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ASSEMBLY OF ACANTHAMOEBA ACTIN IN THE PRESENCE OF ACANTHAMOEBA PROFILIN MEASURED BY FLUORESCENCE PHOTOBLEACHING RECOVERY

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Assembly of Acanthamoeba actin, of which trace quantities had been labeled with 5-(iodoacetamido)-fluorescein, was quantified using the modulation detection method of fluorescence photobleaching recovery (FPR). This technique permits explicit determination of the fraction of labeled actin incorporated into filaments and the translational diffusion coefficients of the filaments, from which filament length can be calculated. Addition of Acanthamoeba profilin in molar ratios to actin of about 1.1:1 and 2.3:1 retarded the initial kinetics of assembly (induced by addition of 2mM Mg^{+2}) and reduced the fraction of actin incorporated into filaments. The diffusion coefficients of filaments formed were greatly changed by the presence of profilin at short times, but the differences became increasingly smaller at longer times. After 26 hr. the filaments formed in 1.1:1 profilin were about 12% shorter and in 2.3:1 profilin were about 20% shorter than filaments formed by actin alone under the same conditions.

Profilin is a low-molecular-weight protein that binds to cytoplasmic actin in a 1:1 complex; its presumed function is to stabilize G-actin against assembly to F-actin (1, 2). Although the activity of profilin in slowing the kinetics and reducing the extent of actin assembly has generally been interpreted in terms of its ability to bind to G-actin, there are recent proposals that profilin can also interact directly with actin filaments (3, 4). The profilin from Acanthamoeba castelanii has been studied most extensively for its effect on the assembly of actin from the same organism (2, 4-11). The actin assays employed in these studies include viscometry, light scattering, and fluorescence enhancement. Although each of these assays has been shown to be an indicator of the kinetic course of actin assembly, none of them permits a quantitative distinction between the fraction of actin assembled and the lengths of the filaments formed. Hence the effect of profilin on actin filament lengths has not yet been determined. We report here the effect of Acanthamoeba profilin on the assembly of Acanthamoeba actin as quantified by

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fluorescence photobleaching recovery (FPR) (12, 13). This assay measures the tracer diffusion coefficients of fluorescent labeled species by tracing the recovery of fluorescence into an area of the sample that has been photobleached by an intense light pulse. The FPR data record can be interpreted to determine the fraction of actin that has been incorporated into filaments and the average diffusion coefficients of the filaments, from which an average filament length may be calculated.

Materials and Methods

Acanthamoeba castellanii kindly supplied by Dr. E. D. Korn, was grown in 15-liter aerated carboys (14). Acanthamoeba profilin and actin were prepared by minor modifications of the method of Reichstein and Korn (2). Actin was labeled with 5-(iodoacetamido)-fluorescein (15). Unlabeled actin concentrations were determined by absorbance at 290 nm using $\varepsilon = 0.62$ ml mg^{-1} cm^{-1}. Labeled actin concentration was determined by the Bradford assay (16) and extent of labeling by absorbance at 495 nm using $\varepsilon = 6 \times 10^4$ M^{-1} cm^{-1}. Profilin concentrations were determined by absorbance at 280 nm using $\varepsilon = 1.4 \times 10^4$ M^{-1} cm^{-1}. Purified actin (labeled or unlabeled) was polymerized and stored as a pellet at 0° C in solution containing 5 mM imidazole, 0.75 mM β-mercaptoethanol, 3 mM NaN_3, pH 7.5. In preparation for experiments, the homogenized actin pellet was dialyzed against buffer G (5 mM imidazole, 0.5 mM ATP, 0.1 mM $CaCl_2$, 0.75 mM β-mercaptoethanol, 3 mM NaN_3, pH 7.5) for at least 48 hr, and the resulting solution was passed through Sephadex G-150 (50 x 1 cm). Central fractions from the G-actin peak were pooled. Labeled and unlabeled actin were combined to yield 9% labeled actin in all samples. Total actin concentration was 14 μM. G-actin was stored in solution for no longer than 2 d after Sephadex chromatography.

Experiments were performed by combining actin, labeled actin, and profilin in the specified ratios, then adding $MgCl_2$ to a final concentration of 2 mM. Samples were immediately loaded into microcuvettes of 100 μm thickness, sealed to a black microscope slide with Permount, and mounted onto the stage of a Zeiss Universal microscope for analysis using the modulation detection method of FPR (17). In this approach a square-wave modulation pattern is photobleached in the sample, and a monitoring beam passing through a translated grating produces a modulation of the fluorescence. The modulation decays as the pattern contrast is reduced by translational diffusion of the labeled species. The data form for a single labeled species is an exponential decay with time constant given by $(DK^2)^{-1}$, where D is the tracer translational diffusion coefficient of the labeled species and K is the wave vector of the photobleached pattern, given by $2\pi/L$, where L is the pattern spacing. For actin assembly experiments the data form consists of a rapid exponential decay, corresponding to the diffusion of G-actin, followed by a much slower decay, corresponding to the diffusion of actin filaments (12, 13). The two characteristic parameters reported here are the fraction of labeled actin in the low-mobility component, f_{LM}, and the average diffusion coefficient of the low-mobility fraction, D_{LM}, obtained as described previously (13). The f_{LM} values were taken primarily from data with $K = 841$ cm^{-1} and the D_{LM} values were taken primarily from data with $K = 2127$ cm^{-1}. All measurements were made at 20° C.

