Dynamic Light Scattering from Spatially Inhomogeneous Cross-Linked Actin Gels

Many biologically important macromolecules form gels. Examples include collagen, DNA, agarose, sickle hemoglobin, and actin. Most of these systems are formed from shear-sensitive filamentous components and are difficult to study without unintended sample disruption. Light scattering methods, both static and dynamic, are extremely useful tools for the study of gelling systems because they are nonperturbative, require no labeling of the macromolecules, and can supply information on the microscopic structure and interactions within the gel. Recently, many systems have been studied by dynamic light scattering using probe particles (typically monodisperse polystyrene latex spheres, PLS) dispersed within networks of concentrated sols or gels.[1-13] The diffusion of the probes can be determined under a variety of sample conditions, and information on the structure of the networks can be deduced. One complication in such studies of gelling systems is the problem of nonergodicity arising from spatial inhomogeneities within the gel. This usually leads to large variances in the scattered light intensity and apparent diffusion coefficient obtained from a standard cumulant analysis. In fact, the extent of such inhomogeneities as detected by fluctuations in the scattered light intensity has been used as a test for the degree of gelation.[14]

Recent theoretical developments by Pusey and van Megen[15] in the area of dynamic light scattering from nonergodic media have shown that, given a system satisfying several assumptions, it is possible to obtain the ensemble-average field autocorrelation function from a measurement of the time-average intensity autocorrelation function obtained at a single spatial location within the sample, if the ensemble-average intensity has been determined. In this model, it is assumed that the total scattered field is divisible into a time-fluctuating component and a static or constant component. The time-average intensity autocorrelation function obtained from a limited region of the sample is then related to the ensemble-average field correlation function, assuming heterodyning between the fluctuating and constant portions of the scattered field, to obtain an equation for the ensemble field autocorrelation function

$$g_E^{(1)}(k,t) = \frac{I_t}{I_E}\left\{\left[\frac{I_E}{I_t} - 1\right] + [g_t^{(2)}(k,t) - g_t^{(2)}(k,0) + 1]^{1/2}\right\} \quad (1)$$

where I_t and I_E are the measured time- and ensemble-average scattered intensities, respectively, and $g_t^{(2)}(k,t)$ is a measured spatially localized time-average intensity correlation function. Thus, using eq 1, from a single time-average measurement of the local intensity autocorrelation function and the mean intensity during that measurement, one can calculate the ensemble-average field correlation function if the ensemble-average scattered intensity has been determined.

This formulation has received limited experimental verification using only polyacrylamide gels with PLS added as diffusion probes.[16] In this study, we have tested the Pusey and van Megen theory using the weakly gelling actin biopolymer system with PLS diffusion probes in the absence and presence of an actin-specific cross-linking protein, actin-binding protein (ABP).

Actin was extracted from rabbit muscle acetone powder and purified according to published procedures using a final column purification step.[17,18] Monomeric actin was adjusted to a concentration of 1.3 mg/mL and 0.2-μm-diameter monodisperse PLS were added, to serve as diffusion probes, at a concentration of 0.007%, sufficient to ensure that the scattering from the PLS dominated that from the actin and thus that diffusion coefficients determined were those of the probe. The actin was then polymerized by the addition of 100 mM KCl. An actin-specific cross-linking protein, actin-binding protein (ABP), purified from rabbit lung macrophage according to standard procedures,[19,20] was then added to some samples in a molar ratio of 1:150 ABP/actin monomers. ABP has been shown to form isotropic actin gels with cross-links forming at near right angles at the molar ratio used here.[19,21-23] The resulting samples behaved like weak gels which slowly flowed on inverting the optical cell even with the added ABP. Dynamic light scattering measurements were standard[17,18] except that the optical cell was scanned vertically, by a micrometer drive, at a rate of several microns per second and micrometer drives in the horizontal directions were also manually controlled. Measurements were performed at six angles in the range 10–90°, at room temperature, and consisted of several 40–100-min scans or 100–300 experiments of 20 s duration. The autocorrelation functions were initially analyzed by third-order cumulants,[24] the shorter experiments were later analyzed using eq 1 to obtain an estimate for the ensemble-average field autocorrelation function. Experiments using latex probes in water verified that no artifacts were introduced by scanning the optical cell vertically and determined the free-particle amplitude of the field autocorrelation function to be 0.92 for the optics of the experiment. The use of eq 1 assumes that the free-particle amplitude is equal to 1; however, Pusey and van Megen indicate that, with lower amplitude values, expected fractional errors are on the order of the difference between 1 and the free-particle amplitude, which in our case is about 8%.

