CHAPTER 10

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

Guanine oxidation and crosslinking studies

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The MutY K142A mutant was prepared by S.D.W. and wildtype MutY was prepared by members of the David laboratories at the University of Utah. MutY kinetics were performed by M.A.P.

INTRODUCTION

Oxidative damage to DNA from a distance has now been observed in many laboratories using a variety of DNA-bound photooxidants (1-10). Injection of a hole into the π-stack of DNA from a site specifically bound oxidant results in migration of the hole to sites of low oxidation potential, namely the 5′-G residue of 5′-GG-3′ doublets, where the radical can be trapped by water and oxygen, resulting in a permanent alkali-labile lesion (11-16). Long range oxidation of 5'-GG-3' sites has been demonstrated over distances of 200 Å (17-18), but this charge migration relies heavily on the integrity of the intervening base stack (19-24). Long range oxidation of 5′-GG-3′ sites was first shown with a phi complex of rhodium (5) but has since been observed with a variety of other DNA-bound photooxidants (3-10).

If there are two guanine doublet sites within a given oligonucleotide, arranged proximal and distal to the tethered oxidant, the ratio of damage at these sites provides a means to assay the efficiency of charge transport between the guanine doublets. As such, DNA-mediated charge transport has proven to be a sensitive probe of DNA conformation and stacking (19-23). For example, in assemblies containing a tethered rhodium intercalator as the photooxidant and two sets of guanine doublets placed before and after an intervening A-T-A bulge, which is known to distort the base stack, distal guanine oxidation was reduced by 75% compared to the proximal doublet (21). Hence, perturbations in the intervening base stack greatly affect DNA-mediated charge transfer.

Similarly, guanine oxidation ratios as measures of charge transport have been used to probe DNA-protein interactions (25-27). Recently this methodology was used to examine base flipping by the methyltransferase *Hha*I (26). In vivo, M.*HhaI* methylates the 5'C on each strand in 5'-GCGC-3' sequences by flipping the cytosine into its active site pocket and inserting Gln237 in its place, effectively creating a □-plug within the π-stack of the DNA (28,29). When the binding site for M.*Hha*I was placed between two guanine doublets, guanine oxidation at the distal site was greatly diminished. In identical experiments using a mutant enzyme that inserts the aromatic, heterocyclic residue tryptophan instead of glutamine, distal damage is restored. Thus, long range DNA charge transport can be modulated by DNA-binding proteins as a direct result of protein induced base stacking perturbations.

DNA charge transport may also be used mechanistically to probe DNA-protein interfaces from a distance. It is well known that DNA-protein crosslinks can be generated as a result of oxidative damage to DNA (30-36). Recently, DNA-protein crosslinks were generated from the oxidation of guanine in DNA charge transport experiments (37). In these experiments, noncovalent ruthenium intercalators were used to generate guanine radicals that subsequently resulted in covalent adducts with histone proteins. Ruthenium binding was not restricted in these experiments, but given the wealth of information known about oxidizing guanine from a distance (1-23,25-27), these results suggest that information about specific DNA nucleobase-protein amino acid contacts may be acquired without any modification of the DNA-protein interface. Using guanine oxidation ratios as

a measure of DNA base stacking perturbations and charge transfer generated DNA-protein crosslinking to discover DNA-protein contacts, DNA-mediated charge transport experiments have the potential to yield valuable information on DNA-protein interactions.

Here we describe the application of DNA charge transport to probe interactions of MutY with DNA. The *Escherichia coli* MutY base excision repair enzyme binds to 8-oxo-G:A and G:A mismatches in double stranded DNA and removes the adenine residue (38-41). MutY provides an ideal candidate for these types of studies based on two recent reports. The structure of the catalytic core of MutY was solved with an adenine residue bound in the enzyme active site, suggesting that MutY may be a base flipping enzyme (42). NMR evidence has further suggested that MutY may employ a double base flipping mechanism, flipping adenine and 8-oxo-G from the helix during repair (43). Crosslinking between the binding site 8-oxo-guanine and MutY has also been recently reported (31). These studies suggest that $IrCl_6^{2-}$ mediated oxidation of 8-oxo-guanine results in a covalent adduct between 8-oxo-G and K142 in the MutY active site, thus implicating this lysine residue in 8-oxo-G recognition. Here we present results that expand on these studies using oxidation promoted from a distance with a tethered rhodium intercalator. These results provide further insight into the catalytically active MutY-DNA complex and extend the methodology for examination of DNA-protein interactions using DNA-mediated charge transport.

MATERIALS AND METHODS

Materials

All reagents for DNA synthesis were obtained from Glen Research. Single stranded $Rh(phi)_2(bpy')^{3+}$-tethered oligonucleotides were prepared according to published procedures (44). The complement oligonucleotide strand of each assembly was radiolabeled with [γ-^{32}P] by T4-polynucleotide kinase according to published protocol (45). MutY was purified as reported previously (46) and diluted to the desired concentrations using dilution buffer (20 mM Tris HCl, pH 7.5, 10 mM Na_2EDTA, 20% glycerol).

