Photoinduced DNA Cleavage via Electron Transfer: Demonstration That Guanine Residues Located 5′ to Guanine Are the Most Electron-Donating Sites

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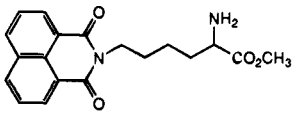
There has long been great interest in the one-electron oxidations occurring in DNA in connection with DNA damage caused by ionizing radiation, oxidizing agents, and photoirradiation with endogenous photosensitizers.[1] While long-range electron transfer between intercalated reagents through a DNA helix has recently been demonstrated in several systems,[2] only a few studies have appeared describing direct electron transfer from nucleobases in DNA,[3] although certain photoinduced DNA cleavage reactions are suggested to proceed via a one-electron transfer process.[1a,4] Here we report direct evidence for electron transfer from a guanine base in duplex deoxyoligonucleotides to an acceptor by means of laser flash photolysis and demonstrate that the most readily oxidizable sites in duplex DNA are the guanine (G) residues located 5′ to guanine, due to the π-stacking interaction of the two guanine bases.

In an effort to design a DNA-cleaving amino acid, we prepared water-soluble L-lysine derivative **1** possessing a naphthalimide chromophore.[5] Irradiation with 366 nm light in the presence of **1** induced a highly selective DNA cleavage at the 5′ side of 5′-GG-3′ steps of duplex DNA together with a lower yield cleavage at the 5′ side of 5′-GA-3′ steps.[5] In order to obtain more quantitative data on the −GG− selective photoreaction, we examined the photoreaction of **1** with oligodeoxynucleotides containing a −GG− step in the middle.

Irradiation of **1** and duplex hexamer $^{5'}T_1T_2G_3G_4T_5A_6$ (**2**)/$^{5'}$TACCAA (**3**) in sodium cacodylate buffer (pH 7.0) with a transilluminator (366 nm) at 0 °C followed by treatment with piperidine (90 °C, 20 min) and subsequent dephosphorylation with alkaline phosphatase (AP) produced TT and GTA as the major products together with small amounts of TTG and TA,



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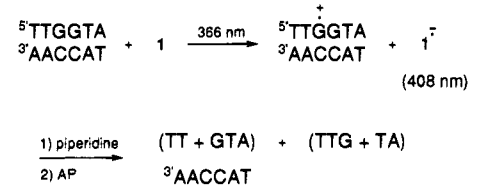
while the complementary strand **3** was unchanged (Scheme 1).

Scheme 1



The formation of TT and GTA from **2** implies that hexamer **2** was degraded by reacting with photoexcited **1** to give an alkali-labile site at G_3, whereas TTG and TA were derived from the photoreaction at G_4 as a minor pathway.[6] The G_3:G_4 ratio (84:16) determined by HPLC was not significantly changed whether the photoirradiation was conducted under aerobic conditions or under a nitrogen atmosphere. Riboflavin-sensitized photoreaction of duplex hexamer **2/3** under identical conditions exhibited a similar G_3 selectivity with a G_3:G_4 ratio of 74:26. It should be noted here that photoirradiation of **1** with single-stranded hexamer **2** alone resulted in a nonselective cleavage at G_3 and G_4 after piperidine treatment as already described[5] (Table 1).

The limiting quantum yield ($\phi = 3.0 \times 10^{-4}$) for the disappearance of **2** in duplex **2/3** in the presence of **1** was determined under anaerobic conditions using 350 nm light isolated with a monochromator, from the quantum yield as function of oligomer concentration.[7] Photoirradiation of **1** with other deoxyhexanucleotides containing a −GT− or a −GC− step in the middle, such as self-complementary duplex (TAC*G*-*TA*)$_2$, (AT*GC*AT)$_2$, and (TA*GC*TA)$_2$, under similar conditions resulted in no appreciable consumption of the hexamers, whereas (ATC*GA*T)$_2$ was reacted with a quantum yield of 1.5×10^{-4} to give ATC and AT almost quantitatively after piperidine and AP treatment. Photoreaction of **1** with duplex heptamer TT*GGG*TA (**4**)/TACCCAA (**5**) under the conditions proceeded more efficiently ($\phi = 5.2 \times 10^{-4}$) to give G_3- and G_4-cleavage products from **4.** Thus, the reactivity of G-containing duplex oligomers toward photoexcited **1** increased in the order, −GGG− > −GG− > −GA− ≫ −GC−, −GT−.

