MOLECULAR MECHANICS OF CYTOSKELETAL COMPONENTS

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<u>*Summary*</u> Semiflexible polymers are of great biological importance in determining the mechanical properties of cells. We have used laser interferometry to passively trap and detect the motions of pairs of micron sized silica spheres in solutions of semiflexible polymer. The single- and dual-bead frequency dependent complex shear moduli were extracted from the auto- and cross-correlation response of the beads respectively, with the response being derived from position fluctuation data using dispersion relations from linear response theory.

INTRODUCTION

One of the characteristic lengths that describes a polymer in a solution is the persistence length L_p, which is the length traversed along the filament contour before the tangent vector thermally randomizes its orientation. L_p is proportional to the bending stiffness of the polymer. Flexible polymers are characterized by $L_p << L$, rigid rod polymers by $L_p >> L$. Semiflexible polymers are in the intermediate regime, characterized by $L_p \approx L >>$ filament diameter. The dynamics of polymer systems greatly depends on the degree of flexibility of the individual polymers. Recent theoretical treatment has been made of semiflexible polymer networks [1-4]. Such polymers are found in cells where they form networks that determine the mechanical properties of the cells.

There are clear advantages to the miniaturization of a rheology experiment: only small amounts of material are necessary, spatial inhomogeneities can be studied, and the bandwidth of the measurement can be high. We have developed a microrheology technique using laser interferometric tracking of the Brownian motion of micron sized beads embedded in viscoelastic materials [5]. We have studied the dynamics and compared single- and dual-bead results for wormlike micelle, semiflexible fd virus and actin network solutions. In the semiflexible systems, which are a first approximation to the cytoskeleton of cells, we explore the rich multitude of length and time scales in the dynamic behaviour of these networks, which are usually not accessible to macrorheology.

PRINCIPLE OF THE TECHNIQUE

The microrheology technique used is a passive one in which the thermal fluctuations of pairs of micron sized beads are used to calculate the auto- and cross-correlation response functions of the beads by the fluctuation-dissipation theorem [5, 6]. The complex response function $\alpha_{ij}^{(n,m)}(f)$ relates the Fourier transform $r_i^{(n)}(f)$ of the displacement of the nth bead $r_i^{(n)}(t)$ to the Fourier transform $F_j^{(m)}(f)$ of the force $F_j^{(m)}(t)$ acting on the mth bead (i and j are either 1 or 2 for the parallel and perpendicular directions to the line joining the bead centers, respectively), $r_i^{(n)}(f) = \alpha_{ij}^{(n,m)}(\vec{r},f)F_j^{(m)}(f)$. The response must first be corrected for the effect of the traps [7]. The fluctuation-dissipation theorem [5] provides the link between the generalized, single sided Power Spectral Density (PSD) [8] $S_{ij}^{(n,m)}(f) = \lim_{t' \to \infty}(2/t) r_{t'i}^{(n)}(f)r_{t'j}^{(m)*}(f)$, and the imaginary part of the response function, where $r_{t'i}^{(n)}(f)$ is the Fourier transform of the position $r_i^{(n)}(t)$, defined over a finite time t'. A Kramers-Kronig relation can then be used to calculate the real part of the response function, provided that the imaginary part is known over a large enough frequency range. The connection between the auto correlation response $\alpha_{ii}^{(n,n)}(f)$ of a bead and the corresponding complex shear modulus $G_{ii}^{(n,n)}(f) = G_{ii}'^{(n,n)}(f) + iG_{ii}''^{(n,n)}(f)$ of the viscoelastic medium surrounding the bead is assumed to be provided by the generalized Stokes-Einstein relation (GSER) [5] $G_{ii}^{(n,n)}(f) = 1/[6\pi a\, \alpha_{ii}^{(n,n)}(f)]$, where G' and G'' are the elastic and loss moduli respectively, and a is the radius of the bead. The cross-correlation shear modulus of the solution is derived from the parallel $\alpha_{//} \equiv \alpha_{11}^{(1,2)}$ response by [9] $G_{cross}(f) = 1/[4\pi r\, \alpha_{//}(f)]$, where r is the distance between the centres of the beads.

