radius. Thus, if the observed effect of TM plus TN is ascribed entirely to a change in the radius of F-actin, the radius must have increased by more than 50 %. This is likely not the case according to a recent electron microscopic observation (Toyoshima & Wakabayashi, 1985). Regulated F-actin is therefore torsionally more rigid than F-actin, though the difference is rather small. We see that regulated F-actin is still more flexible against twist than against bend (Table 2). Neither calcium ions nor ATP changed the torsional motion of regulated F-actin beyond the experimental errors, though the bending is strongly suppressed by removal of calcium ion from the solution (Oosawa, 1983).

3.1.3. Effect of Myosin Head Binding.

Addition of myosin subfragment-1 (S1) to regulated F-actin slowed down the anisotropy decay (Fig. 4). In this case, calcium ions modulated the effect; that is, addition of an excess amount of EGTA either before or after the S1 addition partially reversed the effect of S1 in the presence of calcium ions. A greater effect was observed when the same number of myosin heads were added in the form of heavy meromyosin (HMM). In the case of acto-S1 rigor complex, if heads attach perpendicularly to F-actin at a ratio of one head per ten actin protomers, the head increases the hydrodynamic friction about 3 fold, since myosin head is approximated by a prolate ellipsoid of revolution with a major axis of 16 nm and the radius of F-actin is approximately 5 nm. In the actual rigor complex, myosin heads protrude obliquely into solution with a tail bending at several tens degrees. The expected increase in the friction is therefore somewhere between one and three-fold. The observed reduction in the decay rate, on the other hand, was about two-fold in the presence of calcium ions(Fig. 4). Thus the reduction is largely accounted for by the friction increase due to the attached myosin heads.

Table 2. Flexibility Parameters of Regulated F-actin.

Rigidity (10^{-17} dy cm²)	High-Ca	Low-Ca
bending	3	17
twisting	0.3	0.3

Bending parameters from Fujime & Ishiwata (1979) and twisting parameters from the present study.

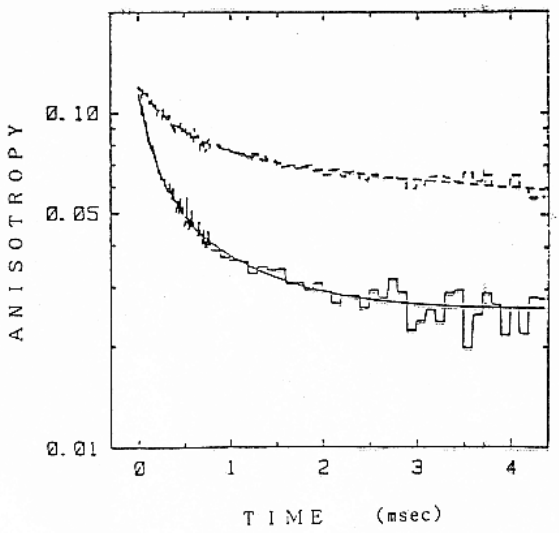

Fig. 3. The Effect of Tropomyosin-Troponin Complex on the Phosphorescence Anisotropy Decay of EIA-F-actin. Lower curve, pure F-actin; upper curve, regulated F-actin (the molar ratio of actin to tropomyosin plus troponin equal to 5). Actin concentration 6 μM. 20°C. 90 mM KCl, 2 mM $MgCl_2$, 0.2 mM ATP, 15 mM Tris-acetate (pH 7.6). The phosphorescence anisotropy is lower than the absorption anisotropy since the transition moment of phosphorescence emission makes a large angle with the moment of absorption.

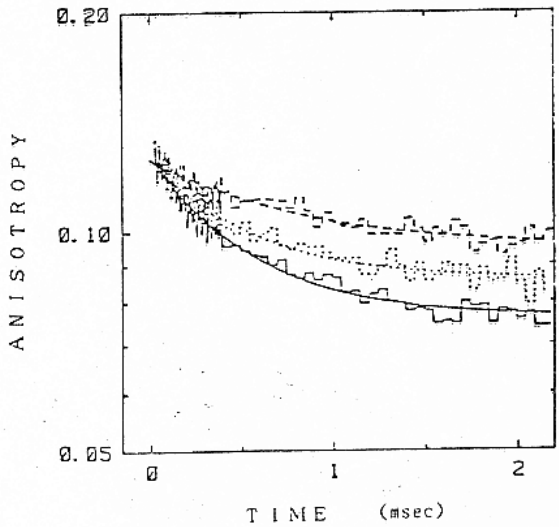

Fig. 4. The Effect of Myosin Subfragment-1 (S1) on the Phosphorescence Anisotropy Decay of regulated EIA-F-actin. Solid line, in the absence of S1; broken line, S1 added at a molar ratio of S1 to actin equal to 0.1; dotted line, 1 mM EGTA added. Other solvent conditions are the same as in Fig. 3 except that ATP is absent and 50 μM $CaCl_2$ is present. Actin concentration 7 μM, at 20°C.

