Measuring the Effects of RAD51 Assembly on dsDNA with Magnetic Tweezers

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Introduction:

RAD51 is a protein in eukaryotic cells that is involved in the repair of double-strand breaks in deoxyribonucleic acid (DNA) by homologous recombination. RAD51 assembles on the resected single strands of the damaged region of the DNA, finds a homologous sequence of an intact double-stranded DNA (dsDNA) molecule, and promotes single-stranded DNA (ssDNA) invasion into this sequence [1]. Studying this protein can help us to understand the activity of RAD51 within the nucleus and properties of RAD51-dsDNA filaments during crucial repair processes. Magnetic tweezers provides a means to conduct experiments with RAD51 and dsDNA.

Magnetic tweezers are a single-molecule technique that allows us to apply a torque and an upward force to DNA molecules. The conventional magnetic tweezers setup consists of two cubic magnets above a flow cell, inside which paramagnetic beads are tethered to the bottom via a DNA molecule. As the magnets are rotated, raised, or lowered, the paramagnetic bead will rotate or experience a lesser or greater magnetic force, respectively. The magnetic torquetweezers (MTT) setup is similar to conventional magnetic tweezers, however it consists of a cylindrical magnet that applies an upward force to the tethered bead and a small side magnet that applies a torque. The torsional trap stiffness of MTT is much weaker than that of conventional magnetic tweezers, allowing for greater angular fluctuations of the tethered bead and the measurement of torque [2]. In our experiment, we use MTT to calculate the torsional modulus of RAD51-DNA filaments under torsional stress.

Methods:

Anti-digoxigenin (100 μg/ml, Roche) and bovine serum albumin (BSA; Sigma) were incubated to allow for DNA tethering and to passivate the surface, respectively. The 8 kB dsDNA, functionalized with digoxigenin on one end and with biotin on the other, was first bound to 1.4 μm radius paramagnetic M-270 beads (Invitrogen). The tethered beads were then incubated inside the flow cell to allow attachment of DNA to the bottom surface. Most tethered beads were bound to 0.5 μm radius biotinylated latex Fluosphere fiducial beads (Invitrogen). We used 1.5 μm radius nonmagnetic latex beads (Life Sciences) that were bound nonspecifically to the bottom surface of the flow cell as reference beads to correct for drift in the magnetic tweezers microscope.

The flow cell was placed over an oil-immersion objective (Olympus ACH 100X, numerical aperture = 1.25) connected to a CCD camera (Pulnix TM-6710CL), and illuminated with collimated LED. Preliminary measurements were taken with conventional magnetic tweezers to ensure that both strands of the molecule of interest were intact and that the paramagnetic bead was tethered by only one molecule. The flow cell surface was passivated with BSA again, and MTT were then used. RAD51 was incubated in the flow cell while the magnet was incrementally rotated to -300 turns to prevent loops, or supercoils, from forming in the DNA molecule while the RAD51 assembled. To track the z-height of the paramagnetic bead, the diffraction pattern calibration profiles of one paramagnetic and one reference bead were used [3]. Angular tracking was made possible by the attachment of a small marker bead to the tethered bead [2] (Figure 1).

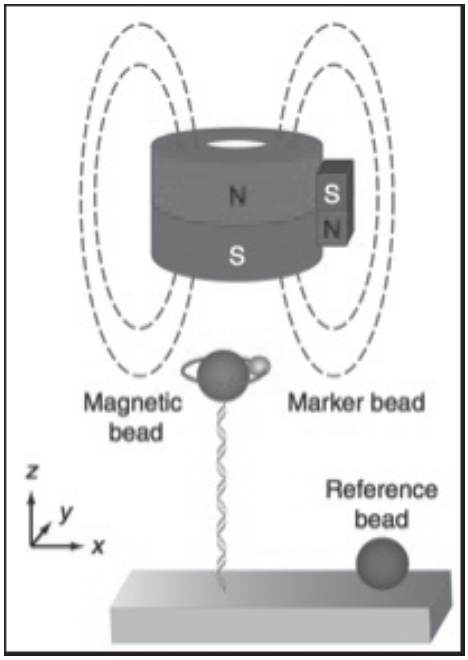

Figure 1: The MTT setup is shown with the dashed lines representing magnetic field lines.

