8-Methoxydeoxyguanosine as an Effective Precursor of 2-Aminoimidazolone, a Major Guanine Oxidation Product in One-Electron Oxidation of DNA

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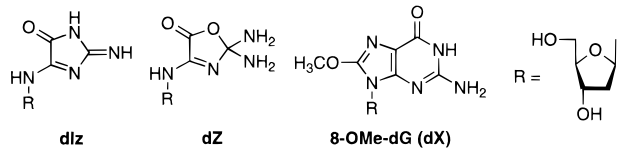
Guanine (G) base is a major target for oxidative DNA damage because it has the lowest oxidation potential among the DNA bases.[1] It has recently been demonstrated that the oxidative guanine damage induced by one-electron oxidations is concentrated on lower oxidation potential sites, such as stacked GG doublets and GGG triplets.[2] The damage is the consequence of oxidation of G to a guanine radical cation ($G^{\bullet+}$) that reacts further with water or molecular oxygen.[1c,3] It has long been known that 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodG) is one of the prominent 2′-deoxyguanosine (dG) decomposition products.[3,4] The 8-oxodG lesion is thought to be involved in mutagenesis, carcinogenesis, and aging.[4] Cadet et al. demonstrated that 2-aminoimidazolone (dIz) and its hydrolysis product oxazolone (dZ) are also major products of one-electron photooxidation of monomer dG.[5] More recently, we have demonstrated that dIz is actually a major isolable product from $G^{\bullet+}$ in the photooxidation of duplex oligodeoxynucleotides (ODN).[6] Efficient formation of dIz without forming 8-oxodG has also been reported in the oxidation of dG by Mn-TMPyP/$KHSO_5$.[7]

Long-range G oxidation through DNA base stack has attracted much attention in recent years, and oxidative G damage at a long distance has been observed with a range of tethered oxidants.[8] The G lesions are usually revealed by cleavage at G residues with piperidine treatment. Both dIz and dZ have been shown to be alkali-sensitive, resulting in a smooth strand cleavage at these sites,[5b,6a] whereas the 8-oxoG lesion was found not to be directly alkali labile.[5b,9] The products of further oxidation of 8-oxoG by available oxidants are suggested to be responsible for the piperidine-sensitive cleavage.[6a,9,10]

Chart 1



Having established that dIz-containing site is a major precursor of piperidine-sensitive cleavage in one-electron oxidation of DNA and that dIz is readily detectable by enzymatic digestion of oxidized ODN mixture followed by HPLC analysis,[5b,6] it is critically important to establish the preparation method of dIz-containing ODN at predetermined sites in order to investigate chemical and biological (mutagenesis, repair) features of this important oxidized DNA base lesion. The site-specific incorporation of dIz into ODN by conventional phosphoramidite method is precluded due to the high alkali liability of the lesion. The protocol for the preparation of ODNs containing dIz at desired sites has not yet been demonstrated.[11] We now report that (i) 8-methoxydeoxyguanosine (8-OMe-dG) is a very effective precursor for introducing dIz site-specifically into ODN and (ii) dIz in duplex is significantly stable as compared with monomer dIz, enough to survive for mutation under physiological conditions.

8-OMe-dG (dX) was prepared according to a slight modification of the reported procedure.[12] After photoirradiation of dX in the presence of riboflavin (Rf) for only 2 min, the peak of dX completely disappeared, to give a new peak which was spectroscopically identified as that of dIz.[13] The yield of dIz as determined by HPLC analysis was approximately 80%, while the yield of dIz from dG under the same conditions was below 30%, implying that the photooxidation of dX is much faster and cleaner. This is consistent with the oxidation potentials of dX ($E° = 1.08$ V vs Ag/AgCl) and dG ($E° = 1.33$ V vs Ag/AgCl) obtained by cyclic voltammetry.

