Guanine Oxidation in Double-Stranded DNA by Mn-TMPyP/$KHSO_5$: 5,8-Dihydroxy-7,8-dihydroguanine Residue as a Key Precursor of Imidazolone and Parabanic Acid Derivatives

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Abstract: The mechanism of oxidation of guanine residues on double-stranded oligonucleotides (ODNs) by the chemical nuclease Mn-TMPyP/$KHSO_5$ is reported. By using HPLC coupled to an electrospray mass spectrometer (ESI/MS) the different oxidized ODN strands were directly analyzed, and labeling experiments in $H_2{}^{18}O$ allowed us to propose a two-electron oxidation mechanism for guanine residues engaged in double-stranded DNA. We found that the imidazolone derivative (dIz) was formed by trapping of a guanine-cation by a water molecule. Two reaction intermediates on the pathway of the formation of dIz were observed: 5,8-dihydroxy-7,8-dihydroguanine and an oxidized guanidinohydantoin intermediate. Furthermore, a secondary route of guanine oxidation leading to parabanic acid was also evidenced. The mechanism of the different routes of guanine oxidation in double-stranded DNA has been discussed in detail.

Introduction

Due to the importance of oxidative degradation of nucleic acids in mutagenesis, carcinogenesis, and aging, a large number of chemical and biological investigations have been recently published.[1−8] Guanine is the most oxidizable base of DNA[9] and constitutes the main target of one-electron oxidants. Guanine damage can also be mediated by singlet oxygen or hydroxyl radicals. These modified guanines are usually detected by DNA cleavage occurring at oxidized sites upon alkali treatment. However, the determination of the exact nature of all the different oxidized guanines responsible for alkali-lability in damaged DNA needs further molecular investigations. Although 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) was easily detected in oxidized DNAs,[10−13] it cannot account for these alkali-labile lesions since it has been recently shown not to be alkali-labile.[2,12,14] Since the redox potential of 8-oxo-dG is significantly lower than that of 2′-deoxyguanosine nucleosides,[2,15] 8-oxo-dG within DNA is readily further oxidized into products that proved to be alkali-labile.[12,16−18] Structures of some oxidation products of 8-oxo-dG in DNA have been recently proposed. A single residue 8-oxo-dG-modified oligonucleotide oxidized by $IrCl_6^{2-}$ (a one-electron oxidant)[19] gave primarily the guanidinohydantoin residue via 5-hydroxy-8-oxo-7,8-dihydroguanine (Scheme 1). Only the guanidinohydantoin derivative was proposed to be alkali-labile. 8-oxo-dG can be oxidized by a 1,2-dioxetane derivative (equivalent to a type I photosensitized reaction) to oxazolone (dZ)[20] within calf thymus DNA.[17] Oxazolone and its precursor imidazolone (dIz)[20,21] proved to be unstable under alkaline conditions.[22,23] Thus, up to now, good candidates to account for the alkali-labile G-lesions obtained through 8-oxo-dG formation are dIz, dZ, and guanidinohydantoin.

However, alkali-labile lesions at oxidized guanines are not necessarily due to the intermediate formation of 8-oxo-dG. Imidazolone and/or oxazolone were recently identified as the major products of guanine oxidation in single-stranded (ss)[23]

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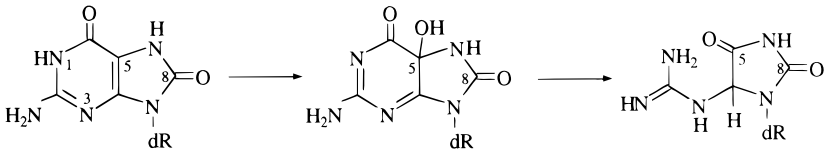
Scheme 1. 8-oxo-dG Oxidation within a ds ODN.[19]



Scheme 2. Sequences of Oligodeoxynucleotides I and II.

ODN I: 5'-d(CAGCTG) **ODN II: 5'-d(TGGTGCACCA)**

and double-stranded (ds)[24] DNA upon type I photosensitized oxidation, whereas 8-oxo-dG was only a minor product. It is to be noted also that 8-oxo-dG was not detected either, within ds DNA, where guanine radical cations were generated by a long-range electron transfer from a 2-deoxyribose radical cation.[25]

Here, we report data on guanine oxidation products within ds DNA that did not involve 8-oxo-dG. Among the different systems able to induce guanine oxidation, the cationic manganese porphyrin Mn-TMPyP[26] activated by $KHSO_5$ behaves in an original way. The high-valent porphyrin Mn(V)═O species[27−29] mediates a two-electron oxidation of guanine leading to a guanine cation. We found that the oxidation of 2′-deoxyguanosine with the chemical nuclease Mn-TMPyP/$KHSO_5$ led within one minute to a nearly quantitative amount of dIz without the formation of 8-oxo-dG.[30] On DNA, the guanine oxidation products were also alkali-labile.[31,32] We report here studies of the mechanism of oxidation of guanine residues on two ds oligonucleotides (ODNs) (Scheme 2) by Mn-TMPyP/$KHSO_5$. Imidazolone dIz and its precursors were the major products. By using HPLC coupled to electrospray mass spectrometry (ESI/MS) the different oxidized DNA strands were analyzed directly and labeling experiments in $H_2{}^{18}O$ allowed us to propose a new mechanism for the formation of dIz. We found that dIz was formed by the trapping of the guanine cation by a water molecule. Two reaction intermediates on the pathway of the dIz formation have also been observed: an oxidized guanidinohydantoin intermediate which has been only previously identified at the nucleoside level,[30] and a second one, namely, a 5,8-dihydroxy-7,8-dihydroguanine intermediate, was tentatively identified here for the first time. Furthermore, a secondary route in guanine oxidation, leading to parabanic acid, has been evidenced. The different mechanisms of guanine oxidation have been discussed in detail.

