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Internal Motion of F-Actin in 10^{-6}–10^{-3} s Time Range Studied by Transient Absorption Anisotropy: Detection of Torsional Motion

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By means of laser flash photolysis, the transient absorption anisotropy (TAA) of the triplet probe, 5-iodoacetamide-Eosin, labeling rabbit skeletal F-actin was measured in the 10^{-6}–10^{-3} s time range. The TAA curve at 20°C showed a relatively slow decay phase covering several hundred microseconds and a large residual anisotropy (~0.1 at 2 ms). After analysis with Barkley & Zimm's formula, it was concluded that the TAA of Eosin-F-actin can be approximated by the anisotropy decay due to torsional motion of F-actin.

So far the internal motion of F-actin has been studied both in the 10^{-9}–10^{-7} s time range by measuring transient fluorescence anisotropy (*1*, *2*) and in the 10^{-3}–10^{-2} s time range by quasi-elastic light scattering and some other methods (*3*). Ikkai *et al.* (*2*) ascribed the transient fluorescence anisotropy of F-actin to some limited motion of the actin protomer or a group of actin protomers. On the other hand, quasi-elastic light scattering measurement detected the bending motion of F-actin as a whole (*4*). In comparison, knowledge on the 10^{-6}–10^{-3} s range is quite deficient. Only a paper on saturation transfer electron paramagnetic resonance spectroscopy of a spin probe labeling F-actin (*5*) reports that F-actin undergoes rotational motion having an effective correlation time of the order of 10^{-4} s. However, the authors found it difficult to assign precise correlation times and specific models. In order to enlarge these previous studies, we have undertaken measurement of transient absorption anisotropy (TAA) of a triplet probe, Eosin, labeling F-actin using laser flash photolysis which is suitable for detecting rotational motion of macromolecules in the 10^{-6}–10^{-3} s range.

Actin was extracted from acetone dried powder of rabbit skeletal muscle according to previous work (*6*) and purified chromatographically (*2*). 5-Iodoacetamide-Eosin (Eosin-IA) purchased from Molecular Probe was dissolved in 10% acetone solution and mixed gently with F-actin solution containing 90 mM KCl, 2 mM $MgCl_2$, 0.2 mM ATP, and 1 mM $NaHCO_3$, at 4°C with a molar ratio of Eosin to actin of 0.7. Five hours after mixing, the reaction was terminated by addition of 2-mercaptoethanol (1 mM) and the solution was centrifuged for 120 min at $10^4 \times g$. The pellet of labeled F-actin was dissolved in ATP solution containing 0.2 mM ATP, 0.1 mM $CaCl_2$, and 0.5 mM NaN_3. The molar ratio of the labeled Eosin to actin was 0.25. In a separate experiment, NEM-F-actin, in which Cys-373 was blocked with

N-ethylmaleimide, was reacted with Eosin-IA under the same conditions of above. We found no appreciable amount of Eosin bound to NEM-F-actin. This indicates exclusive binding of Eosin to the Cys-373 residue in our preparation of Eosin-F-actin. The critical concentration of F-actin was not altered significantly after labeling as judged by viscosity measurement, while the specific viscosity was reduced by about 20%, suggesting that the average length of F-actin became shorter.

Laser flash photolysis was carried out with an instrument principally as described by Cherry (*7*). So only a brief description will be given here. The excitation source was a Phase R DL 1400 dye laser which emitted a light pulse of up to 200 mJ and 0.25 μs duration. The dye Coumarine 540 AF showed emission at 540 nm which lies in the absorption of Eosin labeled F-actin. The excitation light was vertically polarised with a Glan-Taylor prism. Transient absorption change of the labeled Eosin after flash excitation arising from ground-state depletion was monitored with continuous light from a 100 W tungsten-halide lamp. The wavelength of the monitor light was set at 515 nm by use of a Zeiss monochromater. Two components of the absorption change, the vertical one $A_V(t)$ and the horizontal one $A_H(t)$, were separated with a beam splitting polariser and then detected with two photomultipliers. The output of the photomultipliers was fed into a Nicolet 1170 after amplification. Signals from 512 flashes were averaged and transferred to a DEC microcomputer LSI-11/2 with which analysis was carried out. The anisotropy parameter $r(t)$ was generated from the two components

$$r(t)=[A_V(t)-A_H(t)]/[A_V(t)+2A_H(t)]\,.$$

Just prior to measurement, oxygen in the solution was removed by blowing argon gas through for more than 10 min.

The TAA curve of Eosin-F-actin (14 μM) at 20°C, as typically shown in Fig. 1, showed an initial slow decay phase covering a several hundred microseconds range which was followed by a so slow decay that the anisotropy became an almost constant value of approximately 0.1 at 2 ms. This indicates that the rotation observed is a strongly restricted one. In order to determine whether the length distribution of F-actin affects the TAA, the following experiments were done. First, the concentration of Eosin-F-actin was changed to 7 μM, 23 μM, and 28 μM, and left standing for more than 4 h. TAA of these solutions did not show any systematic concentration dependence. Next, since the average length of Eosin-F-actin is likely to be shorter than the unlabeled one as was suggested by the viscosity measurement mentioned above, TAA curves were compared between two solutions, one containing 14 μM labeled plus 9 μM unlabeled F-actin and the other containing 23 μM labeled F-actin. They were indistinguishable from each other and identical to the TAA of a 14 μM solution of labeled F-actin within experimental error. We also found that an Eosin-F-actin solution which was prepared by dissolving a pellet of Eosin-F-actin gave the same TAA as before centrifugation. These results together indicate that the TAA of Eosin-F-actin is not affected by the presence of the length distribution of F-actin. This means that the decay of TAA did not arise from rotation of F-actin as a whole but instead reflected internal motion of F-actin. Of possible modes of internal motion in the time range studied, the most probable one is torsional motion. Then we analyzed the TAA with Barkley and Zimm's formula which was developed for the description of anisotropy decay due to torsional motion of a thin flexible rod (*8*).

