negatively charged groups on G-actin, thus facilitating the approach of neighboring protomers to form the weak bonds between the adjacent protomers in the actin filament. This presumed importance of the role of electrostatic charge in the assembly process makes the assembly of actin an ideal candidate for experimentation by electrophoretic light scattering.

We present here preliminary data to illustrate the study of actin assembly by ELS. Fig. 1 shows three ELS spectra of actin. In the top spectrum, G-actin (8.3 mM) at 20°C was subjected to an electric field strength of 104 V/cm, and the ELS spectrum was measured at a scattering angle of 9°. The ELS spectrum is essentially a single peak. The peak shift of 139 Hz corresponds to an electrophoretic mobility of 2.4 $cm^2/V \cdot s$ in the standard G-actin stabilizing buffer. The spectral width of the top spectrum can be accounted for almost completely by the diffusion of the G-actin monomer. The middle spectrum in Fig. 1 was taken 30 min after the addition of $MgCl_2$ to a G-actin solution, raising the total Mg^{+2} concentration to 0.5 mM. Clearly, the addition of Mg^{+2} induced the formation of several species at lower electrophoretic mobility. With the passing of time, the spectrum evolved into two narrow peaks at lower mobility. The spectrum taken 12 h later is shown at the bottom spectrum in Fig. 1. The higher mobility peak has a mobility of 1.2×10^{-4}. The very low mobility peak may be attributable to actin filaments that attach to the chamber window. Although a detailed interpretation of these spectra would be premature at this point, we believe they serve well to illustrate the exciting potential of ELS for the study of actin assembly. It is clear that the addition of Mg^{+2} reduces the electric charge on actin perceptibly and that the assembly process involved intermediate species that are electrophoretically distinct.

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MECHANISM OF ACTIN FILAMENT SELF-ASSEMBLY AND REGULATION OF THE PROCESS BY ACTIN-BINDING PROTEINS

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The polymerization of actin filaments is one of the classic examples of self-assembly in biology and the mechanism is now understood in some quantitative detail. Using kinetic methods, we and others (see Frieden, 1985 for a review) have identified the major steps in the process and measured the rate constants for the reactions.

Activation

Starting with monomers having bound ATP and Ca^{++}, there is a moderately fast ($k_+ \sim 0.05\ s^{-1}$) but rate-limiting first-order activation reaction (Cooper et al., 1983) that is thought to be the exchange of Mg^{++} for bound Ca^{++} (Frieden, 1983), because the exchange reaction has a similar rate constant (Frieden, 1982). Activated monomers form nuclei (Cooper et al., 1983) and elongate filaments (Pollard, 1983) more rapidly than unactivated monomers.

Nucleation

The overall polymerization reaction is limited by the slow, unfavorable formation of nuclei consisting of three actin

molecules (Cooper et al., 1983; Tobacman and Korn, 1983; Frieden, 1983). The individual reactions (e.g., monomer-to-dimer and dimer-to-trimer) have not been evaluated because all of the studies have made use of a simplifying assumption (Wegner and Engel, 1975) that both reactions are rapid equilibria governed by the same rate constants. This is undoubtedly an over-simplification. The affinity of the subunits in the dimer and trimer for each other is exceedingly low: in Mg^{++} the K_d is ~0.1 M and in Ca^{++} the K_d is ~1 M (Cooper et al., 1983). The trimers are transient intermediates present for only a few milliseconds in very low concentrations for two reasons: first, the trimers are unstable; and second, they are rapidly consumed by the subsequent rapid elongation reaction.

Elongation

Trimers or longer filaments grow rapidly in both directions, providing the monomer concentration exceeds the critical concentration, ~0.1 μM under physiological conditions. At the barbed end the large forward rate constant (10^7 $M^{-1}s^{-1}$) (Pollard and Mooseker, 1981; Bonder et al., 1983) suggests that the polyermerization rate is limited by diffusion. However, the activation energy (7–10 Kcal/mol) (Pollard, unpublished data) is larger than expected for a diffusion-limited process, so some unknown chemical reaction may limit the rate. The association rate constant at the pointed end is 5–10 times lower than at the barbed end. The dissociation rate constants are on the order of 1–2 s^{-1} at both ends (Pollard and Mooseker, 1981; Bonder et al., 1983). Consequently, the critical concentration is higher at the pointed end than at the barbed end.

