# Influence of the Local Helical Conformation on the Guanine Modifications Generated from One-Electron DNA Oxidation†

Annick Spassky* and Dimitar Angelov‡

*Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France*

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ABSTRACT: Two major products, 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentafuranosyl)amino]-5-(2*H*)-oxazolone and its imidazole derivative have been generated from one-electron oxidation of the free 2′-deoxyguanosine. The formation of 7,8-dihydro-8-oxoguanine (8-oxodG), not detected in this case, has been observed from DNA exposed to oxidizing agents. Since these compounds are thought to reflect, respectively, either deprotonation or hydration of the transient guanyl radical cation, these findings suggested that the helical structure could influence the chemical decomposition pathway of the guanine moiety. In the present study, we have photoionized DNA sequences by exposure to high-intensity UV (266 nm) laser pulses. Homo- or heteroduplexes, including guanines in various environments as well as $G_n$ runs, were used as templates. Lesions were analyzed, at the nucleotide level, by taking advantage of the specific removal of 8-oxodG from DNA by the formamidopyrimidine DNA glycosylase (Fpg protein) and of the differential sensitivity of 8-oxodG and oxazolone to piperidine. Variations were observed in the relative yield of each type of lesion at individual guanines of the DNA sequences. We found that the Fpg lesions predominate in regions of stable double helix but are decreased in favor of the piperidine ones in regions of destabilization of the helix. Results are discussed in terms of a relationship between intramolecular rearrangements of the guanyl radical cation and the DNA helical conformation and dynamics.

Oxidative damage of DNA can be caused by diverse biological pathways and probably constitutes the most varied class of DNA lesions [for a review, see Demple and Harrison (1994)]. In particular, purines can undergo oxidation of the ring atoms to form various products (Cadet et al., 1996). We were interested, in the present study, in DNA one-electron oxidation products of guanine. The guanyl radical from the free 2′-deoxyguanosine, transiently generated, has been shown to undergo a rapid deprotonation (Candeias & Steenken, 1992). Two major oxidation products have been identified: the 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentafuranosyl)amino]-5-(2*H*)-oxazolone and its imidazole derivative (Cadet et al., 1994). Their formation is accounted for by the initial deprotonation of the guanyl radical [for a recent review, see Cadet et al. (1996)]. Interestingly, in a recent experiment of photosensitization of calf thymus DNA by riboflavin, the formation of 7,8-dihydro-8-oxoguanine (8-oxodG)[1] has been observed, indicating that the guanyl radical cation underwent hydration as the first step of its decomposition (Kasai et al., 1992). Taken together, these findings led to the conclusion that the helical structure prevents, at least in part, the deprotonation from occurring and suggested that local conformational and dynamics variations, induced by the primary sequence arrangements [reviewed in Wells (1988)], could modulate the relative probability of hydration versus deprotonation of the guanyl radical cation. We were thus interested in analyzing the oxidation products at individual guanines from the ionization of defined DNA sequences.

Exposure of DNA to high-intensity ($10^9-10^{13}$ W/m$^2$) UV (240−270 nm) laser pulses (nanosecond) provides a direct and convenient way of photoionizing the nucleic bases; indeed, under these irradiation conditions, nucleic bases can successively absorb two photons, whose cumulative energy (8−9 eV) exceeds the ionization threshold, thus giving rise to the ejection of an electron and consequently to the formation of the radical cation (Nikogosyan, 1990; Schulte-Frohlinde et al., 1990). After submitting selected DNA sequences to such a photoprocess, we have characterized the guanine oxidation products by taking advantage on the one hand of the active removal of 8-oxodG from DNA by the formamidopyrimidine DNA glycosylase (Fpg protein) (Ravanat 1993; Tchou et al. 1994) and on the other hand of the differential sensitivity of 8-oxodG (Cullis et al., 1996) and oxazolone to piperidine (Cadet et al., 1994). Their quantification has been performed in terms of DNA cleavage events using the footprinting technique (see Experimental Procedures). Two types of sequences have been selected: a 37 bp DNA sequence, including the target site of the endonuclease I-Sce1 (Colleaux et al., 1988) (sequence I in Figure 1), and several guanines in various environments. Irradiation has been performed on the duplex or on each single strand separately. In order to define the influence of the sequence context, we have introduced base changes in the two runs of three guanines, occurring in sequence I; the first GGG was changed into GTG (sequence II) and the second one

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* To whom correspondence should be addressed.

‡ Present address: Institut de Physique du Solide, Académie des Sciences, Sofia, Bulgaria.

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[1] Abbreviations: oxazolone derivatives, 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentafuranosyl)amino]-5-(2*H*)-oxazolone and its imidazole derivative; 8-oxodG, 7,8-dihydro-8-oxoguanine.

