Product Analysis of GG-Specific Photooxidation of DNA via Electron Transfer: 2-Aminoimidazolone as a Major Guanine Oxidation Product

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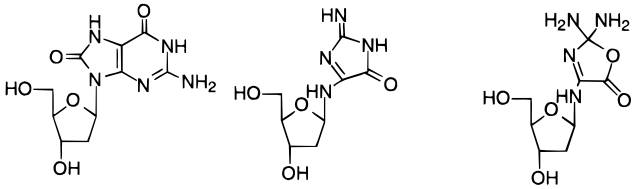
Considerable interest has recently arisen in the one-electron oxidations of DNA in connection with DNA damage caused by ionizing radiation, oxidizing agents, two-photon photoionization by a high-intensity laser pulse, and photoirradiation with photosensitizers.[1] We and others have found a common feature in that one-electron oxidation of DNA selectively generates piperidine-sensitive alkaline-labile sites at the 5′-guanine (G) of 5′-GG-3′ sequences.[2,3] 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoG) has been repeatedly claimed to be the major oxidation product ultimately leading to the piperidine-dependent G cleavage during the one-electron photooxidation of duplex DNA in the presence of various electron-accepting photosensitizers.[4] Recently, Cullis et al. have demonstrated that the 8-oxoG-containing site is not efficiently cleaved by hot piperidine treatment (90 °C, 20 min).[5] While the alkaline-labile sites have been suggested to be 2,2-diaminooxazolone (Z) and/or 2-aminoimidazolone (Iz) containing sites,[1f,5] which were already identified as the oxidation products of deoxyguanosine by Cadet et al.,[6] the direct evidence for the formation of these sites in double-strand DNA has not been documented.



To elucidate the real structure of the alkaline-labile site derived from the guanine cation radical, we have carried out the detailed product analysis of photoirradiated 5′-d(TTGGTA)-3′ and calf thymus DNA in the presence of riboflavin. We found that the Iz-containing oligomer is produced as a major isolatable product and the 8-oxoG-containing oligomer is only a minor product.

Figure 1a shows the reverse phase HPLC profile of photoirradiated single-stranded 5′-d(TTGGTA)-3′ in the presence of riboflavin, showing the formation of two major peaks (peaks 1 and 2) eluted at 35.1 and 37.9 min, respectively.[7] Prolonged low temperature digestion (0 °C, 15 h) of both products provided exactly the same HPLC profile showing the formation of dG, dT, dA, and Iz in a ratio of 1:1:3:1.[8] Electrospray mass spectra (1783, ESMS) confirmed that one G of 5′-d(TTGGTA)-3′ is oxidized to Iz in both products. Hot piperidine treatment (1 M, 90 °C, 20 min) of peak 1 gave TTGp and pTA, whereas the same treatment of peak 2 gave TTp and pGTA, indicating that the structures of peaks 1 and 2 were 5′-d(TTGIzGTA)-3′ (**1**) and 5′-d(TTIzGTA)-3′ (**2**), respectively. A similar procedure for the isolated peak 3 demonstrated that its structure is 5′-d(TTIzIzTA)-3′ (**3**). ESMS indicated that both peaks 4 and 5 have the same molecular weight of 1826. These were also observed in the photoirradiation of the 8-oxoG-containing hexamers 5′-d(TT^{8OXO}-GGTA)-3′ and 5′-d(TTG^{8OXO}GTA)-3′ in the presence of riboflavin. However, further characterization of these products has not been successful due to their thermal instability.

