CHAPTER 9

# DNA-Mediated Charge Transport as a Probe of MutY-DNA Interaction

## Electrochemical and transient absorption studies

Eric Stemp (Chemistry Department, Mount St. Mary's College) assisted in transient absorption experiments. Various members of the David laboratory at the University of Utah prepared the MutY used in these studies. These experiments are ongoing in our laboratory.

## INTRODUCTION

DNA-mediated charge transport been the subject of intense debate for years. Experiments from many different laboratories have now confirmed that DNA-mediated charge transport can proceed over long molecular distances and that the reaction is sensitive to sequence as well as sequence dependent DNA structure (1). Recently researchers have begun to look for a physiological role for this chemistry. DNA-mediated charge transport has been demonstrated to proceed within *Hela* cell nuclei when incubated with a rhodium photooxidant (2). Similar long range charge transport chemistry to generate oxidative damage has been demonstrated on restriction fragments as well as in nucleosome core particles (3). These studies have suggested that the physiological range for charge migration may be on the order of 100 Å. Additionally, DNA binding proteins have been demonstrated to modulate long range charge transport chemistry (4-7). Electrochemistry experiments to sense mismatches and lesions in DNA also suggest that this chemistry might be valuable as a sensing device within the cell (Chapters 2,3,7,8, refs. 8-14). Within the cell, DNA is nearly always associated with proteins and thus one interesting opportunity for physiological DNA charge transport is between proteins and DNA. Particularly intriguing for investigation of charge transport between DNA and proteins are DNA binding proteins that contain redox active cofactors such as FeS clusters.

Iron sulfur clusters are known to exhibit a range of functions in biological systems (15). For example, aconitase (16), an enzyme involved in

the citric acid cycle, is an FeS protein where the cluster mediates substrate binding. MutY and endonuclease III are two proteins that repair DNA damage in *E. coli* (17,18). Although they repair very different lesions, they exhibit remarkable sequence homology (19) and they both contain $[4Fe4S]^{2+}$ clusters, the functions of which are not known. In fact, there are now many examples of FeS cluster containing DNA binding proteins, including MutY (17), endonuclease III (18), SoxR (20), FNR (21), spore photoproduct lyase (22), and a uracil DNA glycosylase (23), among others. Ferredoxins constitute the largest class of FeS proteins, however. These $[4Fe4S]^{2+/+}$ clusters participate in electron transfer reactions as a part of their in vivo function (24). Thus, as most FeS proteins participate in electron transfer reactions, and DNA is an efficient medium for the transport of charge with sensitivity to stacking perturbations such as mismatch and base lesions (1), it is very intriguing that a prosthetic group usually associated with charge transport processes would be incorporated and conserved in proteins responsible for DNA repair.

Endonuclease III (Endo III) from *E. coli* is a DNA repair enzyme that removes pyrimidines damaged by ring saturation, contraction, or fragmentation (25). As purified, Endo III contains 3-4 iron and sulfide atoms per monomer (26). The FeS cluster of this protein has been studied using spectroscopic methods (26,27). Oxidation of this enzyme with ferricyanide produces a species that exhibits an EPR signal near $g_{av} = 2.01$, characteristic of $[3Fe4S]^{+}$ clusters. The protein has a Mössbauer spectrum characteristic of $[4Fe4S]^{2+}$ clusters (single symmetrical quadrupole doublet; isomer shift = 0.44 mm/s; quadrupole splitting value = 1.18 mm/s). These data demonstrate that the native enzyme contains a $[4Fe4S]^{2+}$ cluster with all of the iron atoms

in a similar environment. The fact that the cluster loses an iron atom upon oxidation suggests the $[4Fe4S]^{3+}$ oxidation state is not physiologically relevant (26). The $[4Fe4S]^{2+}$ cluster in Endo III is resistant to reduction with sodium dithionite (at pH 10) and deazaflavin-mediated photochemical reduction suggests a midpoint potential of <-600 mV for the $[4Fe4S]^{2+,+}$ couple, suggesting this is also an unlikely in vivo redox couple (27). Direct electrochemical measurement of the FeS cluster in Endo III has not been reported.

The x-ray crystal structure of endonuclease III shows that the four iron atoms of the cluster are ligated by cysteine residues with unusual spacing (Cys-$X_6$-Cys-$X_2$-Cys-$X_5$-Cys), making this a distinct FeS cluster from other FeS proteins of known structure. This cluster is located in a loop at the C-terminal end of this protein, referred to as the FeS Cluster Loop (FCL) (28). The protein provides a sulfur ligand to each iron atom in the FeS cluster. No changes in the resonance Raman or Mössbauer spectra are observed upon substrate or inhibitor binding to endonuclease III, suggesting that substrate does not interact with the FeS cluster (26,27)

On the basis of the analysis of the crystal structure of the enzyme, Kuo et al. have proposed that the FCL is involved in aligning conserved positively charged residues for interaction with the DNA (28). This proposal is consistent with mutational studies in which changing one of the positively charged residues in this loop was to a negatively charged residue results in a mutant enzyme with a >100 fold $K_m$ and an essentially unchanged $k_{cat}$ (29). Furthermore, based on sequence alignment arguments, Thayer and coworkers have proposed that the FCL might be a common structural

element of DNA binding proteins (29). The motif Cys-$X_6$-Cys-$X_2$-Cys-$X_5$-Cys is conserved in a number of DNA binding proteins including the MutY protein of *E. coli* (30,31), a protein from *Salmonella typhimurium* homologous to MutY (32), a putative thymine DNA glycosylase that has been identified in the archaebacterium *Methanobacterium thermoformicicum* (33), the ultraviolet endonuclease from *Micrococcus luteus* (34), and a homolog of endonuclease III purified from calf thymus (35). Moreover, analysis of translated amino acid sequences suggests that *Caenorhabditis elegans*, *Homo sapiens*, and *Rattus* sp. also have endonuclease III-like proteins that contain this novel FCL DNA binding motif (36). It appears, therefore, that DNA binding proteins that also contain FeS clusters close to the DNA binding interface are present throughout phylogeny.

In contrast to Endo III, the base excision repair (BER) enzyme MutY has only been characterized with respect to its biological function (17,19,32,36-44). It is a 350 residue, 36 kDa protein that acts as a glycosylase to remove adenine from G:A and 7,8-dihydro-8-oxo-2-deoxyguanonsine (8-oxo-G):A mismatches. MutY thus prevents mutations resulting from oxidative damage by removing misincorporated adenine residues opposite 8-oxo-G. MutY is homologous to endonuclease III, despite drastically different substrate recognition features (19) and also contains an FeS cluster bound in a Cys-$X_6$-Cys-$X_2$-Cys-$X_5$-Cys loop near the C-terminus that is thought to make ionic contacts with the DNA backbone to aid in binding (28). Interestingly, MutY possesses an additional C-terminal domain that extends beyond the FCL, which is not present in Endo III. This extra domain has been implicated in recognition of 8-oxo-G, although nothing is known about the residues

involved or the mechanism of recognition. The crystal structure of a truncated version of MutY has been solved (without the extra C-terminal domain), confirming that MutY has a fold similar to Endo III and binds an $[4Fe4S]^{2+}$ cluster in an FCL motif loop (17). No crystallographic data is available for either Endo III or MutY in complex with DNA, however.

