Protein-Modulated DNA Electron Transfer

Scott R. Rajski,[†] Sanjay Kumar,[§] Richard J. Roberts,[§] and Jacqueline K. Barton*,[†]

The Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California 91125
New England Biolabs, 32 Tozer Road
Beverly, Massachusetts 01915

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Long-range oxidative damage to the 5′-guanine of 5′-GG-3′ sequences in DNA readily occurs as a result of electron migration through the π-stacked DNA base pairs.[1,2] Oxidative DNA damage has been demonstrated over distances of 200 Å,[3] yet charge migration relies heavily upon the integrity of the DNA π-stack.[4−6] The *Hha*I methyltransferase presents an opportunity to explore the effect of gaps within the π-stack on long-range electron transfer, since upon binding, the protein induces and stabilizes a π-gap using a novel DNA base-flipping mechanism.[7]

Long-range oxidation of 5′-GG-3′ sites was first shown with a rhodium intercalator.[8−12] The rhodium photochemistry bound to DNA yields base photooxidation upon irradiation at low energy (365 nm),[8,13] whereas irradiation at high energy (313 nm)[11] leads to direct strand scission, marking the sites of intercalation. Other DNA-bound photooxidants also promote DNA damage at long range.[4,6,14−17]

The methyltransferase *Hha*I−DNA complex is a well-characterized DNA−protein assembly in which the structure of DNA is significantly, but locally distorted.[7] The enzyme recognizes 5′-G***C**GC-3′ and effects methylation by extruding the target cytosine (***C**) completely out of the DNA duplex; the remainder of the B-form duplex is left intact. The DNA gap created by base-flipping is filled by insertion of the side chain of glutamine 237[7,18,19] (Gln237).

Construction of a DNA assembly incorporating a M.*Hha*I binding site between two 5′-GG-3′ doublets containing a tethered photooxidant would permit study of protein-induced π-stack distortions (Figure 1). Interruption of electron transfer would be observed as differences in the extent of damage at the respective 5′-GG-3′ sites. Two such substrates where the 5′-GG-3′ doublets were incorporated on the strand complementary to the 5′ Rh-tethered oligonucleotide were synthesized. These differed only in the M.*Hha*I DNA binding domain (DBD) **1A** and **1B**.[20]

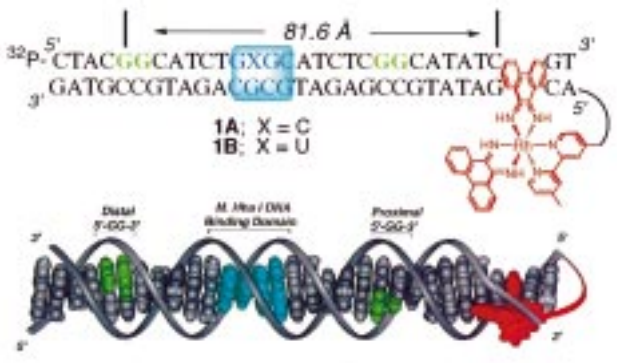

Figure 1. DNA duplexes containing M.*Hha*I binding site 5′-GXGC-3′ placed between two oxidizable 5′-GG-3′ sites. Each duplex contains a Rh-tethered complement to the radiolabeled "target" strand. The complement was selectively functionalized[9] at the 5′ end with the Rh^{3+} intercalator and used as the racemic mixture of Δ and Λ diastereomers. Assemblies contained either matched **1A** or mismatched **1B** protein-binding sites. For both sequence-coded (top) and space-filling diagrams (center), the 5′-GG-3′ sites are shown in green with the M.*Hha* I site 5′-GCGC-3′ in cyan. The photooxidant $[Rh(phi)_2bpy']^{3+}$ (in red) is schematically intercalated into the DNA duplex at a site determined by photocleavage.

Upon photooxidation of these duplexes in the presence of protein, a significant decrease in distal 5′-GG-3′ damage occurred in an enzyme-dependent fashion (Figure 2). Both **1A** and **1B** displayed diminutions of distal 5′-GG-3′ damage at all enzyme concentrations; similarly, both DNAs showed significant protein binding in gel shift assays under parallel conditions.[21−23] Interestingly, analysis of **1A** indicated an average diminution in distal G-oxidation of 42% and a corresponding formation of 25% reversibly gel-shifted DNA. Thus, in the case of **1A**, DNA-mediated charge migration revealed a higher degree of sensitivity to DNA−enzyme contacts than did the complementary gel-shift assay.

