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ATP–stimulated DNA–mediated Redox Signaling by XPD, a DNA Repair and Transcription Helicase

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Abstract

Using DNA-modified electrodes, we show DNA-mediated signaling by XPD, a helicase that contains a [4Fe-4S] cluster and is critical for nucleotide excision repair and transcription. The DNA-mediated redox signal resembles that of base excision repair proteins, with a DNA-bound redox potential of ~80 mV versus NHE. Significantly, this signal increases with ATP hydrolysis. Moreover, the redox signal is substrate-dependent, reports on the DNA conformational changes associated with enzymatic function, and may reflect a general biological role for DNA charge transport.

To protect the genome, a variety of proteins with various functions must act in concert.[1–3] One such protein, XPD is a super family 2 helicase critical to nucleotide excision repair (NER) and important to transcription.[4–6] Helicases are responsible for unwinding DNA in an ATP-dependent fashion in order to access individual bases to allow the other proteins to repair DNA damage and to both replicate and transcribe DNA. In humans, XPD is part of the TFIIH machinery, with single site mutations leading to human diseases with increased cancer risk or premature aging: Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS), Trichothiodystrophy (TTD) or combinations thereof.[4,5,7] Recent chemical analyses and crystal structures of archaeal XPD homologues, which have ~22% sequence identity with the human homologue, reveal the presence of a [4Fe-4S] cluster.[8–10] Furthermore, combined structural, biochemical and mutational analyses show that the catalytic core of XPD is conserved from archaea to humans and has functional relevance for understanding human disease.[5] Mutational analyses of [4Fe-4S] coordinating cysteines have established the importance of the [4Fe-4S] cluster in DNA unwinding activity,[10–12] yet a role for XPD as a redox-active protein remains to be established.

DNA charge transport (CT), where electrons are transferred between proteins bound to DNA in a path through the DNA bases, has been proposed as a first step in localizing a family of base excision repair (BER) proteins containing [4Fe-4S] clusters in the vicinity of damage.[13] DNA CT chemistry facilitates electron transfer over long molecular distances through the

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Supporting Information. Experimental procedures and supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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DNA duplex[14,15] but is remarkably sensitive to perturbations in base pair stacking, as, for example, arise with damage.[16,17]

DNA-mediated CT signaling by proteins was first explored in studies of a class of *E. coli* BER glycosylases that contain [4Fe-4S] clusters.[18,19] Electrochemistry on DNA-modified electrodes showed that DNA binding shifts the cluster potential to ~80 mV, well within the physiological range for redox signaling. Biophysical experiments were then used to examine the redistribution of BER enzymes in the vicinity of damage, and genetic experiments, to explore cooperative signaling between two BER enzymes, endonuclease (Endo) III and MutY.[20,21] These results coupled with DNA electrochemistry linked the ability of Endo III (i) to relocalize near a DNA mismatch, (ii) to cooperate in helping MutY repair lesions in vivo, and (iii) to carry out DNA CT. If CT is generally important for DNA repair, we hypothesized it should be detected in repair pathways besides BER, including NER, which is the major pathway for chemically modified bases that disrupt the DNA double helix.

To test if DNA CT might occur with XPD, we first determined the DNA-bound redox potential of an archael XPD on DNA-modified gold electrodes (Scheme 1, see Supporting Information for Methods). We find that XPD from the thermophile *Sulfolobus acidocaldarius* (SaXPD) has a DNA-bound redox potential of ~82 ± 10 mV versus NHE (Figure 1). This potential, like those found in BER proteins, reflects physiological redox activity and is not sufficient to damage DNA.[18] In the absence of DNA, the potential of the cluster is expected to be significantly more positive and outside of the window of physiological redox activity.[19] To confirm that the measured potential is DNA-mediated and hence reflects the DNA-bound potential, rather than that of the protein directly interacting with the surface without DNA, we compared the signal to that found on a surface with the DNA containing a mismatch close to the surface; with this intervening mismatch, the potential is unchanged yet the redox signal is significantly attenuated, consistent with the protein electrochemistry signal being DNA-mediated (Figure 1).[16] We also observe that the signal intensity exhibits a linear dependence on the square root of the scan rate, which implies that the protein is binding to DNA in a diffusion-limited process.[22] In addition, we observe an electron transfer rate of approximately 1.4 s^{-1} based on Laviron analysis, which is similar to previously published rates that indicate that the rate of electron transfer is limited by tunneling through the carbon linker.[23] Together these data establish that this DNA-mediated signal corresponds to the one-electron redox couple of the [4Fe-4S] cluster of SaXPD bound to DNA and that this redox couple can be physiologically active.