Results

1. HPLC−ESI/MS Analysis of the Oxidation Products of Guanine in 5′-d(CAGCTG) by Mn-TMPyP/$KHSO_5$. The first studied oligonucleotide, 5′-d(CAGCTG) (ODN I, see Scheme 2), is short and self-complementary for simplicity. To target the reactivity of the activated metalloporphyrin, namely a Mn(V)═O species, toward the bases of DNA instead of the sugars,[33] we designed short DNA substrates devoid of any three consecutive AT base-pairs in the sequence. The oxidation of ODN I by Mn-TMPyP/$KHSO_5$ was analyzed by reverse phase HPLC after 5 min of reaction at 0 °C. The chromatographic profiles showed a partially resolved pattern consisting of oxidized strands which were eluted before the undamaged ODN. The latter accounted for 20% of the initial quantity of the starting ODN at a retention time of 44.5 min (Figure 1, upper-left). The DNA material eluted as single strands. The guanine bases were the only targets in this oxidation reaction as detailed later in Section 5. It appeared that oligomers carrying one modified guanine residue eluted between 39.0 and 42.9 min, whereas the oligomers with two modified guanines eluted more rapidly, with a retention time of 34.7 min. A control experiment with ODN I and $KHSO_5$ alone indicated that DNA was stable under the experimental conditions described for the oxidation reaction with Mn-TMPyP/$KHSO_5$.

Relevant structural information on the oxidation products of ODN I was obtained by electrospray ionization mass spectrometry (ESI/MS) coupled to the HPLC separation of the reaction products. The UV detection chart is shown in Figure 1 (upper left). The total ionic current of the ESI source was proportional to the UV absorbance intensity (not shown). Figure 1 shows the $[M - 2H]^{2-}$ species region of the ESI/MS spectra of the different HPLC peaks. This region contained the highest intensity $[M - nH]^{n-}$ signals of all mass spectra under the experimental conditions used. Oxidation of ODN I with Mn-TMPyP/$KHSO_5$ produced oligomers containing several types of guanine lesions. The question concerning which G residue is oxidized in ODN I will be discussed in Section 6. Mass analyses of oxidized strands of ODN I, summarized in Table 1, allowed us to propose the following structural assignments for the observed guanine lesions. The nonmodified ODN I (calculated neutral molecular mass, 1792.2) eluted at a retention time of 44.5 min. Its mass spectrum showed a $[M - 2H]^{2-}$ signal at *m*/*z* 894.9 (Figure 1, upper-right) associated to its sodium adduct at *m*/*z* 905.8. The HPLC peak at 42.9 min gave a $[M - 2H]^{2-}$ signal at *m*/*z* 876.4 (Figure 1 C), indicating a loss of 37 amu with respect to the mass of the starting ODN. We will see later (Section 2) that this modified ODN may correspond to the formation of a parabanic acid derivative.[34] Imidazolone-containing DNA fragments eluted at 40.1 min and showed a $[M - 2H]^{2-}$ signal at *m*/*z* 875.4 (Figure 1 A) (associated sodium adduct at *m*/*z* 886.3) that was typical of a loss of 39 amu from the mass of ODN I. The major HPLC peak at 39.0 min contained a mixture of other oxidation products with an increase of +34 amu or +4 amu compared to the undamaged ODN I ($[M - 2H]^{2-}$ signals at *m*/*z* 911.9 and 896.9

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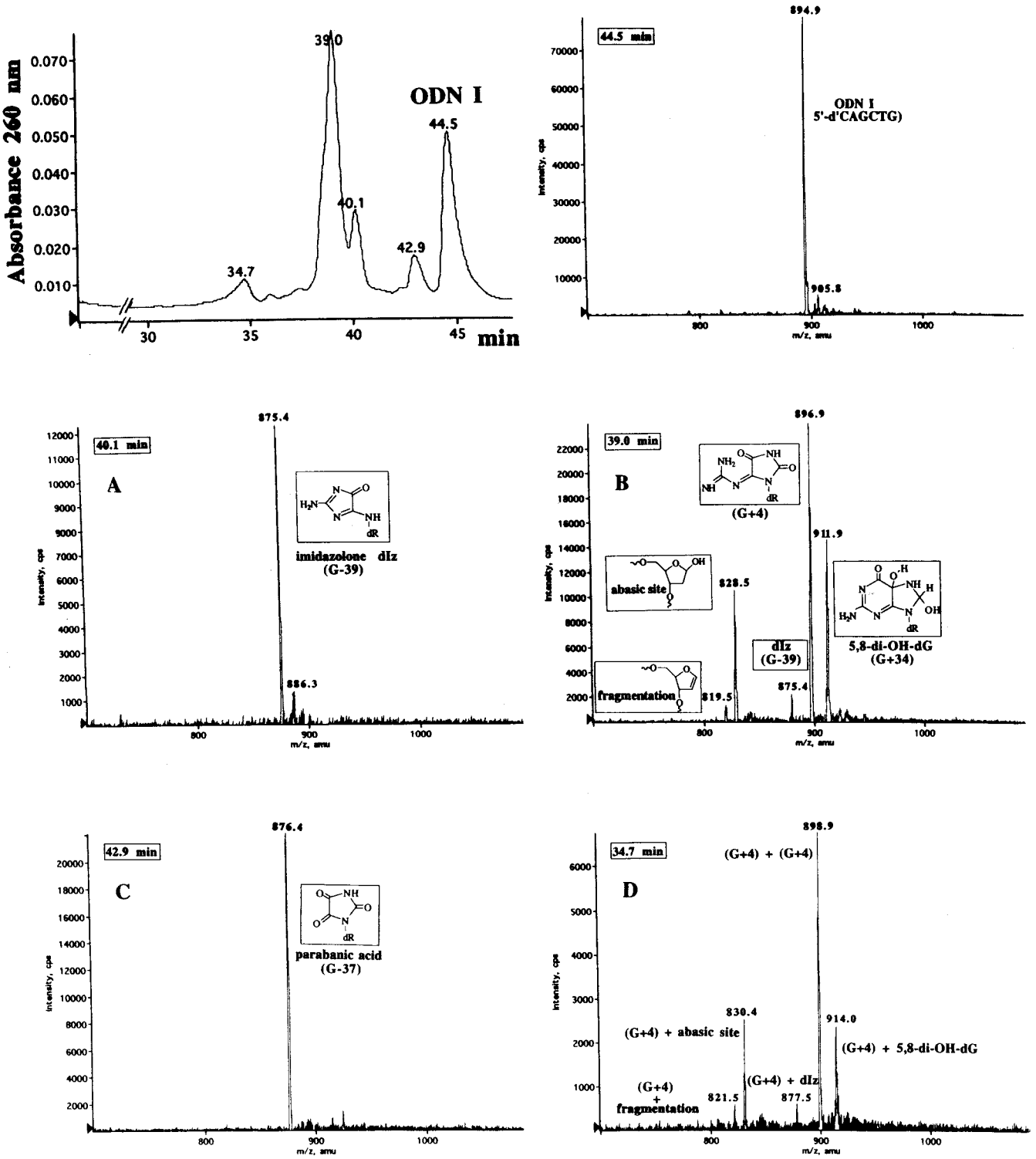

