Investigations of the DNA Repair Properties of Spore Photoproduct Lyase and Characterization of the Iron Sulfur Cluster

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The thymine dimer spore photoproduct (SP) accumulates in sporulating bacteria such as *Bacillus subtillus* when their DNA is exposed to UV light. This type of DNA damage can be repaired via a nucleotide excision repair pathway or by direct reversal. The enzyme that catalyzes the direct reversal pathway is spore photoproduct lyase. Unlike DNA photolyase, which utilizes light for direct reversal of DNA damage, spore photoproduct lyase is light independent. SP lyase instead utilizes S-adenosylmethionine (SAM) and an iron sulfur cluster to initiate radical mediated DNA repair, and as such is a member of the radical-SAM superfamily.

This study focuses on the repair of the spore photoproduct by SP lyase, the binding properties of the SP lyase to both DNA and SAM, and the characterization of the Fe-S cluster of SP lyase using spectroscopic techniques. Gel shift assays with DNA oligomers show that SP lyase is capable of binding DNA in the presence and absence of the iron-sulfur cluster. Equilibrium dialysis experiments show SPL binding with SAM and allow for the calculation of dissociation constants. Repair assays of the spore photoproduct indicate no repair by apo-SP Lyase, which lacks the Fe-S cluster. Assays carried out in the absence of the DNA and spore photoproduct show no SAM cleavage by the enzyme. EPR experiments indicate the presence of a $[3Fe\text{-}4S]^{1+}$ in purified SP lyase which can be converted to $[4Fe\text{-}4S]^{1+}$ under reducing conditions.