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**Homoduplexes**

```
                  1     2    3 4 5        6 7 8
I-     5'-ATAAAAGTTACGCTAGGGATAACAGGGTAATATAACG-3'
       3'-TATTTTCAATGCGATCCCTATTGTCCCATTATATTGC-5'
                     11 10          9

                  1     2    3  5        6 7 8
II-    5'-ATAAAAGTTACGCTAGTGATAACAGGGTAATATAACG-3'
       3'-TATTTTCAATGCGATCACTATTGTCCCATTATATTGC-5'
                     11 10          9

                  1     2    3 4 5        6 7
III-   5'-ATAAAAGTTACGCTAGGGATAACAGGATAATATAACG-3'
       3'-TATTTTCAATGCGATCCCTATTGTCCTATTATATTGC-5'
                     11 10          9

                        1 2          3 4 5
IV-    5'-CGTTATCATTGGTTATCATTGGGTTATCATTCG-3'
       3'-GCAATAGTAACCAATAGTAACCCAATAGTAAGC-5'

                        1 2          3 4 5 6 7
V-     5'-CGTTATCATTGGTTATCATTGGGGGTTATCATTCG-3'
       3'-GCAATAGTAACCAATAGTAACCCCCAATAGTAAGC-5'
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**Heteroduplexes**

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                  1     2    3  5        6 7 8
VI-    5'-ATAAAAGTTACGCTAGTGATAACAGGGTAATATAACG-3'
       3'-TATTTTCAATGCGATCCCTATTGTCCTATTATATTGC-5'
                     11 10          9

                  1     2    3 4 5        6 7
VII-   5'-ATAAAAGTTACGCTAGGGATAACAGGATAATATAACG-3'
       3'-TATTTTCAATGCGATCACTATTGTCCCATTATATTGC-5'
                     11 10          9

                        1 2          3 4 5
VIII-  5'-CGTTATCATTGGTTATCATT-GGG-TTATCATTCG-3'
       3'-GCAATAGTAACCAATAGTAACCCCCAATAGTAAGC-5'

                        1 2          3 4 5 6 7
IX-    5'-CGTTATCATTGGTTATCATTGGGGGTTATCATTCG-3'
       3'-GCAATAGTAACCAATAGTAA-CCC-AATAGTAAGC-5'
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FIGURE 1: Nucleotide sequences of fragments used in this study. The positions of the guanine residues have been numbered on each strand from 5′ to 3′.

into GGA (sequence III). In addition, we used another type of sequence (33−35 bp) containing two, three, or five guanine runs incorporated in the same sequence environment (sequences IV and V). Finally, we have taken advantage of the annealing of the nonhomologous DNA strands to analyze the effect of the destabilization of the helical pairing caused by a single mismatch (sequences VI and VII) or additional unpaired bases on one strand ("bulge") (sequences VIII and IX). We discuss the results in terms of the relationship between intramolecular rearrangements of the guanyl radical cation and the DNA helical conformation and dynamics.

## EXPERIMENTAL PROCEDURES

*Enzymes and Chemicals.* T4 polynucleotide kinase was from Pharmacia; X-ray films were from Kodak and chemicals for developing from Agfa. Fpg protein was kindly supplied by S. Boiteux (CEA, Fontenay aux Roses). [$\gamma$-$^{32}$P]-ATP (specific activity of 3000 Ci/mmol) was purchased from Amersham.

*Oligonucleotides Preparation.* Synthetic oligodeoxynucleotides were purchased from Genset (France). Purification of the crude oligonucleotides was carried out by running on 20% polyacrylamide/7.5 M urea gels in TBE buffer (89 mM Tris base/89 mM boric acid/2 mM EDTA at pH 8.0), cutting out the corresponding band, electrically eluting the DNA, and ethanol precipitation. Oligonucleotides were labeled at their 5′ termini by using T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP. DNA duplexes were prepared by heating a mixture containing equal amounts of the two complementary strands, one labeled and the other unlabeled, at 70 °C for 10 min in 50 mM Tris-HCl (pH 8), 1 mM EDTA, and 10 mM NaCl and then allowing the DNA to cool to room temperature over 3 h.

*Laser Irradiation.* Ultraviolet radiation of high intensity at a wavelength of 266 nm was obtained through successive transformation of the fundamental harmonic (1064 nm) of a $Nd^{3+}$:YAG laser into the second and fourth harmonic in nonlinear DKDP crystals. The duration of the pulses was 15 ns. Irradiation was carried out in small 0.65 mL siliconized Eppendorf tubes, and the diameter of the laser beam was reduced to 0.25 cm ($S = 0.05$ cm$^2$) by means of a circular diaphragm to exactly match the area of the sample. The energy of the laser radiation was measured with a calibrated (±5%) pyroelectrical energymeter, model Rj7100 (Laser Precision Inc.), using the Fresnel reflections (8%) of a parallel fused silica plate, positioned behind the sample.

