Direct Spectroscopic Observation of 8-Oxo-7,8-dihydro-2′-deoxyguanosine Radicals in Double-Stranded DNA Generated by One-Electron Oxidation at a Distance by 2-Aminopurine Radicals

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Oligonucleotide duplexes (15-mers) containing 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) and the base analogue 2-aminopurine (2AP) separated from one another by zero, two, or four thymidine residues on the same strand, were synthesized. Selective two-photon excitation of 2AP with intense 308 nm XeCl excimer laser pulses (fwhm = 12 ns, ∼80 mJ pulse^{-1} cm^{-2}) gives rise to 2AP radical cations that, in turn, oxidize 8-oxo-dG at a distance. Transient absorption techniques allowed for the direct monitoring of the oxidant and product radical intermediates as a function of time on the 100 ns−1 ms time scale and revealed the existence of two-component oxidation kinetics. The evolution in time of the rapid component (<100 ns), attributed to the oxidation of 8-oxo-dG by the radical cation 2AP•$^+$, was not resolved. However, this component was observed only in the case of zero or two intervening thymidine bases and was not evident when the 2AP and 8-oxo-dG residues were separated by four thymidines. A slower kinetic component (>100 ns) is attributed to the oxidation of 8-oxo-dG by the deprotonation product of 2AP•$^+$, the neutral radical 2AP(−H)•. Only the slow, microsecond kinetic component was observed when the 8-oxo-dG and 2AP are separated by four thymidines. The rate constants of electron transfer are (3.8 ± 0.5) × 10^4 s^{-1} and (3.0 ± 0.5) × 10^3 s^{-1} in the case of two and four intervening thymidines, respectively. Because of the pK_a properties of the donor/acceptor couples, this slow, 0.1−1000 μs time scale electron transfer at a distance from 8-oxo-dG to the 2AP(−H)• radicals, is likely to occur via a proton-coupled electron-transfer mechanism.

1. Introduction

Oxidative damage to DNA can be induced by ionizing radiation, photosensitization, or various strong oxidants, giving rise to a variety of DNA lesions (oxidized nucleic acid bases).[1,2] Many of these lesions can be visualized by inducing DNA strand cleavage by either enzymatic or hot alkali treatment (e.g., hot piperidine). One of the most thoroughly studied DNA lesions is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) which can be generated by either hydroxyl radicals,[3,4] singlet oxygen,[5] or one-electron oxidation.[6] However, 8-oxo-dG itself is not hot piperidine-labile, and further oxidation of this lesion is required in order to observe hot alkali-induced strand cleavage.[7] Transformation of the 8-oxo-dG lesion into an alkali-labile site can also be achieved by ionizing radiation,[8] singlet oxygen,[9] and treatment of the damaged DNA with various oxidants.[9−14] Selective oxidation of 8-oxo-dG lesions in DNA is consistent with recent computational[15,16] and experimental[9−11] studies, which have shown that 8-oxo-dG is more easily oxidized than the natural nucleic acid bases (A, C, T, and G) or a guanine present in GG or GGG sequences. Consistent with these observations, 8-oxo-dG is considered to be a deep trap for charge carriers, e.g., mobile holes in DNA, and thus interrupts the migration of oxidative damage in DNA.[17−19]

The further oxidation of 8-oxo-dG initiated by electron abstraction, triggers a cascade of consecutive oxidation reactions thus generating intermediates which are more easily oxidizable than 8-oxo-dG itself.[9−14,20,21] The mechanisms of oxidation of 8-oxo-dG and the characteristics of the intermediates formed are of significant interest. It has been postulated that the 8-oxo-dG•$^+$ radical cation is the primary product of the one-electron oxidation of 8-oxo-dG.[8,9,22,23] Recently, utilizing pulse radiolysis technique, Steenken et al.[24] have shown that the 8-oxo-dG•$^+$ radical cation can be prepared by the oxidation of 8-oxo-dG by various one-electron oxidants. In neutral aqueous solutions, the 8-oxo-dG•$^+$ radical cation (pK_a = 6.6) is in equilibrium with its deprotonated, neutral form, 8-oxo-dG(−H)•. The redox potential of the 8-oxo-dG radical species, determined from electron-transfer equilibria involving known reference redox couples by pulse radiolysis techniques, is E_7 = 0.74 V vs NHE. This is much lower than the value[25] of E_7 = 1.29 V vs NHE for 2′-deoxyguanosine (dG). However, the existence of an 8-oxo-dG one-electron oxidation product has not yet been demonstrated directly, and the lifetime of the resulting radical in double-stranded DNA is not known. Furthermore, the kinetics of one-electron oxidation of 8-oxo-dG by an oxidant species also incorporated within a DNA molecule, but at some distance from 8-oxo-dG, have not been characterized.

