$\langle P_2 \rangle$ and $\langle P_4 \rangle$, is not observed. This also indicates that the T_1 value of the spins in the oriented amorphous region is shorter than 1 s.

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Spontaneous Formation of Long-Range Order in Actin Polymer Networks

Actin is a major protein constituent of cells and, in the filament form, not only helps to define the structure and mechanical properties of cytoplasm but also plays a role in many aspects of cellular motility, as well as in muscle contraction. Our understanding of the details of structure/function relationships in cellular processes is in its infancy, although a large body of knowledge on the interactions of the many actin-binding proteins with actin from in vitro experiments[1-6] has been assembled. These experiments have shown quite complex and varied mechanisms control the polymerization state of actin.

Purified actin forms a network of filaments when polymerized in vitro with salt. The mechanical properties of this network and the diffusion of individual actin filaments or of added probes within the network have been studied by several groups by viscoelastic[7-11] and fluorescent photobleaching methods.[12-15] There are conflicting interpretations in the literature as to whether or not individual actin filaments in the network form noncovalent cross-links in the absence of added actin-binding proteins which are known to form a cross-linked gel with actin.[1-6]

We have recently reported on dynamic light scattering measurements of the diffusion of inert spherical probes within actin solutions at concentrations below about 1 mg/mL.[16] These measurements found a progressive decrease in the diffusion of the probes at increasing actin concentrations for a fixed probe radius or with increasing probe radius at fixed actin concentrations. In attempting to extend this work to higher actin concentrations and lower scattering angles, we observed a new type of behavior which is the subject of this report.

Actin was extracted from rabbit muscle acetone powder and purified according to published procedures using a final column purification step.[16] Monomeric actin was filtered into optical cuvettes and monodisperse polystyrene latex spheres (PLS) were added, to serve as local microviscosity probes, at concentrations which ensured that the diffusion coefficients determined were those of the probe.[16] The probes have been shown to act as inert, non-actin-binding spheres.[16] Prior to polymerization of the actin with either 1 mM $MgCl_2$ or 100 mM KCl, 50 μM Mg-EGTA was added and the sample was incubated for 5 min. Diffusion coefficients were determined by dynamic light scattering with a previously described system.[16-18] Experiments of typically 20-s duration determined the average diffusion coefficient of the probes, the average scattered intensity, and the second cumulant parameter, which is a measure of the polydispersity of local microviscosity in this case. At concentrations of actin above about 1 mg/mL, after an incubation time of 20–90 min (depending upon the salt) a series of dynamic light scattering measurements were made at varying scattering angles (in the range 15° to 90°). When these experiments had been performed at actin concentrations below about 1 mg/mL, the results were reproducible, in the sense that fluctuations in the scattered intensity of light and in the measured diffusion coefficients of the probes were small.[16] At higher concentrations, we observed the onset of large (up to factors of 5) and slow (several minute) fluctuations in the intensity of scattered light and corresponding large fluctuations in the measured diffusion coefficients so that a record of repeated 20-s experiments gave seemingly erratic results (see Figure 1). These results clearly indicate the presence of large spatial inhomogeneities in the PLS probe concentration. When the average diffusion coefficients of the PLS, average scattered intensities during the duration of the experiment, and "polydispersity" measure indicating the range of different local microviscosity environments were cross-correlated in successive experiments, a very strong correlation was found as shown in Table I.

For these same samples, at lower scattering angles, we viewed on a screen a quasi-stationary diffraction pattern (see Figure 2), which had the following characteristics. The pattern consisted of a set of diffraction maxima, randomly arrayed with a circular trend due to the optical arrangement, which had persistence times of several minutes. At higher concentrations or with larger PLS probes, the

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Table I
Results of a Correlation Analysis of Probe Diffusion, Scattered Intensity and Microviscosity Polydispersity[a]

				diffusion			
				intensity		polydispersity	
salt	probe radius, nm	angle	no. expts	corr coef	corr prob	corr coef	corr prob
$MgCl_2$	100	15	5	−0.87	0.95	−0.94	0.98
	265	30	18	−0.82	0.94	−0.93	0.99
	265	90	15	−0.64	0.79	−0.98	0.99
KCl	100	90	12	−0.83	1.0	−1.0	1.0
	265	90	14	−0.62	0.84	−0.99	1.0

[a] Actin concentrations were in the range 2.0–2.2 mg/mL. Correlation coefficients either between the measured probe diffusion and average scattered light intensity during the 20–100-s experiments or between the diffusion and normalized second cumulant parameter, Q (which here reflects the microviscosity polydispersity), were determined in the usual manner. For the given number of experiments the probability for correlation of the two quantities was also determined.[27]

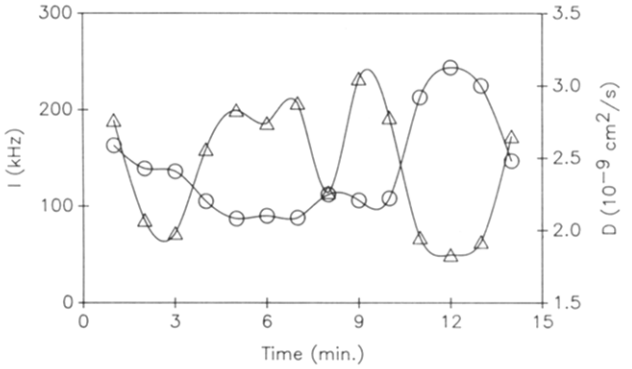

Figure 1. Repeated measurements of the scattered intensity recorded at a 90° scattering angle (○) and probe diffusion coefficient (△) for a sample of 2.1 mg/mL actin polymerized with 100 mM KCl with added 265-nm radius polystyrene latex sphere probes. The large fluctuations over periods of minutes can be attributed to slow and extensive concentration fluctuations of the probes induced by a spatial decomposition or reversible clumping of the actin polymer.

pattern appeared almost immediately after addition of the polymerizing salt and persistence times of many minutes were observed. At lower actin concentrations, as low as about 0.5 mg/mL, or with smaller probes, periods of up to tens of minutes were required for the contrast in the pattern to increase and stabilize and the persistence times of the diffraction maxima were somewhat shorter. The spatial separation of the diffraction maxima is consistent with a spatial ordering of tens of micrometers.

