Jason D B Sutin, Chen-Yuan Dong, Peter T C So, L Mahadevan, and Enrico Gratton.
Magnetic micromanipulation of single molecules of exonuclease during DNA degradation.
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Abstract

We have developed a magnetic manipulation microscope for single molecule micromechanical experiments using magnetic microspheres. The principal feature of magnetic micromanipulation is that the force is inherently applied under isotonic conditions, without the need for feedback. This is particularly advantageous in the low force regime where feedback is difficult. In addition, forces can be applied over large distances limited only by the size of the microscope field and multiple microspheres can be manipulated simultaneously. We are using the magnetic micromanipulator to probe the molecular dynamics of a single molecule of exonuclease as it digests DNA. A 6-histidine mutant of lambda exonuclease is coupled to a 4.5 micron streptavidin coated superparamagnetic bead via a biotin-NTA linker. The beads are introduced to amino-modified lambda DNA that is covalently attached to a coverslip via a silane linkage. Since the contour length of lambda DNA is -16 microns, the motion of the exonuclease as it digests long sections of DNA can be determined by the calculating the centroid of the image of the attached bead. We have measured single molecules of lambda exonuclease degrade double stranded DNA at 6.3 bp/s with processivity greater than 15,000 bp under no load. We are currently using the micromanipulator to apply forces opposing the action of the exonuclease to measure the corcevelocity curve of this enzyme. This work supported by NSF MCB-9604382 and PHS 5 P41-RRO3155.