Figure 1. HPLC−ESI/MS analysis of ODN I oxidized by Mn-TMPyP/$KHSO_5$ after 5 min of reaction at 0 °C. (Upper left) HPLC profile, (upper right) ESI/MS spectrum of ODN I. ESI/MS spectra of HPLC peaks eluting respectively at 40.1 min (A), 39.0 min (B), 42.9 min (C), and 34.7 min (D). dR stands for an intra-strand 2-deoxyribose unit so that the lesions described are observed within an ODN. Only the region of $[M - 2H]^{2-}$ species are shown.

(Figure 1 B), respectively. The +34 molecular mass of this new oxidation product was tentatively attributed to the formation of 5,8-dihydroxy-7,8-dihydroguanine (proposed abbreviation for the corresponding nucleoside: 5,8-di-OH-dG). The G+4 product was attributed to an oxidized guanidinohydantoin residue by analogy with the similar reaction intermediate observed during the formation of dIz in the oxidation of 2′-deoxyguanosine by Mn-TMPyP/$KHSO_5$.[30] It could be noted that these two products had slightly different retention times because their ionization maxima were at 39.4 min for the oxidized guanidinohydantoin-containing ODN and 39.1 min for the 5,8-di-OH-dG ODN (not shown). A third lesion was associated to these two oxidation products of guanine residues. It corresponded to the hydrolysis of the glycosidic bond at a guanine residue. The $[M - 2H]^{2-}$ signal of this abasic site was at *m*/*z* 828.5 (Figure 1 B) indicating a loss of 133 amu from ODN I. The $[M - 2H]^{2-}$ signal at *m*/*z*

Table 1. Selected HPLC-ESI/MS Data[a] of Oxidized Products of ODN I (5′-CAGCTG)[b]

m/z					m/z ($H_2{}^{18}O$)[e]
obsd[c]	calcd	ΔM[d]	Rt (min)	proposed structure	LC/MS or (direct MS[f])
894.9	895.1		44.5	ODN I	894.9
				One Lesion	
876.4	876.6	−37	42.9	one parabanic acid	878.4
875.4	875.6	−39	40.1	one dIz	876.4
911.9	912.1	+34	39.0	one 5,8-di-OH-dG	912.9 (913.9)
896.9	897.1	+4	39.0	one (G+4)	898.9 (not obsd)
828.5	828.6	−133	39.0	one abasic site	828.5 (not obsd)
				Two Lesions	
914.0	914.1	+38	34.7	one (G+4) and one 5,8-di-OH-dG	916.9 (not obsd)
898.9	899.1	+8	34.7	two (G+4)	903.1 (not obsd)
830.4	830.6	−129	34.7	one (G+4) and one abasic site	832.5 (not obsd)

[a] Only the $(M - nH)^{n-}$ peaks of the $n = 2$ series for ODN I are included in this Table. The charge state was deduced from increments of +0.5 in the isotopic distribution of mass peaks. [b] Analyses were performed after a 5 min reaction at 0 °C (without heating step). See also Figures 1 and 2. [c] Mean value, standard deviation ±0.1. [d] ΔM corresponds to the mass of the oxidized ODN minus the mass of the unmodified ODN. The calculated mass of ODN I is 1792.2 (neutral form). [e] Two experimental values. Only the major species are indicated (see text for details). [f] Only if different.

819.5 (Figure 1 B) was due to a base elimination induced by a β-elimination at 2′ in oligonucleotides analyzed by ESI/MS.[35] It was also present on the ESI/MS spectrum of the undamaged ODN I under more drastic ionization conditions.

ODN I sequence contains two guanine residues. Doubly damaged oligomers eluted at a retention time of 34.7 min. This peak increased with extensive degradation of the starting ODN (not shown). It was composed of three main species with $[M - 2H]^{2-}$ signals at *m/z* 914.0, 898.9, and 830.4, respectively (Figure 1 D). These signals fitted with an increase of +4 amu to the previously described lesions at 39.0 min of the HPLC chromatogram. They may correspond to a second oxidized guanidinohydantoin lesion added to a first lesion on the other guanine residue. With respect to the initial mass of ODN I, these doubly damaged oligomers showed a difference of +38, +8 or −129 amu, respectively.

The oxidative degradation of ODN I was first studied in ammonium acetate buffer for mass analyses convenience and the results in this buffer were shown on Figures 1 and 2. However, the same products were obtained in Tris/HCl buffer (not shown). The only difference was an increase of imidazolone at a retention time of 40.1 min compared to the peak at 39.0 min containing both 5,8-di-OH-dG and oxidized guanidinohydantoin intermediates. It must be noted that the coupling of the ESI/MS analysis with an HPLC separation in triethylammonium acetate buffer as eluent allowed to analyze the oxidation reaction initially performed in Tris/NaCl buffer. The sodium cations were eliminated during the HPLC separation of the oligomers and thus coupled ESI/MS analyses of the ODNs could be operated in good conditions.