It is well-known that polymerized concentrated actin solutions which are mechanically undisturbed develop large shear-sensitive apparent macroviscosities. We observed that the low-angle quasi-static diffraction patterns lost contrast and had much shorter persistence times after gently rotating the optical cuvette so that the actin solutions flowed like a fluid. Immediately after observing this fluidlike flow behavior, the higher angle scattered intensity and diffusion coefficient fluctuations stabilized as they had at lower actin concentrations, indicating a spatially more homogeneous population of PLS probes.

Our interpretation of these results is that the actin polymer undergoes a phase separation (probably early stages of a spinodal decomposition) in which regions of higher and lower actin concentration form and dissolve with characteristic persistence times of minutes. The concentration of the PLS probes, which have a highly restricted diffusion in the network of actin, consequently also becomes inhomogeneous and gives rise to the low-angle diffraction pattern. These inhomogeneities are also observed at higher scattering angles as large slow intensity fluctuations with highly (negatively) correlated diffusion coefficients because of the strong actin concentration dependence of the probe diffusion coefficient. Our data clearly indicate the onset of long-range order, although we cannot further comment on the possible presence of weak noncovalent cross-links between actin filaments. In a separate report,[19] we show that the diffraction patterns remain qualitatively unchanged in the presence of cross-linking actin binding protein which forms isotropically branched gels.

Actin polymers, even at 2 mg/mL, are in a highly congested state. Although the distribution of actin polymer lengths in solution remains unclear, it is now generally

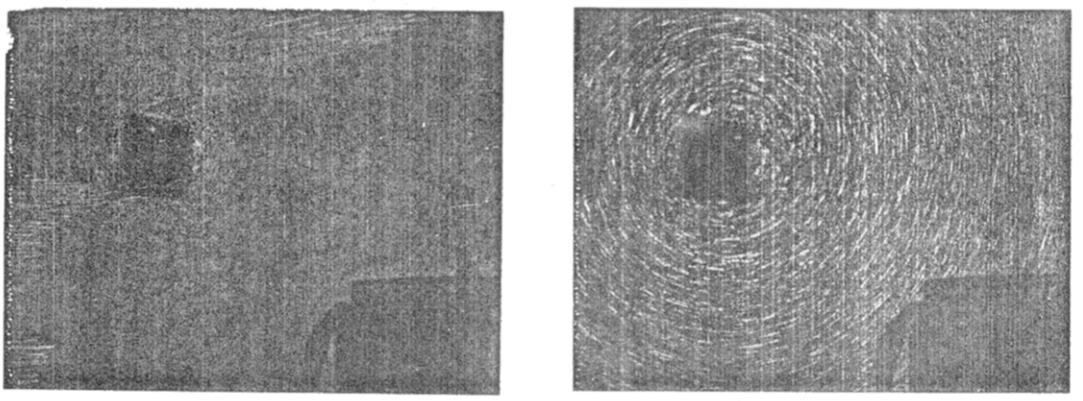

Figure 2. (Left) Photograph of low-angle scattering from a solution of 2.1 mg/mL monomeric actin with 265-nm radius polystyrene latex probes added. (Right) Same conditions as on the left but 5 min after the addition of 1 mM $MgCl_2$ to produce rapid polymerization of the actin. Both photographs were taken with 10-s exposures and a central mask to block the transmitted laser beam. The angular field of view is roughly 5°. Under the given conditions diffraction maxima had persistence times of several minutes. With lower actin concentrations or smaller probe radii, the patterns were similar but had less contrast and somewhat shorter persistence times, while with larger probes the contrast was even greater and persistence times longer.

believed that significant numbers of filaments are longer than 10 μm. Assuming, for purposes of the following calculation only, a uniform population of 10-μm actin filaments, the semidilute regime begins at a concentration of only 0.25 μg/mL, while the concentrated regime (number concentration $> 1/dL^2$, where d and L are the rod diameter and length, respectively) begins at about 35 μg/mL. Two recent studies on filamentous actin solutions at high concentrations[20,21] indicate transitions in the viscoelastic behavior of actin. In one of these,[20] a model was developed in which the immobilized network of actin polymer is thought of as having undergone a glass transition. In the other study,[21] a model is proposed in which liquid-crystal domains are formed which are connected by polymer strands extending from one domain to another to form a global network in the container. In this work, evidence for these domains at very high actin concentrations (8–25 mg/mL) was presented from polarization micrographs. Our study at somewhat lower concentrations suggests a mechanism for the microcrystallization of such domains. A similar mechanism was recently proposed as the trigger for gelation of agarose sols[22,23] and, as such, may provide a general method for controlling the onset of structural ordering in other supramacromolecular systems. Although our work has been for an in vitro system, there may be particular relevance for the formation of microfilaments in local cellular regions. In recent years the role of actin in many aspects of cellular motility, structure, and regulation of internal viscoelasticity has been studied in vivo.[24,25] We are currently extending our studies to the interactions and effects of various actin-binding proteins on the actin network.[19,26]

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