DNA samples (10 $\mu$L, 0.05 pmol) were exposed to a dose of about 1 kJ/m$^2$ of UV (266 nm) radiation in a single pulse (intensity of $0.66 \times 10^{11}$ W/m$^2$) in standard buffer [20 mM Tris-HCl (pH 7.5) and 10 mM NaCl]. The optical density of the irradiated layer was $<<0.1$ at 266 nm.

*Determination of the Yield of Lesions at Individual Sites.* After irradiation, DNA samples were either submitted to Fpg enzymatic treatment [0.5 $\mu$g/mL final concentration in 20 mM Tris-HCl (pH 8), 1 mM EDTA, 50 mM NaCl, and 100 $\mu$g/mL BSA] for 30 min at 37 °C or incubated with piperidine to a final concentration of 1 M for 30 min at 95 °C. In some cases both treatments were successively carried out. Samples were then lyophilized. Because control experiments showed that the presence of the Fpg protein does not influence the migration of the oligonucleotides, no phenol extraction was carried out. Piperidine-treated samples were redissolved five times in 20 $\mu$L of distilled water and lyophilised again. Samples were then dissolved in 3 $\mu$L of deionized formamide with 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, heated briefly to 90 °C, cooled, and then run on a denaturing 15% polyacrylamide gel. Note that, when single-stranded DNA is irradiated, it was annealed before the Fpg treatment. Following electrophoresis, gels were dried and autoradiographed.

The autoradiograms were scanned by using the Phosphorimager and the Image Quant software (Molecular Dynamics). The amount of the radioactivity in each band was determined by the difference between the integration rectangles of the appropriate bands for irradiated and nonirradiated DNA submitted to the same treatments. The depletion of the intact oligonucleotide at the top of the gel, assessed in each case, was found to be less than 10%. We defined the yield $R_i$ of a lesion at individual base $i$ as the ratio between the amount of the radioactivity $r_i$ in band $i$ and the total radioactivity $r_0$ loaded in the lane. Measurements are normalized at a fixed irradiation dose (1kJ/m$^2$) to take into account the fluctuation (found, <10%) of the pulsed energy, $E$. Finally, $R_i = Er_i/r_0$. Through comparison of the results of several different experiments, the measurement accuracy was estimated to be 5%.

## RESULTS

*Enzymatic and Chemical Treatments Reveal Two Different Guanine Modifications.* Duplex DNA fragment (I in Figure

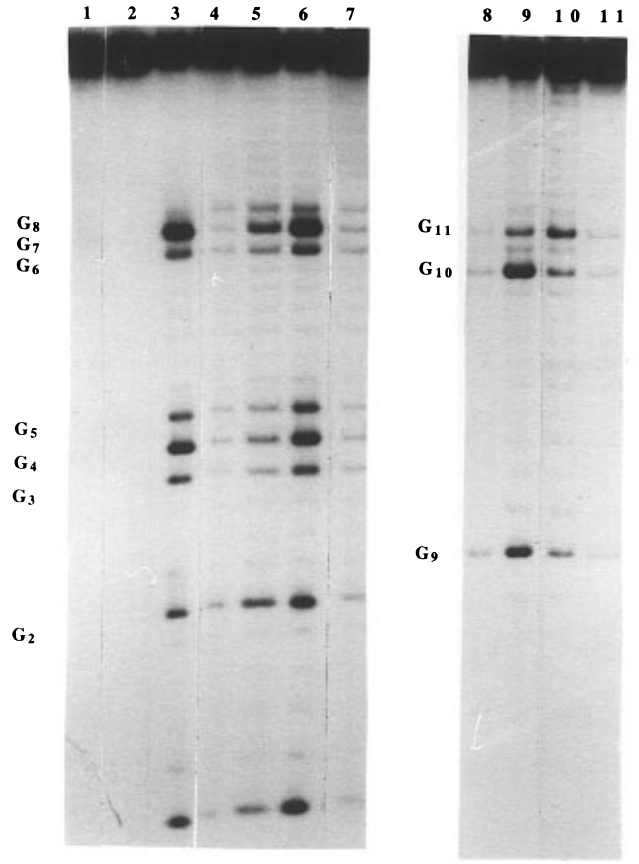


FIGURE 2: High-intensity ($0.66 \times 10^{11}$ W/m$^2$) UV (266 nm) laser-irradiated DNA duplex (sequence I), uniquely 5′ end-labeled at either the upper strand (lanes 1−7) or the lower strand (lanes 8−11), has been submitted either to Fpg digestion (lanes 3 and 9) or to 90 °C piperidine treatment (lanes 5 and 10). Nonirradiated DNA duplex submitted to Fpg treatment (lanes 4 and 8) or to piperidine treatment (lanes 7 and 11). For control, uniquely 5′ end-labeled upper strand DNA duplex nonirradiated and nontreated (lane 1) and irradiated and nontreated (lane 2), submitted to both Fpg and piperidine treatments (lane 6).