In Figure 1 representative data are shown for the field autocorrelation functions obtained from PLS in cross-linked actin samples. Good agreement between the measured ensemble-average and the corrected (using eq 1) time-average field autocorrelation function is seen over the entire time range spanned, but particularly at the short times used to obtain the initial decay rates of the cumulant analysis below.

In a series of similar experiments using cross-linked F-actin solutions, the scattered intensity and time-average field autocorrelation functions were determined within 100 different local regions of the sample. Mean apparent diffusion coefficients were obtained from a cumulant analysis, and their distribution is displayed in Figure 2a as the solid bars in the histogram, while the scattered intensities are shown in Figure 2b. Large fluctuations are apparent in both quantities indicating large spatial inhomogeneities. Such fluctuations are also observed over time periods of many minutes at a fixed spatial location, presumably due to local rearrangement of actin filaments of the weak gels resulting in local redistribution of PLS which dominate the scattering. Three long-time-average experiments were performed by scanning a large portion of the sample, and the mean result, the ensemble-average diffusion coefficient, is shown as the solid line in Figure 2a. The 100 time-average experiments were analyzed by eq 1 and replotted in Figure 2a as the outlined bars. It is apparent that eq 1 results in a sharply peaked distribution of diffusion coefficients within about 10% of the correct ensemble average value. Thus, apparent diffusion coefficients obtained from single spatial locations in the case of inhomogeneous scattering media are not directly

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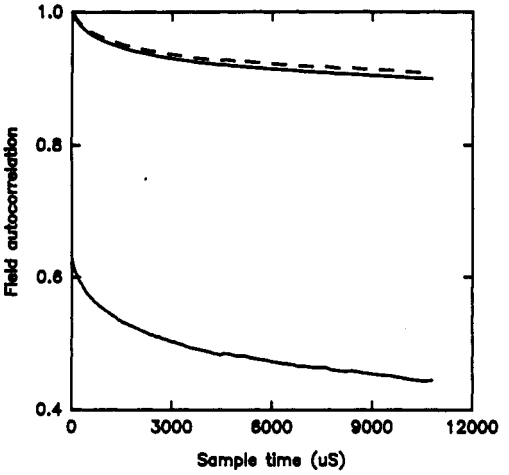

Figure 1. Estimates of the field autocorrelation function obtained from a 30 μM (1.3 mg/mL) F-actin sample polymerized by the addition of 100 mM KCl with the actin-specific cross-linking protein ABP added in a molar ratio of 1:150 actin monomers and 0.2-μm-diameter PLS diffusion probes. Data were recorded using 136 sample channels using the multi-τ option of a Brookhaven 2030AT correlator to span a large delay time. The lower curve represents $[g_t^{(2)}(t) - 1]^{1/2}$, obtained from a single 20-s experiment at a single spatial location within the sample. These data, when corrected using eq 1 and the measured ensemble-average scattered intensity, become the dashed upper curve. The solid upper curve represents the ensemble-average field autocorrelation function, $[g_E^{(2)}(t) - 1]^{1/2}$, normalized to 1, which was obtained from a 40-min experiment while scanning through the sample. Uncertainties in the data, based on the accumulated background levels, are 0.2% for the 20-s experiment and 0.01% for the 40-min experiment. Similiar results were obtained at other scattering angles and with other prepared samples of actin.

related to the true dynamics of the system.

As had been observed previously with high concentrations of actin solutions without cross-linking protein added,[2] the scattered intensities from the short-time-average experiments with ABP present are strongly correlated with the apparent diffusion coefficients determined from a cumulant analysis. This correlation is shown in Figure 3 (open triangles) for the 100 short local experiments. Upon correction of the autocorrelation functions using eq 1 and subsequent determination of diffusion coefficients, the strong dependence on scattered intensity is eliminated, as shown in the same figure (solid triangles).

Similar measurements with actin samples which were not cross-linked with ABP (not shown) did not have as large variations in measured scattered intensities or apparent diffusion coefficients, although these two quantities were still highly correlated. However, apparent diffusion coefficients, corrected using eq 1, did not lead to values in good agreement with the independently obtained ensemble-average diffusion coefficients. A probable explanation for this is that PLS trapped in these actin networks may not produce a truly static contribution to the scattered intensity due to a greater degree of flexibility of the actin filaments without the cross-linking protein. With added cross-linking protein, these trapped PLS are slowed to time scales sufficiently long compared to the experiment duration that they may be considered static.