dX-containing ODN **1**, 5′-d(TGACTGACXTACTGA)-3′, was prepared by the standard phosphoramidite method using Expedite phosphoramidite units. ODN **1** was purified by reversed-phase HPLC and characterized by MALDI-TOF MS.[14] After photoirradiation of ODN **1** for 2 min in the presence of Rf, a new peak close to that of ODN **1** appeared. Enzymatic digestion of the crude photooxidation mixture indicated that the dX residue was cleanly converted to dIz. Furthermore, the time course for the photooxidation of ODN **1** showed that dX was completely consumed to give rise to dIz almost quantitatively, whereas other nucleobases including G were not consumed under the same conditions (Figure 1). The formation of 8-oxodG has not been observed. These results indicate that dIz-containing DNA can be prepared postsynthetically from the corresponding dX-containing

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(13) HRMS (FAB, glycerol/water) calculated for $C_8H_{13}N_4O_4$ $[M + H]^+$ m/z 229.0936, found 229.0944; UV λ_{max} (H_2O) 252 nm.[5a,6a] See Supporting Information for HPLC profiles and spectral data of dIz.

(14) MALDI-TOF MS for ODN **1**: m/z 4620.68 (calcd for $[M - 1]^+$ 4621.07).

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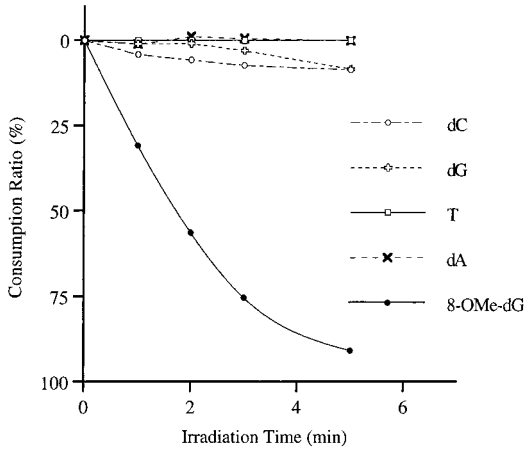

Figure 1. Time course for the photooxidation of ODN **1** in the presence of Rf as assayed by HPLC after enzymatic digestion. A reaction mixture containing ODN **1** (1.0 mM, base concentration) and Rf (50 μM) in 50 mM sodium cacodylate (pH 7.0) was photoirradiated at 366 nm at 4 °C under aerobic conditions. The photooxidized mixture of ODN **1** was digested with snake venom phosphodiesterase and alkaline phosphatase for 30 min at 25 °C.

DNA without decomposition of G residues by Rf-sensitized photooxidation.

We have measured the half-lives of dIz-containing ODN **2**, 5′-d(TGACTGACIzTACTGA)-3′, obtained by the photooxidation of ODN **1**, in the presence and absence of complementary strand.[15] Half-lives of dIz at 37 °C in single-stranded ODN **2** (16.8 h) and in the duplex (20.4 h) were significantly longer than that of monomer dIz (2.5 h),[5a] suggesting that dIz in cellular DNA is sufficiently stable to survive for mutation under physiological conditions.

We next examined the PAGE analysis of photoirradiated 5′-d(TCTTCTTGGTT*NN*TTG-TTCTTCTA)-3′ [*NN* = XG (ODN **3**), GX (ODN **4**), and GG (ODN **5**)]. ^{32}P-End-labeled ODNs **3**−**5** were photooxidized in the presence of Rf after annealing with the corresponding complementary strand (Figure 2a). Only photoirradiation (lane 3) or piperidine treatment (lane 4) did not induce DNA cleavage, whereas photoirradiation followed by piperidine treatment induced GG-specific cleavage (lane 5). As expected, 5′-XG-3′ (ODN **3**) and 5′-GX-3′ (ODN **4**) sites were more effectively photooxidized than the 5′-GG-3′ site (ODN **5**) to give 5′-IzG-3′ and 5′-GIz-3′ sites, respectively (lanes 6 and 7). This is the first demonstration of the preparation of pure ODN containing 5′-GIz-3′ which could hardly be obtained from direct photooxidation of 5′-GG-3′-containing ODN, implying that dX can serve as a very effective hole trap in one-electron oxidation of duplex DNA.

