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

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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.

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.

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 Bacteria, 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.

Cloning PriAΔ365-420

Site-directed mutagenesis was used to introduce Scal 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 Scal 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 was successfully used to insert Scal cleavage 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.

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 PriAΔ365-420. 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.

Conclusions

PriAΔ365-420 unwind 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