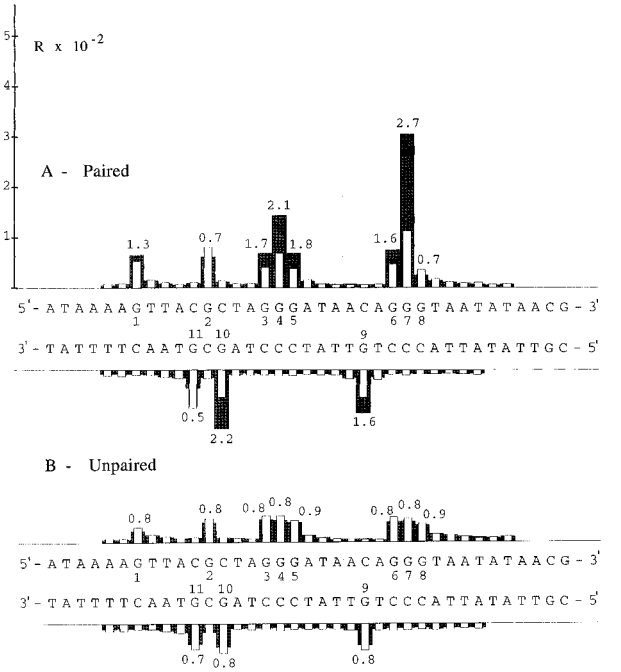


FIGURE 3: Normalized plot of the yield of the high-intensity irradiation lesions generated in sequence I, base paired (A) or unpaired (B), sensitive to Fpg (shaded bars) or hot piperidine (white bars). The yield *R* is represented by the height of corresponding bars (the scale is given on the ordinate of the graph in panel A), and ratios are given above each position.

1) as well as each of the complementary oligonucleotides separately was alternatively submitted to a single pulse of high-intensity ($0.66$-$10^{11}$ W/m$^2$) UV (266 nm) nanosecond laser irradiation, in the standard conditions (see Experimental Procedures). Irradiated and nonirradiated DNA were then either directly loaded on a sequencing electrophoresis gel or incubated, prior to loading, with the Fpg protein or/and hot piperidine. Cleavage positions were identified by comparison with Maxam−Gilbert reactions. No specific splitting of the *N*-glycosylic bond is detected under direct loading of nonirradiated as well as irradiated DNA (lanes 1 and 2, Figure 2). With either Fpg enzymatic digestion or hot piperidine treatment of nonirradiated DNA, there is a low background, predominantly at guanine residues (lanes 4, 7, 8, and 11, in Figure 2). Sharp bands of variable intensity, located exclusively at guanine residues, arise from treatments applied to double-stranded irradiated DNA (lanes 3, 5, 9, and 10, in Figure 2). None of these new cleavage events was detectable in a control experiment in which DNA was submitted to the same UV irradiation dose at low intensity ($5 \times 10^8$ W/m$^2$), indicating that they originate in the absorption of two photons (Nikogosyan, 1990).

Whatever may be the treatment subsequent to the irradiation, the ratio of damaged versus undamaged DNA does not exceed 10%, indicating that our irradiation conditions satisfy single-hit conditions. That permitted us to use the quantification of the total radioactivity loaded in each lane as well as its distribution per band to determine the yields of each Fpg or piperidine cleavage event, namely $R_{Fpg}$ and $R_{pip}$ (see Experimental Procedures, Figure 3A). In this way, we could analyze the effect of each treatment and both together on the irradiated DNA and observe that the sum of the cutting events induced by each individual treatment exceeds by approximately 10% those resulting from both successive treatments (not shown). It is interesting to note (Figure 3A,B) the variations in the ratio Fpg/pip that can, for exemple, change from 0.7 and 0.5, respectively, for positions $G_2$ or $G_8$ and $G_{11}$ to 2.1 and 2.7 for positions $G_4$ and $G_7$, in contrast to the constancy observed for digestion profiles of the single-stranded irradiated DNA. It is also noteworthy that, when the irradiated nucleotide sequence is single-stranded, not only is the ratio Fpg/Pip decreased ($R_{Fpg}/R_{pip} < 1$ at each position), but the overall yield of damaged DNA is also considerably lessened (see Figure 3B).