Methods

MutY/DNA activity was assayed with 100 nM radiolabeled Rh-DNA duplex (quantitated by UV-vis, ε_{390} = 19,000 M^{-1} cm^{-1}) plus various concentrations of MutY in reaction buffer (20 ± 2°C in 25 mM Tris HCl, pH 8, 1 mM Na_2EDTA, 10% glycerol, 20X (base pairs) poly dA/poly dT). For MutY binding assays, 0-500 nM MutY was incubated with the oligonucleotide under the above conditions for 30 min, followed by electrophoresis on a 5% nondenaturing PAGE gel at 4°C and 100 V. Guanine oxidation (with 0-500 nM MutY) and MutY-DNA crosslinks (800 nM MutY) were promoted by photolysis at 365 nm for 60 min using a 1,000 W Hg-Xe arc lamp equipped with a monochromator. Oligonucleotides that were used to assay for guanine oxidation were treated with 10% piperidine and electrophoresed using a 20% denaturing polyacrylamide gel following irradiation. Conditions for the

proteinase K digestions were 10 mM Tris HCl, pH 7.8, 5 mM EDTA, 0.5% SDS and varying concentrations of proteinase K (0-50 ng/mL). After irradiation (and proteinase K digestion in certain cases), MutY-DNA crosslinking reaction mixtures were electrophoresed on a 5% denaturing gel. All gels were imaged and analyzed by phosphorimagry using ImageQuant, v. 3.3 (Molecular Dynamics).

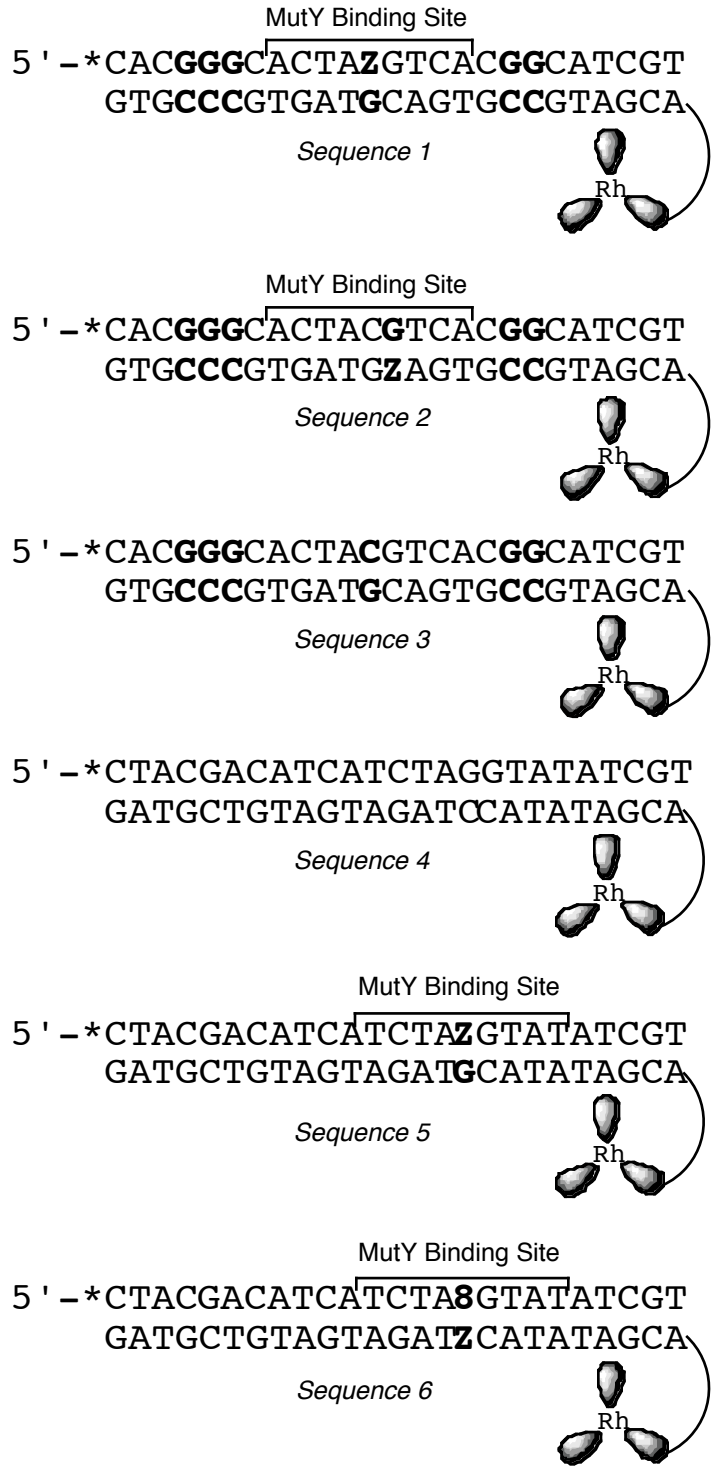
RESULTS

Base flipping assay

Sequence design

MutY preferentially cleaves adenine from A:8-oxo-G and A:G mismatches (38-41). To insure that MutY binds to but does not cleave the test DNA substrates (Figure 10.1), we employed 7-deaza-2'-deoxyadenosine (7-deaza-A, Z). It has been shown that Z effectively mimics the recognition properties of dA, but is resistant to glycosylase activity (47). 8-Oxo-guanine was first used in these studies, but it was found to be preferentially oxidized owing to its low oxidation potential (11-16,30). In fact, 8-oxo-G was so heavily oxidized in the absence of protein, that no appreciable damage to 5'-GG-3' and 5'-GGG-3' sites was evident (data not shown). Therefore, 7-deaza-A:guanine mismatches were used as the MutY binding site in the guanine oxidation studies. A 5'-GG-3' doublet (proximal) is incorporated before the binding site and a 5'-GGG-3' triplet (distal) after the binding site as oxidative

Figure 10.1. Schematic illustration of the DNA assemblies used in these experiments. $Rh(phi)_2(bpy')^{3+}$ was covalently tethered to the 5′-end of one strand and the complementary strand was 5′-^{32}P (*) end labeled.