Buffer Conditions:

A TE tethering buffer (200 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 10 mM NaN_3 supplemented with 0.01-0.02% Triton-X during and after the bead-DNA binding process) was used

during flow cell preparation. Preliminary measurements were carried out in 100 mM NaCl buffer (100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10 mM NaN_3 supplemented with 0.01-0.02% Triton-X). The binding of RAD51 to DNA and measurements with MTT were carried out in Ca^{2+} Assembly buffer (25 mM Tris-HCl (pH 7.5), 25 mM KCl, 2 mM $CaCl_2$, 1 mM DTT, 1 mM ATP, 0.5 mg/ml BSA, and 0.02-0.05% Triton-X).

Results:

We derived the torsional modulus from the torque build-up of the RAD51-dsDNA filament after N turns, which was equal to $2\pi Nk_BTC/L_C$ [2]. Here, k_B was the Boltzmann constant, T was temperature, and C and L_C were the torsional modulus and contour lengths of the RAD51-dsDNA molecule, respectively, which were derived from a force-extension curve.

Figure 2 shows the force-extension curve for bare dsDNA, and Figure 3 shows the force-extension curve for a RAD51-dsDNAfilament. L_C is a function of force, and from these figures we see that L_C of the bare dsDNA was 2.88 μm and L_C of the RAD51-dsDNA filament was 4.09 μm. Figure 4 shows the mean angle of the filament versus N, determined by angular tracking of the paramagnetic bead. The torsional modulus was 553 ± 80.2 nm at 3.5 pN.

Discussion:

Previous studies have found that RAD51 assembly results in a ~ 50% increase in L_C of the dsDNA filament [1]. This is consistent with our measurements, which showed a 42.01% increase in L_C. Lipfert, et al. [2], measured the torsional modulus of bare 8 kB dsDNA and RecA-dsDNA (RecA is a protein in bacteria homologous to RAD51) to be ~ 95 nm and 173 ± 5 nm at 3.5 pN, respectfully. Comparing this with our results, we see that RAD51 assembly increased the torsional modulus of bare dsDNA by ~ 580%, in contrast to a 180% increase caused by RecA assembly.

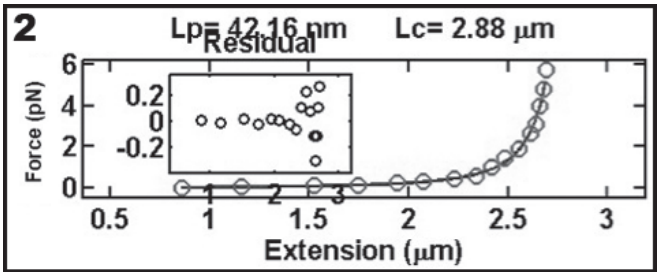

Figure 2: The force-extension curve of the bare dsDNA filament gives LC of that filament.

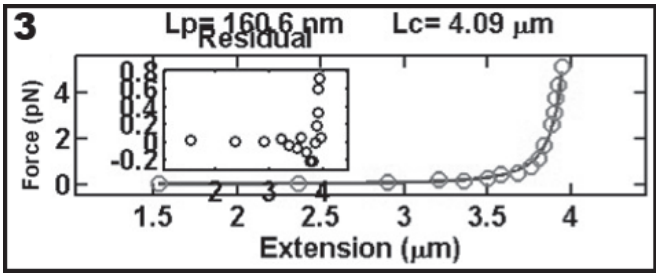

Figure 3: The force-extension curve of the RAD51-dsDNA filament gives LC of that filament.

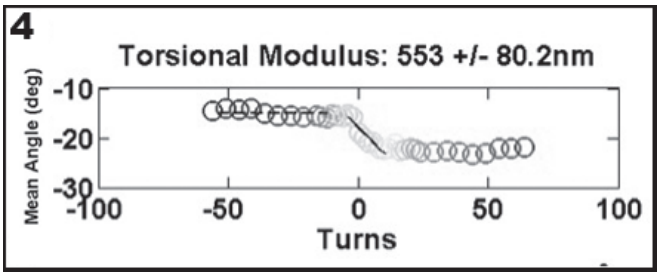

Figure 4: The torsional modulus is derived from the mean angle of the filament versus N.

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References:

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