To pursue these questions, we synthesized 15-mer double-stranded oligonucleotides containing the DNA base analogue 2-aminopurine (2AP) with an 8-oxo-dG positioned zero, two,

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or four bases away (intervening thymidines) on the same strand. The complementary strand had a T and a C opposite the 2AP and 8-oxo-dG, respectively. Excitation of these oligonucleotides with intense 308 nm XeCl excimer laser pulses results in the site-selective two-photon photoionization of the 2AP residues.[26−29] Transient absorption spectroscopy techniques have been employed to monitor the yields and kinetics of oxidation of 8-oxo-dG at a distance by the photoionized 2AP residues in these 15-mer DNA duplexes. It is shown that the kinetics of one-electron oxidation of 8-oxo-dG in double-stranded DNA can extend into the 0.1−1000 μs time scale when the 2-AP radical and 8-oxo-dG are separated by four thymidines and the oxidant is the deprotonated form of the 2AP•$^+$ radical cation, the neutral radical 2AP(−H)•. When the separation between the two species is smaller (zero or two Ts), a prompt, distance-dependent yield (<100 ns) of 8-oxo-dG radicals is also observed. The rates of 8-oxo-dG oxidation by 2AP(−H)• neutral radicals are larger by factors of 5−10 than the rates of oxidation of GG doublets in 15-mer double-stranded oligonucleotides of the same design.[29]

2. Experimental Section

Materials. The site-specifically modified oligonucleotides containing 8-oxo-dG and 2AP and the complementary 15-mer oligonucleotides (with T opposite 2AP and C opposite 8-oxo-dG bases) were synthesized in ~2 μmol quantities by phosphoramidite chemistry. The tritylated oligonucleotides were removed from the solid support and deprotected with concentrated ammonium hydroxide of 0.25 M β-mercaptoethanol to prevent further oxidation of 8-oxo-dG. The crude oligonuclotides were purified by reversed-phase HPLC, detritylated in 80% acetic acid according to standard protocols, and characterized by ESI-MS and UV spectroscopy.

The 2AP-modified oligonucleotides and the complementary strands (10 mol % excess over the concentration of the 2AP-modified oligonucleotide) were dissolved in 20 mM phosphate buffer, pH 7, containing 100 mM NaCl. Annealing of the two strands was accomplished by heating the samples to 80 °C for about 10 min, and then allowing the samples to cool slowly back to room temperature overnight.

Routine UV absorption spectra and UV melting profiles were measured on an HP 84453 diode array spectrophotometer with an HP 89090A Peltier temperature control unit (Hewlett-Packard GMBH, Waldbronn, Germany).

Laser Flash Photolysis. The transient absorption spectra were recorded on a kinetic spectrometer system (~7 ns response time) described earlier.[26] Briefly, this system consists of a pulsed Lambda Physik EMG 160 MSC XeCl excimer laser (308 nm, fwhm = 12 ns, ~80 mJ pulse^{-1} cm^{-2}, 0.1 Hz) or a Q-switched NY81-20 Nd:YAG laser (266 nm, fwhm ≈ 6 ns, ~200 mJ pulse^{-1} cm^{-2}, 20 Hz) excitation sources, and a 75 W xenon pulsed lamp to probe transient absorption kinetics. The probe flash was passed through a McPherson monochromator and monitored by a Hamamatsu R928 phomultiplier; its output was recorded at 5 nm intervals and was digitized by use of a Tektronix TDS 620 oscilloscope. All experiments, including data collection and analysis, were controlled by a computer.

The double-stranded oligonucleotide sample solutions were saturated with oxygen. By use of a positive O_2 pressure, a 0.5 mL quartz flow cell with an optical path length of 1 cm aligned parallel to the direction of the probe light beam was filled with the sample solution. After 10 shots of the excimer laser, the irradiated sample solution was automatically replaced by a fresh portion of the sample solution by use of a computer-controlled Teflon solenoid valve (Parker Hannifin Corp., Fairfield, NJ).

TABLE 1: Melting Points of Duplexes (T_m), Experimental Rate Constants (k_1), and Rate Constants of Oxidation of 8-Oxo-dG by 2AP(-H)• (k_{ag})

sequence[a]	T_m (°C) [h (%)][b]	k_1[c] (s^{-1})	k_{ag}[d] (s^{-1})
[2AP][8-oxo-dG]T$_{13}$	37.6 ± 0.7 [30]	>10^7	
[2AP]T$_2$[8-oxo-dG]T$_{11}$	36.0 ± 0.6 [27]	(4.0 ± 0.5) × 10^4	(3.8 ± 0.5) × 10^4
[2AP]T$_4$[8-oxo-dG]T$_9$	35.8 ± 0.8 [28]	(5.0 ± 0.5) × 10^3	(3.0 ± 0.5) × 10^3

[a] Oligodeoxyribonucleotide sequences are written in the 5′ → 3′ direction. [b] The average values of melting points, T_m, and the hyperchromicity, h, were obtained from absorbance measurements at 260 nm during heating−cooling cycles. Melting points were determined on 5 μM solutions of duplexes. [c] The experimental rate constant k_1 was taken from eq 2 and was determined for 50 μM double-stranded oligonucleotides. [d] $k_{ag} = k_1 - k_a$. The value of $k_a = 2 \times 10^3$ s^{-1} was taken from ref 29.

The concentrations of the 2AP duplex were generally ~50 μM. All laser flash photolysis experiments were performed at 20 °C.