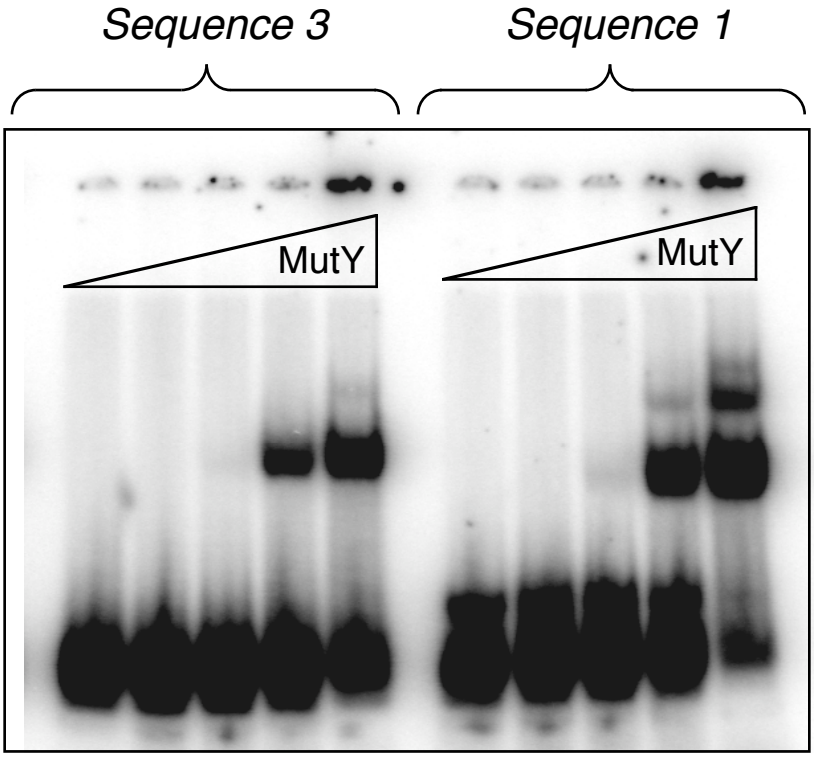
hot spots to provide indicators (via distal/proximal damage ratio) of the efficiency of charge transported through the MutY binding site from the tethered intercalating photooxidant, $Rh(phi)_2(bpy')^{3+}$ (17-23). It has previously been shown that phi complexes of rhodium(III) intercalate into DNA and readily oxidize guanine (5), and thus sequences 1-3 (Figure 10.1) were synthesized with $Rh(phi)_2(bpy')^{3+}$ tethered to the 5'end of one strand. A ^{32}P (*) label is incorporated on the 5' end of the complement to facilitate visualization of damage after piperidine treatment as strand breaks on a gel. Sequences 1 and 2 contain a specific MutY binding site and sequence 3 contains no specific MutY binding site.

Importantly, MutY is catalytically competent and can release adenine from mismatches in these sequences. For a G:A mismatch within sequence 1 (Figure 10.1), $k_2 = 2.5 \pm 0.4$ min^{-1} and $k_3 = 0.04 \pm 0.02$ min^{-1}, where k_2 is the chemical reaction and k_3 is product release. With the OG:A analog of sequence 1, $k_2 > 10$ min^{-1} and $k_3 = .0015 \pm 0.0008$ min^{-1}. With a G:A mismatch in sequence 2, $k_2 = 1.0 \pm 0.1$ min^{-1} and $k_3 = 0.01 \pm 0.01$ min^{-1}; with the OG:A analog, $k_2 > 10$ min^{-1} and $k_3 = 0.0017 \pm 0.0004$ min^{-1}. These kinetics are similar to those previously published for removal of adenine from guanine mismatches within a different sequence context (40).

MutY binding assay

A series of gel retardation assays using sequences 1 and 3 were executed to demonstrate MutY binding under $Rh(phi)_2(bpy')^{3+}$ photochemistry conditions (Figure 10.2). Using these photochemistry conditions, as described in Materials and Methods, efficient MutY binding is

Figure 10.2. Phosphorimage of a 5% nondenaturing polyacrylamide gel illustrating the formation of a MutY/Rh-DNA complex (shifted band). Oligonucleotide concentration is 100 nM, and MutY concentrations are 0, 50, 100, 200, and 500 nM.



observed, comparable to that reported previously (47). It is noteworthy that at high MutY concentrations, the stoichiometry of MutY bound to the DNA duplex may be greater than one, also as observed previously (47).

Guanine oxidation assay for base flipping

Sequences 1-3 were incubated with various concentrations of MutY for 30 min followed by irradiation at 365 nm for 60 min, piperidine digestion, and 20% PAGE (Figure 10.3). Quantitation of the base damage by phosphorimagery revealed little variation in the distal/proximal damage ratio as a function of protein binding (Figure 10.4). In the absence of protein, the damage ratio is already particularly low, 0.04, compared to that on other sequences, where the ratio can be 1-2. In the presence of protein over a concentration range of 0-200 nM, the damage ratio varies only from 0.05 to 0.1. This small variation reflects little perturbation to the intervening base stack. In fact, over this concentration range the distal/proximal ratio increases, indicating maintenance of the π-stack. At 500 nM MutY, a significant increase in the damage ratio is observed that is consistent with multiple proteins bound per duplex. A similar increase was observed as a result of nonspecific binding of ANTP to DNA (25). This has been attributed to decreased dynamical motion in the base pairs as a result of multiple protein binding events that stiffen the duplex.

