Title
Exonuclease motility studied by single-molecule magnetic force assays

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Authors
Sutin, JDB
Dong, C
Mahadevan, L
et al.

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Jason D B Sutin, Chen-Yuan Dong, L Mahadevan, Enrico Gratton, and Peter T C So.

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Abstract

Single molecule force manipulation is an important tool for investigating how biochemical reactions produce mechanical effects, since mechanical properties are difficult to measure by conventional biochemical techniques. We are investigating the mechanism of mechanochemical coupling between DNA hydrolysis and enzyme translocation during DNA degradation by lambda exonuclease. We have developed a magnetic manipulation microscope for single molecule micromechanical experiments using magnetic microspheres. The principle feature of magnetic micromanipulation is that the force is inherently applied under isotonic conditions, without the need for feedback. We have coupled a 6-histidine mutant of lambda exonuclease to 4.5 micron streptavidin coated superparamagnetic beads via a biotin-NTA linker. The beads are introduced to amino-modified lambda DNA covalently attached to a coverslip via a silane linkage. Since the contour length of lambda DNA is ~16 micron, the motion of the exonuclease as it digests long sections of DNA can be determined repeatedly by the calculating the centroid of the image of the attached bead. We have measured the velocity of single molecules of lambda exonuclease at 14 bp/s and processivity greater than 15,000 bp under no load. Under the application of 1.5 pN of force in opposition to the exonuclease, the exonuclease velocity slows to 1.3 bp/s. We are currently measuring the complete force-velocity curve of this enzyme. This work supported by NSF MCB-9604382 and PHS 5 P41-RRO3155.