Figure 1b shows the HPLC profile of the riboflavin-sensitized photooxidation of 5′-d(TTGGTA)-3′ in the presence of the complementary oligomer 5′-d(ATACCAAA)-3′.[9] The results of the quantitative product analysis are summarized in Table 1. It

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(7) A reaction mixture (30 μL) containing 5′-d(TTGGTA)-3′ (1 mM base concentration) and riboflavin (50 μM) with or without complementary 5′-d(ATACCAAA)-3′ (1.33 mM base concentration) in 50 mM sodium cacodylate buffer (pH 7.0) was irradiated with a transilluminator (366 nm, Funakoshi 3UV) under aerobic conditions at 0 °C for 2 min from a distance of 6 cm. The reaction mixture was directly analyzed by HPLC. For enzymatic digestion the same photoreaction was repeated 10 times. After irradiation each peak was collected by HPLC and the combined fraction was desalted on a Cosmosil 5C18 column and lyophilized. For ESMS analysis the same photoreaction was repeated 5 times. After irradiation each peak was collected by HPLC and directly subjected to ESMS.

(8) The samples were digested with alkaline phosphatase (23 unit/mL), snake venom phosphodiesterase (0.07 unit/mL), and P1 nuclease (14 units/mL) at 0 °C for 15 h. Under the present conditions the decomposition of dIz was less than 10% as judged by HPLC. An authentic sample of dIz was prepared as reported and the structure was firmly confirmed by ^{1}H and ^{13}C NMR.[6b]

(9) Since product **1** and 5′-d(TACCAA)-3′ have the same retention time under the HPLC separation conditions, we used 5′-d(ATACCAAA)-3′ to obtain the partially duplex oligomer. The T_m value of the 5′-d(TACCAA)-3′ and 5′-d(ATACCAAA)-3′ duplex (8.3 μM each strand) in 50 mM sodium cacodylate buffer (pH 7.0) was 8.0 °C.

(10) Ab initio (6-31G*) calculations of the base pair system indicated that the stabilities of the Iz−G base pair and Watson−Crick G−C base pair are 18.1 and 20.4 kcal/mol, respectively.

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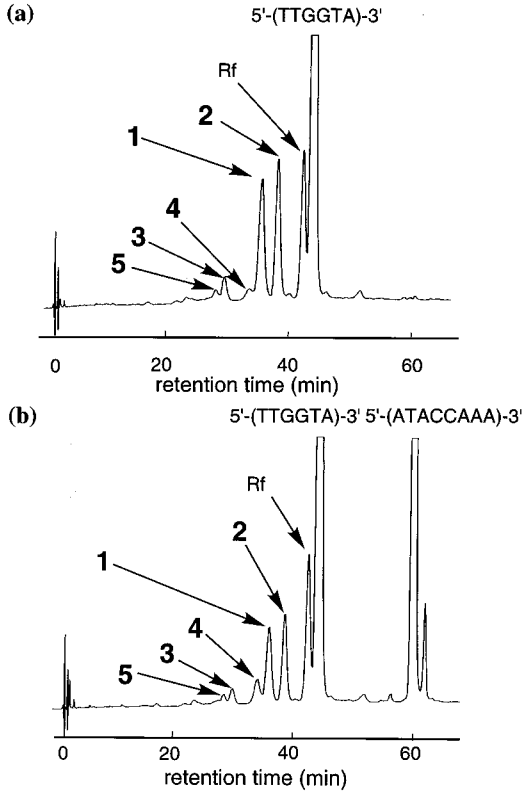

Figure 1. HPLC analysis of photoirradiated 5′-d(TTGGTA)-3′ with riboflavin (Rf) in the absence of 5′-d(ATACCAAA)-3′ (a) and the presence of 5′-d(ATACCAAA)-3′ (b). A reaction mixture containing 5′-d(TTGGTA)-3′ (1 mM base concentration) and riboflavin (50 μM) with or without complementary 5′-d(ATACCAAA)-3′ in sodium cacodylate buffer (pH 7.0) was irradiated at 366 nm under aerobic conditions at 0 °C for 2 min. The reaction mixture was analyzed by HPLC. Analysis was carried out on a CHEMCOBOND 5-ODS-H column (4.6 × 150 mm) (elution with a solvent mixture of 50 mM triethylammonium acetate, 7% (isocratic) acetonitrile/30 min, 7−9%/30−60 min, and 9% (isocratic)/60−65 min at a flow rate of 1.0 mL/min).