It is also noteworthy that strong oxidation is observed at the 7-deaza-A of the binding site in sequence 1. The oxidation potential of 7-deaza-A is expected to be lower than that of adenine and probably comparable to that of guanine (48). The oxidation of 7-deaza-A is likely further lowered by

Figure 10.3. Phosphorimage of a 20% denaturing polyacrylamide gel illustrating the results of an assay for base flipping by guanine oxidation following irradiation at 365 nm and piperidine digestion. Maxam-Gilbert sequencing lanes, and dark controls (DC; no irradiation) are indicated. The lanes showing results with oligomers containing MutY have 0, 50, 100, 200, and 500 nM protein, respectively. All samples contain 100 nM of the indicated oligonucleotide in reaction buffer and were irradiated for 60 min at 365 nm (see Materials and Methods). The site of rhodium intercalation appears as a band doubling near the native DNA band. Sites of proximal and distal 5'-GG-3' damage (⇨) as well as the MutY binding site (✳) are shown.

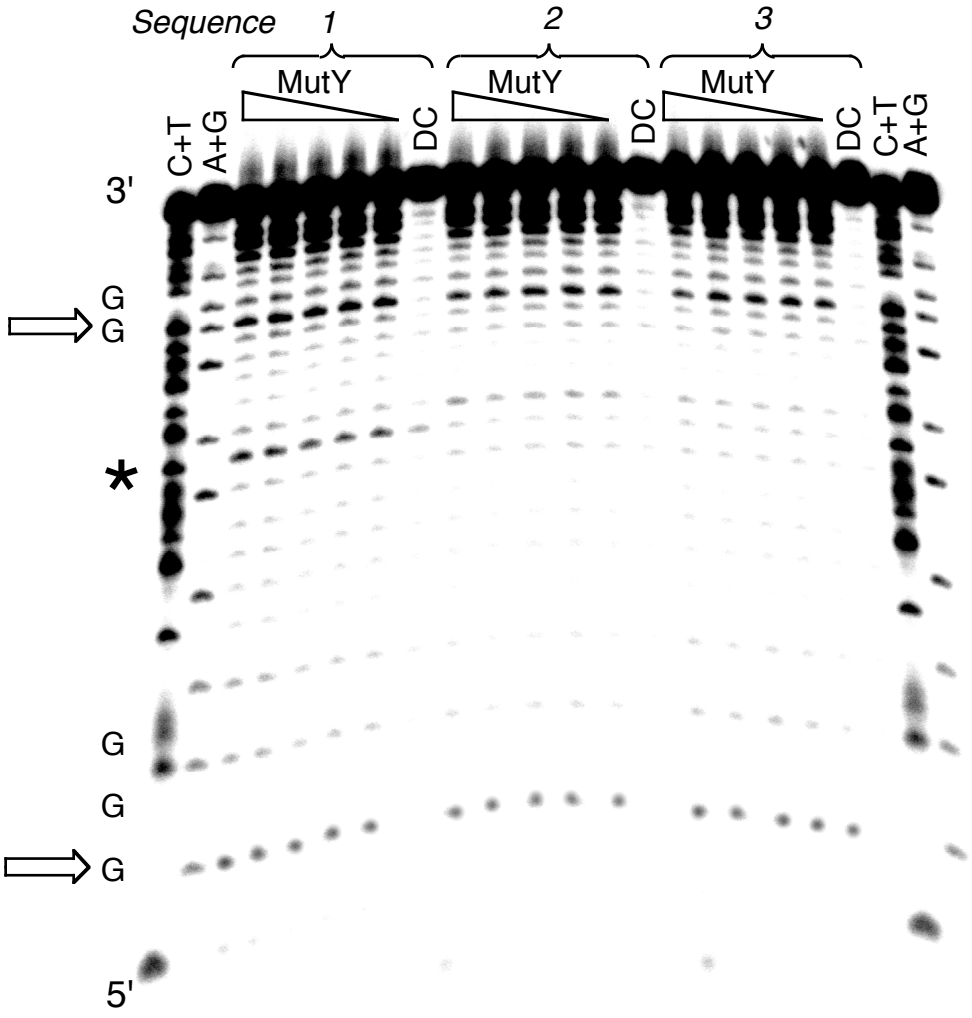
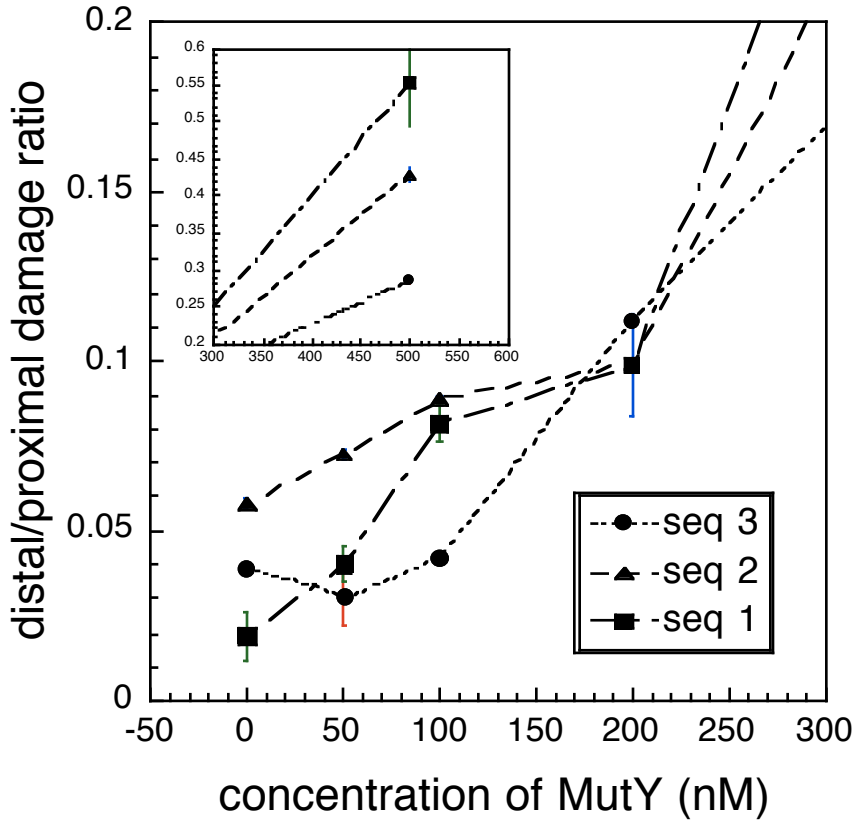