3. Results and Discussion

Oligonucleotides Site-Specifically Modified with 2AP and 8-Oxo-dG Bases: General Aspects. Three oligonucleotides (**1**−**3**), containing one 2AP base and 8-oxo-dG adjacent to the 2AP (**1**) or separated by two (**2**) or four (**3**) thymidine bases, were used in the double-stranded form in complexes with their complementary strands, with T opposite 2AP and C opposite 8-oxo-dG bases. In all of these oligonucleotides the 2AP residues were positioned at the 5′-ends, because fraying of the duplexes at the ends allows some dynamic motion, thus enhancing the lifetime of the excited singlet state, ^{1}2AP. The efficiency of 2AP photoionization[26−29] is thus enhanced because it occurs via the absorption of a second photon by ^{1}2AP, i.e., consecutive two-photon excitation process (see below). In double-stranded oligonucleotides with 2AP embedded near the middle of the duplexes,[26] the yields of 2AP radical were too low for kinetic measurements (data not shown). The following oligonucleotides, paired with their complementary strands (with T and C opposite 2AP and 8-oxo-dG, respectively), were employed:

(**1**) 5′-[2AP][8-oxo-dG]TTTTTTTTTTTTT-3′
([2AP][8-oxo-dG]T$_{13}$)

(**2**) 5′-[2AP]TT[8-oxo-dG]TTTTTTTTTTT-3′
([2AP]T$_2$[8-oxo-dG]T$_{11}$)

(**3**) 5′-[2AP]TTTT[8-oxo-dG]TTTTTTTTT-3′
([2AP]T$_4$[8-oxo-dG]T$_9$)

These duplexes, dissolved in 20 mM phosphate buffer solutions, pH 7, containing 100 mM NaCl, exhibit well-defined cooperative melting behavior during heating−cooling cycles (20−60−20 °C), with typical melting temperatures T_m = 35−37 °C (Table 1). These are smaller than the values of T_m = 42−43 °C for similar 15-mer duplexes[29] with one GG doublet instead of the [8-oxo-dG]T dinucleotide sequence.

The absorption spectra of the aqueous solutions of the oligonucleotides (**1**−**3**) exhibit a characteristic shoulder in the spectral range of 300−330 nm due to absorption of 2AP (λ_{max} = 305 nm[30]) and 8-oxo-dG (λ_{max} = 295 nm[31]). In contrast, the oligonucleotides with the normal DNA bases (A, C, G, and

T) are characterized by negligible absorbances at wavelengths ≥300 nm. This particular feature of the absorption spectra of 2AP-modified oligonucleotides provides the site-selective photoionization of 2AP induced by 308 nm XeCl excimer laser pulse excitation.[26−29] In the case of the oligonucleotides (**1**−**3**), the 308 nm excitation wavelength also overlaps with a weak absorption band of 8-oxo-dG; however, as shown below, this feature does not impact on the results of the studies reported here (see the next section). Before discussing in more detail the consequences of exciting the 2AP and 8-oxo-dG-containing oligonucleotides with 308 nm excimer laser pulses, the effects of intense UV laser pulse excitation on free 8-oxo-dG in aqueous solutions are described.

Two-Photon Photoionization of 8-oxo-dG in Aqueous Solutions. Recently, Steenken et al.,[24] using pulse radiolysis techniques, published the transient absorption spectrum of 8-oxo-dG in aqueous solution. Here we show that the identical transient absorption spectrum, attributable to the 8-oxo-dG radical, is observable by two-photon excitation of 8-oxo-dG with intense 266 nm laser pulses.

The photoexcitation of 8-oxo-dG in aqueous buffer solutions (pH 7) with intense nanosecond 266 nm Nd:YAG laser pulses (∼200 mJ pulse^{-1} cm^{-2}) results in the photoionization of the 8-oxo-dG aromatic residues. The photoionization of 8-oxo-dG was clearly evident from the prompt appearance of the absorption band of hydrated electron, e_h^-, characterized by a broad maximum near 700 nm (data not shown). Other absorption bands in the 300−500 nm spectral range due to 8-oxo-dG radicals were also observed. The yield of e_h^- depended on the laser power in a superlinear manner (data not shown), which is indicative of a consecutive two-photon ionization of 8-oxo-dG at the laser pulse fluence rates utilized.[26,32−35] Absorption of the first photon results in the formation of the 8-oxo-dG excited state, and the absorption of the second photon by *8-oxo-dG (singlet or triplet excited state) causes photoionization:

$$\text{8-oxo-dG} \xrightarrow{h\nu} {}^*\text{8-oxo-dG} \xrightarrow{h\nu} \text{8-oxo-dG}\bullet^+ + e_h^- \qquad (1)$$

In oxygen-saturated solutions, the hydrated electrons, e_h^-, that are formed decay within ∼200 ns after a laser pulse.[26] The 8-oxo-dG radicals decay on millisecond time scales, and their lifetimes were found to be insensitive to the absence or presence of O_2 in aqueous solutions (∼1 atm partial pressure). Thus the 8-oxo-dG radicals remain the sole transient species that decay, like other purine radicals,[36] e.g., dG(−H)•, on millisecond time scales.[37]