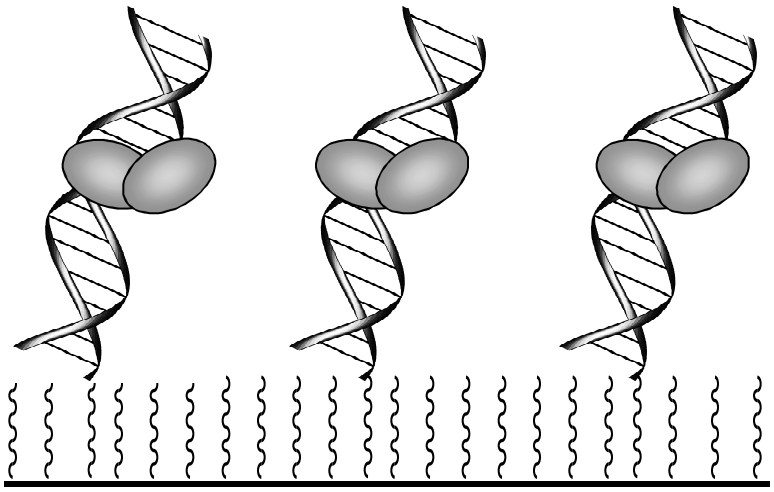
Even though there is now a great deal of information about the structure and function of Endo III and MutY, intriguing questions remain. Specifically, the role of the $[4Fe4S]^{2+}$ remains unsolved. It has been proposed that the FCL is simply a structural element used in binding DNA (28,29). Interestingly, however, MutY is capable of folding independent of cluster assembly (the cluster can be incorporated by self-assembly before or after folding) and the FeS cluster does not seem to aid in overall stabilization of the enzyme, but it is critical for enzyme turnover and substrate binding (43). These data argue against a primarily structural role for the FeS in MutY, but more evidence is needed before the role of this unusual FeS is clarified.

Because both of these enzymes contain an FeS cluster and share remarkable amino acid sequence and protein folding homology, but functionally only share the ability to recognize and repair DNA lesions, perhaps the cluster is involved in this aspect of their function. Perhaps these enzymes utilize FeS clusters for DNA-mediated charge transport, taking advantage of the sensitivity of this chemistry to stacking perturbations, to facilitate location of their binding sites. Interestingly, if the FCL is involved in substrate recognition, the active site and substrate recognition domains of MutY and Endo III are considerably removed from one another.

The previously described spectroscopic characterization of the FeS clusters in these proteins has generally been performed without bound DNA. If the FeS cluster is redox active only in substrate recognition processes, these previous studies may not have been sufficient to detect this activity. Especially as the cluster in each of these enzymes is bound in this FCL loop that is thought to participate in DNA binding, it is entirely possible that the environment around the cluster will be dramatically different in complex with DNA, both with respect to solvent accessibility and hydrogen bonding. These changes would reasonably result in different redox potentials for DNA-bound versus free MutY.

In this chapter we describe spectroscopic and electrochemical characterization of MutY in complex with DNA as a preliminary foundation for probing protein-DNA charge transport chemistry in this complex. As the FeS cluster in MutY is thought to be in proximity of the DNA binding site, and DNA-modified surfaces provide an ideal medium to measure the electrochemistry of molecules that bind DNA, electrochemistry at DNA films provides a platform to study this interesting FeS cluster. We have developed DNA-modified electrode surfaces for probing protein-DNA interactions (Figure 9.1, Chapter 8). Here we apply this assay to measure the cyclic voltammetry of MutY bound to a DNA-modified electrode. The use of monolayers of adsorbed species has been used previously to investigate the electrochemistry of metalloproteins. For example, monolayers of thiol-modified bipyridine (bis(4-pyridyl)bisulphide) on gold electrodes were instrumental in electrochemical measurements of cytochrome c (45,46) and derivitized electrodes have also been used to obtain electrochemical data for

**Figure 9.1.** Schematic illustration of a protein bound to a DNA-modified electrode.



FeS proteins (47,48). In fact, attempts to perform electrochemical experiments of proteins at metal electrodes without the use of a redox inert molecule to orient the protein and keep it from adsorbing on the electrode surface, often result in no or an irreversible response (49).

In addition to electrochemical investigations, we also examine the MutY-DNA complex spectroscopically using flash quench transient absorption spectroscopy. This technique was first used to study electron transfer reactions in proteins (50), but it has also been used to study DNA-protein and -peptide charge transport chemistry (6-7). Intercalated tryptophan and tyrosine residue from tripeptides Lys-Trp/Tyr-Lys have recently been shown to be effectively oxidized by guanine radicals generated by flash quench of $Ru(phen)_2dppz^{2+}$ using $Ru(NH_3)_6^{3+}$ as an oxidative quencher (6). Furthermore, in similar flash quench transient absorption experiments using a mutant methyltransferase *Hha*I (M.*Hha*I) enzyme that inserts a tryptophan residue into DNA at its binding site, a transient tryptophan radical was observed in spectroscopic experiments of DNA-mediated charge transport (7). Thus there is precedence for the transport of radicals generated on DNA to bound proteins, although only to intercalated aromatic amino acid residues, not into a protein metal cofactor. Here we utilize similar flash quench transient absorption spectroscopy methods to study MutY bound to DNA.

## MATERIALS AND METHODS

*Materials*

All DNA synthesis reagents were obtained from Glen Research. MutY was expressed either as a fusion protein with maltose binding protein (MutY-MBP) or in a truncated form (Stop 225). Stop 225 has been crystallographically characterized (17), but not in complex with DNA. Both of these forms of the wildtype enzyme are stable at concentrations much higher than the native form and thus are preferable for spectroscopic and electrochemical studies. Importantly, both proteins also have the same activity as the wildtype on perfectly matched DNA and GA mismatches. The Stop225 protein has reduced activity on oxidized guanine mispaired with adenine, however (51). Stop 225 was used in all transient absorption experiments and MutY-MBP was used in electrochemical studies. A mutated version of Stop 225, C199H, was also expressed. All forms of MutY were purified as reported previously (52) and diluted to the desired concentrations using dilution buffer (20 mM Tris HCl, pH 7.5, 10 mM $Na_2EDTA$, 20% glycerol). Racemic $[Ru(phen)_2dppz]Cl_2$ was synthesized using published procedures (53). All other materials were purchased in their highest available purity and used as received.

*Preparation of DNA-modified surfaces*

Thiol-modified oligonucleotides were prepared using phosphoramidite synthesis as described in the Appendix. Thiol-terminated

linkers were attached to single stranded oligonucleotides, HPLC purified, and hybridized to their complements. Self-assembly was carried out (100 $\mu$M duplex, 5 mM phosphate, 50 mM NaCl, pH 7, ambient temperature) without added $Mg^{2+}$, as described in Chapter 8, in order to create loosely packed DNA films. After assembly of the duplexes, the remaining exposed surface was filled with mercaptohexanol (100 $\mu$M). After backfilling, the electrode was rinsed in 5 mM sodium phosphate buffer containing 50 mM NaCl, pH 7, and used for protein binding and electrochemical experiments.

