Protein Nucleic Acid Interactions II

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Probing DNA Unwinding By Single Helicases

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DNA helicases are multi-functional motor proteins which translocate along DNA using the energy released from ATP hydrolysis. Translocation is coupled to diverse enzymatic activities, such as duplex unwinding, strand degradation and protein displacement. Resolving such complex processes requires our ability to detect rare, or short-lived intermediates and observe their dynamics. To this end, we have developed a generic fluorescence-based assay, which enables monitoring of DNA unwinding by single helicases in vitro. Biotinylated double-stranded DNA fragments are specifically immobilised on poly-ethylene glycol-coated surfaces through biotin-neutravidin interaction. Total internal reflection fluorescence (TIRF) microscopy is used to achieve high signal-to-noise ratio. To probe DNA unwinding, we use a fluorescently labelled mutant of the E.Coli single-stranded DNA-binding protein (SSB), which specifically binds single-stranded DNA. DNA unwinding events mediated by single helicases are observed as fluorescent spots of increasing intensity, as increasing numbers of SSB molecules bind to the ssDNA product of the helicase. Using an objective-based TIRF microscope, these events are recorded at video rate. The increase in fluorescence intensity directly correlates to the rate at which single helicases unwind DNA. Here, we show that the assay is a powerful tool for probing DNA unwinding by various helicases as a function of DNA sequence, ATP and salt concentration. In comparison with bulk measurements, our single molecule data demonstrate that DNA unwinding involves multiple phases which are masked during ensemble averaging. Hence, this assay adds to the single molecule toolbox available for studying DNA processing enzymes.

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Helicase Activity As Monitored By Dual Colour Fluorescence Correlation Spectroscopy

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Helicases are responsible for the unwinding of DNA. These proteins play a key role in the maintenance of the genomic integrity, their defect in humans lead to several disorders including Bloom syndrome (cancer predisposition) and Werner syndrome (premature ageing). The E. coli RecQ helicase activity was studied by Fluorescence Cross Correlation Spectroscopy under single turnover and Michaelis-Menten conditions. The influence of the DNA substrate size on the unwinding activity was clearly different depending on the condition, excess of enzyme or DNA substrate. Moreover, addition of single-stranded DNA of varying size, complementary to one of the DNA substrate strand, displays both stimulating and inhibitory effects depending on the concentration range with efficiencies depending on the size. Our results indicate distinct mechanisms of action of RecQ helicase depending on the enzyme:DNA ratio used.

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Single-Molecule Fluorescence Resonance Energy Transfer Studies of Hjm/Hel308 DNA Helicase in Mesophilic Archaeon, *Methanosarcina acetivorans*

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One class of superfamily-2 (SF2) helicases, the RecQ family, plays crucial roles in DNA repair and homologous recombination in order to maintain the genome integrity. Archaeal RecQ-like DNA helicases, such as Hjm/Hel308, have been biochemically characterized in hyperthermophilic and thermophilic archaea. The studies suggest that Hjm/Hel308 helicases target stalled replication forks and have specificity for unwinding lagging strands. In this study, we cloned and purified Hjm/Hel308 homologue (MacHjm) from a mesophilic archaeon, *Methanosarcina acetivorans*. Single molecule fluorescence resonance energy transfer (smFRET) assay was used to study the single-stranded DNA (ssDNA) binding ability and behavior, the double-stranded DNA (dsDNA) unwinding kinetics, and Holliday junction migration activity induced by MacHjm. By this method, we determined that four MacHjm molecules were able to bind to 17-nucleotide ssDNA with each MacHjm occupying three to four nucleotides of the DNA. In addition, we were able to observe the binding and unwinding activity of MacHjm on DNA in real time. The MacHjm was observed to bind to ssDNA and translocate on ssDNA in 3′ to 5′ in ATP dependent manner. Within three seconds MacHjm was able to complete the unwinding of 18 base-pair dsDNA. The results from our smFRET studies on MacHjm provide important insights into DNA unwinding, stalled replication fork processing, and Holliday junction migration mechanisms for SF2 helicase.

