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ASSEMBLY OF ACTIN FILAMENTS STUDIED BY LASER LIGHT SCATTERING AND FLUORESCENCE PHOTOBLEACHING RECOVERY

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One of the most fascinating macromolecular assemblies found in nature is the contractile apparatus responsible for cytoplasmic motility. All living cells possess the capacity for generating directed force to accomplish active motion. This capacity is manifest in a number of fundamental cellular functions such as cell division, secretion, phagocytosis, and locomotion. Despite over three centuries of fascination with cytoplasmic motility, the molecular mechanism of force generation is still a subject of more conjecture than understanding. Though analogies with the mechanism of the contraction of skeletal muscle are compeling and often instructive, the essential difference is the absence of a static structure in the cytoplasm. The transient assembly of a fragile molecular apparatus for force generation in the cytoplasm must require a complicated and dynamic regulation of even greater subtlety than its counterpart in muscle; and a study of the structure and dynamics of the transient assembly responsible for cytoplasmic contractility will surely require an even wider range of sophisticated physical techniques.

It is agreed that the primary structural component of the cytoplasmic contractile apparatus is actin. Cytoplasmic actin is virtually identical to the actin of skeletal muscle, and the self-assembly of cytoplasmic globular actin (G-actin) to actin filaments (F-actin) appears to be a fundamental event of cytoplasmic motile processes (1). In addition, there are a host of proteins in the cytoplasm whose role seems to be regulation of the assembly and cross-linking of actin filaments into a supramolecular network capable of generating force, presumably by interaction with cytoplasmic myosin. Because of the transience, fragility, and complexity of the supramolecular complex associated with cytoplasmic motility, the common structural techniques that have been successful in studying other macromolecular assemblies are often inappropriate for addressing the fundamental issues of the structure and function of the cytoplasmic contractile network.

Our research group has addressed itself to the study of the supramolecular complexes associated with cytoplasmic motility by the measurement of transport coefficients in simple solutions, in complex mixtures, and in native cytoplasm. The transport coefficients measured are (*a*) the mutual diffusion coefficient in solution, which we measure by quasi-elastic light scattering (QELS) (2, 3); (*b*) the tracer diffusion coefficient either in solution or in complex mixtures, which we measure by fluorescence photobleaching recovery (FPR) (4); and (*c*) the electrophoretic mobility in solution, which we measure by electrophoretic light scattering (5). These transport coefficients can be used to characterize the molecular dimensions and electrical charge of the components of the cytoplasmic supramolecular complexes and, in some cases, are able to provide pertinent information regarding the association of specific components with the assemblies and/or the mobility of specific components or probe particles within and through the assembled networks.

RESULTS AND DISCUSSION

The study of the diffusion coefficient of G-actin has been useful in resolving an earlier issue regarding the molecular assembly of the globular form of this protein. FPR measurements in our laboratory (6, 7) and QELS measurements in other laboratories (8, 9) establish the diffusion coefficient to be in the range 7.1–8.1 $\times$ 10^{-7} cm^2/s. This value of the diffusion coefficient is consisent with the sedimentation coefficient and partial specific volume of G-actin and is roughly the value to be expected for a nearly spherical particle of this mass (42,000 d). Therefore, the earlier speculation regarding the molecular assembly of G-actin based on a lower measured value of the diffusion coefficient can now be dismissed. Using FPR (6), we have recently observed, as another group have observed using QELS (9), that the diffusion coefficient of G-actin is significantly lower when the standard low-salt buffer (2 mM Tris-HCl, 0.2 mM $CaCl_2$, 0.2 mMATP, 0.5 mM 2-mercaptoethanol, pH 8.0) used to stabilize G-actin includes Mg^{+2} as a cation in place of Ca^{+2} (concentrations range between 50 mM and 200 mM). Our data indicate that the presence of Mg^{+2} leads to the reversible formation of a stable aggregate, roughly the size of a tetramer. We have shown further that Mg^{+2}-incubated G-actin contains species which either are assembly nuclei or are much more readily converted to assembly nuclei than normal G-actin, so that trace quantities of Mg^{+2}-incubated actin serve to stimulate the assembly kinetics of G-actin after the addition either of ~1 mM Mg^{+2} or ~100 mM KCl. The use of these transport techniques has provided the first physical evidence for the existence of Mg^{+2}-stabilized oligomers in G-actin solutions.