We then carried out *ab initio* calculations of lowest ionization potentials of stacked nucleobase models in a geometry for the standard B DNA structure at the 6-31G* level.[9] The calculated lowest ionization potentials of such stacked nucleobase models are in the following order: $^{5'}$GGG (7.07 eV) < $^{5'}$GG (7.28 eV) < $^{5'}$GA (7.51 eV) < $^{5'}$GC (7.68 eV) ~ $^{5'}$GT (7.69 eV) < G (7.75 eV). This order is in good agreement with the experimentally observed reactivity. Of special interest is that the HOMO of the stacked $^{5'}$GG molecule is largely localized on the G at the 5′ side, which is also compatible with the observed G_3/G_4 selectivity.

The laser flash photolysis studies of transient intermediates in the photoreaction of **1** with DNA oligomers were next

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(6) While the structures of several products obtained in 1-mediated photooxidation of 2′-deoxyguanosine still remain to be clarified, two major stable products other than 8-oxo-dGuo have recently been characterized in riboflavin-photosensitized one-electron oxidation of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine.[4f]

(7) Quantum yield measurements were carried out at 0 °C by using phenylglyoxylic acid ($\phi = 0.70$[8] at 334 nm in acetonitrile−water) as an actinometer.

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Table 1. Products Formed in the Photoreaction of **1** with Various Deoxyoligonucleotides Together with Quantum Yields[a]

run	oligonucleotide	product (μM)	selectivity	quantum yield[b]
1	TTGGTA (**2**)/TACCAA (**3**)	TT (37 μM), GTA (32 μM), TTG (7 μM), TA (11 μM)	G_3:G_4 = 84:16	
2	(**2**)/(**3**), riboflavin[c]	TT (17 μM), GTA (15 μM), TTG (6 μM), TA (9 μM)	G_3:G_4 = 74:26	
3	(**2**)/(**3**), under argon	TT (14 μM), GTA (14 μM), TTG (3 μM), TA (6 μM)	G_3:G_4 = 82:18	3.0×10^{-4} [b]
4	$(ATCGAT)_2$	ATC (6 μM), AT (5 μM)	100% selectivity at G_4	1.5×10^{-4}
5	TTGGGTA (**4**)/TACCCAA (**5**)	TT (19 μM), TTG (27 μM), GGTA + GTA (57 μM), TTGG (2 μM), TA (4 μM)	G_3:G_4:G_5 = 40:56:4	5.2×10^{-4}

[a] Each of the reaction mixtures (100 μL) containing oligomer (800 μM base concentration for each oligomer) and **1** (100 μM) in sodium cacodylate buffer (pH 7.0) was irradiated under aerobic conditions or under argon with a transilluminator (366 nm) for 120 or 180 min (run 3) at 0 °C. After irradiation the reaction mixture was treated with 1 M piperidine (90 °C, 20 min), digested with alkaline phosphatase, and then subjected to HPLC analysis. [b] The limiting quantum yield for the disappearance of oligonucleotides was determined at 350 nm under argon. [c] Riboflavin was used as a photosensitizer.

examined. Fortunately, the luminescence and transient properties of 1,8-naphthalimide derivatives have already been studied,[12] and the triplet–triplet absorption (λ_{max} 475 nm) and the radical anion (λ_{max} 415 nm, ϵ = 27 000 ± 2500 dm^3 mol^{-1} cm^{-1} in dry acetonitrile) of *N*-phenyl-1,8-naphthalimide have already been assigned by time-resolved spectroscopy.[12a] The nanosecond laser flash photolysis was then conducted with argon-purged solutions containing **1** and various donors.[13] The laser flash photolysis of **1** (0.1 mM) and DABCO (1.0 mM) in acetonitrile–water (1:1) resulted in the formation of the radical anion $\mathbf{1}^{\bullet-}$ (λ_{max} 408 nm) as a transient species which decayed on a time scale longer than that of the triplet (λ_{max} 475 nm) as previously reported.[12a] A very similar transient–time profile has been obtained in the laser flash photolysis of **1** in the presence of duplex hexamer **2**/**3** in acetonitrile–water at pH 7.0 (Figure 1). The growth of the absorption of $\mathbf{1}^{\bullet-}$ at 408 nm occurred exactly in the same time interval as did the triplet decay, implying that the triplet state is the precursor of the radical anion. The quenching rate constant of the triplet state

