Sensitivity of DNA-Mediated Electron Transfer to the Intervening π-Stack: A Probe for the Integrity of the DNA Base Stack

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Double-helical DNA, with its well-ordered array of π-stacked base pairs, may serve as a novel medium to facilitate long-range electron transfer.[1−7] Recently, we have demonstrated oxidative damage to DNA from a remote site through long-range hole migration.[7] These DNA-mediated electron transfer reactions are found to be exquisitely sensitive to stacking of donor and acceptor in the helix.[4,6,7] Here, we demonstrate the sensitivity of DNA-mediated hole transfer to the intervening DNA π-stack, and we utilize this DNA-mediated electron transfer chemistry to probe qualitatively the disruption in DNA base stacking.

Our approach uses DNA bulges to introduce perturbations into the DNA double-helical structure. DNA bulges, resulting from errors in recombination and replication, have been widely studied due to their role in frameshift mutagenesis.[8] DNA bulges reduce the thermodynamic stability of the helix,[9] and bases within the bulge become accessible to chemical modification.[10,11] Biophysical experiments show that bulges result in a rigid, globally bent structure.[11,12] NMR studies of oligonucleotides containing single nucleotide bulges have shown that bulged purines stack within the helix while pyrimidines either loop out or stack, depending on the sequence and temperature.[13] A high-resolution NMR structure of a DNA duplex containing an ATA-bulge showed continuous stacking of the bulged bases within the helix despite extensive bending of the DNA helix (50−60°) across the bulge.[14]

Figure 1 shows metal−oligonucleotide conjugates prepared to assess long-range electron (hole) transfer chemistry in bulged DNA. DNA duplexes containing a tethered rhodium complex,[7] two 5′-GG-3′ sites of potential oxidation,[15] as well as bulges of varying size were synthesized. Hole migration to guanine doublets was initiated by photoexcitation of the tethered $Rh(phi)_2(bpy')^{3+}$ (phi = 9,10 phenanthrenequinone diimine, bpy′ = 4′-methylbipyridine-4-butyric acid) at 365 nm. The yield of oxidative damage to guanine was examined by treatment of the 5′-^{32}P-labeled oligonucleotide reaction mixture with hot piperidine, resolution of the cleaved products was by denaturing polyacrylamide gel electrophoresis, and quantitation was by phosphorimagery.[16] Previous work had shown that the photooxidation is intraduplex and does not involve diffusible $^1O_2$.[7] Since oxidation of the distal 5′-GG-3′ doublets requires DNA-mediated hole transfer, the yield of damage at the 5′-GG-3′ doublet distal to the tethered rhodium complex relative to that at the proximal 5′-GG-3′ doublet provides a probe of π-stacking intervening the two sites. The proximal and distal 5′-GG-3′ doublets are 17 and 40 Å away, respectively, from the site of rhodium intercalation, but the yield of oxidative damage is found to be independent of distance over this range for the DNA duplex containing no intervening bulge.[7,17] It is important to note in this context that the through-space distance between the rhodium complex and the distal 5′-GG-3′ doublet actually decreases upon introduction of a bulge, due to the kinking of the helix, while the distance to the proximal 5′-GG-3′ remains unchanged.

The results are shown in Figure 2 and Table 1. In no case does the introduction of a bulge lead to increased oxidative damage at the distal site. Instead, the ratio of damage at the distal to proximal site decreases with perturbation to the intervening π-stack. The ATA-bulge has a dramatic effect on the oxidation of the distal 5′-GG-3′ doublet, leading to more than a 4-fold diminution in damage. A single A-bulge leads to a smaller but substantial diminution in damage at the remote 5′-GG-3′, while damage at the proximal 5′-GG-3′ remains strong. Across the series of A_n-bulges, the reduction in damage at the distal site is comparable (60% reduction).[19,20] It is noteworthy along this series that not only does damage decrease at the distal 5′-GG-3′ upon introduction of a DNA bulge, but damage also increases somewhat at the proximal 5′-GG-3′. This suggests that the two sites may be oxidized in competition with

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(20) Melting temperature for unmetalated duplexes, 4 μM in 25 mM NH_4OAc, pH = 9: no bulge, 64 °C; T-bulge, 55; TT-bulge, 52; TTT-bulge, 49; A-bulge, 55; AA-bulge, 53; AAA-bulge, 47.