*Influence of the Local Helical Features on the Ratio $R_{Fpg}/R_{pip}$.* In order to further investigate the effect of the local helical features induced by the surrounding primary sequence on the relative distribution of each type of lesions, we repeated the experiment with sequences II and III, which differ from sequence I by a point mutation either at position $G_4$, changing $G_3G_4G_5$ in $G_3TG_5$, or at position $G_8$, changing $G_6G_7G_8$ in $G_6G_7A$. Second, we used two new sequences, IV and V (Figure 1), in which guanine runs are incorporated in the same environment. Results are shown in Figure 4. The prevalence of Fpg with respect to piperidine sensitive guanine modifications is confirmed in runs of contiguous guanines, as early as two guanines (as shown by $G_1$ of sequence IV, see Figure 4) and particularly in the middle of the run (see in figure 4, $R_{Fpg}/R_{pip}$ for $G_4$ of sequence IV or for $G_4$, $G_5$, or $G_6$ of sequence V). Note that we found similar $R_{Fpg}$ and $R_{pip}$ values at positions $G_8$ of sequence I (Figure 3), $G_2$ and $G_5$ of sequence IV, $G_7$ of sequence V (Figure 4),

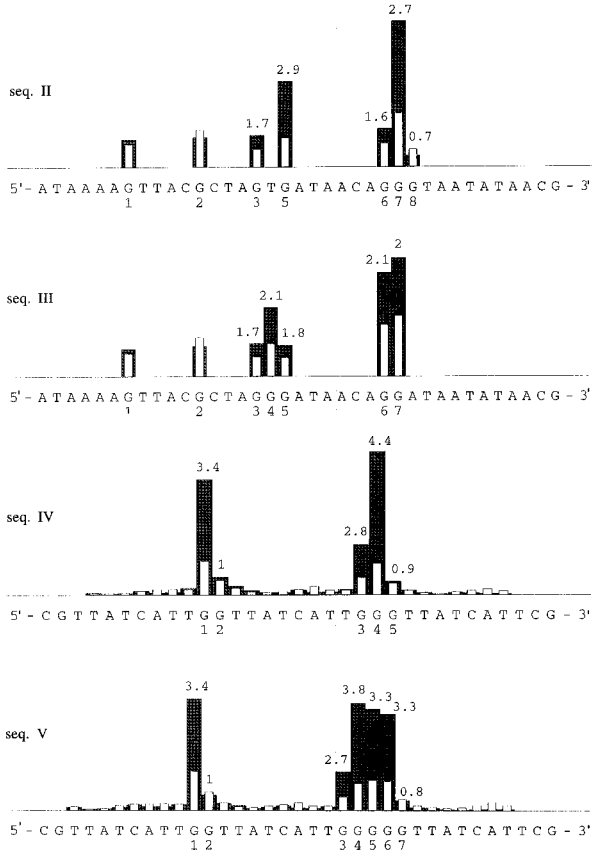


FIGURE 4: Normalized plot of the yield of the lesions, sensitive to Fpg (shaded bars) or hot piperidine (white bars) treatments, generated from high-intensity ($0.66 \times 10^{11}$ W/m$^2$) UV (266 nm) laser irradiation of duplex-irradiated sequences II−V (see Experimental Procedures). The yield $R$ is represented by the height of corresponding bars (the scale is given on the ordinate of Figure 3A), and ratios are given above each position.

$G_8$ of sequence VI, $G_5$ of sequence VIII, and $G_7$ of sequence IX (Figure 6), being all 3′ of a run of guanines and 5′ nearest neighbor of a thymine; in contrast, $R_{Fpg}$ is 2−3 times higher than $R_{pip}$ at positions $G_5$ of sequence II and $G_7$ of sequences III and VII, also 3′ of a run of guanines but 5′ nearest neighbor of an adenine (see Figures 4 and 6).

The influence of the perturbation in the stacking of base pairs was checked by analyzing the ratio $R_{Fpg}/R_{pip}$ for the sequences resulting from the annealing of the noncomplementary strands; on the one hand, the upper strands of sequences II and III with the lower strands of sequences III and II give rise, respectively, to sequences VI and VII (including mismatches, see Figure 6), and on the other hand, the upper strands of sequences IV and V with the lower strands of sequences V and IV give rise, respectively, to sequences VIII and IX (including extra bases, see Figure 6). The use as template of sequences VIII and IX in place of IV and V results in a dramatic decrease of the ratio $R_{Fpg}/R_{pip}$, as shown by the Figures 5A,B and 6.