Unlike BER glycosylases, the principal activity of the XPD helicase is ATP-dependent, and as such, we could also investigate the effect of ATP on the DNA-bound signal. After the protein was allowed to equilibrate on the DNA-modified surface, various concentrations were added of ATP or ATP-γ-S, a markedly more slowly hydrolysable ATP analogue (Figure 2A).[24] Interestingly, as ATP was titrated onto the surface, a noticeable ATP-dependent increase in the current was observed. No shift in potential was evident, indicating that the cluster is neither degrading nor markedly changing in its environment; instead DNA coupling appears to increase. In contrast, the slowly hydrolysable analogue shows little effect on the electrochemical signal of the protein. Thus, the electrochemical signal reports on the ATPase activity of XPD. This sensitivity in signal to ATP hydrolysis is remarkable given that, based on the crystal structure of the protein without DNA bound, the distance between the cluster and the ATP binding site is 30 Å.[10] The XPD structure revealed that the [4Fe-4S] cluster domain is tightly linked to the ATP binding site by β-sheets that could provide a mechanism for mechanical coupling of the motions of the cluster domain to those in the ATP site as a result of hydrolysis. This increase in electrochemical signal must be reporting on motions at the protein/DNA interface as the protein carries out ATP hydrolysis.

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We next examined the effect of DNA substrate (Figure 2). XPD has shown a preference as a 5′-3′ helicase;[25] modeled on a surface, the 5′-3′ helicase would be expected, therefore, to move downward towards the surface. Since the protein concentration is well above the dissociation constant (66 nM),[10] the protein should bind DNAs with either 3′- or 5′-overhangs, as well as duplex DNA. Indeed, we see differences in the DNA electrochemistry based on substrate. When protein is placed on the surface modified with DNA with a 3′ ssDNA overhang, with each addition of ATP, the signal increases temporarily, likely reflecting increased coupling associated with reaction, but then, with each additional ATP jump, decreases with time. This decay may result from the protein sliding off the small segment of DNA bound to the surface. Notably, the absolute signal could not be compared among the surfaces because of the variability in surface coverage using different substrates. On the fully duplexed surface, which has no directionality bias, we see after the ATP addition, the signal is mainly flat, as expected for binding by XPD in both orientations.

XPD mutations in humans are associated with several often fatal diseases: XP, CS and TTD.[4,5,7] One such mutant, G34R, shows attenuated ATPase and helicase activity relative to wild type (WT) protein in biochemical assays (Table 10).[10] Interestingly, this SaXPD mutant exhibits a redox signal comparable to that of WT in the absence of ATP (Table 1). However, the rate of electronic signal increase with ATP for the G34R mutant is significantly lower compared to WT, further demonstrating the sensitivity of our assay to ATP hydrolysis (Figure 3). While there is certainly not a simple linear relationship between activity measured electrically and biochemically, the electronic signal appears to be a sensitive reporter of changes in protein/DNA coupling that result from ATP hydrolysis. This assay complements well a fluorescence helicase assay seen earlier, but without the need for DNA labeling.[26]