Figure 10.4. Plot of distal/proximal guanine damage ratio versus concentration of MutY. The inset shows results for high concentrations of MutY.



stacking with a guanine residue to its 3′-side (11-16); thus, ready oxidation at this site, 3′-GZ-5′, is not surprising. It is therefore possible to obtain oxidative damage both within and through the MutY-DNA interface. Based upon these data, MutY appears not to greatly disrupt the local DNA structure or stacking.

Crosslinking assay

Sequence design

Sequences 4-6 were designed with and without specific MutY binding sites (Figure 10.1) to probe 8-oxo-guanine crosslinking to MutY from a distance. The stable oxidation product of guanine oxidation, 8-oxo-guanine, has a very low oxidation potential, estimated at 580 mV (30). Thus it is expected that radicals generated on DNA should preferentially migrate to 8-oxo-G. As MutY specifically recognizes 8-oxo-guanine sites (38-41), we considered that the 8-oxo-G radical, once formed, would be in very close proximity to the protein sidechains and thus in prime position for formation of a crosslink between MutY and DNA. Oxidation of a base within the DNA-MutY interface is possible, as demonstrated by oxidation of 7-deaza-A in the guanine oxidation studies described above (Figure 10.3). 7-Deaza-A was again used to prevent enzyme turnover during these experiments (47). As above, $Rh(phi)_2(bpy')^{3+}$ is tethered to the 5′end of one strand, and the complementary strand contains a 5′-^{32}P-end label (*).

MutY-DNA crosslinking

Several experiments were carried out to determine if crosslinking between MutY and DNA could result from long range oxidation of 8-oxo-G (Figure 10.5-10.8). Otherwise identical samples were incubated with or without protein, irradiated at 365 nm, or left in the dark, and electrophoresed on a denaturing 5% polyacrylamide gel. As can be seen in Figure 10.5, a shifted band appears that depends upon the presence of MutY, light, and 8-oxo-G. In all cases (Figures 10.5-10.8), under conditions where crosslinking is observed, we always observed some material that is well shifted. This material may also be crosslinked material, or it may be due to MutY binding in general, but in our analyses we have only considered the lower band that runs into the gel. The data in Figure 10.5 imply that photoexcited Rh can generate an electrophilic radical on 8-oxo-G from a distance that results in a permanent protein adduct; 8-oxo-G has by far the lowest potential of any species in the assembly (30). Although the band is clearly visible on a gel, only about 10% of the radiolabel is incorporated in the shifted band, indicating this is not an efficient reaction. Notably, this level of crosslinking is similar to other reported crosslinking yields, however (49).

Crosslinking to sequence 5 is not observed despite evidence of oxidation at 7-deaza-A in the guanine oxidation studies. In guanine oxidation studies on duplexes with an incorporated 8-oxo-G nucleotide, very high yields of oxidative product are formed at that site (data not shown). Thus if only 10% crosslinking is observed at 8-oxo-G, based on guanine oxidation comparisons, the amount of crosslinking at 7-deaza-A should not be sufficient to be detectable. It should be noted that the native DNA band

Figure 10.5. Phosphorimage of a 5% denaturing polyacrylamide gel illustrating the formation of a MutY-DNA crosslink (only shifted band considered, not well shifted material) that depends upon the presence of light, protein, and 8-oxo-G. The contents of each lane are indicated on the gel. D = dark control (100 nM duplex plus 800 nM protein, incubated at ambient temperature in the dark for 60 min); L = light (100 nM duplex plus 800 nM protein, irradiated at 356 nm for 60 min). B = buffer control (100 nM duplex, no protein); MYB = MutY buffer control (100 nM duplex plus 800 nM dilution buffer, no protein); MY = MutY (100 nM duplex plus 800 mM protein).

