Mapping of the Hot Spots for DNA Damage by One-Electron Oxidation: Efficacy of GG Doublets and GGG Triplets as a Trap in Long-Range Hole Migration

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There has been much current interest in the long-range oxidative damage to DNA through the DNA duplex caused by one-electron oxidations.[1] Hole (radical cation) migration through the DNA duplex has been suggested to play a crucial role in mutagenesis and carcinogenesis caused by carcinogenic agents, ionizing radiation, and high-intensity laser irradiation.[1,2] As is well-known, guanine (G) is the most easily oxidized base,[3] and the electron-loss center created in duplex DNA ultimately ends up at G residues via hole migration through the DNA duplex. Several years ago, we demonstrated both experimentally and by ab initio calculations that 5′-G residues of 5′-GG-3′ steps in B-form DNA are the most easily oxidized due to the GG stacks and can act as thermodynamic sinks in hole migration across the DNA π stack.[4] We also demonstrated that the highest occupied molecular orbital (HOMO) of a GG stack is especially high in energy and concentrated on the 5′-G.[4b] Thereafter, examples of 5′-G selective oxidations have been reported in many different systems. These include (i) photooxidation using different types of DNA-binding agents such as Rh(III)-metallointercalators,[1a−c,f] substituted anthraquinones,[1d,e,5] riboflavin,[6] naphthalimide derivatives,[4] a *p*-cyano-substituted benzophenone,[7] and pterins;[8] (ii) chemical oxidation by Ru(III)-metallointercalators[1c,9] and Ni(II)-ligand/sulfite system;[10] (iii) two-photon photoionization of DNA with a high-intensity laser pulse (266 nm);[11] and (iv) direct irradiation with a powerful 193-nm excimer laser.[12] Notably, the 5′-G selectivity of 5′-GG-3′ steps is irrelevant to the structural features of the photosensitizers or the oxidizing agents, as is most typically exemplified by the two-photon photoionization of duplex DNA without any additive.[11]

Base radical cation, formed initially by one-electron oxidation, "hops" through the duplex DNA until it is localized at a GG step (low oxidation potential site) where the radical cation reacts irreversibly with molecular oxygen or water. It is therefore very important to know accurately the relative efficiency of various G- and GG-containing sequences that act as a trap in long-range hole migration through the DNA helix, since oxidation potentials of GG steps are strongly influenced by adjacent 3′- and 5′-base pairs. To execute precise mapping of such G-rich hot spots, we first examined the photoinduced one-electron oxidation of various G- and GG-containing DNA segments. Photoinduced DNA cleavage of double-stranded ^{32}P-end-labeled ODN 30-mers possessing two different G-containing sequences (5′-TXGYT-3′) and a 5′-TTGGT-3′ step as a standard ($k^{rel} = 1.0$) on the same strand has been carried out using riboflavin as an electron-accepting photosensitizer. A typical result of 5′-CGTACTCTTTGGTGGGTCGGTTCTTTCTAT-3′ is shown in Figure 1. Under the low conversion photoirradiation conditions, only the cleavage bands of 5′-Gs of the two GG steps and of the middle G of the GGG triplet were observed by hot piperidine treatment. Quantitative densitometric assay of the DNA cleavage bands in several lanes (lanes 2−8) provided the average values of the relative reactivity of each G-containing sequence. To obtain more accurate data, the positions of these two 5′TXGYT-3′ steps were exchanged with each other, but the position of standard 5′-TTGGT-3′ remained unchanged on an alternative ^{32} P-end-labeled ODN 30-mer, which was similarly photooxidized with riboflavin after annealing with its complementary strand. The average values of the relative efficiency of DNA cleavage in both runs were calculated, and the entire data set of the 5′-TXGYT-3′ sequences are listed in Table 1.[13] It is clear from Table 1 that the susceptibility of the G-containing sequences to photoinduced one-electron oxidation increases in the following order: GGG > CGG > AGG ≈ TGG > GGT > GGA > GGC > CGA ≈ AGA > TGA > AGT > AGC.[14] Pyrimidine-G-pyrimidine sequences such as TGT, TGC, CGT, and CGC are almost unreactive under the photoirradiation conditions.