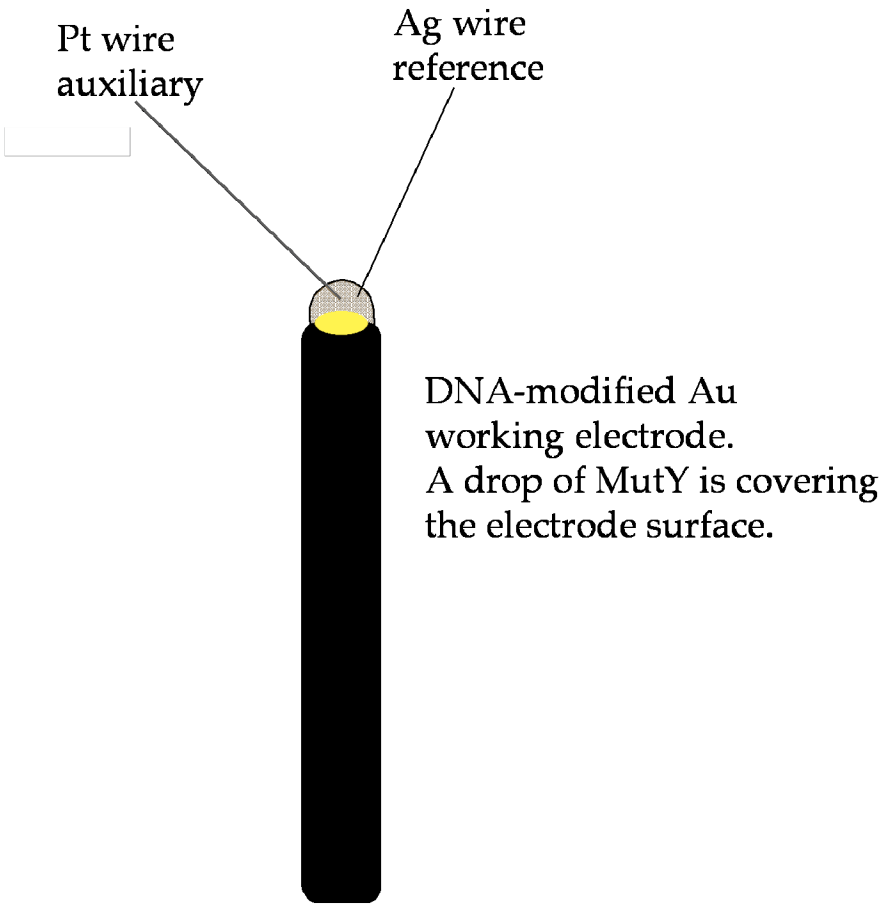
*Electrochemical measurements*

Cyclic voltammetry (CV) was carried out on 0.02 $cm^2$ gold electrodes using a BAS Model CV-50W electrochemical analyzer. Buffer and electrolyte conditions were 20 mM Tris HCl, pH 7.5, 10 mM $Na_2EDTA$, 20% glycerol, ambient temperature (+/- 390 $\mu$M MutY). The solution of MutY was balanced on the electrode surface and a Ag wire reference electrode and a Pt wire auxiliary electrode were inserted into this drop (Figure 9.2). Ag wire was used rather than a saturated calomel electrode due to very low volume constraints in the protein experiments.

*Laser spectroscopy*

Time resolved emission and transient absorption measurements used an excimer pumped dye (Coumarin 480) laser ($\lambda$ = 480 nm) or a YAG-OPO laser ($\lambda_{exc}$ = 470 nm) (54). Laser powers ranged from 1 to 2.5 mJ/pulse. The emission of the dppz complexes was monitored at 610 nm, and the emission

**Figure 9.2.** Diagram of electrochemical setup for these low volume, high protein concentration experiments. A drop of MutY is balanced on top of the upright DNA-modified gold electrode (0.02 $cm^2$). Pt wire auxiliary and Ag wire reference electrodes are inserted into this drop.



intensities were obtained by integrating under the decay curve for the luminescence. MuY was incubated with DNA at room temperature for 20 minutes prior to laser spectroscopy experiments.

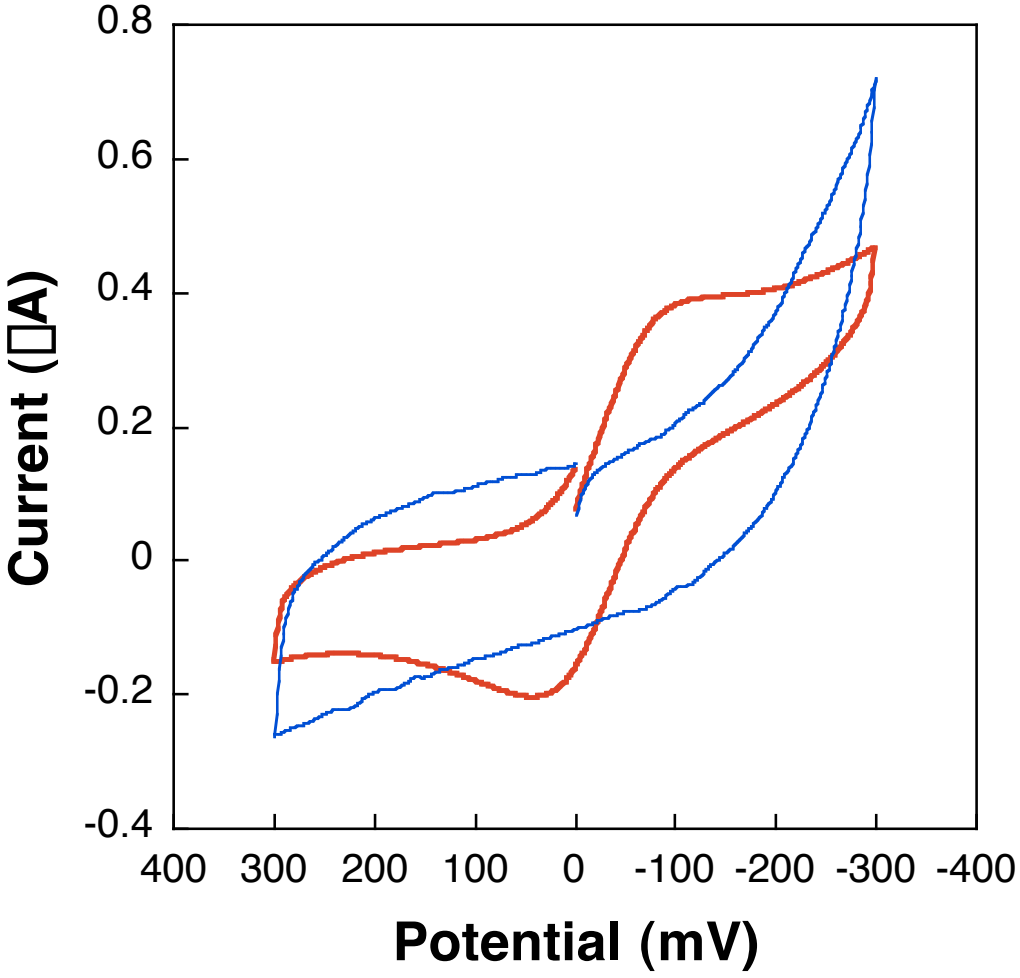
## RESULTS AND DISCUSSION

*Electrochemistry*

We have developed an electrochemical assay based on charge transport through double stranded DNA-modified gold electrodes (Chapters 2-8, refs. 8-14). Modified electrodes are prepared by self-assembly of prehybridized duplexes. Significantly, we have most recently begun to study electrochemistry of bound proteins at loosely packed DNA-modified surfaces (self-assembled mixture of DNA and mercaptohexanol, Figure 9.1) (Chapter 8, reference 14). Electrochemical reduction and oxidation of MutY at these loosely packed DNA films provide a means to access the DNA-bound redox potential of the FeS cluster and also to explore whether reduction of the FeS cluster is possible in a DNA-mediated charge transport reaction.

In order to measure the DNA-bound redox potential of MutY, a gold electrode surface was modified with the thiol-terminated duplex SH-5'-CACGCTGACGTAGCG (nonspecific DNA binding site, MutY dissociation constant ~ 250 nm (55)). After backfilling with mercaptohexanol, this electrode was incubated with 390 $\mu$M MutY for 20 minutes (Figure 9.2). Cyclic voltammetry of MutY (Figure 9.3) was then recorded. A pronounced

**Figure 9.3.** Cyclic voltammetry of 390 $\mu$M MutY (red) or MutY storage buffer (blue) at a gold electrode modified with the DNA duplex SH-5'-CACGCTGACGTAGCG-3′ ( = 100 mV/s, A = 0.02 cm$^2$).



electrochemical signal is observed at about –20 mV vs. SCE. Cyclic voltammetry of MutY storage buffer yielded no signal.

This potential of ~ 250 mV vs. NHE is within the range of known high potential FeS clusters (HiPips), but if the enzyme starts out in the 2+ oxidation state, as suggested by spectroscopic experiments without bound DNA (26,27,43), then the reversible reduction in the CV suggests a $[Fe_4S_4]^{2+/+}$ couple, as is typical for ferredoxins, making it a very unusual FeS cluster. This may be consistent with its unusual iron ligation and location near the C-terminus, in the FCL motif, however. Ferredoxins cycle between 2+ and 1+ with potentials ranging between –200 mV to –700 mV and HiPips have 2+/3+ couples with potentials typically in the range of +50 mV to +500 mV vs. NHE (24). Although the structurally identical [4Fe4S] clusters are capable of being in a 1+, 2+, or 3+ oxidation state, only one of these couples is physiologically available, owing to the dramatic effects of the surrounding protein matrix. HiPips are usually located in hydrophobic pockets and have five NH-S hydrogen bonds. Fdns are generally in more hydrophilic environments with eight NH-S hydrogen bonds (47). Hydrogen bonds are thought to stabilize the lower oxidation states.