XPD, a critical helicase for NER, thus contains a redox-active [4Fe-4S] cluster that is sensed electronically as a reporter of activity. The DNA-bound redox potential is similar to previously reported BER proteins. Here, DNA electrochemistry is seen to provide a sensitive means for detecting ATP-dependent signaling that may be generally useful in screening the activity of DNA-binding proteins containing redox centers. The activity can be distinguished between the WT protein and ATP-deficient activity of the G34R mutant as well as between the native and non-native DNA substrates. Additionally, these results prompt the question as to how this electronic signaling of XPD activity might be utilized *in vivo*, and further, to which proteins XPD may be signaling inside the cell. Various repair and replication proteins, including FancJ and Dna2, which act in maintaining genomic stability, also have an associated [4Fe-4S] cluster.[27,28] We suggest that it will be important to test other proteins acting at the interface of repair with major DNA processes of replication and transcription for their CT ability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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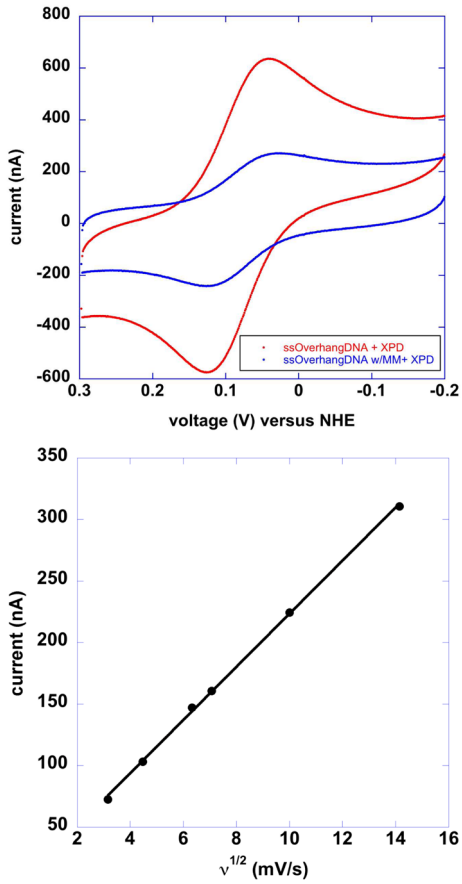

Figure 1.

Electrochemistry of DNA-bound WT SaXPD. (Top) Cyclic voltammogram (CV) of SaXPD [120 μM] on a well-matched DNA-modified electrode (red) and on DNA with a CA mismatch located near the gold surface as in Scheme 1 (blue). (Ag/AgCl reference electrodes; Pt auxiliary electrode, 50 mV/s scan rate, NHE = normal hydrogen electrode). (Bottom) Plot of current versus $\nu^{1/2}$ (square root of scan rate). The data indicate that our signal is obtained through a diffusion-limited process.

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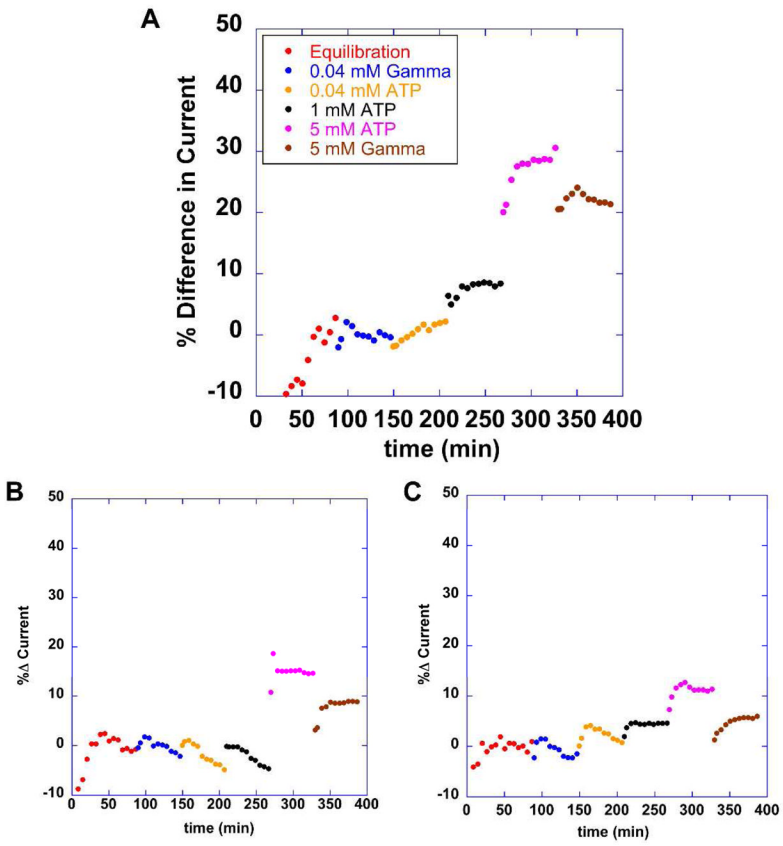