A typical transient absorption spectrum of 8-oxo-dG radicals in oxygen-saturated aqueous solutions (pH 7), recorded at a delay time of $\Delta t = 10\ \mu$s after the laser flash, is shown in Figure 1 (trace 1). This spectrum is characterized by a narrow absorption band with a maximum at 320 nm and a shoulder near 400 nm. For comparison, the transient absorption spectrum resulting from the photoionization of 2′-deoxyguanosine 5′-monophosphate (dGMP), generated under identical conditions of laser excitation is also shown in Figure 1 (trace 3). In good agreement with the literature data,[24,25,39] except for the ∼10 nm red shift, the transient absorption spectrum of the 8-oxo-dG radical (trace 1) is similar to the normalized spectrum of the dGMP(−H)• neutral radicals (trace 3). Like the dGMP•$^+$ radical cation (pK_r = 3.9) which rapidly (∼0.5 μs) dissociates at pH 7 to form the neutral radical dGMP(−H)•,[39] the 8-oxo-dG•$^+$ radical cation partially deprotonates to form the neutral 8-oxo-dG(−H)• radical.[24] The pK_a of 8-oxo-dG•$^+$ is equal to 6.6 and, at pH 7, the contribution of the 8-oxo-dG(−H)• form is ∼70%.

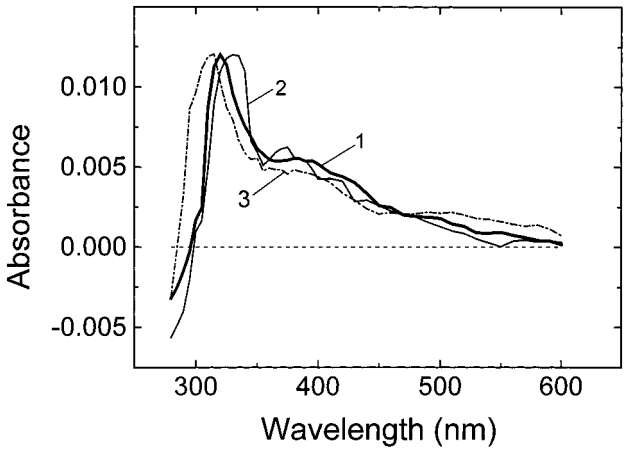

Figure 1. Transient absorption spectra of 8-oxo-dG, or dGMP in *oxygenated* aqueous solutions measured at a delay time, $\Delta t = 10\ \mu$s, after excitation with 266 nm Nd:Yag laser pulses (∼200 mJ pulse^{-1} cm^{-2}, fwhm ≈ 6 ns, 20 Hz). The spectra were normalized to one another at their respective maxima. Trace 1, [8-oxo-dG] = 0.1 mM, pH 7; trace 2, [8-oxo-dG] = 0.1 mM, pH 2; trace 3, [dGMP] = 0.1 mM, pH 7.

Here, we denote the 8-oxo-dG radical species as 8-oxo-dG•$^+$/8-oxo-dG(−H)• in view of the equilibrium between the protonated and unprotonated forms at pH 7. In support of this concept, it is shown that the normalized spectrum of the 8-oxo-dG intermediate generated at pH 2 (Figure 1, trace 2) is red-shifted with respect to the spectrum measured at pH 7 (trace 1).[24] In contrast to excitation at 266 nm, the photoexcitation of 8-oxo-dG in aqueous buffer solutions (pH 7) with intense nanosecond 308 nm XeCl excimer laser pulses (∼80 mJ pulse^{-1} cm^{-2}) does not induce photoionization of 8-oxo-dG. The lack of the 8-oxo-dG photoionization at the laser fluences used was clearly evident from the absence of hydrated electrons and other absorption bands in the 300−500 nm spectral range that could have been due to 8-oxo-dG radicals. This particularly favorable feature of 8-oxo-dG opens the opportunity for studies of the site-specific photoionization of 2AP bases in oligonucleotides containing both 2AP and 8-oxo-dG bases with intense nanosecond 308 nm XeCl excimer laser pulses.

Transient Absorption Spectra of Double-Stranded Oligonucleotides Containing Single 8-Oxo-dG and 2AP Bases. In air-saturated aqueous solutions, the hydrated electrons released by the site-selective photoionization of the 2AP residues are rapidly scavenged by oxygen,[40] and the 2AP radicals are sufficiently long-lived to cause the oxidation of 8-oxo-dG at a distance in the oligonucleotides (**1**−**3**). Other nucleic acid bases are not directly involved in this reaction at a distance, because our previous experiments with d([2AP]T_{14})·d(A_{14}T) have shown that 2AP radicals do not react with or oxidize T and A bases.[29]

The transient absorption spectra of the 2AP radicals are characterized by a bleaching of the 2AP band at 310 nm, and the appearance of the absorption bands at 360 and 510 nm.[26−29] In contrast, the 8-oxo-dG•$^+$/8-oxo-dG(−H)• radical species exhibit the characteristic narrow absorption band at 320 nm (trace 1, Figure 1). Therefore, the time dependence of the transient absorption spectra of the oxidized forms of 8-oxo-dG and 2AP residues in the oligonucleotides provide evidence for the distance-dependent electron-transfer reactions between these two species in double-stranded DNA.