As the only structural information available for MutY includes neither the extra C-terminal domain nor bound DNA, it is difficult to predict exactly what the environment of this interesting FeS cluster might be. It is quite possible that the potential is shifted upon binding DNA, however. In fact, if DNA binding stabilized the $[4Fe4S]^{3+}$ form, then DNA binding could promote oxidation to the 3+ state so that perhaps in electrochemistry experiments we are monitoring the 3+/2+ couple. Very important future experiments include

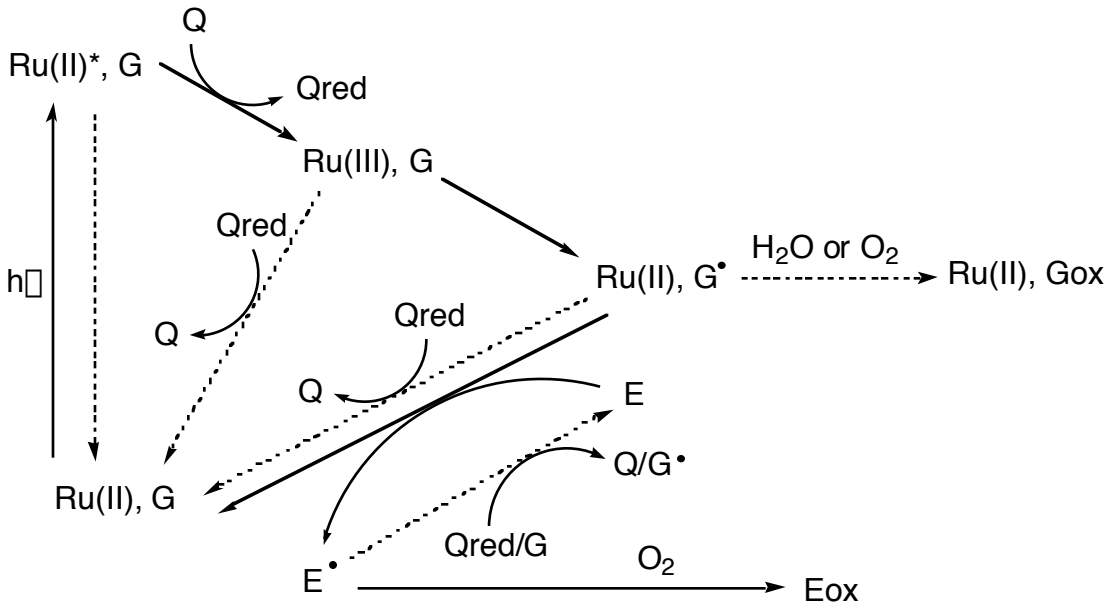
measuring the potential at an alkanethiol modified gold electrode (no DNA on the surface). We will also further explore the electrochemistry of MutY by determining the peak potential as a function of scan rate to determine if the protein is indeed surface bound. Finally, we plan to study MutY mutants where one of the cluster cysteines has been changed to a histidine to determine if there is an effect on the redox potential, consistent with this change in the ligating atom.

*Transient absorption spectroscopy*

While the electrochemistry experiments are important to establish the possibility for flow of charge from DNA to the bound FeS cluster, in order to assay for whether the FeS cluster can participate directly in a DNA-mediated charge transport reaction, we also performed transient absorption spectroscopy experiments.

We examined the flash quench reactions of $Ru(phen)_2dppz^{2+}$ bound to poly dGdC with and without bound MutY (Figure 9.4). Excitation of $Ru(phen)_2dppz^{2+}$ bound to poly dGdC by nanosecond laser pulses leads to an emission decay at 610 nm that can be fit biexponentially. This excited state is oxidatively quenched by $Ru(NH_3)_6^{3+}$ in the presence (~ 70% quenched) and absence (~ 90% quenched) of MutY. Quenching is less efficient with bound MutY, however, likely due to restricted access of the quencher ($Ru(NH_3)_6^{3+}$) to $Ru(phen)_2dppz^{2+}$ when MutY is bound to the helix. MutY alone does not quench the excited state of $Ru(phen)_2dppz^{2+}$, indicating the absence of direct electron transfer from the protein to excited state $Ru(phen)_2dppz^{2+}$.

**Figure 9.4.** Flash quench reaction (G = guanine, Gox = oxidized guanine, Q = quencher, Qred = reducedQ, E = DNA-bound enzyme residues, Eox = oxidized enzyme residues). Excitation of intercalated $Ru(phen)_2(dppz)^{2+}$ with visible light produces the corresponding excited ruthenium complex, $*Ru(phen)_2(dppz)^{2+}$, which can be quenched via electron transfer with a nonintercalating quencher, such as $Ru(NH3)_6^{3+}$, to yield the ground state oxidant $Ru(phen)_2(dppz)^{3+}$. Ground state $Ru(phen)_2(dppz)^{3+}$ can undergo back electron transfer with the reduced quencher or can oxidize guanines within the DNA double helix. Owing to the particularly low p*K*a of the guanine cation radical within the C:G base pair, only the neutral deprotonated guanine radical has been detected spectroscopically in double helical DNA (36). At this point, the guanine radical can either be reduced by quencher to regenerate the whole redox system, or it can oxidize a peptide side chain in the DNA π-stack, such as Trp or Tyr (6,7). Like the guanine radical, the Trp radical can also be reduced by the reduced quencher, yielding the regenerated system. Both radicals can undergo irreversible trapping reactions with water or oxygen.



We assayed these assemblies by transient absorption spectroscopy to obtain the full absorption difference spectrum with and without MutY bound to poly dGdC. At each wavelength, the transient absorption signal was fit biexponentially ($A(t) = C_0 + C_1\exp(-k_1t) + C_2\exp(-k_2t)$) and the coefficients for the fast phase ($C_1$) and the slow phase ($C_0$) were plotted against wavelength (Figure 9.5). The spectrum of the fast phase (Figure 9.5a) looks qualitatively like the spectrum of the guanine radical in double stranded DNA, with broad maxima at 390 and 510 nm (54). There appears to be less of this product, presumably the guanine radical, in the presence of MutY, however.

The spectrum of the slow phase yields an interesting result (Figure 9.5b). In the presence of MutY, there is a definite absorption maximum at about 405 nm. This absorption is not observed using poly dAdT (the spectrum observed with poly dAdT is consistent with Ru(II) bleaching, Figure 9.6) or without inclusion or one or more of the necessary reagents: MutY, $Ru(phen)_2dppz^{2+}$, and $Ru(NH_3)_6^{3+}$. The actual transient absorption traces at 405 nm are shown in Figure 9.7. The rise time of this signal at 405 nm (mostly RuIII) is fast, ~$10^6$ $s^{-1}$, with a very slow decay, ~$10^4$ $s^{-1}$. The traces at 405 nm make it clear that a long lived transient that absorbs at 405 nm is generated only in the presence of both MutY and DNA containing guanine.

The shape this spectrum looks qualitatively like that of a tyrosine radical (Figure 9.8) (56). MutY contains many aromatic residues including many tyrosines in and around its DNA binding pocket (17) (Figure 9.9). While a cocrystal structure of MutY bound to a DNA oligomer has not been solved, it is possible that one of the tyrosine residues in the binding pocket could make contact with and possibly intercalate into bound DNA. Studying

**Figure 9.5.** Transient absorption spectrum of the radical generated in flash quench experiments with (closed circles) and without (open circles) MutY bound to poly dGdC polymers. The individual transient absorption signals were fit to the biexponential function ($A(t) = C_0 + C_1\exp(-k_1t) + C_2\exp(-k_2t)$) and coefficients for the (a) fast ($C_1$, red) and (b) slow ($C_0$, blue) components were plotted as a function of wavelength. The samples contained $Ru(phen)_2dppz^{2+}$ (20 $\mu$M), poly dGdC (2 mM nucleotides), MutY (+/- 20 $\mu$M), and $Ru(NH_3)_6^{3+}$ (400 $\mu$M) in 5 mM phosphate buffer, pH 7.5. $\lambda_{exc}$ = 470 nm.