Additional alkali-labile damage at the 3′-G was found within the 5′-GU**G**C-3′ of **1B** but not of **1A**; the distal/proximal damage ratio was comparable for the two DNAs, however. This damage was found to rely on enzyme, light, and Rh and was revealed only after piperidine digestion. A sensitivity to oxidation of the orphan G in the enzyme-bound mismatched site has been seen.[24] This damage event appears to be diagnostic of protein binding. Notably, we find that the extent to which both DNA damage events occur directly correlates with enzyme−DNA binding activity.

Gln237 insertion into the π-gap is a key feature of the DNA−protein base-flipped complex. We therefore examined also two

* To whom correspondence should be addressed.
[†] California Institute of Technology.
[§] New England Biolabs.

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(20) M.*Hha*I has high affinity for mismatches within its target sequence. See Klimasauskas, S.; Roberts, R. J. *Nucleic Acids Res.* **1995**, *23*, 1388.
(21) The concentrations used were $\geq K_M$ for M.*Hha*I binding to 5′-GCGC-3′ and 5′-GUGC-3′, assuming 100% enzymatic activity.[20]
(22) The efficiency of photooxidative damage at the proximal 5′-GG-3′ site for both **1A** and **1B** was unchanged even with high M.*Hha*I concentrations (Figure 2). Also nonspecific competitor DNA enhances enzyme-dependent inhibition of distal G-oxidation.
(23) Trends in DNA damage observed at [DNA] of 100−200 nM were also unaffected by the addition of S-adenosyl-homocysteine; this analogue lacks the methyl group necessary for DNA methylation but is known to stabilize the DNA-M.*Hha*I complex.[18]
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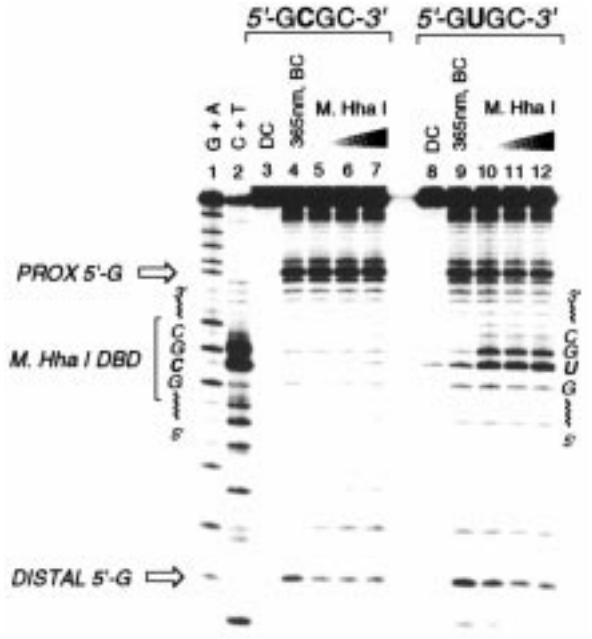

Figure 2. Photooxidative damage as a function of M.*Hha*I concentration and DBD sequence. The 5′-^{32}P-end-labeled DNAs as visualized by phosphorimagery following irradiation, piperidine digestion, and 20% denaturing polyacrylamide gel electrophoresis are shown. Lanes with Maxam−Gilbert sequencing reactions, each assembly (**1A** and **1B**) without irradiation (DC) and the duplex irradiated but without M.*Hha*I (BC) are as noted. Lanes 5−7 (**1A**) and 10−12 (**1B**) contained 100, 400, and 800 nM native M.*Hha*I, respectively. All samples contained 25 mM NH_4OAc (pH 9.0), 1 mM $KHPO_4$ (pH 7.4), 5 mM NaCl, 50 μM EDTA, 5% glycerol, 200 nM SAH, 200 nM **1A** or **1B**, and a 40-fold molar excess (in base pairs) of poly dA−poly dT. Irradiations were performed for 1 h at 365 nm using a 1000-W Hg/Xe lamp with monochromator at 25 °C. The sites of rhodium intercalation appear as the band doubling near the native DNA band. Sites of proximal and distal 5′-GG-3′ damage are shown.

mutants of M.*Hha*I in which Gln237 is replaced either with a purely aliphatic amino acid, isoleucine, or with the aromatic heterocyclic residue, tryptophan.[25] Insertion of Gln237 interrupts the aromatic manifold by which charge migration through the DNA can occur, while tryptophan insertion can be viewed as maintaining the π-stacked array. Isoleucine insertion would provide similar aliphatic−aromatic contacts to those of Gln237, but would lack the hydrogen-bonding network of native M.*Hha*I.