We next examined the ab initio calculations of sixteen sets of base paired G- and GG-containing 5-mers at the HF/6-31G* level using GAUSSIAN94.[15] Double strands of B-form DNA with the 5′-TXGYT-3′ sequence were built using the Insight II program with standard B-form parameters. For quantum mechanical calculations, all of the sugar backbones of the duplex 5-mer were removed from the coordinate file, keeping the positions of all of the atoms fixed, and were replaced by methyl groups.[4b] Calculated ionization potentials (IPs) estimated by Koopmans' theorem are

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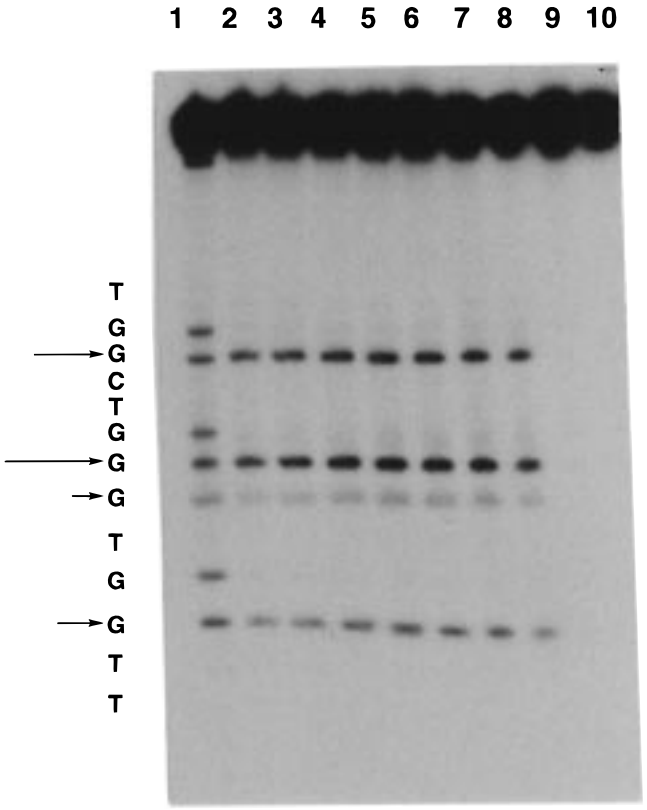

Figure 1. (a) Autoradiograms of a denaturing 12% polyacrylamide/7 M urea gel for the ^{32}P-5′ end-labeled ODN 5′-CGTACTCTTTGGTGGGTCG-GTTCTTTCTAT-3′ sequence after photooxidation of the duplex in the presence of riboflavin. The ^{32}P-5′-end-labeled ODN 30-mer was hybridized to its complementary strand (5.0 μM, strand concentration) in an aerated buffer of 10 mM sodium cacodylate at pH 7.0. Hybridization was achieved by heating the sample at 90 °C for 5 min and slowly cooling to room temperature. The ^{32}P-5′-end-labeled ODN duplex (2.0×10^5 cpm) containing riboflavin (50 μM) and calf thymus DNA (10 μM, base concentration) was irradiated at 366 nm with a transilluminator at 0 °C for 20 min. After piperidine treatment (90 °C, 20 min), the sample was dried and electrophoresed through a denaturing 12% polyacrylamide/7 M urea gel. (Lane 1) Maxam−Gilbert sequencing reactions G + A; (lanes 2−8) irradiated DNA in the presence of riboflavin; (lane 9) irradiated DNA with no riboflavin, irradiated at 366 nm for 20 min; (lane 10) DNA, dark control, no piperidine treatment. Relative reactivity of 5′-TXGYT-3′ sites was estimated from the average values of lanes 2−8.

Table 1. Relative Reactivity of 5′-TXGYT-3′ sequences in B-Form DNA toward Photoinduced One-Electron Oxidation[a]

X (5′ side)	Y (3′ side) G	A	T	C
G	TGGGT 2.7 ± 0.10	TGGAT 0.8 ± 0.02	TGGTT 0.9 ± 0.03	TGGCT 0.7 ± 0.03
A	TAGGT 1.0 ± 0.06	TAGAT 0.4 ± 0.20	TAGTT 0.2 ± 0.04	TAGCT 0.1 ± 0.04
T	TTGGT 1.0	TTGAT 0.3 ± 0.07	TTGTT n.d.[b]	TTGCT n.d.[b]
C	TCGGT 2.0 ± 0.20	TCGAT 0.4 ± 0.20	TCGTT n.d.[b]	TCGCT n.d.[b]