We previously reported the mapping of "GG hot spots" for DNA damage by one-electron photooxidation and estimated the relative hole-trapping efficiency of various dG-containing sequences.[16] Based on the previous result that the hole trap efficiency increased in the order GGG > CGG > AGG > TGG, we selected the 5′-CXG-3′ sequence as an effective hole trap. As shown in Figure 2b, dX of 5′-CXG-3′ sequence was highly

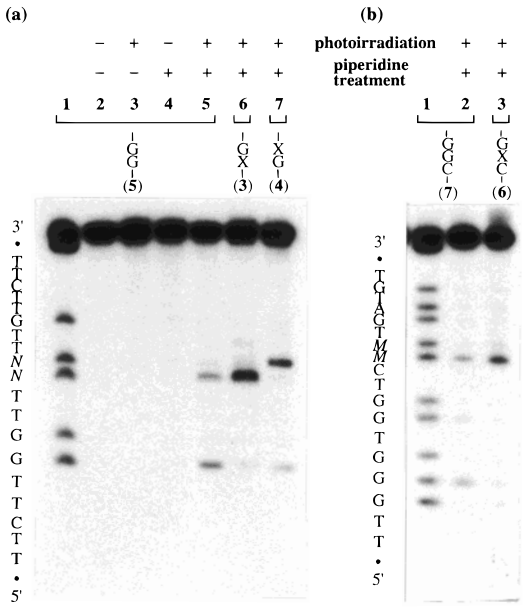

Figure 2. (a) Autoradiogram of ^{32}P 5′-end-labeled 24-mer ODNs, 5′-d(TCTTCTTGGTT*NN*TTG-TTCTTCTA)-3′ [*NN* = XG (ODN **3**), GX (ODN **4**), and GG (ODN **5**)]. Each sample solution, which contained ^{32}P 5′-end-labeled 24-mer ODN after annealing with complementary strand (5.0 μM, base concentration), calf thymus DNA (5.0 μM, base concentration), and Rf (50 μM), was photoirradiated at 366 nm at 4 °C for 20 min. After piperidine treatment, the sample was dried and subjected to electrophoresis on 8 M urea−15% polyacrylamide gel. (b) Autoradiogram of ^{32}P 5′-end-labeled 24-mer ODNs, 5′-d(TTTTGGGTGGTC*MM*TGATGTTTTA)-3′ [*MM* = XG (ODN **6**) and GG (ODN **7**)]. Each sample solution was photoirradiated under the same conditions as described above. Lane 1 was Maxam−Gilbert G + A reaction.

selectively photooxidized to give the cleavage band at this site (lane 3).[17] These results indicate that the incorporation of 5′-CXG-3′ sequence into DNA may lead to a selective oxidation of the dX site to give rise to a site-specific formation of dIz without significant damage on GG doublets and GGG triplets.

In summary, we have demonstrated that (i) dX is a very effective precursor for introducing dIz site-specifically into DNA and (ii) dX stacked with dG such as 5′-CXG-3′ may serve as a super hole trap in one-electron oxidation of duplex DNA. We previously proposed that dIz in a template DNA functions just like cytosine, indicating an intriguing possibility of G−C transversion.[6a] The present method for site-specific incorporation of dIz into DNA template would facilitate more intensive investigation on both chemical and biological features of this important oxidized DNA base lesion.

Supporting Information Available: ^{1}H and ^{13}C NMR, UV, and HRMS spectra of dIz, HPLC profiles of photooxidation of dX-containing ODN, proposed mechanism for the photooxidation of dX, HPLC analyses of enzymatic digestion mixtures of photoirradiated ODN **1**, table of half-lives, and autoradiograms of denaturing gels of ODNs **1**, **6**−**9** showing the effective hole trap (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) dIz-containing ODN **2** was used directly without further purification after the photooxidation of ODN **1**. ODN **2** annealed with equimolar complementary strand was used for double-stranded DNA.

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