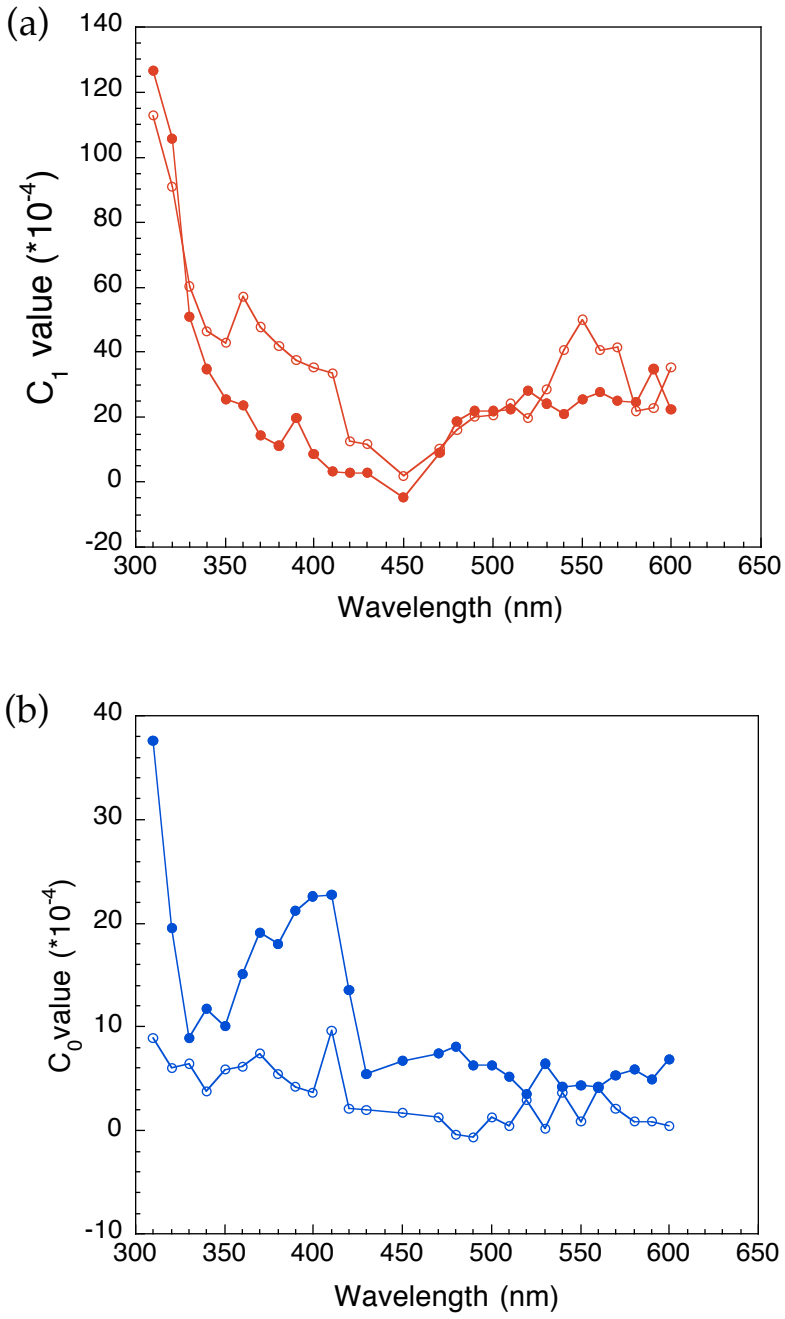


**Figure 9.6.** Transient absorption spectrum of the radical generated in flash-quench experiments using 20 $\mu$M MutY bound to poly dAdT DNA. The individual transient absorption signals were fit to the biexponential function ($A(t) = C_0 + C_1\exp(-k_1t) + C_2\exp(-k_2t)$) and coefficients for the fast ($C_1$, red) and slow ($C_0$, blue) components were plotted as a function of wavelength. The samples contained $Ru(phen)_2dppz^{2+}$ (20 $\mu$M), poly dGdC/dAdT (2 mM nucleotides), MutY (20 $\mu$M), and $Ru(NH_3)_6^{3+}$ (400 $\mu$M) in 5 mM phosphate buffer, pH 7.5. $\lambda_{exc}$ = 470 nm.

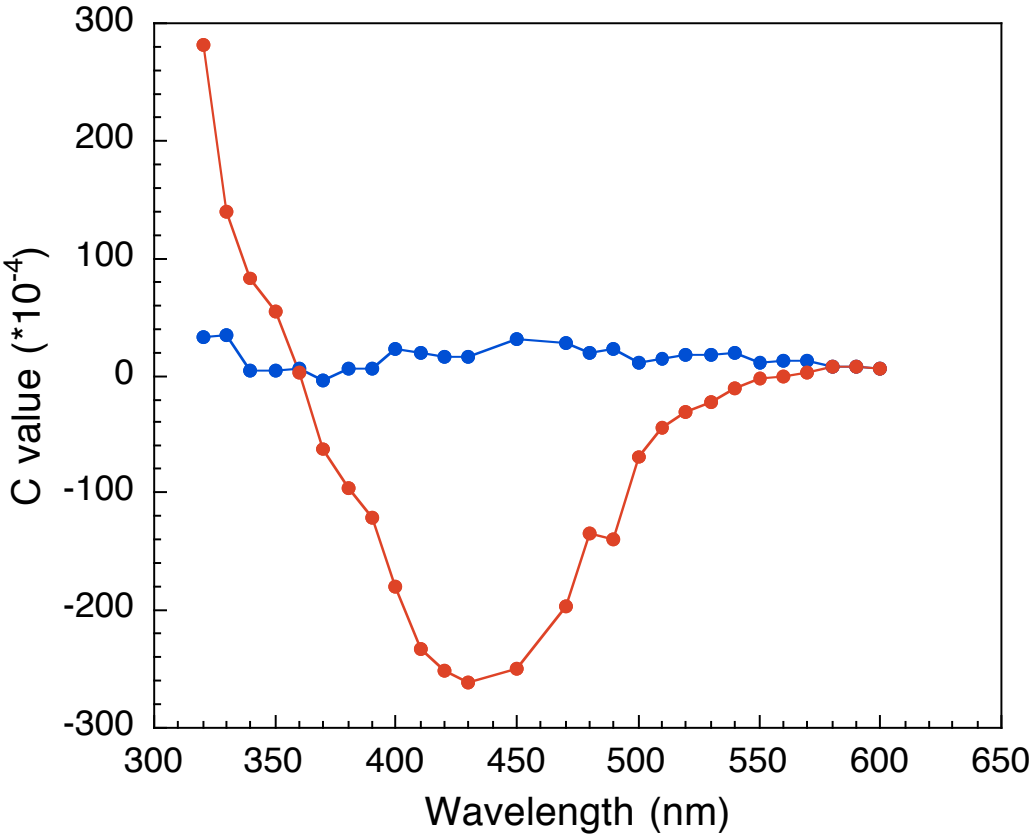


**Figure 9.7.** Transient absorption at 405 nm with formation of the transient intermediate with (red) and without (blue) MutY bound to poly dGdC and unbound in solution (black). The samples contained $Ru(phen)_2dppz^{2+}$ (20 $\mu$M), poly dGdC (+/- 2 mM nucleotides), MutY (+/- 20 $\mu$M), and $Ru(NH_3)_6^{3+}$ (400 $\mu$M) in 5 mM phosphate buffer, pH 7.5. $\lambda_{exc}$ = 470 nm. The rise time of the signal at 405 nm (mostly RuIII) is fast, ~$10^6$, with a very slow decay, ~$10^4$.