At this stage, after 5 min of reaction at 0 °C, we propose that the main guanine oxidation products generated by Mn-TMPyP/$KHSO_5$ consisted of 5,8-dihydroxy-7,8-dihydroguanine and oxidized guanidinohydantoin lesion (G+4) since they eluted under the major HPLC peak. Exact quantification of products that coeluted under the same HPLC peak, and consequently analyzed by mass spectrometry as a mixture, is not possible. An approximate quantification of the various HPLC peaks can just be obtained from the UV chart (Figure 1, upper-left). Imidazolone and parabanic acid were also formed (see Scheme 3). The following ^{18}O-labeling experiments and stability studies corroborated these proposed structures.

2. $H_2{}^{18}O$ labeling on the products of guanine oxidation by Mn-TMPyP/$KHSO_5$ on 5′−d(CAGCTG). The observation of a product whose molecular mass corresponded to an increase of +34 amu compared to the starting ODN, and that was attributed to 5,8-di-OH-dG was not consistent with the former proposed mechanism of dIz formation with Mn-TMPyP/$KHSO_5$.[30] We previously showed that excess $KHSO_5$ reacted as a nucleophile on the guanine cation and dIz was formed through a peroxide intermediate generated at C5 of guanine. We checked that the oxygen atom found in dIz came from monopersulfate, when working at the level of the nucleoside dG. The 5,8-di-OH-dG derivative was the signature of a guanine cation being, at least partly, trapped by a water molecule. The lower concentration of $KHSO_5$ used under the present experimental conditions with a double-stranded DNA could explain the nonquantitative formation of imidazolone from the nucleophilic attack of $KHSO_5$ onto the guanine cation. Furthermore, the anionic character of the peroxide may reduce its approach to DNA. To confirm this hypothesis, we performed the oxidation of ODN I in labeled water ($H_2{}^{18}O$, 96.5 atom % labeled) and the oxidized ODNs were analyzed by HPLC−ESI/MS or directly by simple ESI/MS. The results are reported in Table 1 and on Figure 2. The chromatogram of the $H_2{}^{18}O$ experiment was identical to that performed in nonlabeled water (Figure 1, upper-left).

The $[M - 2H]^{2-}$ signal of starting ODN I remained unchanged at *m/z* 894.9 as expected. The imidazolone containing oligomer now showed a $[M - 2H]^{2-}$ signal at *m/z* 876.4 (Figure 2 A) compared to the previous *m/z* 875.4 (Figure 1 A) which indicates an increase of two amu in the oligomer corresponding to the incorporation of one atom of ^{18}O from $H_2{}^{18}O$. Expansion of the $[M - 2H]^{2-}$ composite peak showed that the maximum peak of the isotopic distribution was at 876.4 but the intensity of the 875.4 peak was above the theoretical isotopic distribution of the imidazolone product. A significant amount of nonlabeled imidazolone oligomer at *m/z* 875.4 was also present. Thus, the guanine cation was trapped by a nucleophilic attack of water as a major pathway but about 40% reacted with $KHSO_5$ (see Scheme 4 for the addition of a water molecule and ref 30 for the addition of $KHSO_5$ on guanine cation).

The attack of water at C5 of the guanine cation was further confirmed by the mass analysis of the damaged ODN strand carrying the putative 5,8-di-OH-dG lesion. The former $[M - 2H]^{2-}$ signal of this species previously observed at *m/z* 911.9 (Figure 1 B) appeared at *m/z* 912.9 (Figure 2 B) (one atom of ^{18}O incorporated), when the mass analysis was performed on the HPLC eluted product. The *m/z* 913.9 was not the major species but was significant (30%). Direct injection of the crude reaction mixture in the mass spectrometer source showed a unique $[M - 2H]^{2-}$ signal at *m/z* 913.9 (two atoms of ^{18}O incorporated) (not shown). One of the two ^{18}O atoms of the 5,8-di-OH-dG product exchanged with solvent during the liquid chromatography. The difference in O-atom exchange is due to the fact that direct injection of the sample limits the number of exchangeable oxygen atoms (O-exchanges are favored with the aqueous eluent used in HPLC coupled to ESI/MS).

Consistent also with the addition of water molecules during the oxidation of guanine was the incorporation of two ^{18}O atoms

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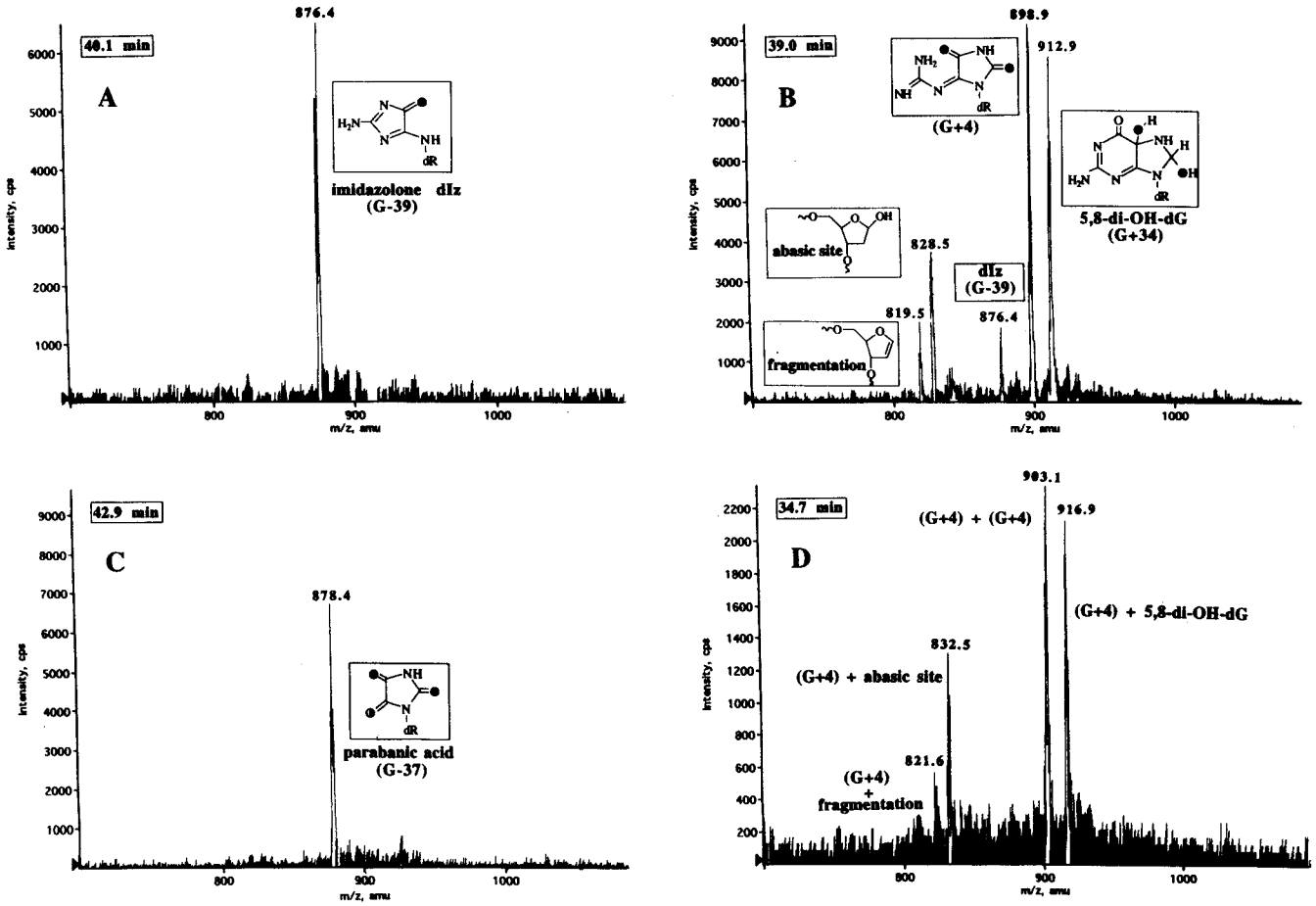