## DISCUSSION

The present study points out two main types of biphotonic guanine modifications generated, with high quantum efficiency (on the order of $10^{-2}$), during DNA high-intensity ($0.66 \times 10^{11}$ W/m$^2$) UV (266 nm) laser irradiation. Previous studies have shown that photoionization of nucleic bases is the main photoprocess induced in such experimental conditions (Nikogosyan, 1990; Schulte-Frohlinde et al., 1990). The most likely situation is, thus, that these lesions arise from the chemical decomposition of the transient guanyl radical cation. Significant participation of free radical-mediated reactions is highly improbable. It has, indeed, been shown

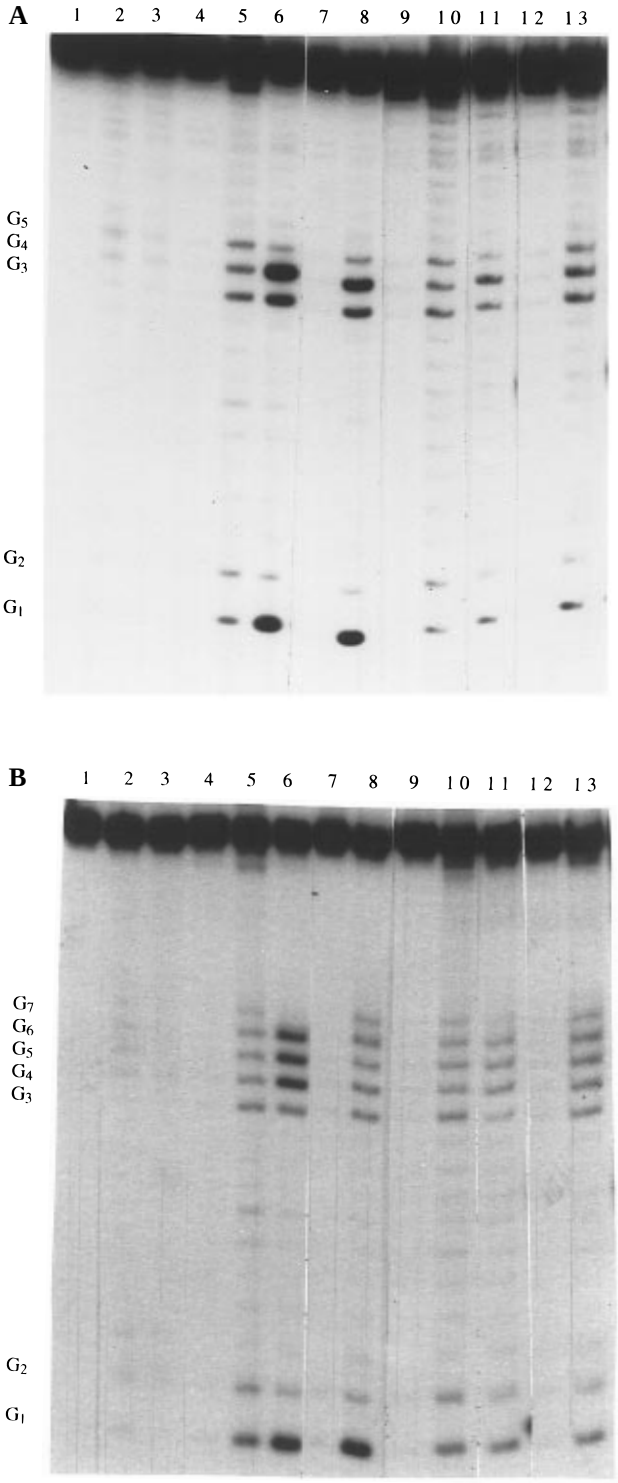


FIGURE 5: Sequence gel analysis of fragments generated from uniquely 5′ end-labeled upper strand DNA sequences IV and VIII (A) and V and IX (B). First exposed to high-intensity ($0.66 \times 10^{11}$ W/m$^2$) UV (266 nm) laser irradiation and then submitted to Fpg (lanes 6 and 11) or piperidine (lanes 8 and 13) treatments. Irradiated single-stranded DNA sequences IV and V treated with either Fpg (lane 5) or piperidine (lane 10). Irradiated double-stranded sequences IV and VIII and V and IX directly loaded, respectively, in lanes 2 and 3. Nonirradiated sequences IV and VIII and V and IX submitted, respectively, either to Fpg in lanes 4 and 9 or to piperidine in lanes 7 and 12. Lane 1, direct loading of DNA sequences IV (A) and V (B).