Figure 3 shows long-range oxidative damage of mismatch substrate **1B** with Ile237 and Trp237 M.*Hha*I mutants along with native M.*Hha*I. Here, optimally wild-type M.*Hha*I showed 45% inhibition of damage at long range. M.*Hha*I−Ile237 inhibited DNA-mediated electron transfer, although not to the same extent as native methylase. In parallel, much less damage at the orphaned 3′-G within the DNA-binding domain was observed with Ile237. Notably, both damage patterns for Ile237 indicate decreased complex formation compared to the wild type, which was confirmed by gel-shift assay.

More significantly, with Trp237, distal oxidative damage was retained, and hence the variant appeared to restore charge migration through the DNA duplex. The distal:proximal ratios for Trp237 closely paralleled the ratio obtained in the absence of

(25) K_M values for the mutants are considered to be within an order of a magnitude to that of the wild-type based upon restriction assays. All mutants methylate DNA in vitro and in vivo.[18]

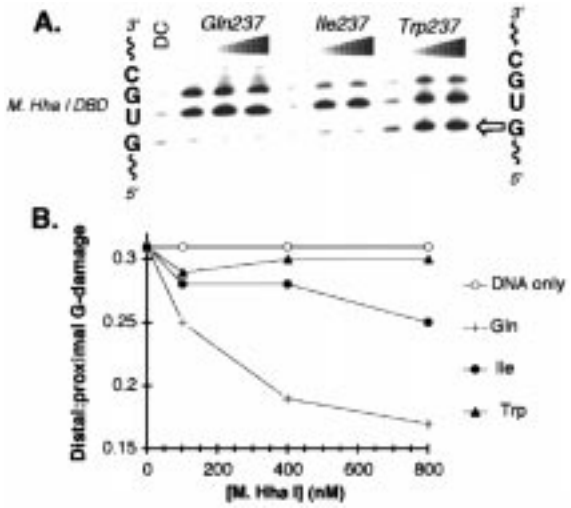

Figure 3. Photooxidative damage with bound mutants. (A) As in Figure 2 but showing only a closeup of DBD region using mismatched DNA **1B**. (B) Plot of distal to proximal 5′-GG-3′ damage ratio versus concentration of M.*Hha*I mutants.

enzyme. This retention of distal damage with Trp237 supports the notion that decreased distal damage by the wild type enzyme arises as a result of a protein-induced π-stacking disruption rather than through competitive hole-trapping by the protein. In fact, Trp radical formation would be thermodynamically feasible but appears kinetically not to yield significant trapped products.[26] That retention of long-range damage in the Trp237 mutant was not due to failure of the protein to bind is supported by an intense piperidine-labile G-reaction at the *first* 5′-G contained within the 5′-**G**UGC-3′ site, as well as at the 3′-G (Figure 3). This enhanced 5′-G damage is unique to the tryptophan variant and relies upon the presence of Rh complex, light (365 nm) and piperidine digestion. Hence, this reaction provides another indication of intimate DNA−protein contact. This damage may be the result of a lowered oxidation potential for the 5′-G because of the inserted tryptophan to its 3′-side, as suggested by molecular modeling.[2] Importantly, this 5′-G damage as well as the retention of distal 5′-GG-3′ oxidation both demonstrate that DNA-binding proteins can also activate long-range electron transfer through DNA.

These results indicate that long-range oxidative damage to DNA is highly sensitive to protein-induced DNA distortions. Here for the first time we observe that protein binding can modulate electron-transfer chemistry in DNA. Not only can protein−DNA interactions serve to inhibit electron transfer through the π-stack, but such interactions can also activate oxidative damage to DNA. These experiments demonstrate the potential utility of DNA electron transfer as a useful diagnostic and lend support to the intriguing idea that DNA-mediated charge transport may play a role in cellular events.

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(26) $E° = 1.2$ V for the Trp cation radical,[27] comparable to the guanine cation radical.[4] Transient absorption studies of Lys-Trp-Lys bound to partially oxidized DNA duplexes indicate the formation of the Trp radical which decays on the ms time scale. Wagenknecht, H. A.; Stemp, E. D. A.; Barton, J. K., unpublished results.

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