Investigations of DNA-Mediated Protein Oxidation

Thesis by

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Abstract

DNA charge transport (CT) involves the efficient transfer of electrons or electron holes through the DNA π-stack over long molecular distances of at least 100 base-pairs. Despite this shallow distance dependence, DNA CT is sensitive to mismatches or lesions that disrupt π-stacking and is critically dependent on proper electronic coupling of the donor and acceptor moieties into the base stack. Favorable DNA CT is very rapid, occurring on the picosecond timescale. Because of this speed, electron holes equilibrate along the DNA π-stack, forming a characteristic pattern of DNA damage at low oxidation potential guanine multiplets. Furthermore, DNA CT may be used in a biological context. DNA processing enzymes with 4Fe4S clusters can perform DNA-mediated electron transfer (ET) self-exchange reactions with other 4Fe4S cluster proteins, even if the proteins are quite dissimilar, as long as the DNA-bound $[4Fe4S]^{3+/2+}$ redox potentials are conserved. This mechanism would allow low copy number DNA repair proteins to find their lesions efficiently within the cell. DNA CT may also be used biologically for the long-range, selective activation of redox-active transcription factors. Within this work, we pursue other proteins that may utilize DNA CT within the cell and further elucidate aspects of the DNA-mediated ET self-exchange reaction of 4Fe4S cluster proteins.

Dps proteins, bacterial mini-ferritins that protect DNA from oxidative stress, are implicated in the survival and virulence of pathogenic bacteria. One aspect of their

protection involves ferroxidase activity, whereby ferrous iron is bound and oxidized selectively by hydrogen peroxide, thereby preventing formation of damaging hydroxyl radicals via Fenton chemistry. Understanding the specific mechanism by which Dps proteins protect the bacterial genome could inform the development of new antibiotics. We investigate whether DNA-binding *E. coli* Dps can utilize DNA CT to protect the genome from a distance. An intercalating ruthenium photooxidant was employed to generate oxidative DNA damage via the flash-quench technique, which localizes to a low potential guanine triplet. We find that Dps loaded with ferrous iron, in contrast to Apo-Dps and ferric iron-loaded Dps which lack available reducing equivalents, significantly attenuates the yield of oxidative DNA damage at the guanine triplet. These data demonstrate that ferrous iron-loaded Dps is selectively oxidized to fill guanine radical holes, thereby restoring the integrity of the DNA. Luminescence studies indicate no direct interaction between the ruthenium photooxidant and Dps, supporting the DNA-mediated oxidation of ferrous iron-loaded Dps. Thus DNA CT may be a mechanism by which Dps efficiently protects the genome of pathogenic bacteria from a distance.

Further work focused on spectroscopic characterization of the DNA-mediated oxidation of ferrous iron-loaded Dps. X-band EPR was used to monitor the oxidation of DNA-bound Dps after DNA photooxidation via the flash-quench technique. Upon irradiation with poly(dGdC)$_2$, a signal arises with g = 4.3, consistent with the formation of mononuclear high-spin Fe(III) sites of low symmetry, the expected oxidation product of Dps with one iron bound at each ferroxidase site. When poly(dGdC)$_2$ is substituted with

poly(dAdT)$_2$, the yield of Dps oxidation is decreased significantly, indicating that guanine radicals facilitate Dps oxidation. The more favorable oxidation of Dps by guanine radicals supports the feasibility of a long-distance protection mechanism via DNA CT where Dps is oxidized to fill guanine radical holes in the bacterial genome produced by reactive oxygen species.

We have also explored possible electron transfer intermediates in the DNA-mediated oxidation of ferrous iron-loaded Dps. Dps proteins contain a conserved tryptophan residue in close proximity to the ferroxidase site (W52 in *E. coli* Dps). In comparison to WT Dps, in EPR studies of the oxidation of ferrous iron-loaded Dps following DNA photooxidation, W52Y and W52A mutants were deficient in forming the characteristic EPR signal at g = 4.3, with a larger deficiency for W52A compared to W52Y. In addition to EPR, we also probed the role of W52 Dps in cells using a hydrogen peroxide survival assay. Cells containing W52Y Dps survived the hydrogen peroxide challenge more similarly to those containing WT Dps, whereas cells with W52A Dps died off as quickly as cells without Dps. Overall, these results suggest the possibility of W52 as a CT hopping intermediate.

DNA-modified electrodes have become an essential tool for the study of the redox chemistry of DNA processing enzymes with 4Fe4S clusters. In many cases, it is necessary to investigate different complex samples and substrates in parallel in order to elucidate this chemistry. Therefore, we optimized and characterized a multiplexed electrochemical platform with the 4Fe4S cluster base excision repair glycosylase Endonuclease III (EndoIII). Closely packed DNA films, where the protein has limited surface accessibility, produce

EndoIII electrochemical signals sensitive to an intervening mismatch, indicating a DNA-mediated process. Multiplexed analysis allowed more robust characterization of the CT-deficient Y82A EndoIII mutant, as well as comparison of a new family of mutations altering the electrostatics surrounding the [4Fe-4S] cluster in an effort to shift the reduction potential of the cluster. While little change in the DNA-bound midpoint potential was found for this family of mutants, likely indicating the dominant effect of DNA-binding on establishing the protein redox potential, significant variations in the efficiency of DNA-mediated electron transfer were apparent. On the basis of the stability of these proteins, examined by circular dichroism, we proposed that the electron transfer pathway in EndoIII can be perturbed not only by the removal of aromatic residues but also through changes in solvation near the cluster.

While the 4Fe4S cluster of EndoIII is relatively insensitive to oxidation and reduction in solution, we have found that upon DNA binding, the reduction potential of the $[4Fe4S]^{3+/2+}$ couple shifts negatively by approximately 200 mV, bringing this couple into a physiologically relevant range. Demonstrated using electrochemistry experiments in the presence and absence of DNA, these studies do not provide direct molecular evidence for the species being observed. Sulfur K-edge X-ray absorbance spectroscopy (XAS) can be used to probe directly the covalency of iron-sulfur clusters, which is correlated to their reduction potential. We have shown that the Fe-S covalency of the 4Fe4S cluster of EndoIII increases upon DNA binding, stabilizing the oxidized $[4Fe4S]^{3+}$ cluster, consistent with a negative shift in reduction potential. The 7% increase in Fe-S covalency corresponds to an

approximately 150 mV shift, remarkably similar to DNA electrochemistry results. Therefore we have obtained direct molecular evidence for the shift in 4Fe4S reduction potential of EndoIII upon DNA binding, supporting the feasibility of our model whereby these proteins can utilize DNA CT to cooperate in order to efficiently find DNA lesions inside cells.

In conclusion, in this work we have explored the biological applications of DNA CT. We discovered that the DNA-binding bacterial ferritin Dps can protect the bacterial genome from a distance via DNA CT, perhaps contributing to pathogen survival and virulence. Furthermore, we optimized a multiplexed electrochemical platform for the study of the redox chemistry of DNA-bound 4Fe4S cluster proteins. Finally, we have used sulfur K-edge XAS to obtain direct molecular evidence for the negative shift in 4Fe4S cluster reduction potential of EndoIII upon DNA binding. These studies contribute to the understanding of DNA-mediated protein oxidation within cells.

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