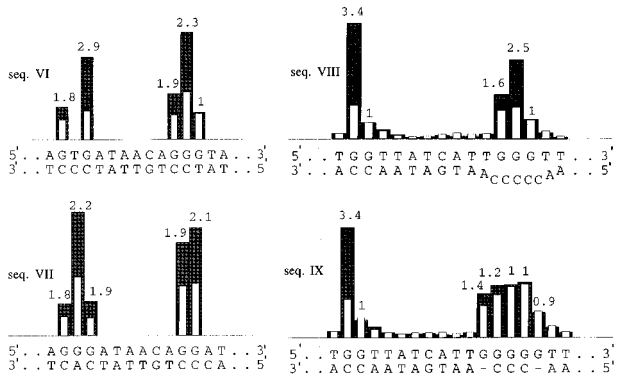


FIGURE 6: Normalized plot of the yield of the lesions, sensitive to Fpg (shaded bars) or hot piperidine (white bars) treatments, generated from high-intensity ($0.66 \times 10^{11}$ W/m$^2$) UV (266 nm) laser irradiation of laser-irradiated sequences VI−IX (see Experimental Procedures). The yield $R$ is represented by the height of corresponding bars (the scale is given on the ordinate of Figure 3A), and ratios are given above each position.

that direct ionization of water does not occur under nanosecond laser irradiation conditions (Nikogosyan, 1990). Even if hydroxyl radicals could be produced as a result of indirect ionization of water (Nikogosyan, 1990), the yield of photoproducts thus induced would be expected to be 2−3 orders of magnitude lower (Angelov et al., 1997) than that observed here. Neither singlet oxygen leading only to monophotonic oxidative damage (Cadet et al., 1996) nor the superoxide ion, possibly arising from the reaction between the hydrated electron and oxygen dissolved in water (Nikogosyan, 1990), but not known to efficiently react with nucleic acids (Cadet et al., 1996), nor the optical breakdown process whose threshold is well above the fluence used in our irradiation conditions (Hefetz et al., 1990) could reasonably play an important role in the present results.

These lesions are sensitive either to the enzymatic activity of the Fpg protein or to the hot piperidine treatment. Although several lesions can be excised by the Fpg protein (Tchou et al. (1994), the first type of guanine modification is identified as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG); indeed, its weak piperidine susceptibility agrees well with the weak sensitivity characterizing this compound and excludes the possibility that it could be either an imidazole ring-opened residue (FAPyGua) or an apurinic site, since these lesions are very labile in piperidine (Boiteux et al., 1992). This assignment is supported by the comparison between the amount of the total Fpg cleavage events, quantified by densitometric analysis of bands from the autoradiographs, and that of the 8-oxodG monitored by the HPLC/electrochemical method (Floyd et al., 1986) (not shown; Angelov et al., 1977). An additional observation strengthens the identification of this lesion as 8-oxodG; we have found that, contrasting with the similar amount of cleavage occurring from the Fpg treatment of duplexes containing the modified VI-$G_8$ paired with either dT or dC, the amount of cutting of duplexes containing dA opposite the modified VII-$G_4$ is significantly lower than that one in which dC is the opposing base (not shown). This agrees well with the result showing that 8-oxodG is efficiently excised by 8-oxodG DNA glycosylase when opposite dC, dT, and dG but is resistant when positioned across from dA (Plum et al., 1995). Together, these findings provide convincing evidence that the guanine modification removed by Fpg is 8-oxodG, at least predominantly (>90%). According to the chemical mechanism proposed for the process, the frequency of Fpg cutting at individual guanine reflects the probability of the hydration of the radical cation transiently formed at the site (Cadet et al., 1996). Note that this conclusion would not be changed even if a low amount of FAPy was present, since its formation involves the same initial conversion step of the radical cation (Cadet et al., 1996).

On the basis of data obtained with free nucleoside bases, it appears that the second type of lesions, not recognized by the Fpg protein, should be assigned to the other major oxidative guanosine lesion resulting from the deprotonation of the radical guanyl, namely 2,2-diamino-4-[(2-deoxy-$\beta$-D-*erythro*-pentafuranosyl)amino]-2*H*-oxazolone or its precursor (Jovanovic et al., 1986). This proposal is consistent with the strong piperidine lability of the oxazolone derivative (Cadet et al., 1994).