We have used FPR for the investigation of the assembly of G-actin to form F-actin filaments (7, 10). The FPR technique can measure independently the fraction of actin assembled into filaments, the diffusion coefficients of the filaments (from which the filament lengths can be inferred), and the state of aggregation of the actin protomers that are not assembled into filaments. Thus, the data obtained from this approach provide the molecularly defined parameters that are of direct relevance for mechanistic distinctions. A recent illustration of the power of this approach is provided by the study of the effects of cytocholasin-D upon actin assembly (11). The cytocholasins are a class of fungal metabolites that are known to have a disruptive effect on the motile apparatus of many different cell types and have been shown to reduce the viscosity of actin filament solutions in vitro. This activity could result from the binding of cytocholasins to the growing ends of actin filaments, leading to a net depolymerization from the slower-growing end or from a cleavage activity of simply reducing the length of actin filaments in solution. The FPR approach can distinguish readily between these two types of activity. A controlled study of the effects of cytocholasin-D on actin filaments, using FPR, showed that the activity of cytocholasin-D can be ascribed solely to a binding to the growing end when the assembly of actin has been induced by the presence of K^+. However, in the presence of Mg^{+2}, cytochalasin-D induces a dose-dependent increase in the diffusion coefficient of actin filaments up to a factor of ~10, indicating a shortening of filaments consistent with considerable filament cleavage.

The results cited above plus an extensive literature on the assembly of G-actin into F-actin have prompted a considerable interest in the role of cations in promoting actin self-assembly. It has generally been assumed that the cations function to reduce electrostatic repulsions between

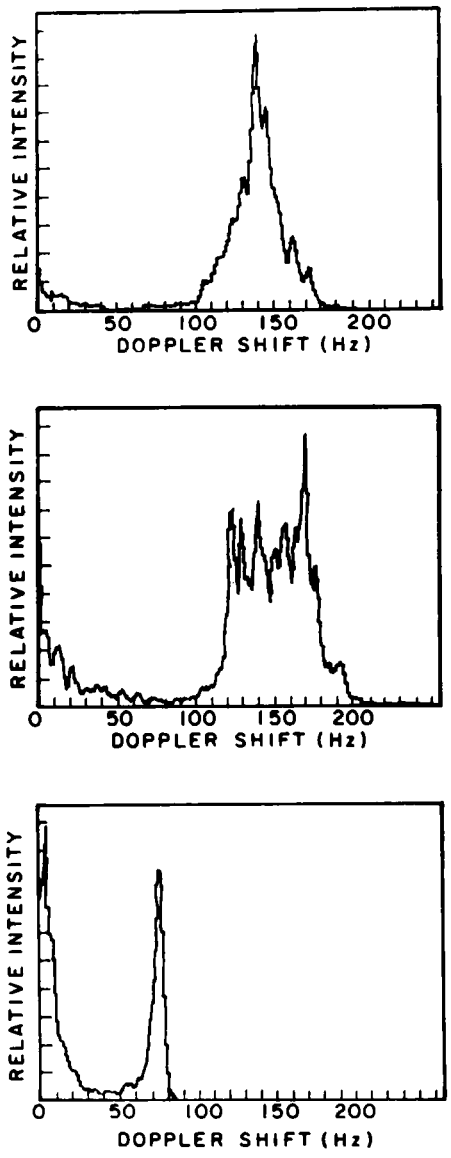

FIGURE 1 Three ELS spectra of actin in solution. For the *top* spectrum the sample was G-actin. The *middle* spectrum was taken 30 min after raising the concentration of Mg^{+2} to 0.5 mM and the *bottom* spectrum was taken 12 h later. For all three spectra the actin concentration was 8.3 mM, the temperature was 20°C, and the scattering angle was 9°. The electric field strength was 104 V/cm for the *top* spectrum and 113 V/cm for the other two.

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