Figure 2. HPLC−ESI/MS analysis of ODN I oxidized by Mn-TMPyP/$KHSO_5$ in labeled water, after 5 min of reaction at 0 °C. ESI/MS spectra of HPLC peaks eluting respectively at 40.1 min (A), 39.0 min (B), 42.9 min (C), and 34.7 min (D). ●, ○, and ◑ correspond to oxygen atom 100% ^{18}O, 100% ^{16}O, and partially labeled, respectively. dR stands for an intra-strand 2-deoxyribose unit so that the lesions described are observed within an ODN. Only the region of $[M - 2H]^{2-}$ species are shown.

on the oxidized guanidinohydantoin modified oligomer (G+4) (see Scheme 4). The mass spectrum of this species, as analyzed under HPLC−ESI/MS conditions showed a major $[M - 2H]^{2-}$ signal at *m*/*z* 898.9 (Figure 2 B) compared to *m*/*z* 896.9 (Figure 1 B) (implying an incorporation of two ^{18}O atoms). Expansion of the *m*/*z* scale showed that the *m*/*z* 897.9 (one ^{18}O atom incorporated) accounted for about 20% of the signal. This result will be discussed later (see Discussion).

The $[M - 2H]^{2-}$ signal of the abasic site-containing strand remained unchanged at *m*/*z* 828.5 (Figure 2 B). This signal was absent on the mass spectrum of direct introduction of the reaction mixture, and thus it was not possible to discriminate between an exchange of the anomeric hydroxyl group during the chromatography or an abasic site generated by fragmentation.

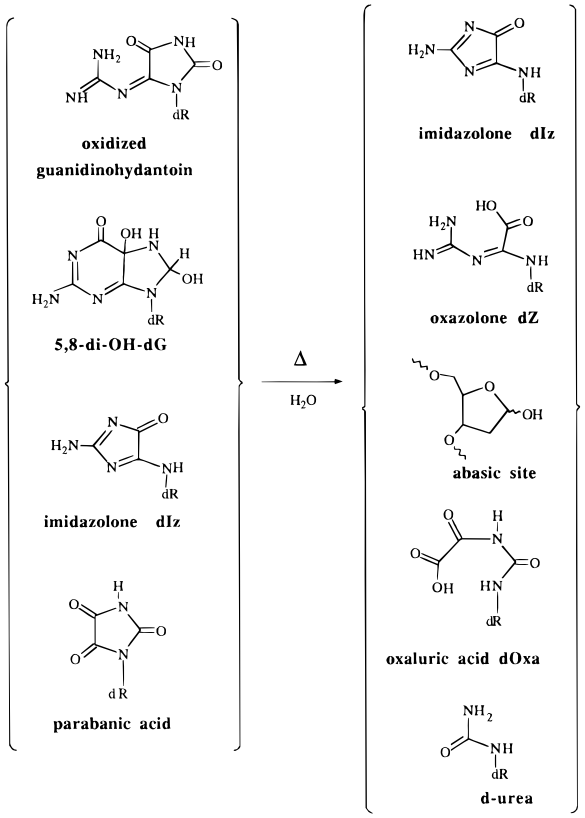
The HPLC−ESI/MS analysis of the doubly damaged oligomers followed the same general rules for labeled oxygen incorporation. The $[M - 2H]^{2-}$ signal corresponding to a DNA strand containing one oxidized guanidinohydantoin associated with one 5,8-di-OH-dG, another oxidized guanidinohydanthoin or with an abasic site appeared at *m*/*z* 916.9, 903.1, 832.5 (Figure 2 D) compared to *m*/*z* 914.0, 898.9, or 830.4 (Figure 1 D). These data confirmed that the modified oligomers contained 3, 4, or 2 labeled oxygen atoms, respectively (these signals were absent on the mass spectra obtained by direct introduction).

Labeling experiments have been also very useful in the determination of the structure of the product eluting at 42.9 min. This lesion was tentatively attributed to a parabanic acid derivative due to the observation of a major $[M - 2H]^{2-}$ signal corresponding to the incorporation of two ^{18}O atoms. This major $[M - 2H]^{2-}$ signal was at *m*/*z* 878.4 (Figure 2 C) under $H_2{}^{18}O$ compared to *m*/*z* 876.4 (Figure 1 C) in nonlabeled water. Although the mass of this species appeared to increase by 4 amu under $H_2{}^{18}O$, it resulted probably from the incorporation of three atoms of oxygen on the structure as it will be discussed later (see Discussion).