Results and Discussion

Figure 1 shows the kinetics of assembly of 14 μM actin, 14 μM actin with 16 μM profilin, and 14 μM actin with 32 μM profilin, where time 0 is defined as the time of addition of 2 mM Mg^{+2}. The parameter f_{LM} can be interpreted as the fraction of actin that has assembled into filaments. It is clear that the presence of profilin retards the kinetics of assembly in a dose-dependent manner. Since the slope of the assembly curves is everywhere smaller in the presence of profilin and is everywhere reduced at the higher profilin concentrations, it appears that the presence of profilin inhibits both the nucleation and elongation phases of filament growth. Profilin also appears to have a slight effect on the fraction of actin assembled in the steady state. However, the long-time f_{LM} from this experiment must be interpreted with caution. It has been shown that the binding of profilin to actin is reduced by roughly one order of magnitude when the actin is labeled by fluorophore or otherwise modified at Cys 374 (10, 18). We have confirmed these observations independently using FPR to measure K_d of the fluorescein-actin-profilin complex and have obtained a value of 48 μM (Plank and Ware, to be published), roughly ten times greater than reported values for modified actin-profilin (6-9). Thus, we may anticipate that the reduction in the fraction of assembled actin could be significantly greater for unlabeled actin than for labeled actin. The trace in Fig. 1 for actin in the absence of profilin is

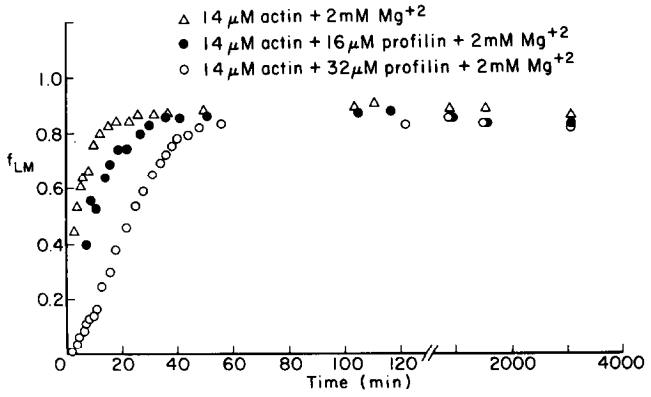

Fig. 1. Kinetics of actin assembly as followed by the fraction of low-mobility fluorescence, f_{LM}, in an FPR experiment, for three samples as specified. Time zero is defined as the time of addition of $MgCl_2$ to the actin or actin-profilin solutions.

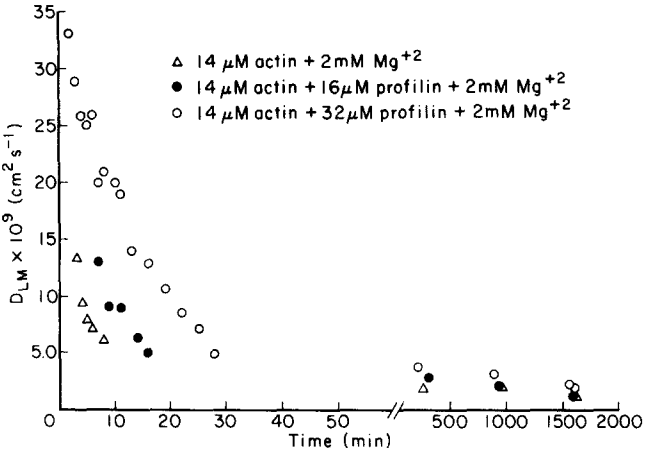

Fig. 2. Kinetics of actin assembly for the same experiment depicted in Fig. 1. The parameter plotted is the average low-mobility translational diffusion coefficient, D_{LM}, determined as described in ref. (13).

remarkably similar to the initial work on characterization of the assembly of Acanthamoeba actin using viscometric assays for similar solution conditions (19).

The changes of average filament diffusion coefficient, D_{LM}, with time for the same experiments are shown in Fig. 2. The kinetic effect of profilin from these measurements is completely consistent with the independent f_{LM} data of Fig. 1. The rate of elongation ($-dD_{LM}/dt$) is clearly reduced by the presence of profilin in a dose-dependent manner. The D_{LM} values reflect the translational diffusion coefficient of filaments into which labeled actin has been incorporated; hence they should be reliable indicators of filament length irrespective of the altered assembly propensity of labeled actin discussed in the preceding paragraph. Perhaps the most interesting aspect of the data in Fig. 2 is the slight extent of the increase in the steady-state value of D_{LM} brought about by the addition of profilin. Although in each case for the three sets of long-time data the diffusion coefficient is increased by addition of profilin in a dose-dependent manner, the difference decreases with increasing time. At the longest time measured, ~1600 min, the values of D_{LM} for the actin filaments in the absence of profilin, in the presence of 16 μM profilin, and in the presence of 32 μM profilin were $1.36 \pm .07 \times 10^{-9}$ $cm^2 s^{-1}$, $1.65 \pm .11 \times 10^{-9}$ cm^2 s^{-1}, and $2.00 \pm .18 \times 10^{-9}$ cm^2 s^{-1}, respectively. If we

calculate filament lengths based on a model of independent diffusion of stiff rods (13), the weight-average filament lengths obtained are about 25 μM, 22 μM, and 20 μM, respectively. These values must be considered upper limits for filament lengths, since effects of filament-filament interactions would always be to reduce the measured diffusion coefficient for a given filament length. Similarly the changes in filament lengths are upper limits. We conclude that the presence of 1.1:1 profilin:actin reduces filament lengths by at most 12% and 2.3:1 profilin:actin reduces filament lengths at most 20% under the conditons of our experiments.

Acknowledgements

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