3.2. Subunit Incorporation at Both Ends of F-actin.

The above results of flexibility study showed that the interprotomer interaction of regulated F-actin is Ca-dependent associated with the Ca-receptive state of troponin-C in the filament. This suggests that the s b nit exchnage at terminal of regulated F-actin may be also Ca-dependent. To test this possibility following experiments were designed to study the effect of Ca ions on the rate of subunit incorporation at the terminal of regulated F-actin under the steady state condition in the presence of ATP.

3.2.1. Ca-dependent incorporation of pyrenyl-G-actin into regulated F-actin.

For the purpose of this study, we used a potential fluorescence assay involving labeling of actin with N-pyrenyliodoacetamide (PIA) at the site of Cys-374 near the C-terminal of the peptide. As described in the previous paper (Kouyama & Mihashi, 1981), the fluorescence signal of the probe increases approximatel 25 times when the labeled G-actin is incorporated into F-actin. Measurements were done as follows; in order to attain the steady state of subunit exchange at the terminal of regulated F-actin, 2 ml o regulated F-actin (10 μM) was firstly incubated at 31°C in the presence of 0.2 mM ATP, 20 mM KCl, 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, 1 mM 2-mercaptoethanol, 1 mM NaN_3, 10 mM MOPS (pH 7.2) for 10 min. Then 0.02 ml of $CaCl_2$ (10 mM solution) or EGTA (100 mM solution) was added to give "high-Ca" or "low-Ca" solution respectively. A few minutes after incubation, 0.05 ml of PIA-G-actin was added and fluorescence increase was monitored (excitation 368 nm, emission 405 nm). The time course of the label incorporation was shown in Fig. 5. The label incorporation was apparently faster in "high-Ca" solution than in "low-Ca" solution. The critical actin concentration under the experimental condition was 0.02 μM irrespect of Ca concentration. The final level of the fluorescence increase was the same in both "high-Ca" and "low-Ca" solutions. Therefore the result indicates that the rate of subunit incorporation is much faster in "high-Ca" than in "low-Ca" condition. We found in a separate experiment that label incorporation of pure F-actin in "high-Ca" condition is slower than in "low-Ca" condition. Thus it is apparent that the Ca-dependent incorporation of subunit is primarily controled by the Ca-receptive state of troponin-C in regulated F-actin. This explanation was supported in the following observation; Addition of an excess amount of $CaCl_2$ during the label incorporation of reglated F-actin in "low-Ca" solution resulted in an instantaneous increase in the rate of label incorporation corresponding to the Ca reception of troponin. It is suggested that the Ca-receptive state of troponin-C is informed very rapidly to the terminal of long regulated F-actin filament.

3.2.2. Distinction between the B-end and P-end of the label incorporation.

An important question arises here. Is there any difference in the label incorporation between the B-end and the P-end with respect to the Ca-dependent regulation. Pollard &

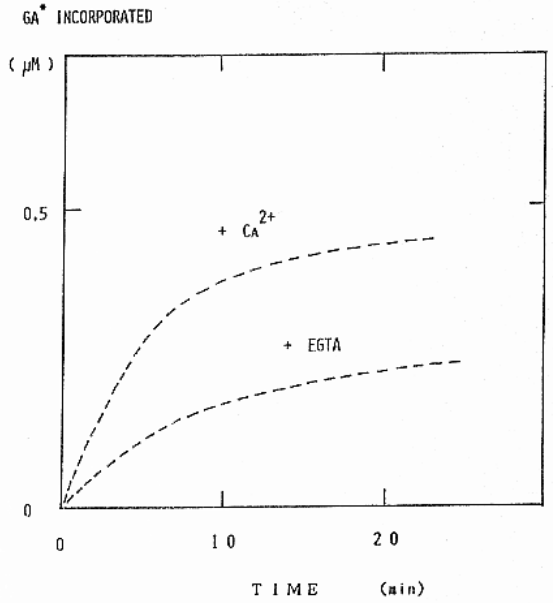

Fig. 5. The Time Course of the Incorporation of the Labeled G-actin into regulated F-actin in "high-Ca"(+Ca) and "low-Ca"(+EGTA) conditions. See test for experimental conditions.

