A Structural Investigation of Polymerase θ Helicase-like Domain

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Background & Significance

DNA double-stranded break (DSB) is regarded as the most lethal and biologically significant type of damage to cells[1-3]. DNA damage, such as DSB, due to increased exposure to ionizing radiation during space expeditions beyond low Earth orbit has been identified as the *single most significant risk* to astronaut health[4], potentially manifested as radiation sickness, cancer, central nervous system defects, and degenerative diseases[5]. Most DSB events are repaired by homologous recombination or non-homologous end-joining pathways[3]. Recently, however, researchers have identified a third DSB repair pathway, theta-mediated end-joining (TMEJ)[6, 7]. The human gene *POLQ* encodes DNA polymerase θ (pol θ) which repairs DSBs as the key enzyme in TMEJ [6, 8, 9]. It is the only known polymerase to contain both a polymerase and helicase-like domain (HLD) as one molecule. Despite belonging to the superfamily II class of helicases based on conserved structural elements, the N-terminal HLD displays robust ATP hydrolysis activity but no overt helicase activity[8, 10]. We aim to further elucidate the role and mechanism of the mysterious HLD as it relates to DSB repair.

Project Goals

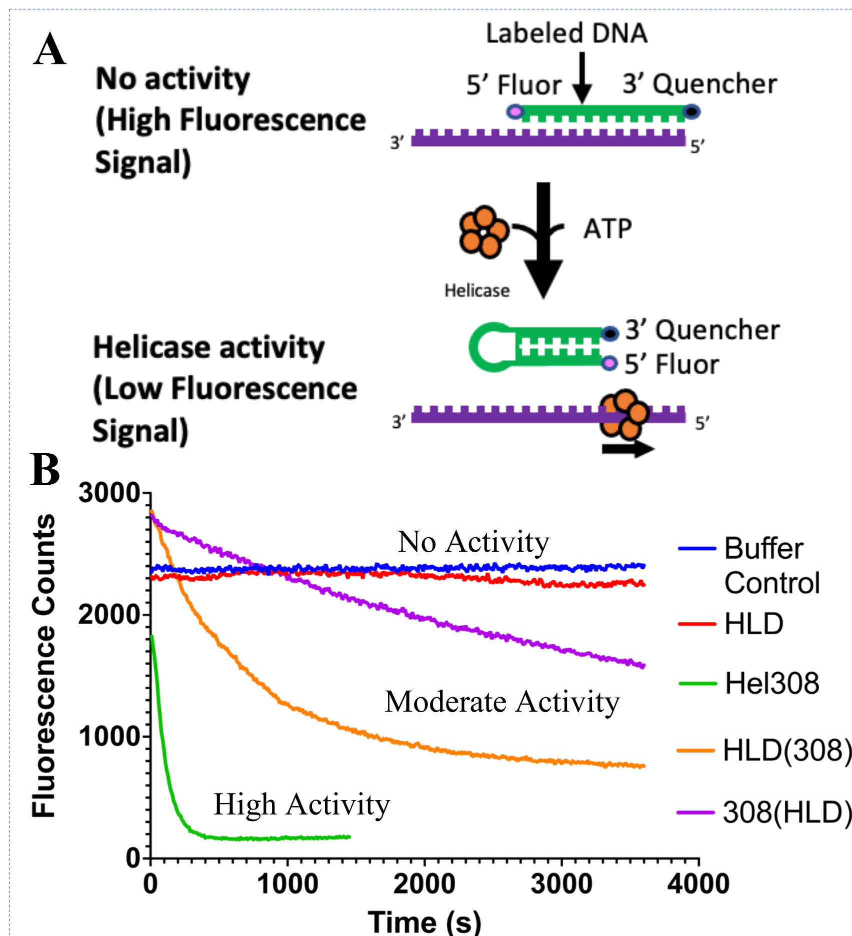
Aim 1. We will visualize the DNA-binding interface of pol θ HLD. Our working hypothesis is that the HLD binds single-stranded (ss) DNA in such a way so as to promote the dissociation of ssDNA stabilizing proteins and the recognition and annealing of complementary sequences between two adjacent, ssDNA substrates. This will be determined using X-ray crystallography to produce high-resolution models of HLD bound to substrate DNA.

Aim 2. Determine the structural basis for the HLD's inability to unwind duplexed DNA. We hypothesize that the stunted separation wedge of the HLD is responsible for its lack of helicase (DNA strand separation) activity. We will purify chimeric proteins in which the separation wedges of the HLD and its helicase-active, archaeal relative Hel308 are swapped. Then, we will perform a molecular beacon-based helicase assay to measure the strand separation activity of the chimeras and the wild types.

Summary of Key Findings

Aim 1. We purified pol θ HLD (spanning amino acids 1-894) to homogeneity. Via the hanging drop vapor diffusion method, we then were able to grow crystals of the HLD in the presence of a partially double-stranded DNA. Crystals were harvested and shipped to the Advanced Photon Source (APS, Chicago) where we remotely collected diffraction data to an approximate resolution of 3.4 angstroms (Å) in March 2021. We used this data to produce a model, however, despite our best efforts, we have so far been unsuccessful in finding the location of and building the DNA molecule. We were, however, able to make the intriguing observation that the HLD crystallized as a dimer (two HLD molecules bound together) which is starkly different than the previously reported crystal structure[10] which was a tetramer (four molecules bound together).

Aim 2. We designed and purified constructs of the HLD and its close relative, Hel308, in which we swapped the larger separation wedge of Hel308 with the equivalent, stunted wedge of the HLD. We then performed a molecular beacon-based helicase assay (see graphic below) to assess the strand separation activity of the HLD, Hel308, and the two chimeric proteins consisting of the HLD with the Hel308 wedge ("HLD(308)") and Hel308 with the HLD wedge ("308(HLD)"). We observed that 308(HLD) had reduced activity compared to Hel308. Amazingly, HLD(308) displayed activity whereas HLD had none. By swapping in the larger separation wedge from Hel308, we were able to add helicase activity to the HLD. This indicates that the evolutionarily stunted wedge of the HLD is the physical basis for its lack of helicase activity.



(A) Schematic diagram of molecular beacon-based helicase assay (MBHA). A DNA strand is labeled with a fluorophore at one end, a quencher at the other, and annealed to an unlabeled strand. When a helicase separates the two, the labeled strand will anneal to itself to bring the quencher close to the fluor and quench the fluorescence signal. (B) MBHA results. Fluorescence signal was measured over time with a fluorometer. HLD(308) and 308(HLD) denote HLD with the longer wedge of Hel308 and vice-versa, respectively.

References

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