Fragmentation

If the overall process is sufficiently slow, filaments can break slowly into shorter fragments (Wegner and Savko, 1982; Cooper et al., 1983).

ATP-hydrolysis

The ATP bound to the subunits is hydrolyzed after their incorporation into filaments. The reaction is slow, appears to be first-order, and has a rate constant of ~0.05 s^{-1} (Pollard and Weeds, 1984; Carlier et al., 1984). Because of the absolute rates of the assembly reactions, ATP-actin occupies the ends of most filaments even at steady state. During rapid elongation in the presence of high concentrations of ATP-actin, there may be hundreds of ATP-actins at the ends of a polymer.

Actin monomers with bound ADP also polymerize, but the critical concentration for polymerization is ~50 times higher than for ATP-actin because of differences in the elongation rate constants (Pollard, 1984*a*; Carlier et al., 1984). Compared with ATP-actin the association rate constant is 10 times smaller and the dissociation rate constant is five times larger for ADP-actin. Consequently, ATP-actin bound to the ends of actin filaments stabilize them against rapid depolymerization.

The assembly of actin filaments in cells must be controlled by regulatory proteins and we have characterized five purified actin binding proteins from *Acanthamoeba* that may regulate polymerization and cross-link actin filaments in the cell. Similar proteins are found in other cells. The total actin is ~200 μMol/kg cells. Profilin (Reichstein and Korn, 1979) is a small (12,000 mol wt) protein present in high concentration (100 μMol/kg) and distributed throughout the cytoplasmic matrix (Tseng et al., 1984). Profilin inhibits nucleation and elongation at the pointed end by binding to actin monomers with a K_d of ~5 μM and by capping the barbed end of actin filments with a low affinity (K_d = 50 μM) (Pollard and Cooper, 1984). Actophorin is a 15,000 mol wt protein also present in high concentrations (20 μMol/kg cells) (Cooper et al., 1984). It forms a nonpolymerizable complex with actin and can also fragment preformed actin filaments. Together profilin and actophorin can account for most of the unpolymerized actin in the cell. Capping protein (Isenberg et al., 1980) is a heterodimer of 28,000 and 31,000 mol wt subunits that is present in low concentration (2 μMol/kg) and is concentrated in the cell cortex with the bulk of the actin filaments (Cooper et al., 1984). It binds to actin filaments and blocks monomer addition at the barbed end, but also stabilizes actin oligomers and thereby promotes nucleation (Cooper and Pollard, 1985). Gelatin protein (Pollard, 1981) is a rod-shape molecule 50 nm long. It has globlar regions at both ends and in the center, much like α-actinin from muscle. It is composed of two 90,000 mol wt polypeptides and is present at a concentration of 4 μMol/kg. It is found throughout the cytoplasmic matrix, but is concentrated in parts of the cortex. It cross-links filaments into three-dimensional networks that resist high frequency but not low frequency deformation (M. Sato, W. Schwartz, T. D. Pollard, unpublished data). This may be related to the low affinity of gelation protein for actin filaments (K_d > 5 μM). At low frequency the cross-links may rearrange faster than the displacement of the filaments. A 260,000 mol wt subunit of spectrin has also been purified from soluble extracts of the amoeba (Pollard, 1984*b*). It can cross-link actin filaments and is concentrated very near the plasma membrane in the cell.

Because none of these proteins is regulated by Ca^{++}, it is an intriguing possibility that mass action alone is responsible for the assembly of the actin system in the cell and that its reorganization during cell movements is an active process determined entirely by tension exerted by myosin on the network of actin filaments.

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