Thus, the variation in the ratio of the cleavage events $R_{Fpg}/R_{pip}$ would reflect the relative probability that the guanyl radical would undergo either hydration or deprotonation. The oxazolone derivatives are the only product found from the decomposition of the guanyl radical arising from the unpaired nucleoside. Present results show that Fpg sensitive guanine modification appears as soon as the unpaired nucleoside is incorporated in a helical structure. In single-stranded oligodeoxynucleotides, the two chemical pathways, guanyl radical hydration and deprotonation, have a similar probability of occurring at each guanine (irrespective of position). When the same DNA sequence is ordered in a double-helical structure, the ratio $R_{Fpg}/R_{pip}$ can vary by 1 order of magnitude from one guanine to another (Figure 3A). Note in addition that, overall, the sum of these two lesions is greater in this latter case than in the former (compare panels A and B of Figure 3). Thus, the hydration versus deprotonation of the guanyl radical appears to be intimately related either to the solvation of the nucleotide or to local helical features or both. From the comparison of the ratios $R_{Fpg}/R_{pip}$ (Figures 3 and 4), it clearly appears that the hydration of the radical cation prevails with respect to its deprotonation at clustered guanines of double-helical DNA ($R_{Fpg}/R_{pip}$ from 2 to 4.4). On examination of the effect of helix destabilization, by introducing mispaired or unpaired bases, we found that the ratio $R_{Fpg}/R_{pip}$ is modulated in a manner consistent with the extent of the expected helical perturbation. It has been found that mismatches, in general, can be accommodated in B-DNA with relatively small deviations in conformation; even a GA mismatch can be introduced in the DNA double helix with both bases in the anti conformation in a way that does not create a bulge in the structure, but rather can be accommodated by a translation of the base pair into the major groove (Kennard & Hunter, 1989). We found here that mismatches included in sequences VI and VII do not induce significant changes in the ratio $R_{Fpg}/R_{pip}$ with respect to paired sequences II and III. In contrast, the ratio $R_{Fpg}/R_{pip}$ falls sharply for guanines $G_3-G_7$ in the duplex IX compared to those in the duplex V. The same tendency is observed, although to a lesser extent, for guanines $G_3-G_5$, of duplex VIII with respect to IV. In both of these cases, two one-base bulges are included in the double helix. It is not possible, without any direct structural information, to predict the amplitude of the perturbation induced by an extra base; studies have shown that they could result in several alternative secondary structures, either stacked into the helix or in a looped-out conformation (Joshua-Tor et al., 1992). In addition, depending on the context and environmental factors,

either conformation may or may not disrupt base-stacking interactions of the flanking bases. Nevertheless, on the basis of physical measurements and gel mobility studies, some general trends might be identified. In particular, probably because of their larger size, bulged purine bases induce a greater angle of kinking than corresponding pyrimidine bulges (Bhattacharyya & Lilley, 1989). Interestingly, this could be related to the effect observed in the ratio $R_{Fpg}/R_{pip}$ which is greater in the case of sequence IX containing two one-G bulges on both sides of the three paired guanines ($G_4G_5G_6$) than in sequence VIII containing two one-C bulges.

A strict correlation between values of the ratio $R_{Fpg}/R_{pip}$ and firm structural information remains to be established. Nevertheless, present observations strongly suggest that hydration would operate most efficiently in regions of stable double helix and would decrease in favor of deprotonation as a consequence of the destabilization of the helix. The modulation observed in the ratios $R_{Fpg}/R_{pip}$ at individual guanines of the biological sequence could reflect subtle local deviations from the B form induced by the variations in the content and distribution of the associated water molecules as well as in local lifetime and opening of base pairs (Leroy et al., 1988). The prevalence of the piperidine lesions versus Fpg ones occurs at only three positions: $G_2$, $G_8$, and $G_{11}$. The two steps 5′-$CG_2$C-3′/5′-$CG_{11}$T-3′ have been identified as non-B sequence from a study comparing the free energies of the 1,10-phenanthroline−copper ion cleavage reaction and of base pair stacking (Schaeffer et al., 1996). In other respects, at position $G_8$, the ordered structure of the guanine run can be disrupted by the low stacking free energy displayed by the nearby 5′-TA-3′ step. In agreement with that view, the low $R_{Fpg}/R_{pip}$ ratio is not observed at $G_5$, which is 3′ to a cluster of three guanines, but adjacent to a 5′-AT-3′. Local deviation from the B form DNA structure can lead to large changes in the base pair life and opening times. It may be reasonable to expect correlation between the kinetics of disruption of DNA duplexes and the probability of imino deprotonation.

Finally, it should be noted that important variations in the sum of the two types of guanine modifications are observed at individual guanines of the sequence. Although the induction of lesions resulting from the conversion of the guanyl radical cation and not detected in our assay cannot be totally excluded, the most likely conclusion is that this variation reflects the dependence of the amount of the radical cation guanyl upon charge and/or energy transfer along the sequence. This requires further analysis and is currently being studied.

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