[a] 5′-^{32}P-end-labeled ODN 30-mers containing two 5′-TXGYT-3′ segments and a 5′-TTGGT-3′ site as a standard ($k^{rel} = 1.0$) were prepared. Riboflavin-sensitized photooxidation of these oligmers after annealing with complementary strands was conducted as described in Figure 1. The relative reactivity was estimated by the densitometric assay of all G bands resulting from each 5′-TXGYT-3′ segment under the low conversion photoirradiation conditions. [b] No data available.

shown in Table 2.[16] The calculated IPs are in the following order: GGG (6.34 eV) < CGG (6.44 eV) < AGG ≈ GGA (6.50 eV) < TGG (6.52 eV) < GGT (6.59 eV) < GGC ≈ CGA (6.63 eV) < AGA (6.73 eV) < TGA (6.76 eV) < CGT (6.91 eV) < AGT (6.93 eV) < CGC ≈ TGT (6.96 eV) < AGC (7.01 eV) < TGC (7.12 eV). Since the hole is trapped at lower oxidation potential sites on the DNA helix, hole-trapping efficiency in long-range DNA oxidation would follow the same order, i.e., GGG and CGG are deep hole traps (low oxidation potential sites) and AGG is a better hole trap than GGC. The calculated IPs are in fairly good agreement with the experimentally observed relative reactivity. A plot of the experimentally obtained relative reactivity (k^{rel}) versus calculated IPs is illustrated in Figure 2. A linear correlation between the log of the relative rate constants of one-electron oxidation and the calculated IPs has been obtained.

Table 2. Calculated Ionization Potentials (IPs) of Stacked Base Paired Deoxypentanucleotides 5′-TXGYT-3′ (eV)[a]

X (5′ side)	Y (3′ side) G	A	T	C
G	TGGGT 6.34	TGGAT 6.50	TGGTT 6.59	TGGCT 6.63
A	TAGGT 6.50	TAGAT 6.73	TAGTT 6.93	TAGCT 7.01
T	TTGGT 6.52	TTGAT 6.76	TTGTT 6.96	TGC[b] 7.12
C	TCGGT 6.44	TCGAT 6.63	CGT[b] 6.91	CGC[b] 6.96

[a] Ionization potentials were estimated by Koopmans' theorem. The values are the HOMO energies of HF/6-31G* single-point calculations. [b] IPs of the corresponding 5-mer sequences (e.g. TTGCT) could not be obtained accurately since the complementary strand (e.g. AACGA) has a lower IP. Therefore, only three base pairs (e.g. TGC/ACG) were calculated in these cases.

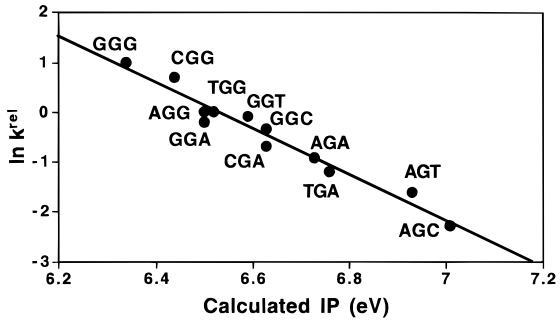

Figure 2. A plot of the log of the relative reactivity (k^{rel}) toward photoinduced one-electron oxidation versus calculated IP. The relative reactivity was obtained from the densitometric assay of G bands with TGG as a standard ($k^{rel} = 1.0$) under single-hit conditions.

The present experimental and calculated data provide a simple way to predict how different sequences of 5′-XGY-3′ alter the IPs and, as a result, the reactivity toward one-electron oxidation. These data are very useful in predicting the favorable sites for one-electron oxidation of DNA and in estimating the relative hole-trapping efficiency of a number of other G-containing sequences in long-range DNA oxidation when the hole is created by oxidizing agents, ionizing radiation, and high-intensity laser irradiation, although the DNA cleavage efficiencies do not necessarily correlate to the hole-trapping efficiencies. The present results shed light on the quantitative aspects of DNA oxidation and the effectiveness of GG-doublets and GGG-triplets as a trap in long-range hole migration.

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Supporting Information Available: Figures of autoradiograms of a denaturing gel showing the relative reactivity of G- and GG-containing seqments toward riboflavin-sensitized photooxidation and the autoradiogram for the photooxidation with other photosensitizers (6 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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