Mutant PriA C-Tev ML346 and its Unwinding DNA Capabilities

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Binding and Unwinding Damaged DNA on a Longer Leash
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Abstract

In DNA replication mistakes can happen where the DNA sequences have been damaged. When that happens it causes the process to stall. There’s a protein called PriA that recognizes this issue, unwinds the duplex DNA at the fork junction and reloads the replisome which will restart the replication process. PriA has multiple structural domains that cooperate with one another to carry out its functions in binding DNA and unwinding it. These structural domains are a very compact unit when they’re all assembled together in the inact protein. The winged helix domain, however, seems to resist this trend. It’s connected by a long, flexible tether to the remaining compact structure. This experiment examined the significance of the winged helix domain’s long, flexible tether by lengthening the C-terminal tether. We hypothesized it would alter its DNA unwinding capability. Through a helicase assay it was observed that lengthening the C-terminal tether didn’t change its capability to unwind duplex DNA.

Goal

The objective of this project was to connect the role of the winged helix’s isolation with PriA’s function. We hypothesized that lengthening the C-terminal tether of the winged helix domain, thus increasing its mobility, in PriA will alter Kpn PriA activity. To test this hypothesis, I introduced the mutagenized PriA into the pET28b plasmid, confirmed the insertion, grew and purified the cells, and then performed a helicase assay to determine its capability of unwinding duplex DNA.

Results

• Construction of the mutant PriA C-Tev ML346 was successful
• Protein purification of mutated PriA showed 90% purity
• Helicase assay results, shown below in Figure 5, showed the PriA C-Tev ML346 unwound duplex DNA about the same as the PriA Wild-Type

Conclusion

Lengthening the C-terminal tether of the winged helix domain in the PriA of the ML346 did not have much effect on altering its Kpn PriA activity. When analyzing the structure of PriA it makes sense that it wouldn’t change PriA activity. The N-terminal tether is shorter than the C-terminal tether, as seen in Figure 4. So when the C-terminal tether is elongated it ultimately doesn’t change the winged helix’s position in relation to the rest of the protein. Since the N-terminal tether is keeping that domain the same distance to the remainder of the protein, PriA’s capability of unwinding DNA doesn’t change.

The winged helix domain is a DNA binding domain. It contributes to PriA being able to bind onto the stalled replication fork.