$$r(t) = r_0[A_1+A_2\exp(-\gamma\sqrt{t}) + A_3\exp(-\gamma\sqrt{t}/4)] \qquad (1)$$

where the decay constant γ is related to the torsional rigidity C of the thin flexible rod as

$$\gamma = 2kT/(\Pi b\sqrt{\eta\cdot C}) \qquad (2)$$

where k is the Boltzman constant, T absolute temperature, η the viscosity of the solvent and b the radius of the thin rod. We assumed $b=4$ nm for F-actin. A_i is the function of the angle ε between the absorption dipole moment of the labeled probe and the long axis of the thin rod.

$$A_1 = (1/4)(2-3\sin^2\varepsilon)^2\,,$$
$$A_2 = (3/4)\sin^4\varepsilon\,,$$
$$A_3 = 3\sin^2\varepsilon\cos^2\varepsilon\,.$$

$r_0=0.32$, which was determined from fluorescence anisotropy of the same solution (excitation at 520

nm and emission at 560 nm) was used as an approximation.

The best fit for TAA of Eosin-F-actin at 20°C (Fig. 1) was obtained with adjustable paramters $C = 1.9 \times 10^{-18}$ dyn·cm² and $\varepsilon = 32.8$ degrees. Fitting was satisfactory over the whole time range of TAA. Since Eq. 1 does not contain contribution of bending motion, the above result indicates that the TAA can be explained by contribution of torsional motion and contribution of bending motion is minor or negligible. It is worthwhile to note that torsional rigidity of F-actin determined above is approximately ten times smaller than the flexual rigidity reported in previous studies (*3*). This means that F-actin is more flexible in twisting motion than in bending motion. The existence of variability of the helical pitch of an actin filament was suggested in 1967 by the electron microscopic study of Hanson (*9*). Recently in a more elaborate electron microscopic study of isolated actin filaments, Egelman *et al.* (*10*) concluded that actin filaments can be described by a helix in which the rotation between actin protomers is considerably variable with a constant axial rise per protomer. The results of our dynamic study are very compatible with the static picture of actin filaments given in these previous works.

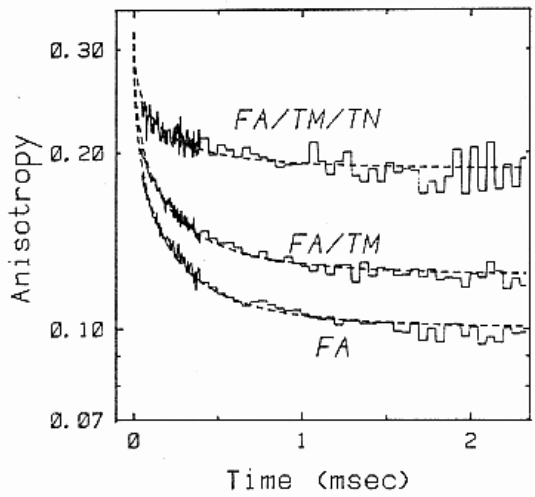

Fig. 1. Transient absorption anisotropy (TAA) of Eosin-F-actin and the effect of tropomyosin and troponin at 20°C. F-Actin (FA) 14 μM, tropomyosin (TM) 1.6 μM, troponin (TN) 1.6 μM. The solvent contained KCl 90 mM, $MgCl_2$ 2 mM, $CaCl_2$ 0.1 mM, ATP 0.2 mM, NaN_3 1 mM; pH 7.6 (Tris-acetate 15 mM). Solid lines are experimental curves and dashed lines are theoretical curves obtained according to Eq. 1 with the adjustable parameters given in Table I.

TABLE I. Parameters used in calculation of the theoretical curves given in Fig. 1.

Actin	C (dyn·cm²)	ε (degree)
F-Actin	1.9×10^{-18}	32.8
F-Actin/tropomyosin	2.3	30.2
F-Actin/tropomyosin/troponin	2.4	23.4

Finally the effect of tropomyosin and troponin was examined. The obtained TAA curves and the results of analysis are given in Fig. 1 and Table I. We can see that the torsional rigidity of labeled F-actin increased slightly with the presence of the regulatory proteins. The torsional rigidity increased slightly further after removal of calcium ions (not shown in Fig. 1). If we consider in the calculation a possible increase in the effective radius b due to the binding of regulatory proteins to actin (*11*, *12*), the difference in the torsional rigidity between pure and regulated actin would become smaller. The change in the angle ε (Table I) may be only in appearance, since it is possible that a very slow decay of the anisotropy (due to an increase in the torsional rigidity) may have not been detected owing to the limitation of the time range of measurement.

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