The transient absorption spectra of the double-stranded oligonucleotides (**1**−**3**) recorded at various time intervals Δt after the actinic laser flashes are summarized in Figure 2. The scattered light from the laser pulses effectively prevented

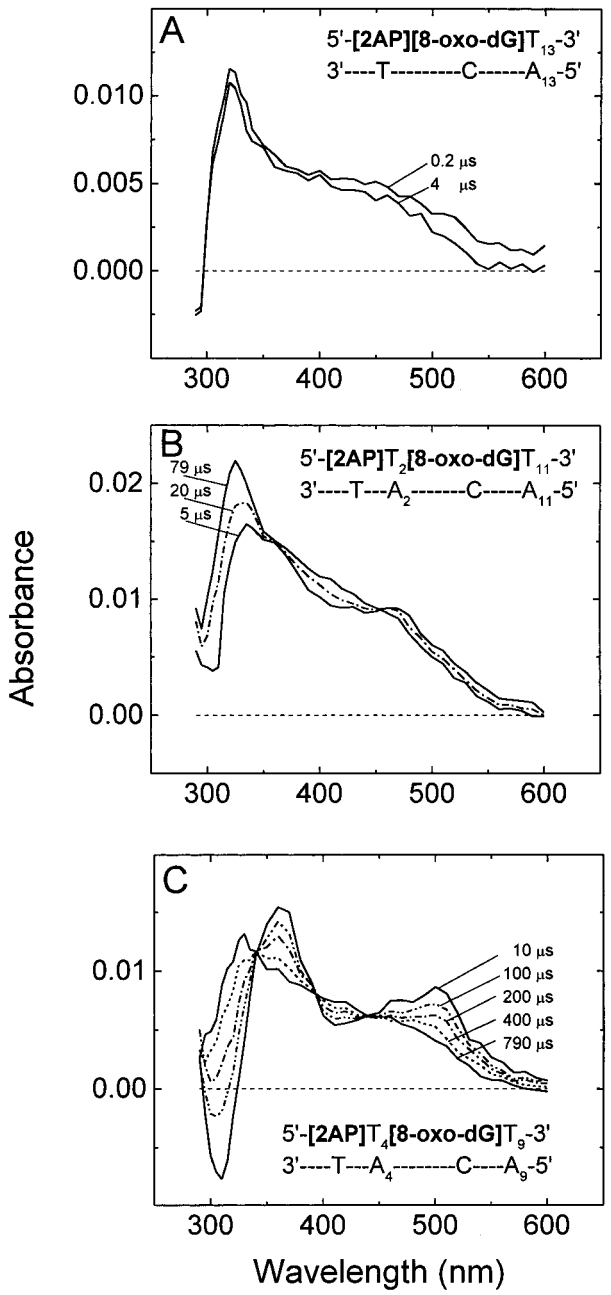

Figure 2. Transient absorption spectra of double-stranded oligonucleotides (50 μM) in *oxygenated* 20 mM phosphate buffer (pH 7) solutions measured at different delay times, Δt, after the 308 nm XeCl excimer laser pulse excitation (80 mJ pulse^{-1} cm^{-2}, fwhm $\approx$ 12 ns, 0.1 Hz).

measurements of the transient absorption spectra or kinetics at short time intervals (Δt < 100 ns).

The transient absorption spectra of the double-stranded oligonucleotide [2AP][8-oxo-dG]T_{13} recorded at delay times Δt = 200 ns and 4 μs are characterized by a strong narrow absorption band at 320 nm and a shoulder near 400 nm (Figure 2A). These absorption spectra are characteristic of the 8-oxo-dG•$^+$/8-oxo-dG(−H)• radical species (trace 1, Figure 1). The absorbance in the region of 350−600 nm recorded at Δt = 0.2 μs is somewhat higher than at Δt = 4 μs (Figure 2A). This difference is attributed to the tail of the e_h^- transient absorbance,[29] which almost completely decays within ~200 ns. Thus, in the double-stranded oligonucleotide [2AP][8-oxo-dG]T_{13} with the 2AP and 8-oxo-dG bases adjacent to one another, the transient absorption spectra indicate that electron transfer from 8-oxo-dG occurs on a time scales faster than the effective time resolution of our experiment (~100 ns). This result is in agreement with our previous experiments with 15-mer double-stranded oligonucleotide[29] [2AP]GGT_{12} containing the GG doublet instead of 8-oxo-dG adjacent to 2AP. It is important to note that the absorbance at 320 nm, attributed to the 8-oxo-dG•$^+$/8-oxo-dG(−H)• radical species, is similar in the 0.2 and 4 μs spectra (Figure 2A), indicating that oxidation of 8-oxo-dG indeed occurs on time scales <100 ns and that there is no additional formation of oxidized guanine products on the longer time scales.

The transient absorption spectra of the double-stranded oligonucleotide [2AP]T_2[8-oxo-dG]T_{11} recorded at delay times of 5, 20, and 79 μs are shown in panel B (Figure 2). In contrast to [2AP][8-oxo-dG]T_{13}, in which the full concentration of 8-oxo-dG•$^+$/8-oxo-dG(−H)• develops within <100 ns (panel A), in the case of [2AP]T_2[8-oxo-dG]T_{11} the transient absorbance at 320 nm continues to increase in the Δt = 5−79 μs range (panel B). In [2AP]T_2[8-oxo-dG]T_{11}, the initial radical yield estimated from the optical density at ~340 nm (an isosbestic point) and measured at Δt ~ 100 ns is larger by a factor of ~2 than in the case of [2AP][8-oxo-dG]T_{13} (panel A). Similar effects have been previously observed[29] in the case of double-stranded oligonucleotides with a GG doublet instead of an 8-oxo-dG at the analogous sites; these observations can be attributed to an efficient quenching of the ^{1}2AP singlet excited state by the adjacent, flanking guanine or 8-oxo-dG residues.