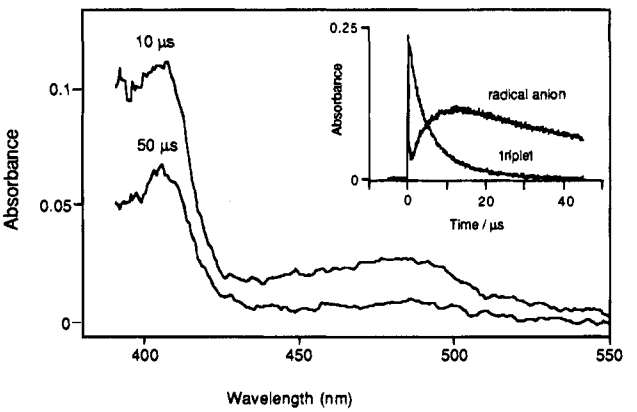

Figure 1. Transient absorbance spectra of the intermediate radical anion ($\mathbf{1}^{\bullet-}$) recorded at 10 and 50 μs delays after a 351 nm excimer laser pulse. [**1**] = 0.1 mM, [**2**] and [**3**] = 4 mM (strand concentration) in acetonitrile–water (1:1). Inset: Transient–time profiles following laser flash excitation. Triplet decay at 475 nm and the radical anion built up at 408 nm and decay.

of **1** by **2** giving $\mathbf{1}^{\bullet-}$ via electron transfer was estimated to be 5.3×10^7 M^{-1} s^{-1}. It is noteworthy that (i) the triplet state of **1** and radical anion $\mathbf{1}^{\bullet-}$ are the only transients detected on the time scale, and that (ii) $\mathbf{1}^{\bullet-}$ decayed according to second-order kinetics ($k_r = 5.6 \times 10^9$ M^{-1} s^{-1}). A similar result has also been obtained in the laser flash photolysis of **1** in the presence of calf thymus DNA.

In conclusion, electron transfer from a –GG– step in duplex DNA to triplet excited **1** was demonstrated for the first time by direct observation of the electron transfer intermediate. While the electron loss center created in DNA has long been known to ultimately end up at guanine due to its lowest ionization potential among the nucleobases,[1,3a] the present studies indicate that the most electron-donating sites in duplex DNA are the guanine residues located 5′ to guanine.

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(9) The ionization potentials were obtained from the HOMO energies of the stacked nucleobase models calculated on a Silicon Graphics IRIS Indigo R4000 using Spartan molecular modeling software (version 3.1) at the 6-31G* level. Geometries of the stacked nucleobase models were constructed as follows. The corresponding di- and trinucleotides were built up using the Insight II program with standard B form helical parameters (pitch, 3.38 Å; twist, 36°; tilt, −1°). All the sugar backbones of the di- and trinucleotides were removed except deoxyribose C1′ carbon and C1′ H. Two H atoms were then attached to the C1′ methine. Calculated lowest ionization potentials of four methylated nucleobases, 9-methylguanine (7.75 eV), 9-methyladenine (8.24 eV), 1-methylcytosine (8.87 eV), and 1-methylthymine (9.14 eV), were in good agreement with the experimentally observed ionization potentials of free bases.[10,11] Ionization potentials of guanine are 7.77 eV (adiabatic)[10] and 8.24 eV (vertical).[11] For *ab initio* calculations of free bases, see: Colson, A.-O.; Besler, B.; Close, D. M.; Sevilla, M. D. *J. Phys. Chem.* **1992**, *96*, 661 and references therein.

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