The labeling experiments allowed us to confirm the proposed structures for the oxidized products of guanine, dIz, 5,8-di-OH-dG, oxidized guanidinohydantoin (G+4), and parabanic acid. Furthermore, the origin of the oxygen atoms on the products yielded insights into the mechanism of the G-oxidation in oligonucleotides. We then studied the modification of these compounds during a thermal step.

3. Stability of Oxidized Guanine Products during a Heating Step. After 5 min of oxidation of ODN I with Mn-TMPyP/$KHSO_5$ at 0 °C the reaction was stopped by the addition of Hepes buffer ($KHSO_5$ is eliminated by reacting with this buffer). The reaction medium (pH 6.5) was then incubated at 90 °C for 15 min. HPLC analyses were performed with a different gradient of solvents (Figure 3, upper left), thus the retention times were not the same as those on Figure 1. Table 2 summarizes the results. Except for the starting ODN I (retention time of 51.8 min), none of the above-described guanine lesions were stable. The 5,8-di-OH-dG-containing oligomers seem to be present after heating, but with a decreased intensity and at a retention time of 42.2 min. Oligomers containing one dIz were observed and showed the typical [M

Scheme 3. Main Guanine Oxidation Products within ds DNA after Oxidation by Mn-TMPyP/$KHSO_5$ and Their Fate during a Heating Step[a]



[a] dR stands for an intra-strand 2-deoxyribose unit.

$-2H]^{2-}$ signal at 875.6 (Figure 3 B). While dIz containing ODNs eluted at 44.2 min, the dZ corresponding ODNs were observed at 42.2 min. Oxazolone (dZ) lesion was identified by a $[M-2H]^{2-}$ signal at *m*/*z* 884.3 (Figure 3 A). It was furthermore subjected to a decarboxylation in the mass spectrometer source as previously described,[32] giving an associated signal at *m*/*z* 862.3. The HPLC peak corresponding previously (Figure 1, upper left) to parabanic acid vanished. One $[M-2H]^{2-}$ signal of the modified oligomers eluting at a retention time of 44.8 min might be attributed to its corresponding hydrolysis derivative at a $[M-2H]^{2-}$ signal at *m*/*z* 885.5 (Figure 3 C and Scheme 3), namely, 3-amino-4-carbonyl-5-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-2-oxoacetic acid (oxaluric acid derivative or dOxa).[36] This latter compound, as well as dZ, was prone to decarboxylation and gave a fragmentation *m*/*z* signal at 863.5 associated also with a second fragmentation peak at *m*/*z* 841.9 corresponding to the additional loss of CONH, thus confirming the proposed open structure for the dOxa derivative. The new major products observed after the heating step, now co-eluted at a retention time of 44.2 min. The corresponding mass spectrum of this peak indicated that it consisted of a mixture of products, a urea derivative (abbreviated d-urea, Scheme 3), the previously observed imidazolone and an abasic site (Figure 3 B). The d-urea lesion containing oligomer showed a $[M-2H]^{2-}$ signal at *m*/*z* 849.5. The imidazolone and the abasic site lesions were attributed to the $[M-2H]^{2-}$ signals at *m*/*z* 875.6 and 828.4, respectively, as before.

(36) Luo, Y.; Henle, E. S.; Linn, S. *J. Biol. Chem.* **1996**, *271*, 21167−21176.

One $[M-2H]^{2-}$ signal remained non-identified at a *m*/*z* of 897.9 (not shown) for a product formed upon heating and eluting with a retention time of 41.2 min. This product has a molecular mass of +6 uma compared to the initial ODN I.

Thus, the four observed guanine lesions (dIz, oxidized guanidinohydantoin, 5,8-di-OH-dG, and parabanic acid) were not stable upon a short heating step at 90 °C (15 min). They were extensively modified even at neutral pH. The new lesions were dIz, dZ, d-urea, abasic site, and dOxa. Scheme 3 summarizes the main different products of guanine oxidation before and after the heating step.

