DNA-mediated Charge Transport in DNA Repair

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ABSTRACT

The double-helical structure of deoxyribonucleic acid (DNA) imparts upon this important biological molecule both the ability to store genetic information within a cell and also the capacity to serve as medium for charge transport. DNA-mediated charge transport is now a very well-studied phenomenon but biological roles for these reactions have not been explored. It has been demonstrated that DNA-mediated charge transport can funnel oxidative DNA damage to sites of low oxidation potential in a number of biologically relevant environments ranging from reconstituted nucleosome core particles, to isolated nuclei and mitochondria from HeLa cells. DNA-mediated charge transport may also play a role in transcriptional activation or repression as modulated by redox-active transcription factors. Here we examine how DNA-mediated charge migration could also provide a pathway for protein-protein communication among DNA repair enzymes, a pathway that might serve as a scheme for rapid lesion detection inside the cell.

DNA-mediated charge transport reactions are modulated by the structure and dynamics of the double helix. Particularly important for fast and efficient charge transport is the integrity of the base-pair π-stack of DNA. The presence of even a single mismatched base-pair causes a dramatic attenuation in the effectiveness of DNA-mediated charge transport. To examine the scope of base-pair structure perturbations that can hinder DNA charge transport, we have investigated a series of duplexes, each containing a single altered base, at DNA-modified electrodes. The efficiency of DNA charge transport in these systems is evaluated electrochemically by monitoring the reduction of an intercalative probe. These experiments reveal that a wide variety of damaged bases can

diminish charge migration through DNA, including those that result from oxidative damage events (8-oxo-guanine, 5-hydroxy-cytosine) and those associated with aberrant alkylation (O4-methyl-thymine, O6-methyl-guanine).

The remarkable sensitivity of charge transport reactions in DNA to a broad range of damaged bases inspired investigation of the role of DNA-mediated charge transport in DNA repair. A class of base excision repair glycosylases exists that contain [4Fe4S] clusters and the function of this cofactor in these enzymes is not well understood, though these clusters are often found in proteins involved in electron transfer reactions. We have used DNA-modified gold electrodes to investigate the properties of the [4Fe4S] cluster in these enzymes and discovered that MutY and Endonuclease III (EndoIII) are redox-active when bound to DNA with midpoint potentials in the 50–100 mV *versus* NHE range, typical of $[4Fe4S]^{2+/3+}$ processes. This redox activity furthermore requires a DNA-mediated path to the [4Fe4S] cluster. Studies of EndoIII on graphite electrodes show that the DNA-bound redox properties of the enzyme are similar to those observed on gold, while in the absence of DNA, the potential for the $[4Fe4S]^{2+/3+}$ couple is shifted positive by ~ 280 mV. This potential shift may indicate a differential binding affinity for DNA by the oxidized and reduced forms of EndoIII; the oxidized form could bind DNA as much as 3 orders of magnitude more tightly than the reduced form of the enzyme. The DNA-mediated redox activity observed in these proteins has prompted us to propose a model for how these proteins might use DNA charge transport as a fast and efficient damage detection method. In this model, a protein binds DNA and becomes oxidized. If the surrounding DNA is undamaged, DNA charge transport will allow another repair protein to reduce the first protein from a distance *via* the DNA base-pair stack. This reduced protein has diminished affinity for DNA and diffuses away; the charge transport

reaction has served as a scan of the intervening genomic region. If, instead, lesion sites are present in the vicinity of the initial protein, this protein is more likely to remain oxidized and tightly bound in the damaged area. Thus, this detection scheme would allow [4Fe4S] DNA repair enzymes to rapidly eliminate undamaged regions of the genome from their search while spending more time bound near lesions.

The DNA-bound redox activity of the [4Fe4S] cluster harbored by MutY has also been examined in solution. DNA-mediated oxidation of the [4Fe4S] cluster *via* a guanine radical intermediate leads to formation of the $[4Fe4S]^{3+}$ cluster as observed by electron paramagnetic resonance (EPR) spectroscopy and transient absorption spectroscopy. Furthermore, gel electrophoresis experiments indicate that MutY can quench guanine radicals, preventing formation of permanent oxidative guanine lesions. EPR experiments also demonstrate that degraded cluster products (e.g., $[3Fe4S]^{1+}$) are formed both by DNA-mediated oxidation and by oxidants in solution. In the latter case, signal intensities are increased in the presence of DNA. These results support the idea that the DNA-bound form of MutY is more easily oxidized than MutY free in solution. The fact that guanine radicals can oxidize MutY may be biologically relevant, as well. Guanine radicals are one of the first products of oxidative DNA damage, thus oxidation of MutY by a guanine radical could serve to not only directly repair this lesion, but also to activate a DNA-mediated charge transport search for damage in the genome in regions undergoing oxidative stress.

DNA-mediated charge transport may also be employed in a cooperative fashion among different [4Fe4S] cluster DNA repair enzymes, allowing them to help each other eliminate undamaged portions of the genome from their search. To explore this

possibility we have calculated that cooperative DNA CT makes possible for MutY, an extremely low copy number enzyme, a full scan of the *Escherichia coli* genome within the doubling time of the cell (~ 20 minutes). The genome scanning time also depends on the proportion of protein initially in the oxidized state, thus allowing the DNA repair response to adjust according to the conditions present in the cell. A simple processive scanning model for lesion detection by MutY is insufficient. This cooperativity between MutY and EndoIII was also tested experimentally in *E. coli.* Inactivation of EndoIII (*nth-*) in a MutY activity reporter strain yields a twofold increase in the mutation rate, indicating a loss of MutY activity in the absence of EndoIII. This loss of activity, or helper function, cannot be attributed to an overlapping substrate specificity. However, investigation of an EndoIII mutant (Y82A) that retains this defect in helper function also exhibits a 50% loss in signal intensity (compared to wt EndoIII) when examined on a DNA-modified electrode. Thus, helper function by EndoIII could involve DNA-mediated redox activity of the [4Fe4S] cluster in EndoIII. This work demonstrates a connection between *in vivo* cooperativity among DNA repair enzymes and DNA-mediated charge transport as well as a biological role for this chemistry in DNA repair.

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