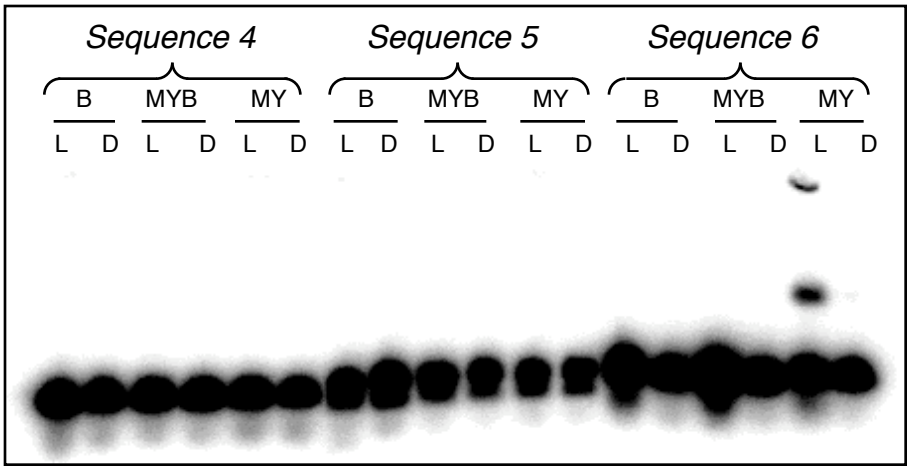
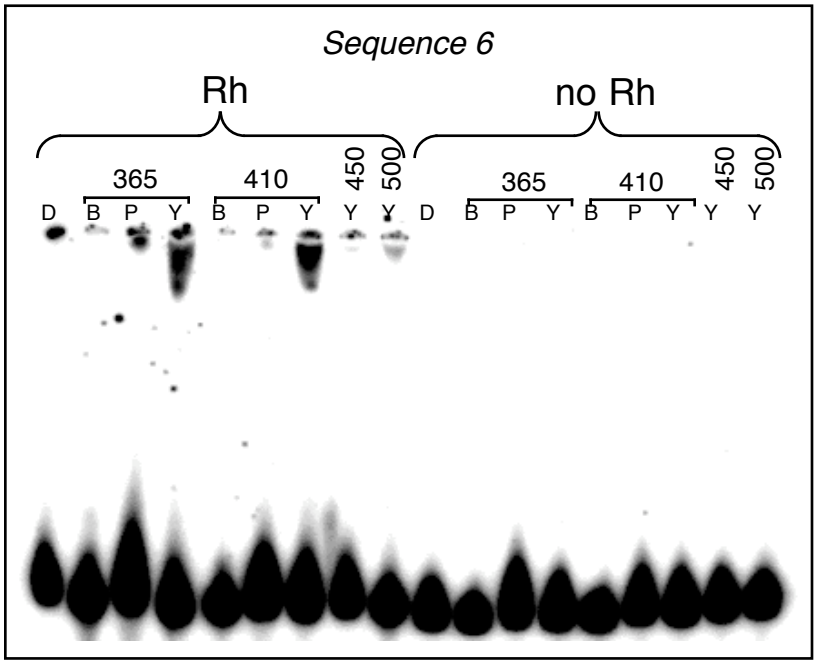


Figure 10.6. Phosphorimage of a 5% denaturing polyacrylamide gel illustrating the wavelength and Rh dependence of a MutY-DNA crosslink (only shifted band considered, not well shifted material). All lanes contain Sequence 6, although as indicated, there is no tethered rhodium in some cases. The contents of each lane are indicated on the gel. D = dark control (100 nM duplex plus 800 nM protein, incubated at room temperature in the dark for 60 min); B = MutY buffer control (100 nM duplex plus 800 nM dilution buffer, irradiated for 60 min); P = MutY post-irradiation control (800 nM protein added post irradiation of 100 nM duplex for 60 min); Y = MutY (100 nM duplex plus 800 mM protein, irradiated for 60 min). 365 = irradiation at 365 nm (λmax of $Rh(phi)_2(bpy')^{3+}$); 410 = irradiation at 410 nm (λmax of $[4Fe4S]^{2+}$ cluster); 450 = irradiation at 450 nm; 500 = irradiation at 500 nm. Rh = $Rh(phi)_2(bpy')^{3+}$ tethered duplex (Sequence 6); no Rh = no metal tethered.



has a mobility shift similar to single strand controls (data not shown), as this would be expected to appear on a denaturing gel.

Further investigation of the purported crosslinking reaction was accomplished by examining the wavelength dependence using sequence 6 (Figure 10.6). Again it is observed that in order for the shifted band to be formed, the rhodium complex must be photoexcited at 365 nm (8% shifted). Interestingly, the shifted band also occurs with irradiation at 410 nm, the wavelength for maximum absorption by the FeS cluster in MutY (50). However, this band is less intense (5% shifted), and probably also reflects Rh photochemistry, given that in experiments without the Rh oxidant, no crosslinking at all is observed. These experiments lend further credence to long range guanine oxidation followed by protein to DNA electron transfer that results in a covalent adduct.

Finally, amino acid side chain lysine 142 is involved in the crosslinking reaction in some way. The shifted band is absent when a mutant protein with an alanine in the active site (K142A) is used in otherwise identical crosslinking reactions (Figure 10.7). Importantly, K142A binds well to oligonucleotides with 8-oxo-G:7-deaza-A mispairs (51-52), so this result cannot be explained by a lack of protein binding.

To demonstrate that the shifted band contains MutY, samples containing sequence 6 were irradiated at 365 nm in the presence of MutY. These reaction mixtures were then digested with increasing amounts of the nonspecific protease, proteinase K; these results are illustrated in Figure 10.8. The shifted band fades and disappears as the concentration of proteinase K is

Figure 10.7. Phosphorimage of a 5% denaturing polyacrylamide gel illustrating lysine 142 as a MutY residue involved in the observed crosslink (only shifted band considered, not well shifted material). All lanes contain Sequence 6. The contents of each lane are indicated on the gel. D = dark control (100 nM duplex plus 800 nM protein, incubated at ambient room temperature in the dark for 60 min); L = light (100 nM duplex plus 800 nM protein, irradiated at 356 nm for 60 min); B = MutY buffer control (100 nM duplex plus 800 nM dilution buffer, irradiated for 60 min). K142A is a mutant protein without the active site lysine residue.

