DNA-Bound Redox Activity of DNA Repair Glycosylases Containing [4Fe-4S] Clusters†

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Abstract:

MutY and endonuclease III, two DNA glycosylases from *Escherichia coli*, and AfUDG, a uracil DNA glycosylase from *Archeoglobus fulgidus*, are all base excision repair enzymes that contain the $[4Fe\text{-}4S]^{2+}$ cofactor. Here we demonstrate that, when bound to DNA, these repair enzymes become redox-active; binding to DNA shifts the redox potential of the $[4Fe\text{-}4S]^{3+/2+}$ couple to the range characteristic of high-potential iron proteins and activates the proteins toward oxidation. Electrochemistry on DNA-modified electrodes reveals potentials for Endo III and AfUDG of 58 and 95 mV versus NHE, respectively, comparable to 90 mV for MutY bound to DNA. In the absence of DNA modification of the electrode, no redox activity can be detected, and on electrodes modified with DNA containing an abasic site, the redox signals are dramatically attenuated; these observations show that the DNA base pair stack mediates electron transfer to the protein, and the potentials determined are for the *DNA-bound* protein. In EPR experiments at 10 K, redox activation upon DNA binding is also evident to yield the oxidized $[4Fe\text{-}4S]^{3+}$ cluster and the partially degraded $[3Fe\text{-}4S]^{1+}$ cluster. EPR signals at $g = 2.02$ and 1.99 for MutY and $g = 2.03$ and 2.01 for Endo III are seen upon oxidation of these proteins by $Co(phen)_3^{3+}$ in the presence of DNA and are characteristic of $[3Fe\text{-}4S]^{1+}$ clusters, while oxidation of AfUDG bound to DNA yields EPR signals at $g = 2.13$, 2.04, and 2.02, indicative of both $[4Fe\text{-}4S]^{3+}$ and $[3Fe\text{-}4S]^{1+}$ clusters. On the basis of this DNA-dependent redox activity, we propose a model for the rapid detection of DNA lesions using DNA-mediated electron transfer among these repair enzymes; redox activation upon DNA binding and charge transfer through well-matched DNA to an alternate bound repair protein can lead to the rapid redistribution of proteins onto genome sites in the vicinity of DNA lesions. This redox activation furthermore establishes a functional role for the ubiquitous [4Fe-4S] clusters in DNA repair enzymes that involves redox chemistry and provides a means to consider DNA-mediated signaling within the cell.