The Effect of the Inserted Sequence in the Helicase Domain of the *Deinococcus radiodurans* PriA Protein

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Abstract

PriA, a replication restart protein found in bacteria, is highly conserved in almost all bacteria. However, it contains extra amino acid sequences in the microbe *Deinococcus radiodurans.* Since *D. radiodurans* is extremely resistant to ionizing radiation, these insertions could play a role in conferring resistance by improving the microbe's ability to continue replication after DNA is damaged. The project investigated the effects of the fifty-six amino acid insertion in the helicase domain of the PriA protein in *D. radiodurans*. To do this, a version of the PriA gene lacking the inserted element was cloned. The recombinant and wild type PriA proteins were over-expressed in *E. coli* and purified. Helicase assays were performed to compare the functions of the forms of the protein. It was hypothesized that the inserted element would enhance the helicase activity of the protein. However, helicase assays showed that the mutant unwound DNA more efficiently. This means that the inserted element inhibits the helicase activity of PriA.

DNA Replication Restart Is Essential to an Organism's Survival

All cells need to duplicate their entire genome before they divide in order to ensure that their daughter cells receive a complete set of DNA. This replication is necessary for the survival of organisms and entire species. The process of DNA replication in bacteria is shown in Figure 1.

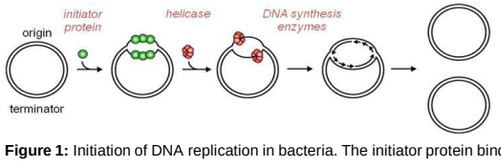

Figure 1: Initiation of DNA replication in bacteria. The initiator protein binds to the origin of replication and recruits the replicative helicase, which unwinds DNA so that DNA synthesis enzymes can replicate the chromosome.

When the DNA replication machinery encounters a break in DNA, DNA replication can stop. DNA breaks can be caused by a variety of factors including reactive oxygen species that are produced during normal metabolism, UV light, and chemicals like polycyclic aromatic hydrocarbons (1). Since this process is so critical to life, cells must be able to restart DNA replication when it is halted because of these breaks. When the replication machinery becomes dislodged from the strand of DNA, it must be reassembled before replication can continue. In bacteria, the DNA replication restart pathway often involves proteins like PriA, PriB, and DnaT. In *Escherichia coli* for example, PriA, PriB, and DnaT form a complex that recruits the replicative helicase DnaB to the fork, allowing the process to begin again (2). DnaB unwinds duplex DNA at the replication fork, which allows the rest of the replisome to access both strands of DNA. DnaB can only bind to single stranded DNA, so PriA unwinds the helix and separates the strands (3). This process is illustrated in Figure 2.

Figure 2: DNA replication restart. When the replication fork stops because of DNA damage, DNA synthesis enzymes must be recruited back to the fork after the DNA is repaired. This is achieved by primosome proteins PriA, PriB, and DnaT.

Deinococcus radiodurans Has an Unusual Ability to Repair DNA Damage

Deinococcus radiodurans is a spherical, non-motile, mesophilic microbe that does not form spores (4). Its most unusual property is an ability to withstand ionizing radiation. In fact, it is one of the most DNA damage-tolerant species currently known. It can survive 5000 Gy of radiation, while a dose of 5 Gy is fatal to a human being (5). This tolerance does not occur because the bacterium protects its DNA from damage. Gamma radiation does induce double-stranded breaks in the DNA of *D. radiodurans*, but the microbe has an extraordinary ability to repair this damage (6).

PriA Is Highly Conserved in Most Microbes, But Not *D. radiodurans*

PriA is a highly conserved protein, meaning that most prokaryotes utilize very similar forms of it. The amino acid sequences of most PriA proteins are of comparable lengths. This is illustrated in Figure 3.

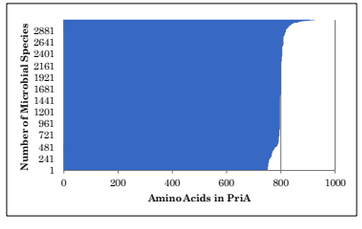

Figure 3. Graph showing the lengths of the PriA protein amino acid sequence in a number of different bacterial species. *D. radiodurans* PriA has 925 amino acids.

The bacterium *Deinococcus radiodurans*, however, possesses extra amino acids, as shown in Figure 4A. This gives the *D. radiodurans* PriA protein 925 amino acids, which Figure 3 shows is extremely unusual.

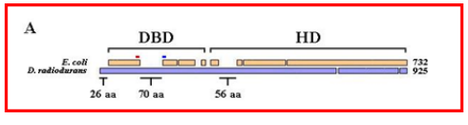

Figure 4: A comparison of the PriA amino acid sequences in *E. coli* and *D. radiodurans,* showing the extra sequences in the DNA-binding domain (DBD) and helicase domain (HD).