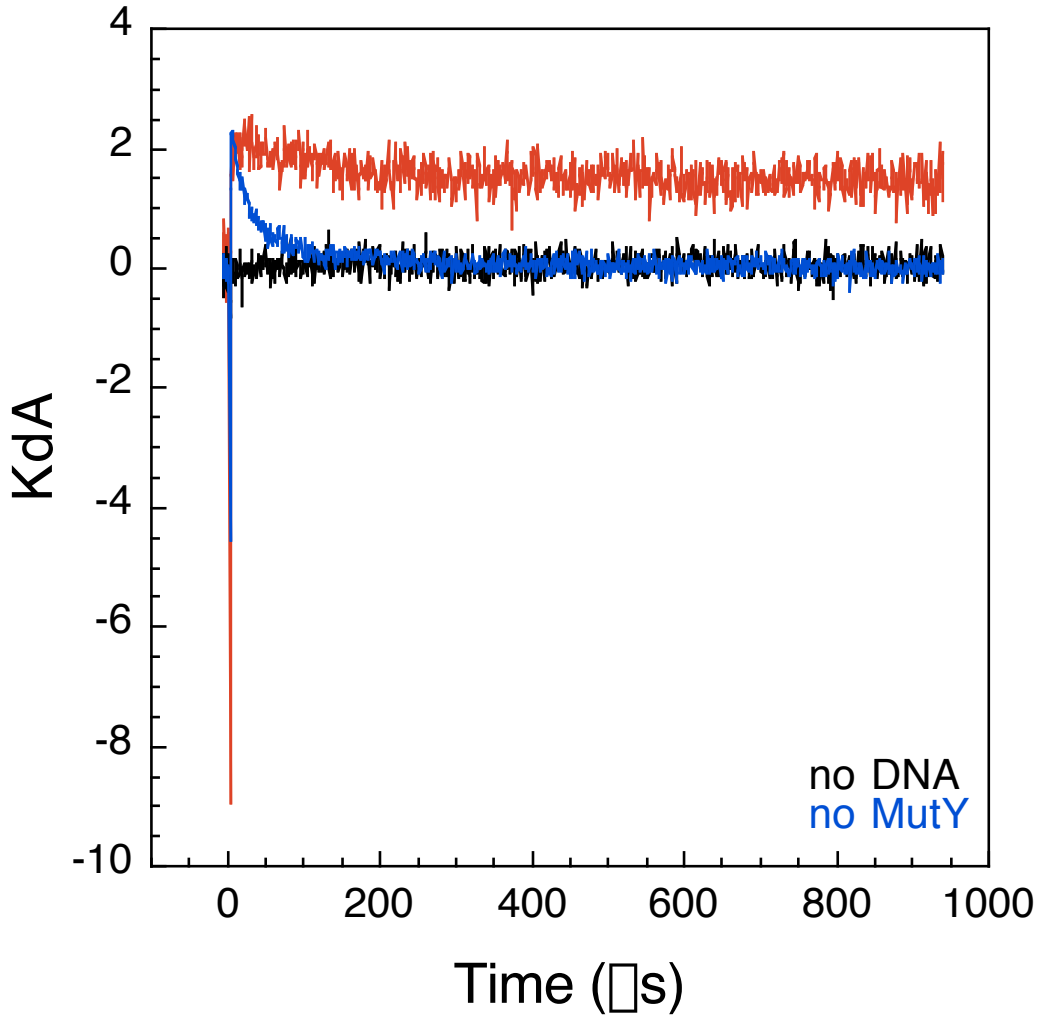


**Figure 9.8.** (a) Literature spectra of tryptophan and tyrosine radicals (37). (b) Spectrum of long lived species generated in these experiments. This data is averaged from four separate trials.

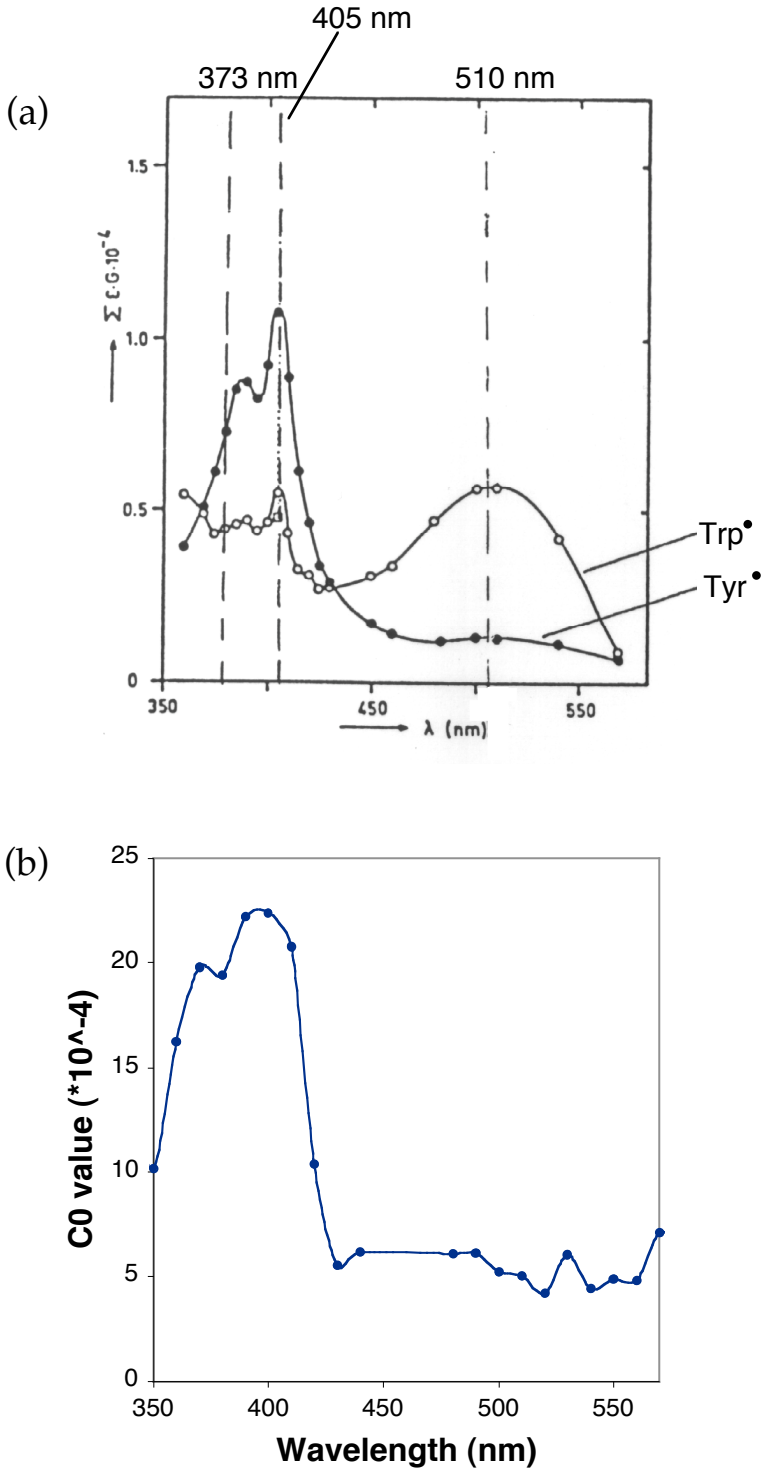
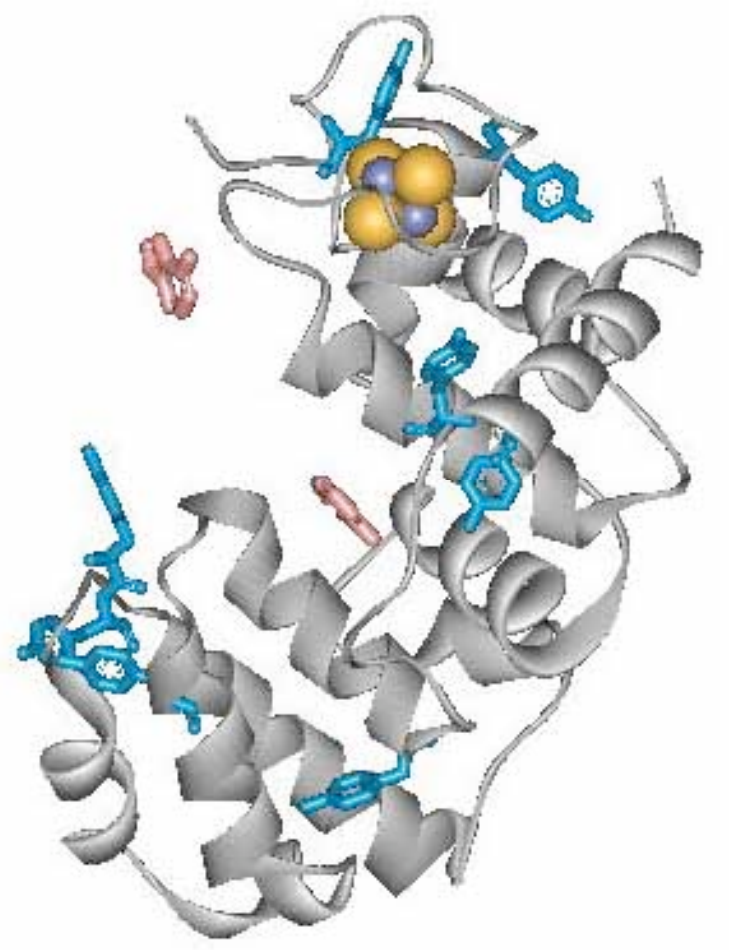