In the double-stranded oligonucleotide [2AP]T_4[8-oxo-dG]-T_9 with four thymine bases separating the 2AP and 8-oxo-dG residues (Figure 2C), the transient absorption recorded at Δt = 10 μs in the range of 300−330 nm is negative due to the bleaching of the 2AP absorption band. The 2AP•$^+$ radical cation generated initially by the laser excitation is a strong Brønsted acid[36,39] and thus rapidly deprotonates (~30 ns) to a neutral radical,[28] 2AP(−H)•, in neutral aqueous solution. Indeed, the transient absorption spectrum recorded at Δt = 10 μs is close to that of the 2AP(−H)• radicals[28] rather than of the 2AP•$^+$ radical cations.[28] The buildup of the positive absorbance at 320 nm, accompanied by the decay of the transient absorption at 510 nm, are clearly evident in the time interval from 10 to 790 μs. In [2AP]T_4[8-oxo-dG]T_9 the initial radical yield estimated from the optical density at ~340 nm (an isosbestic point) measured at Δt ~ 100 ns, is close to the yield in [2AP]T_2[8-oxo-dG]T_{11} (panel B); this suggests that the photoionization efficiencies in these duplexes are close to one another.[29]

Prompt and Slow Kinetic Components of 8-Oxo-dG Oxidation by 2AP Radicals. The transient absorption signal at 325 nm (Figure 3) induced by a 308 nm laser pulse excitation of the double-stranded oligonuclotides (**1**−**3**) is related to a positive signal due to the generation of the 8-oxo-dG•$^+$/8-oxo-dG(−H)• radicals (at this wavelength the changes of the 2AP radical/2AP ground state absorption are negligible). Examination of the early transient absorption profiles (Δt ~ 0.1 μs) reveals wide variations in the value of the transient absorbance at 325 nm, which depends on the number of thymidine residues between the 2AP and 8-oxo-dG bases. For example, the full absorbance at 325 nm develops promptly (within 0.1 μs) in the case of double-stranded oligonucleotide [2AP][8-oxo-dG]T_{13} (Figure 3A), and there is no slower phase of oxidation of 8-oxo-dG. In contrast, in the case of the double-stranded oligonucleohdtide [2AP]T_4[8-oxo-dG]T_9, the prompt phase is absent, and the positive 8-oxo-dG•$^+$/8-oxo-dG(−H)• signal grows slowly within the time interval of ~10−800 μs. In the case of the double-stranded oligonucleotide [2AP]T_2[8-oxo-dG]T_{11},

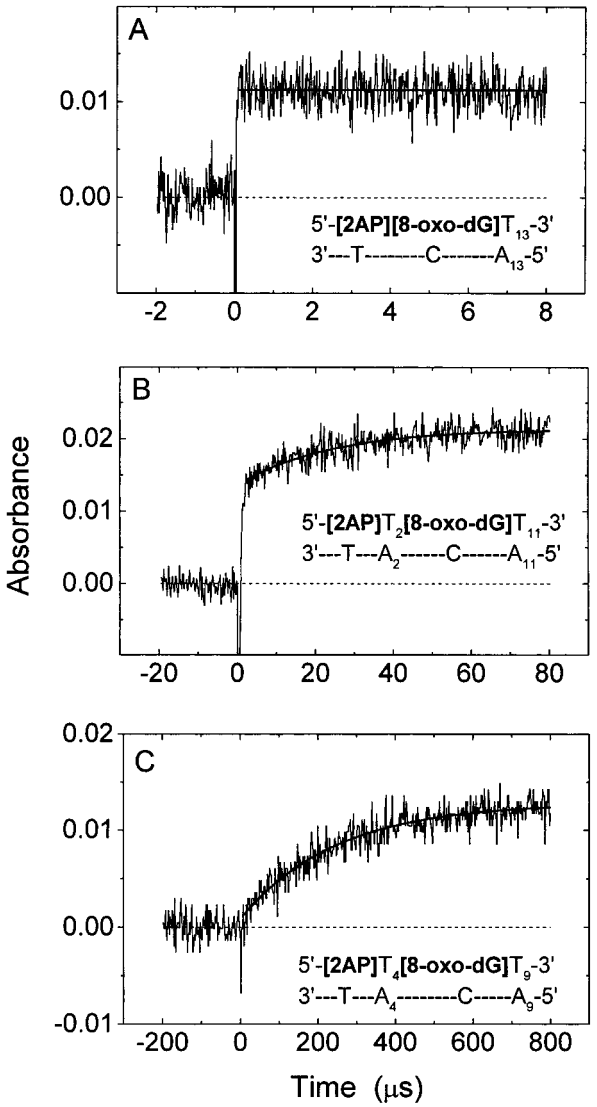

Figure 3. Kinetics of the transient absorbance at 325 nm induced by 308 nm XeCl excimer laser pulse excitation (80 mJ pulse^{-1} cm^{-2}, fwhm ≈ 12 ns, 0.1 Hz) of the double-stranded oligonucleotides (50 μM) in *oxygenated* 20 mM phosphate buffer solution (pH 7). The solid lines are best fits of eq 2 to the experimental data points.