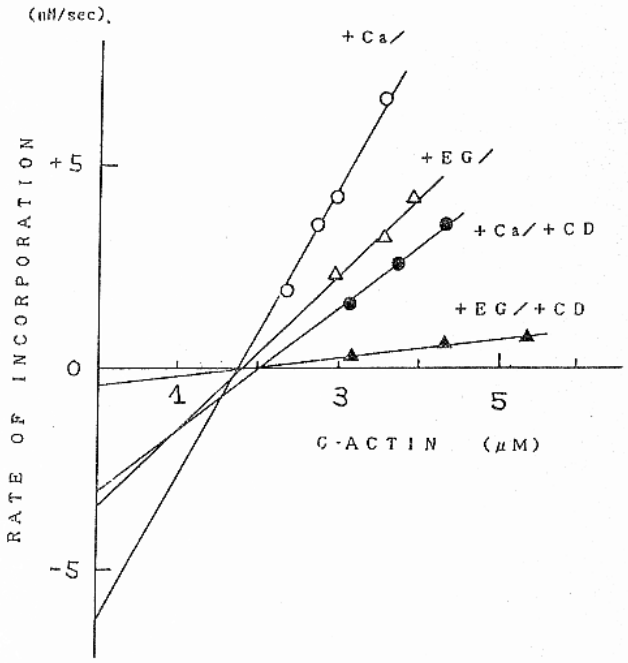

Fig. 6. The Dependence of the Rate of Subunit Incorporation at the B-end and the P-end on the G-actin concentration. To regulated F-actin solution in which the steady state of subunit exchange was established, a small amount of the labeled G-actin was added at various concentrations and the initial rate of label incorporation was determined. The rate of the total incorporation (the labeled plus non-labeled G-actin) was calculated by making correction for the fraction of labeled G-actin to the total G-actin. (+Ca),"high-Ca"; (+EGTA), "low-Ca"; (+Ca/+CD), "high-Ca" with

cytochalasin D (1 μM); (+EG/+CD), "low-Ca" with cytochalasin D (1 μM). The rate of subunit incorporation at the P-end was determined in the presence of cytochalasin D. The rate at the B-end was obtained as the difference between the rates in the absence and the presence of cytochalasin D. The number concentration of regulated F-actin was assumed as 10^{-8} M (Mihashi & Suzuki, unpublished result).

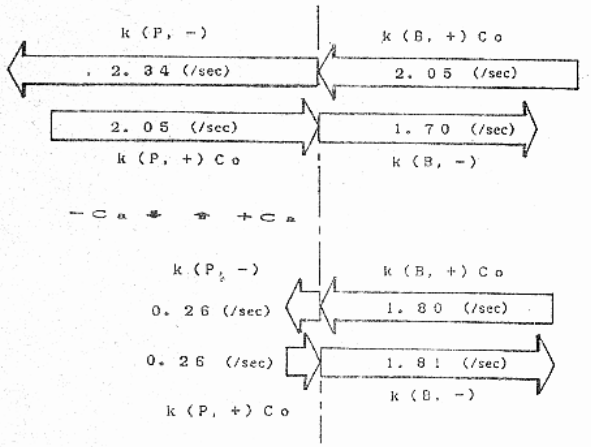

Fig. 7 Illustration of the Subunit Flow (in and out) at the B-end and the P-end of Regulated F-actin under the Steady State Condition. Using the data in Fig. 6, the subunit flow-in and flow-out at the B-end under the steady state condition, $k(B,+)C_o$ and $k(B,-)$ respectively, are calculated. C_o is the steady state concentration of G-actin. Similar calculation was done for the P-end. The values of the flow-in and flow-out obtained ($M^{-1}s^{-1}$) are illustrated as the length of arrows. Solvent conditions; 0.2 mM ATP, 0.5 mM $MgCl_2$, 5 mM Tris-HCl (pH 8.0), 1 mM NaN_3, 1 mM 2-mercaptoethanol. Regulated F-actin 10 μM.

Mooseker (1981) found under the electron microscopic observation that the average rate of elongation of the B-end of pure F-actin is several times higher than that of the P-end under some conditions. The rate of elongation V was linearly dependent on the concentration of G-actin C_m in the following relations;

$$V(B) = k(B,+)C_m - k(B,-)$$
$$V(P) = k(P,+)C_m - k(P,-)$$

They found that addition of cytochalasin D (1 μM) suppressed the elongation of the B-end completely. On the basis of these findings, fluorescence assay of label incorporation at the B-end and P-end was done as follows; without addition of cytochalasin D, the PIA-G-actin was incorporated at both ends, while only the incorporation at the P-end occurs in the presence of cytochalasin D. The difference gives the net incorporation at the B-end. The results given in Figs. 6 and 7 show that the Ca-dependent regulation is more significant at the P-end than at the B-end, indicating that the change in the Ca-receptive state of troponin spreads along F-actin directionally. In a separate experiment, we found that the effect of the active interaction of HMM(ATP) with regulated F-actin in the presence of Ca appears more significantly at the B-end than the P-end (data not shown).

In conclusion, tropomyosin plus troponin of regulated F-actin modulates interprotomer interaction of actin directionally depending on the calcium receptive state of troponin-C. It is suggested that the directional movement of myosin head along F-actin is inherently related with "dynamic polarity" of F-actin (Ebashi & Endo, 1968).

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