**Figure 9.9.** Crystal structure of MutY (pdb file 1mud). The $[4Fe4S]^{2+}$ cluster is shown in yellow, tyrosine residues are shown in blue, and bound adenine residues are shown in red. The lower adenine is thought to be bound in the active site of MutY. Clearly there are several tyrosine residues that could make contact with DNA. There are also tyrosines near the FeS cluster that, in theory, could shuttle charge from the cluster to DNA.



tyrosine replacement mutants would help to identify if this is indeed a tyrosine radical species that we observe in the flash quench experiments. Furthermore, there are also tyrosine residues in the region of the FeS cluster (Figure 9.9). It is also possible that one of these tyrosines may participate as a pathway intermediate in an electron transfer reaction between the FeS cluster and bound DNA. This is an exciting possibility, but we have no data so far that necessarily implies the cluster to be participating in the generation of any of these transient radicals.

In order to probe this issue of whether or not the cluster might be involved, we further examined this long lived signal at 405 nm using a mutated MutY enzyme, C199H (Figures 9.10 and 9.11). In this enzyme, one of the cluster cysteines is replaced with a histidine residue. Biochemically, this enzyme is identical to the wildtype (57), but such a mutation should alter the redox potential of the FeS cluster, and thus the transient absorption spectroscopy should change if the cluster were involved in the mechanism of the observed signals. A transient absorption difference spectrum was generated with C199H and compared to the wildtype. Figure 9.10 illustrates the slow phase spectrum for these two versions of MutY. There seems to be interesting differences in the two enzymes. By comparing the signal at 405 nm of wildtype MutY (Figure 9.7) with C199H (Figure 9.11) it seems that there is less of the long lived transient generated in the presence of the mutant enzyme. Whether the long lived species had the same identity in both enzymes, or if the mutated spectrum contains a mixture of the species observed with wildtype MutY and something else, has not been determined.

**Figure 9.10.** Transient absorption spectrum of the radical generated in flash quench experiments using 20 $\mu$M MutY (blue) or C199H (red) bound to dGdC DNA. The individual transient absorption signals were fit to the biexponential function ($A(t) = C_0 + C_1\exp(-k_1t) + C_2\exp(-k_2t)$) and coefficients for the slow ($C_0$) components were plotted as a function of wavelength. The samples contained $Ru(phen)_2dppz^{2+}$ (20 $\mu$M), poly dGdC (2 mM nucleotides), MutY or C199H (20 $\mu$M), and $Ru(NH_3)_6^{3+}$ (400 $\mu$M) in 5 mM phosphate buffer, pH 7.5. $\lambda_{exc}$ = 470 nm.

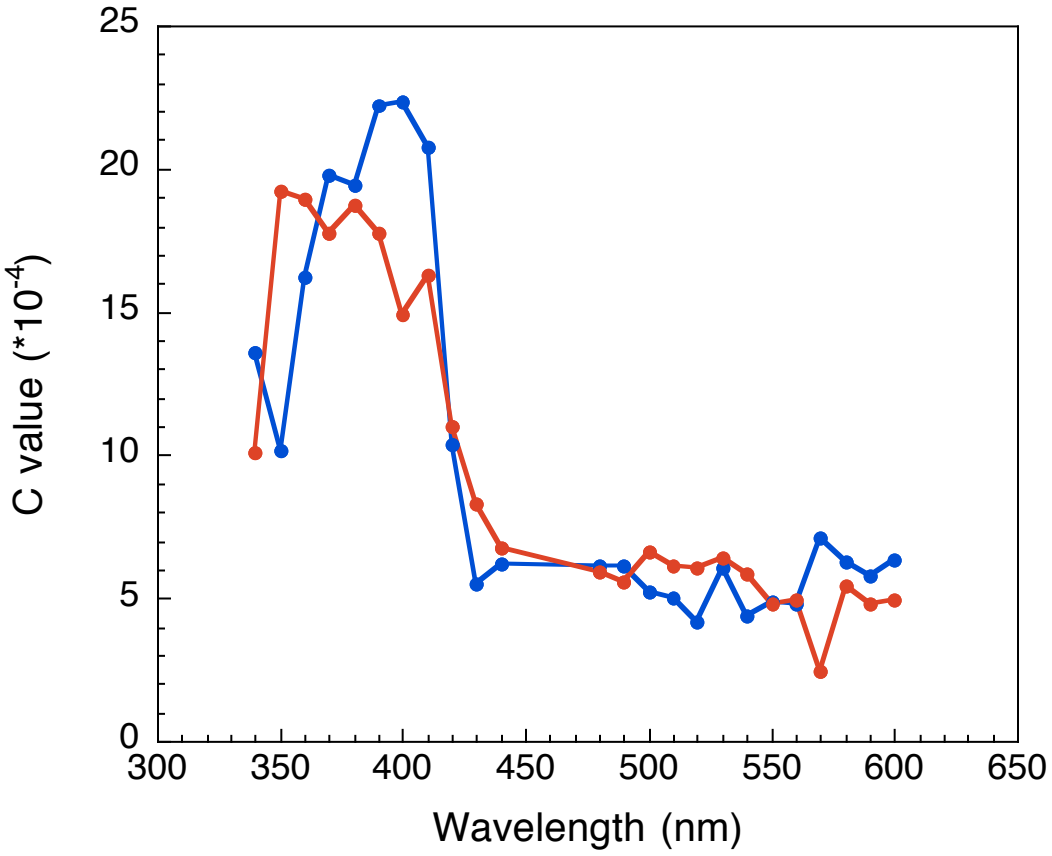
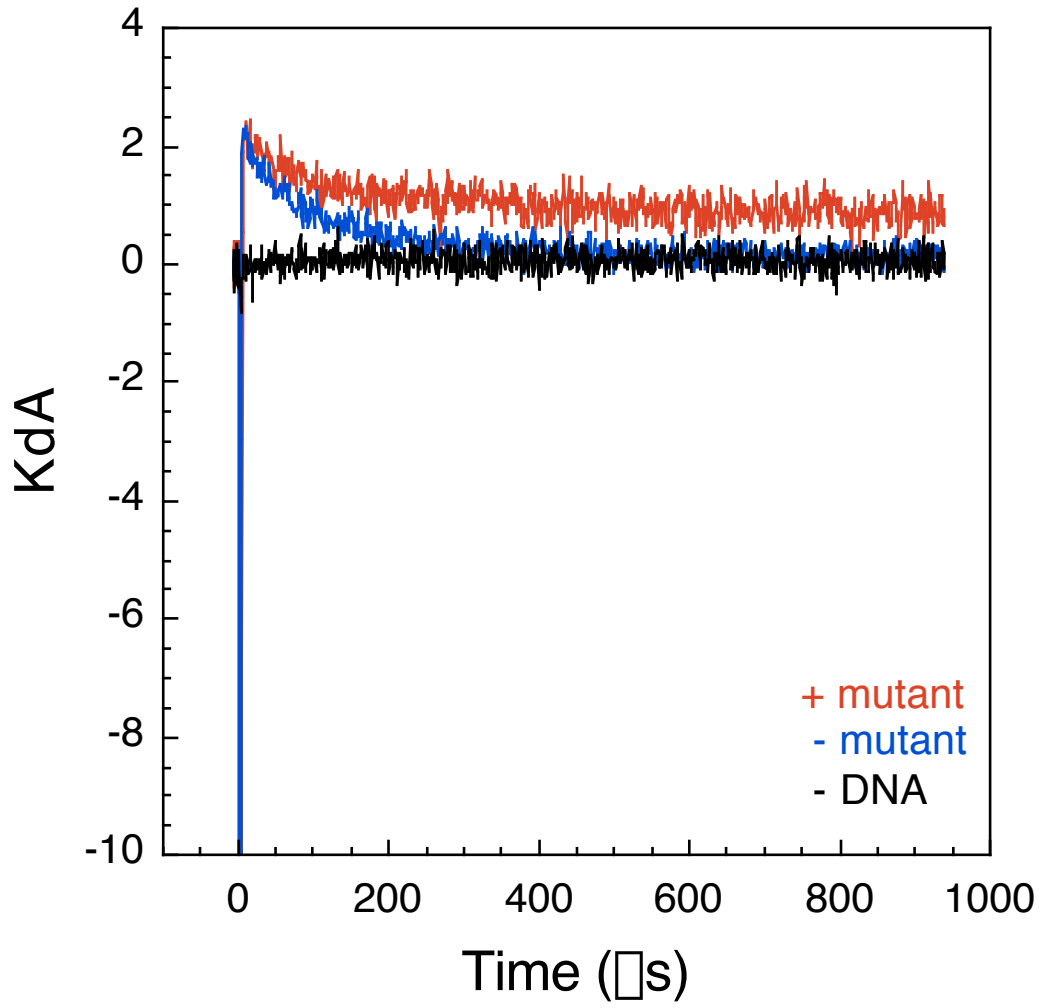