the contribution of the prompt component (Δt < 0.1 μs) is ~60−70% of the total, and a slow increase of the 325 nm signal is evident in the ~5−80 μs time range (Figure 3B). Thus, depending on the number of thymidine bases between the 2AP and 8-oxo-dG bases in these oligonucleotide duplexes, two kinetic components of 8-oxo-dG oxidation of variable proportions, a prompt component (Δt < 0.1 μs) and a slower one (Δt > 0.1 μs), are apparent. Similar prompt and slow kinetic components have also been observed in the case of the double-stranded oligonucleotides with GG doublets instead of the [8-oxo-dG]T dinucleotide sequences in **1**−**3**.[29]

The exact structure of the oxidant responsible for the fast phase of the oxidation of 8-oxo-dG is unknown; depending on the actual rates of the 2AP•$^+$ deprotonation and electron abstraction from 8-oxo-dG in double-stranded DNA, the oxidant could be 2AP•$^+$ or 2AP(−H)•. The oxidant responsible for the oxidation of 8-oxo-dG on the microsecond time scale is the 2AP(−H)• neutral radical. In the double-stranded oligonucleotides, the transient absorption spectrum is due to 2AP(−H)•, not the radical cation[28] (see above). Thus, oxidation of 8-oxo-dG (neutral molecules) by 2AP(−H)• (neutral radicals), with the

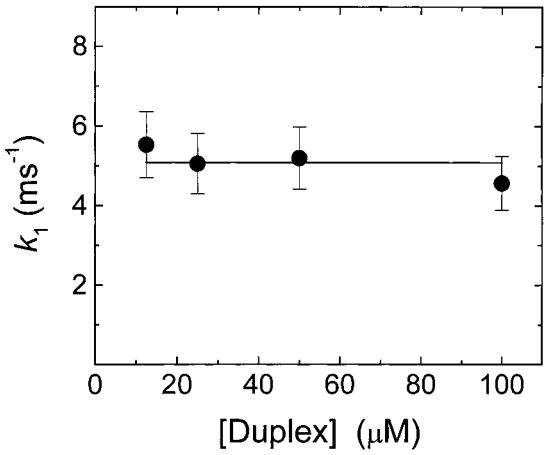

Figure 4. First-order rate constants k_1 (average values calculated from the buildup of the transient absorbance at 325 nm and the decay at 510 nm) induced by 308 nm XeCl excimer laser pulse excitation (80 mJ pulse^{-1} cm^{-2}, fwhm ≈ 12 ns, 0.1 Hz) of the double-stranded oligonucleotide d([2AP]T$_4$[8-oxo-dG]T$_9$)•d(A$_9$CA$_4$T) in *oxygenated* 20 mM phosphate buffer solution (pH 7).

concomitant formation of 8-oxo-dG•$^+$/8-oxo-dG(−H)• and 2AP (neutral molecules), can be viewed in terms of an electron-transfer reaction from 8-oxo-dG to 2AP(−H)• with a coupled protonation of 2AP(−H)$^-$. The latter occurs via proton transfer from water to 2AP(−H)$^-$ and with the deprotonation[24] of 8-oxo-dG•$^+$ at pH > 6.6. Analogous long-distance proton-coupled electron-transfer reactions[41] have been observed in the case of oxidation of tyrosine by tryptophan neutral radical linked to oligoproline bridges.[42−45]

Oxidation of 8-Oxo-dG in Double-Stranded Oligonucleotides Is an Intraduplex Process. In the double-stranded oligonucleotide [2AP]T$_4$[8-oxo-dG]T$_9$ with four thymidine bases separating the 2AP and 8-oxo-dG residues, the buildup of the transient absorbance of 8-oxo-dG•$^+$/8-oxo-dG(−H)• species occurs very slowly (Figure 3C). In the case of such slow processes, the contribution of oxidation processes occurring via interduplex mechanisms, in which 8-oxo-dG base located in a particular duplex is oxidized by a 2AP radical in another duplex, cannot be excluded. To test this hypothesis, we investigated the potential effect of duplex concentration on the formation of the 8-oxo-dG•$^+$/8-oxo-dG(−H)• radical species. Figure 4 shows that the averaged first-order rate constants of the 8-oxo-dG oxidation calculated from the kinetic traces recorded at two representative wavelengths (325 and 510 nm) do not depend on the concentration of the double-stranded oligonucleotides. Thus, the oxidation of 8-oxo-dG is predominantly an intraduplex reaction in which both reaction partners, the 8-oxo-dG and 2AP radicals, are located in the same duplex.