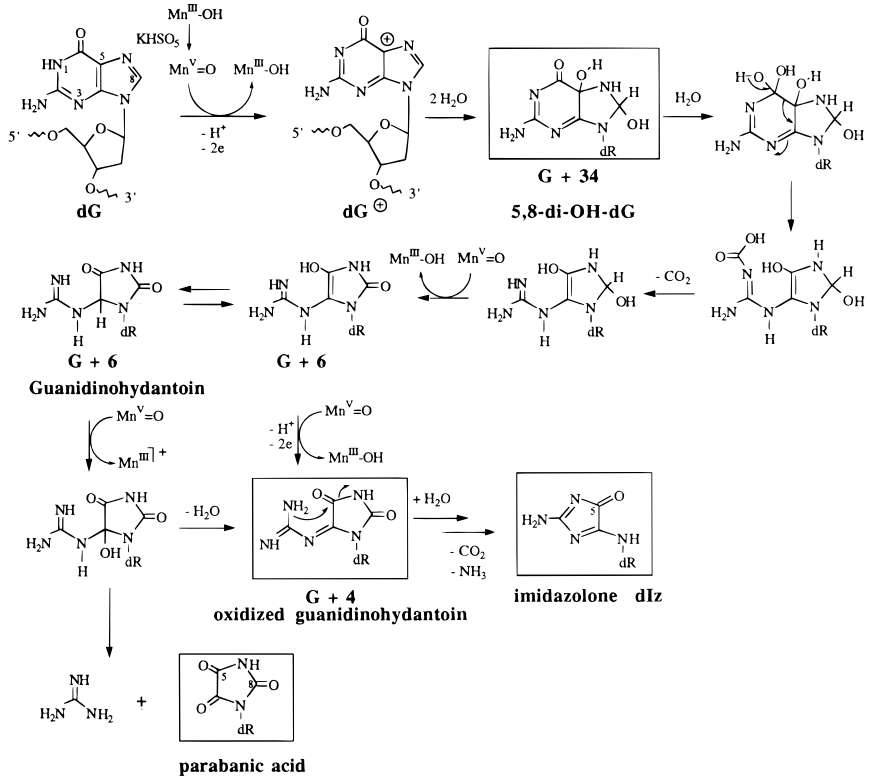
4. $H_2{}^{18}O$ Labeling on the Products of Guanine Oxidation after a Heating Step. A heating step was performed after an oxidation reaction was carried out in labeled water. As described above, the products were analyzed by HPLC coupled to ESI/MS detection. The results are summarized in Table 2. The $[M-2H]^{2-}$ signal of starting ODN I remained unchanged at *m*/*z* 894.9. The d-urea containing ODN was 70% labeled with ^{18}O. It was detected by two $[M-2H]^{2-}$ signals at *m*/*z* 850.4 and 849.5 (Figure 3 B′) with 70 and 30% relative intensities, respectively. The same result was obtained for the abasic oligomer that was observed at *m*/*z* values of 829.3 and 828.5 (Figure 3 B′) with 70 and 30% relative intensities. This indicated a partial incorporation of one ^{18}O atom on these compounds or a partial exchange during chromatography. The $[M-2H]^{2-}$ signal corresponding to the 5,8-di-OH-dG modified oligomer appeared at *m*/*z* 912.8 and 913.8 (Figure 3 A′) with about 30 and 70% relative intensities respectively, instead of *m*/*z* of 911.8 (Figure 3 A) in nonlabeled water. We verified that the dZ containing ODN incorporated two labeled oxygen atoms. The $[M-2H]^{2-}$ signal was at *m*/*z* 886.4 (Figure 3 A′) under $H_2{}^{18}O$ compared to the previous 884.3 value (Figure 3 A). Interestingly, the $[M-2H]^{2-}$ signal associated with dZ decarboxylation was at the same *m*/*z* 862.4 as before, confirming the loss of labeled CO_2. As expected, the parabanic acid derivative reacted with labeled water during the heating step. The $[M-2H]^{2-}$ signal at *m*/*z* 889.3 (Figure 3 C′) under labeled water compared to 885.5 (Figure 3 C) showed the incorporation of four oxygen atoms in dOxa (Scheme 3). The incorporation of four ^{18}O atoms into dOxa may correspond to the exchange of the carboxylic oxygens with water during the heating step. The dOxa modified oligomer showed a fragmentation *m*/*z* signal at 843.0 in Figure 3′ C which is to be compared with the signal at 841.9 as in Figure 3 C (loss of CO_2 and CONH).

The unidentified product incorporated one ^{18}O atom from water since its $[M-2H]^{2-}$ signal was at *m*/*z* 898.9 compared to 897.9 (not shown).

This second set of labeling studies confirmed the proposed structure for the transformations of products upon heating (Scheme 3).

5. Quantification of the Imidazolone Nucleoside by Enzymatic Digestion. Phosphodiesterase and alkaline phosphatase digestion of oxidized ODN I allowed the identification and the quantification of the dIz nucleoside (by comparison of the peak area with a calibration curve obtained from standard solutions prepared as described[30]). It was found that, after 5 min of reaction in Tris/HCl buffer at 0 °C, dIz was produced in a 50% yield based on the consumed guanine residues. When the reaction was performed in ammonium acetate buffer dIz was produced in a 30% yield. The stability of dIz was verified in these conditions, as also reported.[24] dIz appeared only as the β-anomer.[37a] We checked that the 8-oxo-dG nucleoside was not present in the reaction mixture (an authentic sample eluted at 48.3 min). Unfortunately, it must be noted that 5,8-di-OH-dG

Scheme 4. Proposed Mechanism of Guanine Oxidation within ds DNA by Mn-TMPyP/$KHSO_5$[a]



[a] dR stands for an intra-strand 2-deoxyribose unit. Highlighted boxes correspond to experimentaly observed derivatives after 5 min of reaction at 0 °C. Carbon atom numbering corresponds to the one of the initial guanine.

probably elutes with the enzyme peaks in the first minutes of the chromatogram and consequently was not identified. The oxidized guanidinohydantoin nucleoside was not found either, but it can be transformed to imidazolone during the time course of the digestion.[30] Parabanic acid or dOxa were not detected.

Quantitative recovery of dA, dC, and dT nucleosides, determined by means of calibration curves of standards, allowed us to conclude that only guanine oxidation products were obtained (data not shown).

6. Preferential Oxidation of Terminal G of 5′-d(CAGCTG). Piperidine treatment and subsequent dephosphorylation of the DNA fragments with alkaline phosphatase allowed us to address the position of the guanine lesions within the oxidized ODN I together with their alkali-lability. An oxidized G_6 should lead to a 5′-CAGCT DNA fragment, and a G_3 lesion should give two DNA fragments, 5′-CA and 5′-CTG. The G_6/G_3 cleavage ratio was determined by the (5′-CAGCT + 5′-CT)/(5′-CA) oligomer fragments ratio on HPLC chromatograms under gradient conditions. The retention times of 5′-CAGCT, 5′-CT, and 5′-CA were 34.7, 23.6, and 25.1 min, respectively, compared to that of ODN I at 37.5 min. In the early stage of the oxidation reaction (entry 1, Table 3), the hexamer was preferentially oxidized to an alkali-labile site at the terminal G_6 which is probably more weakly paired than G_3 and more accessible. After a longer reaction time, more alkali-labile lesions were obtained at G_3 (entries 2 and 3, Table 3). When ODN I was not hybridized (entry 4, Table 3), both G residues were equally touched. Thus, the oxidation of guanines by Mn-TMPyP/$KHSO_5$ occurred preferentially at unpaired or end-of-duplex guanines, but the oxidation of ODN II allowed us to verify that this was not due to a dehybridization effect and that paired guanines were prone to oxidation as well (see Section 8). This experiment confirmed that all guanine lesions were alkali-labile since no residual oxidized ODN strands were left uncleaved after piperidine treatment.