Previous reports from this laboratory have presented results from experiments either from similar samples at lower concentrations[1] or from samples at comparable concentrations but without ABP cross-linker present.[2] At concentrations above 1 mg/mL, large fluctuations in intensity and diffusion coefficient were measured in the

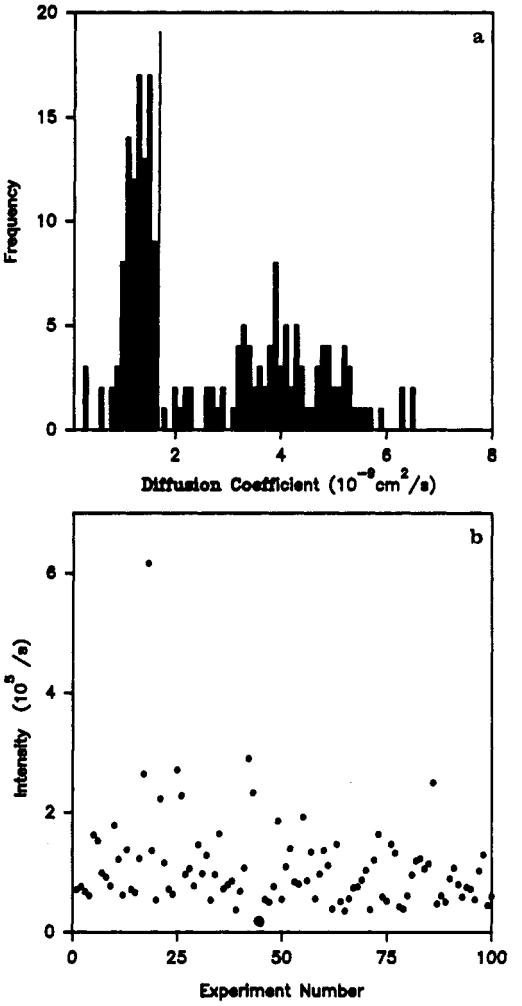

Figure 2. (a) Frequency distribution of the measured apparent diffusion coefficients of 0.2-μm-diameter PLS in 30 μM F-actin with ABP added at a molar ratio of 1:150, obtained in 100 independent short-time-average experiments at different spatial locations within the sample while scanning continuously. The solid bars represent those values obtained from a third-order cumulant analysis of the early time data (first 40 channels) for $g_t^{(2)}(t)$ (mean diffusion coefficient, $\langle D \rangle = (4.0 \pm 1.1) \times 10^{-9}$ cm^2/s). The hatched bars are the same data reanalyzed using the same cumulant analysis after correction after correction of the autocorrelation functions using eq 1 ($\langle D \rangle = (1.3 \pm 0.2) \times 10^{-9}$ cm^2/s). The single solid line indicates the D value determined from the independently measured ensemble-average field autocorrelation function ($\langle D \rangle = (1.7 \pm 0.1) \times 10^{-9}$ cm^2/s). (b) Distribution of scattered intensities for the 100 measurements of Figure 2a.

absence of ABP, and these results were assumed to indicate spatial inhomogeneities even in the un-cross-linked samples. These fluctuations were found to be highly correlated in the same sense as in Figure 3. While these earlier results for diffusion coefficients were not obtained using the protocols used in the current study, we believe the main conclusions drawn from the earlier data remain valid.

In conclusion, this study confirms the reliability of the theory of Pusey and van Megen for the interpretation of dynamic light scattering from diffusion probes in cross-linked actin gels. Either direct measurement of the ensemble-average intensity autocorrelation function by sampling large volumes of the gel or correction of time-average spatially localized measurements is required to avoid large systematic errors in the determination of diffusion coefficients. Data obtained from un-cross-linked actin networks, while exhibiting variations in probe diffusion and scattered intensities to a lesser extent, are

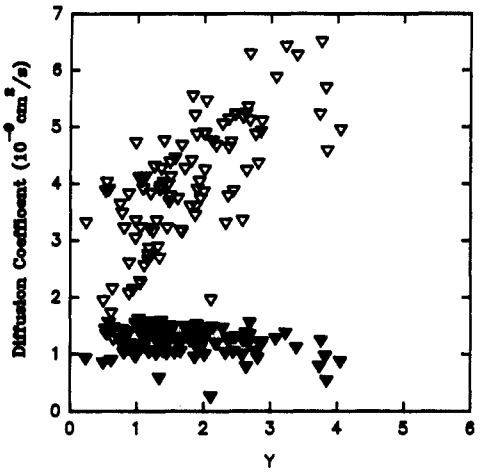

Figure 3. Correlation of apparent diffusion coefficients with Y, the ratio of the ensemble-average to time-average scattered intensity, for the data of Figure 2. The open triangles are the cumulant results for apparent D values from the time-average autocorrelation functions while the solid triangles are the values obtained from the same cumulant analysis on the correlation functions after correction by eq 1.

not as amenable to the Pusey and van Megen analysis.

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