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Protein-Induced DNA Unwinding is An Intrinsic Feature of Certain Sequence-Specific DNA-Binding Proteins

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Protein-DNA interactions play a key role in many fundamental biological processes, such as DNA replication, transcription, and recombination. Very often, the DNA-binding proteins induce structural changes of the target DNA-binding sequences. These DNA structural changes include DNA bending, twisting, and wrapping. Although protein-induced DNA-bending has been widely studied, other protein-induced DNA structural changes have not been fully explored due to the lack of a feasible experimental approach. In this study, we developed a new experimental strategy to probe the protein-induced DNA-unwinding. Our first step is to make a circular plasmid DNA template containing multiple tandem copy of a DNA-binding site of a sequence-specific DNA-binding protein, such as λ O protein and LacI. This plasmid also contains one nicking-enzyme recognition site, such as Nb.Bsm I. Next, we nicked this plasmid using the nicking enzyme, Nb.Bsm I, and then titrated the nicked plasmid by increasing the concentration of the DNA-binding protein. The DNA template was ligated by T4 DNA ligase. The linking number (Lk) of the ligated DNA products was determined by one or two-dimensional agarose gel electrophoresis in the presence of chloroquine. The linking number changes (ΔLK) was calculated using equation $\Delta LK = Lk - Lk^{\circ}$, where Lk and Lk° are the Boltzmann centers of the topoisomers band in the presence of the DNA-binding protein and in its absence, respectively. If we define the apparent protein-induced DNA unwinding angle (α_{obs}) equals to $360 \times \Delta LK$, i.e. $\alpha_{obs} = 360 \times \Delta LK$, then the protein-induced DNA-unwinding angle (α) and the DNA-binding constant (K) can be simultaneously determined. Using this method, we have determined the DNA-unwinding angle and the DNA-binding constant for the following sequence-specific DNA-binding protein: λO, LacI, GalR, AraC, and CRP. Our results indicate that protein-induced DNA-unwinding is a unique feature for these sequence-specific DNA-binding proteins.

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Kinetic Mechanism of Duplex rRNA Unwinding by the DEAD-box Protein, DbpA

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DEAD-box RNA helicases are enzymes that couple cycles of ATP binding, hydrolysis and product release to the unwinding of duplex RNA. We have previously determined the rate and equilibrium constants defining the ATPase cycle of DbpA, a DEAD-box protein from *E. coli* that is specifically activated by rRNA. In this study, we have measured rRNA duplex unwinding using a real-time fluorescence assay. Efficient and rapid unwinding of an 8 base pair duplex RNA requires chemical cleavage of ATP. Strand displacement coincides with product release step. Collectively, our analysis allows us to determine how the unwinding kinetic intermediates are coupled to specific ATPase cycle transitions. Because DEAD-box proteins are highly conserved among prokaryotes and eukaryotes, these results will be applicable to eukaryotic DEAD box RNA helicases involved in fundamental aspects of RNA metabolism in the cell.

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Studying RecBCD Helicase Translocation along Chi-DNA Using Tethered Particle Motion with a Stretching Force

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E. coli RecBCD helicase unwinds blunt-end duplex DNA to repair damaged DNA molecules in the homologous recombination pathway. Previous single-molecule experiments show that RecBCD recognizes an 8 nt DNA sequence, chi, and lowers its unwinding rate afterwards under saturating ATP condition. We have developed a single-molecule Force Tethered Particle Motion (FTPM) method, which is modified from the conventional TPM method, and applied it to study RecBCD motion in details. In the FTPM experiment, a stretching force is applied to the DNA-bead complex, and suppressed bead's Brownian motion, resulting in an improved spatial resolution at long DNA substrates. Based on the equipartition theorem, the mean square displacement (MSD) of the bead Brownian motion measured by FTPM correlates linearly to DNA extension length with a predicted slope, circumventing the difficulties, such as non-