**Figure 9.11.** Transient absorption at 405 nm with formation of the transient intermediate with (red) and without (blue) C199H bound to poly dGdC and unbound in solution (black). The samples contained $Ru(phen)_2dppz^{2+}$ (20 $\mu$M), poly dGdC (+/- 2 mM nucleotides), C199H (+/- 20 $\mu$M), and $Ru(NH_3)_6^{3+}$ (400 $\mu$M) in 5 mM phosphate buffer, pH 7.5. $\lambda_{exc}$ = 470 nm.



Taken together, these transient absorption data suggest that upon oxidative flash quench of $Ru(phen)_2dppz^{2+}$ bound to poly dGdC in the presence of bound MutY, a guanine radical is initially generated, as is expected from previous experiments of flash quench DNA charge transport (54). This radical then leads to a second species that is very long lived. Controls with no MutY, no DNA, or no guanine indicate that this long lived radical is dependent on the presence of guanine as an intermediate species as well as the presence of MutY. Based on the literature spectrum of the tyrosine radical (56) this long lived species might be identified as such. This cannot be confirmed, however, nor can the possibility that the generated species is something else, such as a FeS cluster radical, be dismissed. A cluster mutated form of MutY, C199H, alters this second long lived species, which may be consistent with a change in the redox potential of the MutY FeS cluster. The $[4Fe4S]^{2+}$ cluster of MutY has a broad absorbance maximum at 410 nm (43). Typically, upon reduction this absorbance is bleached in $[4Fe4S]^{2+/+}$ proteins (58). Thus if the cluster were being reduced to $[4Fe4S]^{+}$ over the course of this experiment, the absorbance at 405 nm would probably go down, not up. However, if the cluster is 2+ but upon DNA slowly oxidizes to 3+, several reactions could be feasible with a 3+/2+ couple with a potential of about 250 mV, as measured by CV in these experiments (equations **1**-**4**, below). If this were true, and upon DNA binding the cluster oxidizes to the 3+ state, then in the oxidative flash quench experiments reported here we may be able to observe only guanine and tyrosine radicals in the transient absorption spectra (**1** and **2**), as these radicals would not be predicted to react with the 3+ cluster. This could be one explanation for why we see a long lived tyrosine radical in

these experiments; if tyrosine is an intermediate on a charge transport pathway between DNA and the FeS cluster, the electronic hole might get stuck at the tyrosine if the cluster is in the 3+ state and thus not able to accept the charge. There are other possibilities to explain the transient absorption at ~ 400 nm as well, however. For example, if there is some amount of 2+ cluster that is electronically coupled to guanine radical, and this guanine radical oxidizes the cluster to 3+ (**1**), then an absorption at ~ 400 nm might be expected.

$G^{\bullet+}$ ($E^o$ ~ 1.3 V) + $[4Fe4S]^{2+}$ → G + $[4Fe4S]^{3+}$ ($E^o$ ~ 0.25 V) **1**
repair of guanine radical by reduced cluster, $\Delta E^o$ ~ +1.05 V

$Tyr^{\bullet+}$ ($E^o$ ~ 1.0 V) + $[4Fe4S]^{2+}$ → Tyr + $[4Fe4S]^{3+}$ ($E^o$ ~ 0.25 V) **2**
repair of tyrosine radical by reduced cluster, $\Delta E^o$ ~ +0.75 V

$Ru^{3+}$ ($E^o$ ~ 1.5 V) + $[4Fe4S]^{2+}$ → $Ru^{2+}$ + $[4Fe4S]^{3+}$ ($E^o$ ~ 0.25 V) **3**
reduction of ruthenium by reduced cluster, $\Delta E^o$ ~ +1.25 V

8-oxo-$G^{\bullet+}$ ($E^o$ ~ 0.58 V) + $[4Fe4S]^{2+}$ → 8-oxo-G + $[4Fe4S]^{3+}$ ($E^o$ ~ 0.25 V) **4**
repair of oxidized guanine radical by reduced cluster, $\Delta E^o$ ~ +0.33 V

There is much to be done before any mechanism for MutY can reasonably be proven, but this hypothesis suggests an interesting redox role for the FeS cluster of MutY in vivo. Perhaps the 2+ cluster of MutY can participate in charge transport reactions with 8-oxo-G (**4**) or guanine radial (**1**) via long range DNA-mediated chemistry and this change in oxidation state of the enzyme is the signal that there is oxidized DNA ahead and repair is then initiated.

## SUMMARY AND FUTURE DIRECTIONS

We have investigated the electrochemistry and transient absorption spectroscopy of MutY bound to DNA. Based on cyclic voltammetry, the DNA-bound 2+/+ redox potential of MutY seems to be around 250 mV vs. NHE, making it a very unusual FeS cluster. There are many other examples of proteins with very similar FeS clusters, characterized by the FCL DNA binding motif, so perhaps these represent a new class of FeS proteins. We have also observed a long lived species generated by flash quench spectroscopy in a MutY-poly dGdC complex that may be consistent with a tyrosine radical. These results demonstrate that protein to DNA charge transport is possible in the MutY-DNA complex. Furthermore, as MutY contains a FeS cluster, it is feasible that MutY may be involved in redox chemistry with DNA in vivo and an intercalated tyrosine may be a relay in such a mechanism. The results presented here would be consistent with, but are insufficient alone, to establish such a proposal.

Future experiments are planned to further investigate the electrochemistry of MutY bound to DNA-modified electrodes containing specific as well as nonspecific binding sites. We will also examine the electrochemistry of C199H and other cluster mutants bound to DNA. Furthermore, the transient absorption spectroscopy of MutY bound to oligonucleotide DNA containing specific and nonspecific binding sites are among the future objectives of this study.

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