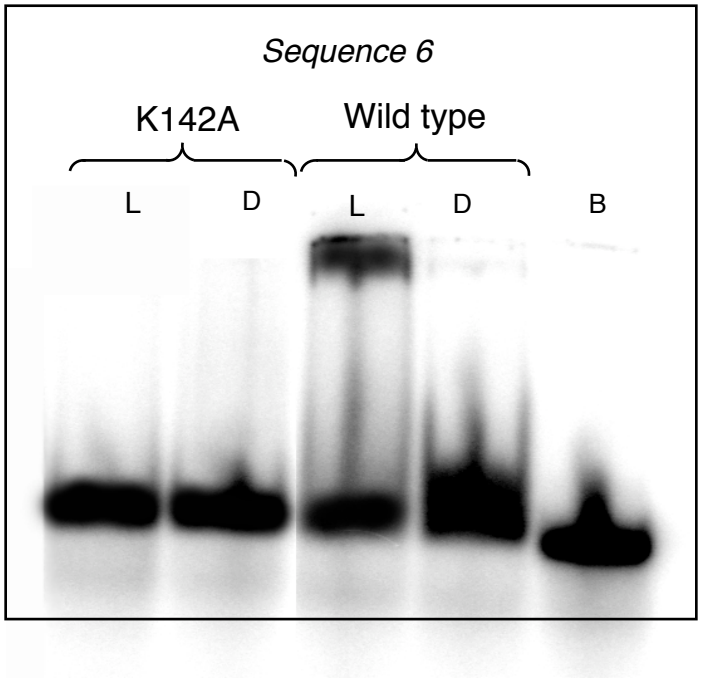
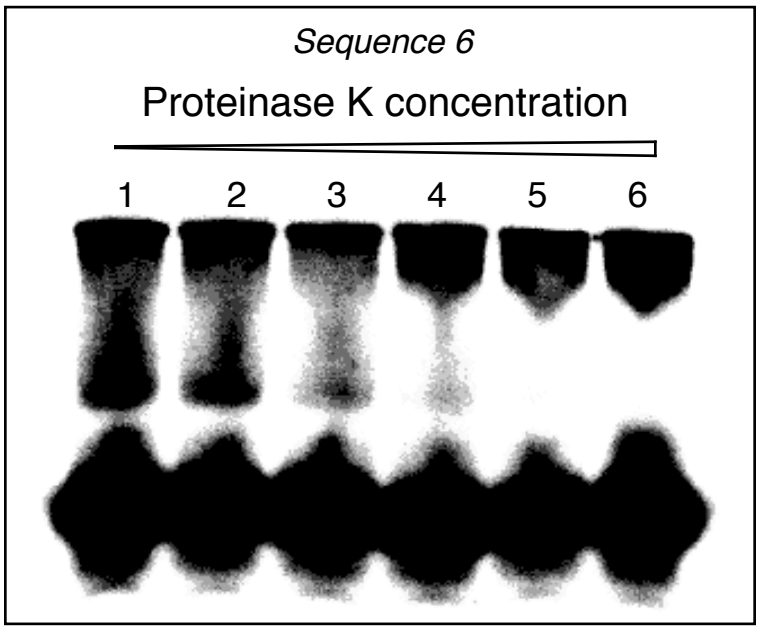


Figure 10.8. Phosphorimage of a 5% denaturing polyacrylamide gel illustrating that the crosslink (only shifted band considered, not well shifted material) contains protein. All lanes contain 100 mM duplex (Rh-tethered) plus 800 nM protein irradiated at 365 nm for 60 min. Proteinase K concentration in post-irradiation digestion is 0, 10, 20, 30, 40, and 50 ng/mL.



increased, indicating that the band shift is due to protein complexation and crosslinking.

We propose that this shifted band corresponds to a crosslink between 8-oxo-G and MutY facilitated by lysine 142. This crosslink could be formed as a result of long range oxidation of 8-oxo-G by $Rh(phi)_2(bpy')^{3+}$ followed by nucleophilic addition to the radical by lysine 142. The crosslink may be specific for lysine 142, implying K142 makes specific contact with 8-oxo-G in the MutY-DNA complex, as earlier proposed by Hickerson and coworkers (31).

DISCUSSION

MutY binding does not perturb long range DNA charge transport

Measurements of DNA charge transport provide a sensitive means to detect protein base flipping activity (2,25-27). Crystallographic and NMR data have been used to propose that MutY may use a base flipping mechanism to find its substrate (42-43). To test this hypothesis, we performed guanine oxidation studies with MutY. We can find no evidence for diminished distal/proximal guanine ratios at protein concentrations up to 200 nM (2:1 ratio of MutY to binding site; Figure 10.3-10.4) despite evidence of protein binding (Figure 10.2).

These results furthermore do not support progressive base flipping as a mechanism for MutY to find its binding site. The lack of protein dependent

change in distal/proximal damage ratio in sequence 3 (no specific binding site; Figure 10.3-10.4) is particularly revealing. If MutY were progressively base flipping to find its substrate, it would be scanning up and down sequence 3, continually flipping out nucleotides. Such a process would result in a diminished damage ratio, since bases flipped from the base stack cannot support long range charge transport (25-27). This diminution is not observed. It is important to note, however, that a remaining possibility is that MutY progressively flips out a base and flips into the helix interior an aromatic, heterocyclic side chain such as tryptophan; then when intercalated into DNA, the Trp moiety could support long range charge transport. While we cannot rule out this possibility, there are no clear candidates for intercalative insertion in the protein (42).