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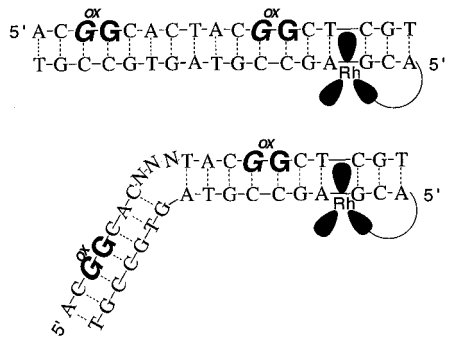

Figure 1. Electron transfer assemblies utilized to probe bulged DNA. Shown schematically are duplexes containing a tethered rhodium intercalator serving as a photooxidant and two 5′-GG-3′ doublets with various intervening bulges (-NNN-). Photoexcitation of $Rh(phi)_2(bpy')^{3+}$ at 365 nm generates a hole at the site of intercalation which can migrate to promote oxidation of the 5′-GG-3′ doublets. By comparing the yield of oxidative damage at the distal 5′-GG-3′, which has an intervening bulge, to that at the proximal 5′-GG-3′, the effect of the bulge on hole transfer can be determined.

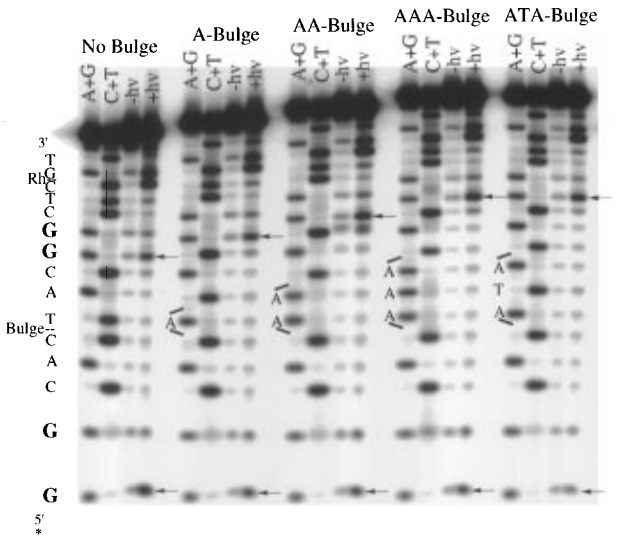

Figure 2. Comparison of the damage at the distal 5′-GG-3′ to the proximal 5′-GG-3′ as a function of the bulge size. The 5′-^{32}P-end-labeled bulge-containing strand as visualized by phosphorimagery after electrophoresis through a 20% denaturing polyacrylamide gel is shown. Shown in sets of four lanes from left to right is the duplex without a bulge, the duplex with a A-bulge, AA-bulge, AAA-bulge, and ATA-bulge, respectively. For each sequence is shown the Maxam−Gilbert A + G and C + T sequencing reactions, the oligonucleotide without irradiation treated with piperidine (−*hν*) and the duplex irradiated with 365 nm light for 1 h followed by treatment with piperidine (+*hν*). The predominant site of rhodium intercalation is shown near the top of the gel (Rh). The damage neighboring the intercalation site does not require piperidine treatment for strand breakage and results from direct abstraction of the C3′ hydrogen atom from the sugar residue.[21] The sites of DNA damage at the 5′-G of the 5′-GG-3′ doublets are indicated with arrows. The bulged bases are indicated next to the sequencing lanes. A 1000 W Hg/Xe lamp equipped with a monochromator was used to irradiate 20 μL samples containing 8 μM DNA duplex in 25 mM ammonium acetate buffer, pH = 9. Quantitation was performed by subtracting intensities for the control sample without irradiation from the intensity of damage of the photolyzed sample (see Table 1).