Figure 2.

ATP-dependent Electrochemistry of SaXPD on different DNA substrates. Time points shown are every 6 minutes. (Ag/AgCl reference electrode; Pt auxiliary electrode, 50 mV/s scan rate). % Difference in Current for SaXPD [9 μM] on a 5′-ssDNA overhang **(A)**, 3′-ssDNA overhang **(B)**, fully duplexed DNA **(C)**. The signal is seen to be ATP-dependent and sensitive to substrate. Note at the high concentration addition [5 mM ATP-γ-S], some ATP still remains; once depleted, the signal levels off.

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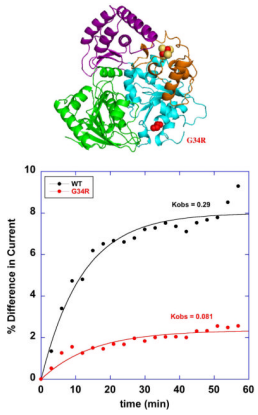
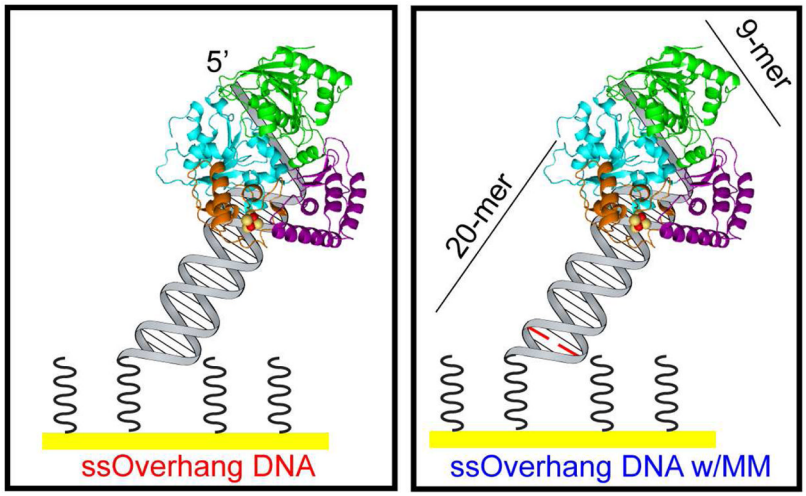


Figure 3.

Electrochemical comparisons of SaXPD mutants after 5 mM ATP addition. (Top) Crystal structure of SaXPD with residues that were mutated shown in space filling model. Also shown in space filling models is the [4Fe-4S] cluster (Bottom) ATP-dependent CT of WT (black) and G34R (red). Observed initial rate constants (first ten minutes) of 0.29 and 0.08 min^{-1} for WT and G34R.



Scheme 1.
Cartoon Schematic of well-matched DNA with a 9 nucleotide single-strand overhang (left) and DNA with a CA mismatch shown in red (MM) (right).

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Table 1

Redox and Biochemical activity of WT and G34R XPD.

Mutant	Redox Signal Intensity	Helicase[a] (bp/min)	ATPase[a] (mol/sec)	ssDNA[a] Kd (nM)
WT	1	20.5 ± 0.7	1.24 ± 0.03	66 ± 5
G34R	1.2 ± 0.09	0.8 ± 0.1	0.0 ± 0.01	67 ± 3

[a] – see Supporting Information for Methods