Table 1. Quantitative Analysis of Products Formed in Photoirradiated 5′-d(TTGGTA)-3′ with Riboflavin in the Absence and Presence of 5′-d(ATACCAAA)-3′

		product yield (%)[a]			
run	oligonucleotide	**1**	**2**	**3**	8-oxodG[b]
1	TTGGTA	20	19	1.9	0.2
2	TTGGTA/AAACCATA	14	16	5.1	0.4

[a] Yields were based on consumed hexamer. [b] The yield of 8-oxodG obtained by enzymatic digestion of the photolyzate was based on consumed deoxyguanosine.

was clearly demonstrated that irradiation of 5′-d(TTGGTA)-3′ at 366 nm with riboflavin produced Iz-containing oligomers **1** and **2** as major detectable products in both single- and double-stranded DNA. 8-OxoG-containing oligomers and their further oxidized products were minor products in both cases.

The formation of dIz was further confirmed by the photooxidation of calf thymus DNA in the presence of riboflavin. Figure 2 shows the quantitative analysis of the enzymatic digestion of photoirradiated calf thymus DNA. It was found that Iz is produced in approximately 25% yield based on the consumed guanine residue, whereas 8-oxoG was obtained only in one-fifth of Iz over the wide range of irradiation periods. These results clearly indicated that Iz is a major identified product of the guanine cation radical in the duplex DNA under atmospheric conditions.

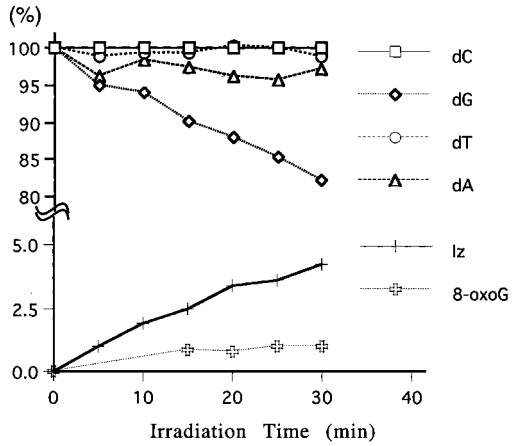

Figure 2. Time course of the photooxidation of calf thymus DNA in the presence of riboflavin. A reaction mixture containing sonicated calf thymus DNA (1 mM base concentration) and riboflavin (50 μM) in sodium cacodylate buffer (pH 7.0) was irradiated with a transilluminator (366 nm) under aerobic conditions at 0 °C for 0, 5, 10, 15, 20, 25, and 30 min from a distance of 6 cm. The irradiated mixtures were subjected to low-temparature enzymatic digestion.[8] The digested sample was analyzed by HPLC. Analysis was carried out on Cosmosil 5C18-MS column (4.6 × 150 mm) (elution with a solvent mixture of 50 mM ammonium formate, 0% (isocratic) acetonitrile/5 min, 0−7%/5−27 min, and 7% (isocratic)/27−30 min at a flow rate of 1.0 mL/min).

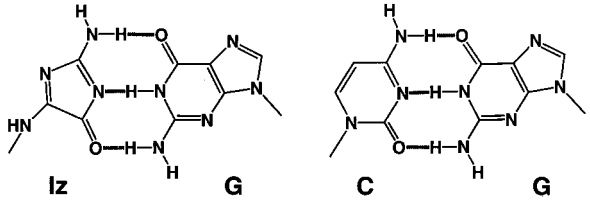

Figure 3. Proposed Iz−G base pair and Watson−Crick C−G base pair.

Preliminary molecular orbital calculations indicated that the Iz−G base pair possesses a stability comparable with the Watson−Crick G−C base pair (Figure 3).[10] These results indicate an intriguing possibility that Iz in a template DNA functions such as C. In fact, G−C transversion has been reported in photosensitizations, γ irradiations, and the Fenton reactions of DNA.[11] Further work to examine this possibility is in progress.

Supporting Information Available: Enzymatic digestion profiles of the photoproduct **1**−**5** and photooxidized calf thymus DNA with riboflavin and ESMS of **1**−**5** (6 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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