each other and not independently.[21] The structural distortions introduced by the bulge are local[14] and do not affect the inherent reactivity of either 5′-GG-3′ site as determined using noncovalently-bound rhodium complex (Table 1). In an assembly prepared with guanine preceding the bulge, we observed significant damage at this base; the nucleotide flanking the bulge at the defect boundary may be sensitive to oxidation. The consistent decrease in distal/proximal damage with an intervening bulge also demonstrates that this chemistry is mediated by the DNA stack rather than a result of a transient helix disruption or bend.

Table 1. Effect of Intervening Base Bulges on Long-Range Oxidative Damage

bulge[a]	ratio [distal/proximal][b]	bulge	ratio [distal/proximal]
Rh-none	1.2 (1)	Rh-A	0.5 (1)
Rh + none	1.3	Rh-AA	0.5 (1)
Rh-T	0.8 (2)	Rh-AAA	0.4 (1)
Rh-TT	0.7 (2)	Rh + AAA	1.3
Rh-TTT	0.5 (2)	Rh-ATA	0.3 (1)
		Rh + ATA	1.5

[a] Duplexes contained the sequence 5′-ACGGCAC**X**TACGGCTCGT-3′, where X is the single-stranded bulge shown (-NNN- in Figure 1). Conditions for photooxidation experiments were as described in Figure 2. Rh-X represents a covalent assembly and Rh + X represents the addition of rhodium complex to an unmodified duplex. [b] The ratio of damage at the distal 5′-GG-3′ to the proximal 5′-GG-3′ was determined for each bulged duplex. The standard deviations in parenthesis were determined on the basis of at least four experiments.

This electron (hole) transfer chemistry may provide a means to compare and contrast stacking within different oligomers. In the ATA-bulge, we see the largest diminution in damage at the distal site. The smaller reduction in damage across the A_n-bulges could reflect the larger surface of adenine compared to thymine.[22] Experiments on the T_{1-3} series of bulges (Table 1 and Supporting Information) indicate that the TTT-bulge shows an effect comparable in magnitude to that of the A_{1-3} series, but a single T-bulge has only a small effect on electron transfer.[23] This long-range oxidative chemistry may therefore serve as a qualitative probe of the integrity of stacking within the DNA helix;[24] as such, it is unique and distinct from chemical reagents that probe nucleic acids on the basis of solvent accessibility.

These results demonstrate that long-range electron (hole) transfer in DNA is sensitive to perturbations in the intervening base pair stack. This assay may prove to be a novel methodology in characterizing systems where the integrity of the π-stack is ill-defined and difficult to probe by other methods, such as in DNA−protein complexes.[25] This modulation of oxidative DNA damage by disruptions in base stacking may also suggest mechanisms for the protection of DNA sequences from long-range radical damage within the cell.

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Supporting Information Available: Quantitation of Figure 2 and phosphorimagery for the series of T-bulges (3 pages). See any current masthead page for ordering and Internet access information.

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(22) Since the NMR structure of the 5′-GATAG-3′ bulge shows that the flanking cytosines of the complementary strand are sheared apart and are not base stacked,[14] we propose that hole transfer occurs through the bulged strand which maintains some stacking.

(23) Gel mobility studies[11,12] have shown that purine bulges bend the DNA helix more than pyrimidine bulges and are consistent with NMR experiments[13] that have shown that single pyrimidine bulges are more likely to loop out of the helix. However, the reduction in oxidative damage is not a measurement of bend angle, *per se*, as shown by the similarity we observe between the A- and AAA-bulges which induce bends of 20−25° and 50−70°,[12] respectively. Instead, our assay reflects differences in the stacking through these bulges.

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