EXPERIMENTAL SETUP

Pairs of probe particles imbedded in solutions of semiflexible polymer were trapped by two focused laser beams. Position fluctuation data was recorded using laser interferometry and quadrant photodiode detection as described in detail previously [5, 10, 11]. Near-infrared 1064 nm laser illumination was used for one trap and an 830 nm laser for

the second trap. The beams are brought to focus in the sample chamber by a high NA objective. The laser light emerging from the condenser lens, after passing through the sample, is projected onto two quadrant photodiodes in such a way that the back-focal plane of the condenser is imaged [10]. The signals from the four quadrants of each photodiode are combined to obtain the X and Y voltages corresponding to the displacements of each bead in these directions in the plane normal to the propagation direction of the laser. The output voltages are, after analog amplification and pre-processing, recorded using an A/D interface and data acquisition software.
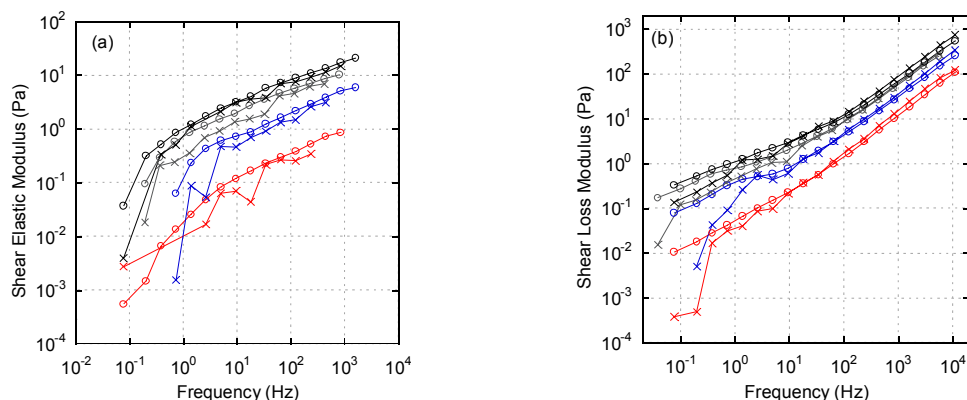
RESULTS

FIG. 1. (a) The concentration dependence of auto and cross correlation elastic modulus data of 1.16 μm diameter beads in 2, 5, 10 and 14 mg/ml fd solutions. (b) The same for the loss modulus. Pairs of auto (circles) and cross (crosses) moduli curves are higher for higher concentrations.

Figure 1a shows the concentration dependence of the frequency dependent single- (circles) and dual- (crosses) bead elastic modulus. The viscous modulus is shown in Fig. 1b. Data are displayed for 2, 5, 10, and 14 mg/ml solutions. The high frequency slope behaviour of the viscous modulus is consistent with single filament dynamics predictions [1, 4]. At such high frequencies, the effect of entanglement of the polymer in its network does not contribute to the modulus. The modulus is proportional to concentration and expected to have a slope of ¾. The elastic modulus is expected to exhibit the same behaviour but due to the cut-off in the Kramers-Kronig integral, the elastic modulus extends to a smaller frequency range below that where high frequency dynamics dominates. The high frequency behaviour is attributed to tension in the filaments due to extension. At lower frequencies, the tension contributes less, and the dominating behaviour is that of bending and orientational contributions to the stress [1, 2]. It has been predicted that single bead measurements will be sensitive to the local properties of the medium surrounding the beads while the dual-bead results would reflect the bulk viscoelastic properties of the solutions probed [9]. Depletion of polymer in the vicinity of the probe particles, or having comparable probe size and network length scales is expected to cause differences in the single- and dual-bead modulus behaviour. We present results for actin networks where a difference between the two techniques is detected, and results from micelle and fd virus solutions (Fig. 1 shows fd virus results) which show agreement between the single- and dual-bead methods.

CONCLUSIONS

We have applied a passive microrheology technique to study the dynamics and compare one- and two-bead complex shear moduli of solutions of various semiflexible polymers. Results were compared to recent theoretical predictions, and one- and two-bead measurements were compared for each polymer system over a large concentration regime.

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