We hypothesize that the inserted sequence in the helicase domain of the *Deinococcus radiodurans* PriA protein enhances the helicase activity of the protein

Since *D. radiodurans* is so efficient at repairing breaks in its DNA, we reasoned that the activity of its DNA replication restart pathway may be enhanced. PriA is a protein in this pathway that has extra elements, so we hypothesized that the inserted sequence in its helicase domain increases the protein's ability to unwind DNA.

To test this hypothesis, we cloned a version of the PriA gene that lacked the helicase domain inserted element. This gene was then over-expressed in bacteria, and the resulting protein was purified. Helicase assays were performed on the wild-type and mutant PriA proteins to assess the effect of the inserted element in the protein's ability to unwind DNA.

Cloning PriAΔ365-420

Site-directed mutagenesis was used to introduce Sca1 sites on either side of the inserted element in the PriA gene. This allowed excision of the helicase domain inserted element because a digest with the enzyme Sca1 would cut the gene on either side of the inserted sequence. First, pET28b was used as the vector, but splice junction insertion was unsuccessful because of the large size of the plasmid. Therefore, the PriA gene was transferred to a pUC19 vector. Then polymerase chain reaction were successfully used to insert Sca1 splice junctions on both sides of the inserted element. The 5' splice junction was added first, followed by the 3' junction. The gel verifying the 3' splice junction addition is shown in Figure 5.

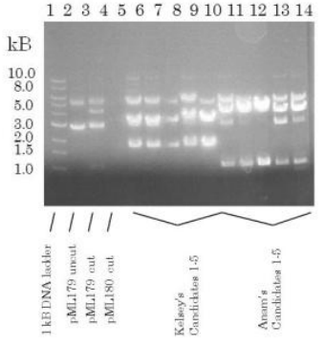

Figure 5: 0.8% agarose gel showing successful 3' junction insertion. Candidate 2, which was chosen for future use, is found in lane 6. After a Sca1 digest, bands were expected at 1,847 and 3,278 kB. Unsuccessful candidtates would have the higher band at 3,383 kB.

After both splice junctions were added, the insert was transferred back to the pET28b vector. This was necessary because the pUC19 vector cannot be used for protein production. Then a Sca1 digest was used to remove the inserted element in the helicase domain. The gel verifying the inserted sequence's removal is shown in Figure 6.

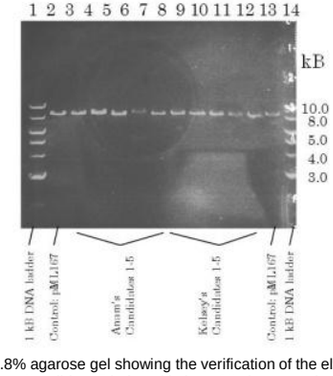

Figure 6: 0.8% agarose gel showing the verification of the element-deleted clone. Candidate 5 is found in lane 12, the second one over from the ladder on the right. It is running slightly faster than the control on its right because the inserted element has been removed.

Purification of Wild-Type PriA and PriAΔ365-420

Wild-type and mutant PriA proteins were overexpressed in codon plus cells. Then the wild-type protein was purified using affinity chromatography, and the mutant was purified using size exclusion chromatography.

Helicase Assays

Helicase assays were conducted on both the wild-type protein and PriA Δ365-420. The results from these assays are shown in Figure 8. The total unwinding by the wild-type protein reached 17.5% at 15 nM, while the unwinding by the mutant averaged 45.7% at the same concentration. This means that the helicase lacking the inserted element unwound almost three times as much DNA as the wild-type at the same concentration.

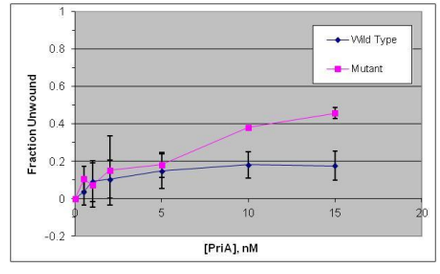

Figure 7: Graph showing the averages of the seven helicase assay trials on both wild-type and mutant PriA. The concentration of PriA is found on the x-axis, and the fraction of the unwinding is placed on the y-axis. Error bars represent one standard deviation from the mean.

Conclusions

PriAΔ365-420 unwound DNA more efficiently than the wild-type protein. Therefore, the inserted element in the helicase domain of the *Deinococcus radiodurans* PriA protein inhibits the helicase activity of the protein.

References

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