Distance Dependence of 8-Oxo-dG Oxidation Rates (Slow Kinetic Component). The kinetic profiles of the 8-oxo-dG oxidation by 2AP radicals on microsecond time scales (Figure 3B,C) can be described by the following equation:

$$A(t) = A - B\exp(-k_1 t) \qquad (2)$$

where k_1 is associated with the appearance of the signal due to the 8-oxo-dG•$^+$/8-oxo-dG(−H)• species. The decay of the 8-oxo-dG radicals in the double-stranded oligonucleotides occurs on millisecond to second time scales[46] and does not interfere with their formation. The values of k_1 were obtained by the best fits of eq 2 to the experimental kinetic traces recorded at 325 nm (Figure 3B,C). The oxidation of 8-oxo-dG by 2AP, with rate constant k_{ag}, competes with the decay of 2AP radicals by

other pathways with rate constant k_a; thus, $k_1 = k_a + k_{ag}$. The value of k_a was measured by use of the double-stranded oligonucleotide [2AP]T_{14}, which contains no 8-oxo-dG residues.[29] These kinetic parameters are summarized in Table 1. The value of $k_{ag} = k_1 - k_a$ decreases as the number of thymine bases between the 2AP and 8-oxo-dG residues increases from two to four by a factor of ~12 (Table 1).

Recently we measured[29] the rate constants of the GG doublet oxidation by 2AP(−H)• radicals in the double-stranded 15-mer oligonucleotides of the same design as the double-stranded oligonucleotides **2** and **3** with two and four thymine bases between 2AP and 8-oxo-dG residues. The values of k_{ag} in the case of the GG doublets are lower by factors of 5−10 than those measured for the oxidation of 8-oxo-dG by 2-AP radicals separated by the same number of thymidine bases. Recently, Meggers et al.[19] studied the oxidation of G and 8-oxo-dG in double-stranded oligonucleotides by a carbohydrate radical cation resulting from the photochemical cleavage of a modified sugar DNA backbone. Instead of kinetic methods, the rate constants of electron transfer were estimated on the basis of the photochemical yields of strand cleavage at varying distances from the radical and from the rate constants of competitive pathways. In the case of double-stranded oligonucleotides containing the same number of intervening thymine bases, the oxidation of 8-oxo-dG by the carbohydrate radical cation occurs on time scales similar to those found in this work. For instance, in the double-stranded oligonucleotide with two intervening thymine bases, the electron transfer in the 3′ → 5′ direction also occurs on the microsecond time scale with $k_{ET} \sim 1 \times 10^6$ s^{-1} (compared with $k_{ag} \sim 4 \times 10^4$ s^{-1}, Table 1). This apparent difference in the rate constants may be related to differences in the thermodynamic driving forces, since the electron acceptors, and the reorganization energies and electronic matrix elements, are probably not identical. Nevertheless, both approaches give very similar ratios of the rate constants. For instance, the rate constants of oxidation of guanines were smaller by a factor of ~4 than the oxidation of 8-oxo-dG in the same sequence, which is quite close to the factor of 5−10 obtained here.

The higher rate constants (this work), or yields of oxidation of 8-oxo-G and G by a different oxidant[19] in DNA, is a consequence of the lower oxidation potential of 8-oxo-dG.[24] However, because the redox potentials of either 8-oxo-dG or GG in oligonucleotides are not established and are likely to depend on sequence,[15,16,49] further quantitative interpretations and comparisons of these results would be premature.

4. Concluding Remarks

Photoexcitaion of double-stranded oligonucleotides containing a strand site-specifically modified with 2AP and 8-oxo-dG bases with intense nanosecond 308 nm XeCl excimer laser pulses results in the selective two-photon photoionization of the 2AP residues. The 2AP radicals generated in the photoionization reaction oxidize 8-oxo-dG at a distance. The dynamics of the 8-oxo-dG oxidation separated from 2AP radicals by zero, two, or four thymine bases was directly monitored by transient absorption spectroscopy and revealed the existence of biphasic kinetics. The efficiency of the fast process (<100 ns) rapidly decreases with increasing distance, and this component is observed only in the case of zero or two intervening thymidine bases. This fast component is no longer apparent when the 2AP and 8-oxo-dG residues are separated from one another by four thymidines. A slower kinetic component (>100 ns) is assigned to the oxidation of 8-oxo-dG by 2AP(−H)• radicals and is kinetically resolvable in the double-stranded oligonucleotide with two bridging thymine bases. When the 8-oxo-dG and 2AP are separated by four thymidines, this slower component (~0.3 ms) is the only measurable decay component. Thus, the long-distance migration of oxidative damage in double-stranded DNA that occurs on millisecond time scales is more likely to be induced by neutral radicals than by radical cations.

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radicals with molecular oxygen and $O_2^{\bullet-}$ radicals, with the latter generated in approximately the same concentrations as 8-oxo-dG radicals by the reaction of e_h^- with O_2. In this work, we did not detect any direct effect of O_2 on the decay rate of 8-oxo-dG radicals; this is not surprising since it is already known that purine radicals, e.g., G(−H)• and A(−H), react with O_2 very slowly[38] ($k \leq 10^2$ M^{-1} s^{-1}). In contrast, the reactions of purine radicals with $O_2^{\bullet-}$ are quite rapid; for example, Candeias and Steenken[36] estimated that the rate constant of reaction of dG(−H)• with $O_2^{\bullet-}$ is $\sim 3 \times 10^9$ M^{-1} s^{-1}.

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