It is noteworthy, with this binding site analog, 7-deaza-A:G, base flipping after specific binding is not observed. It is possible, perhaps even likely, that MutY flips out adenine in order to excise it from a duplex during normal repair. Evidently, however, MutY does not flip 7-deaza-adenine from the duplex, as there is no protein dependent decrease in distal/proximal guanine oxidation ratios for sequences 1 or 2. Furthermore, strong oxidation at the 7-deaza-adenine site is observed, especially when it is 5' to a guanine residue (resulting in a lowered oxidation potential for 7-deaza-A). This indicates that 7-deaza-adenine must remain in the duplex, because oxidation by $Rh(phi)_2(bpy')^{3+}$ cannot occur unless stacking is intact (19-24). Based on this result, it is concluded that MutY makes an important contact with the N7 of adenine during excision repair, either for base flipping or base excision. If in fact MutY were unable to extrude 7-deaza-A from a duplex, this could be

the reason for the lack of change in guanine oxidation ratios in sequences 1 and 2. However, there is no 7-deaza-A in sequence 3, and again, no evidence for base flipping is observed. Therefore, we find no data consistent with base flipping as a method for mismatch recognition by MutY.

DNA charge transport does promote formation of an oxidative crosslink between MutY and 8-oxo-G from a distance

8-oxo-guanine has earlier been demonstrated to crosslink to MutY upon incubation with $IrCl_6^{2-}$ (31). In this system, Ir(IV) presumably directly oxidizes the 8-oxo-G residue that in turn reacts with MutY. Interestingly, the duplexes used for these studies had an 8-oxo-G:apurinic binding site, the product of base excision by MutY. Additionally, it has recently been demonstrated that introduction of an electronic hole in DNA can result in a guanine-protein crosslink via DNA-mediated charge transfer to guanine and subsequent trapping by a protein residue (37). To extend both of these methodologies, we designed a system to investigate oxidatively induced DNA-protein crosslinking without perturbing the DNA-protein interface. To that end, we synthesized oligonucleotides with an 8-oxo-G:7-deaza-A MutY binding site (a *substrate*, not product analog) that was separated from a covalently tethered photooxidant, $Rh(phi)_2(bpy')^{3+}$, by more than 20 Å. Thus oxidized 8-oxo-G is generated in a long range charge transfer reaction and it is then available for direct attack from the bound protein. Notably, 8-oxo-G must also be stacked within the helix for oxidative damage from a distance to occur.

In our experiments, a shifted band is observed on denaturing polyacrylamide gels that controls indicate is dependent on generation of photoactive $Rh(phi)_2(bpy')^{3+}$, potent oxidant, 8-oxo-guanine, an easily oxidized base in MutY binding site, and MutY with a lysine residue at position 142. Furthermore this shifted band is certainly the result of protein binding, as addition of proteinase K leads to the disappearance of the band. Thus it is concluded that oxidation of 8-oxo-guanine results in a covalent crosslink with MutY that involves lysine 142. K142 may be the residue involved in the crosslink itself, or it may facilitate some interaction that gives rise to the observed crosslink. Notably, K142 has been proposed to recognize guanine in the G:A mispair and facilitate protein binding (53), but not to participate in the catalytic base excision reaction (51,53).

Our data confirm the previously published results using $IrCl_6^{2-}$ to covalently crosslink 8-oxo-guanine to MutY involving reside K142 (31). However, these experiments utilize a substrate analog (7-deaza-A), rather than an apurinic site, which is more representative of initial substrate recognition and binding contacts. It is interesting to note, however, that both substrate and product duplexes crosslink to MutY in a fashion that is oxidation and lysine 142 dependent.

Taken together, the base flipping and crosslinking results indicate that K142 may be involved in recognition of oxidized guanine within the duplex. However, this site is not found via a progressive base flipping mechanism. It is interesting to consider that perhaps 8-oxo-G is flipped out to facilitate repair *after* MutY finds the site, especially because 8-oxo-G is the damaged

base and adenine is a normal DNA base. The 8-oxo-G to K142 crosslinking results could support this hypothesis.

Long range charge transport in DNA is thus valuable in obtaining information about DNA-protein interactions, both by probing protein induced DNA distortion and by identifying amino acids that contact guanine residues in DNA. Furthermore, in our laboratory, we have observed the sensitivity of DNA-mediated charge transport to DNA mismatches and lesions, as well as protein binding (23-27). In light of these data, it is interesting to consider whether DNA-mediated charge transport may play a role in cellular events.

SUMMARY

MutY is an *E. coli* DNA repair enzyme that binds to 8-oxo-G:A and G:A mismatches and catalyzes the deglycosylation of the mismatched 2'deoxyadenosine. We have applied DNA-mediated charge transport to probe the interaction of MutY with its DNA substrate. Oligonucleotides synthesized with a tethered rhodium intercalator and guanine doublets placed before and after the MutY binding site are used to assay for base flipping activity by MutY. Based on this assay, we find no evidence that MutY uses progressive base flipping as a means to find its binding site; protein binding does not perturb long range DNA charge transport. DNA-mediated charge transport can be utilized to promote protein-DNA

crosslinking from a distance. Long range oxidation of 8-oxo-G within the MutY binding site using tethered rhodium intercalators promoted crosslinking and yielded information on MutY side chains that interact with this base. Based on photooxidative crosslinking of the wildtype but not K142A mutant, it is evident that within the protein complex, lysine 142 makes important contacts with 8-oxo-G.

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