7. Guanine versus Sugar Oxidation. Finally, we checked that, under the typical 5 min oxidation conditions, the oxidative degradation of deoxyribose units of ODN I by Mn-TMPyP/$KHSO_5$ is a minor process when the oligonucleotide contains a G-rich sequence. The hydroxylation at the C1′ position of deoxyribose units was quantified as previously described.[37b] The amount of 5-methylene-2-furanone (5-MF) released after heating the damaged DNA, indicated that only one sugar over 50 was oxidized under the less drastic experimental conditions used here compared to our previous work.[37b]

8. HPLC−ESI/MS Analysis of the Products of Guanine Oxidation on 5′-d(TGGTGCACCA) by Mn-TMPyP/$KHSO_5$. The self-complementary ODN II (Scheme 2), 5′-d(TGGTGCACCA), was used as a second substrate to study the oxidation of guanines by Mn-TMPyP/$KHSO_5$. It is longer than ODN I and is a double-stranded substrate without a terminal guanine. The oxidation of ODN II allowed us to verify that paired guanines were sensitive to oxidation, the ratio of oxidized G residues on ODN II was 6.5/2.5/1 for $G_2/G_3/G_5$, respectively. This reactivity correlates with the values of the ionization potentials of the considered guanines. The most oxidizable G should be G_2, the 5′G of the 5′-GG sequence with a ionization potential of 6.7 eV.[2,38] Then G_3, the 3′G of 5′-GG sequence will have an ionization potential at 7.1 eV.[15] The less oxidized guanine residue of ODN II should be the isolated G_5 (7.3 eV).

The HPLC−ESI/MS analysis of the oxidation reaction of ODN II by Mn-TMPyP/$KHSO_5$ showed the same reaction products than ODN I. The results are reported in Table 4. dIz-,

(37) (a) Vialas, C.; Pratviel, G.; Meyer, A.; Rayner, B.; Meunier, B. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1201−1205 (b) Pratviel, G.; Pitié, M.; Bernadou, J.; Meunier, B. *Nucleic Acids Res.* **1991**, *22*, 6283−6288.

(38) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068.

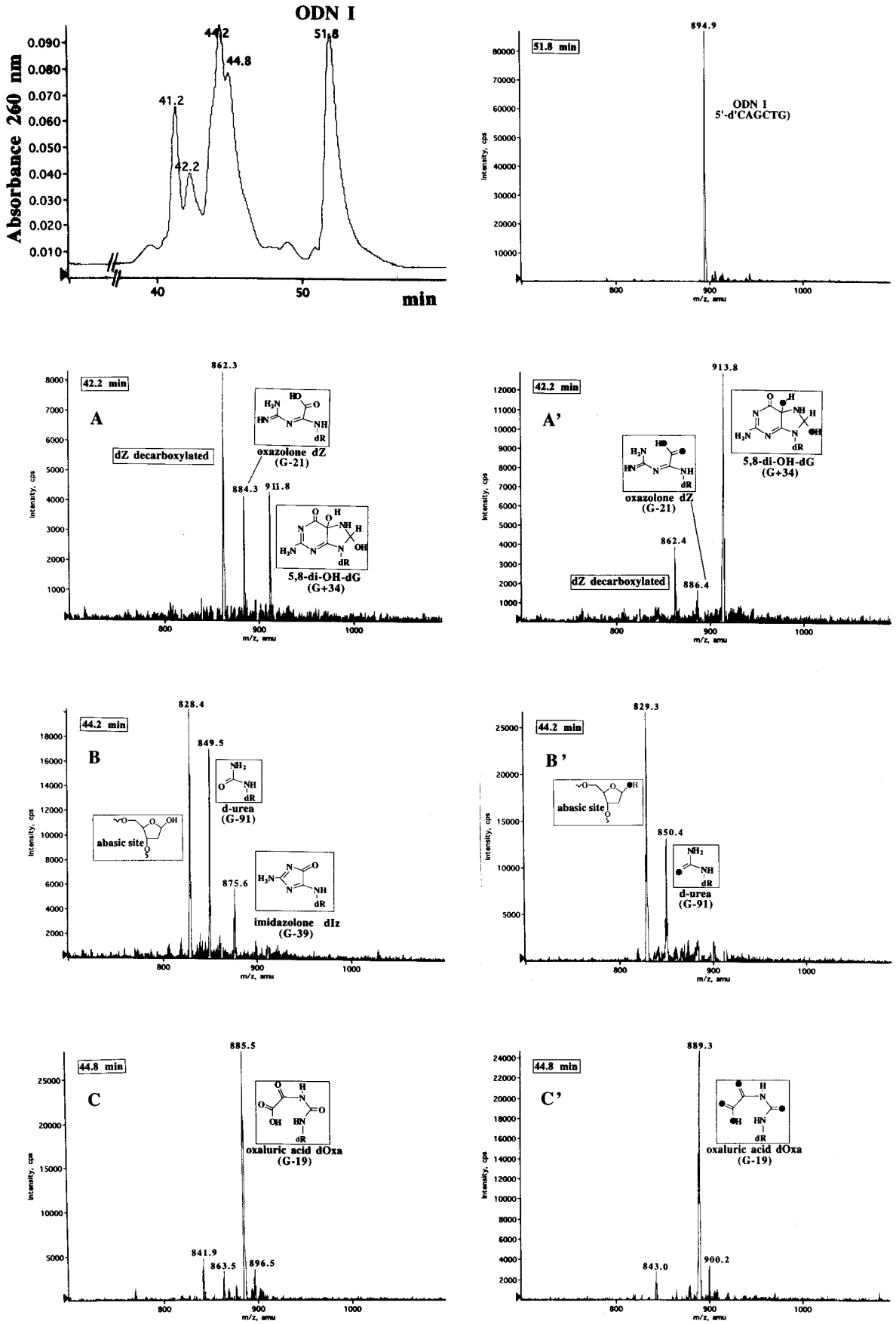


Figure 3. HPLC−ESI/MS analysis of ODN I oxidized by Mn-TMPyP/$KHSO_5$ after a heating step (90 °C, 15 min) in $H_2{}^{16}O$ compared to $H_2{}^{18}O$. (Upper left) HPLC profile, (upper right) ESI/MS spectrum of ODN I. ESI/MS spectra of HPLC peaks eluting respectively at 42.2 min (A and A′), 44.2 min (B and B′), and 44.8 min (C and C′). (A−C) spectra correspond to reactions performed in nonlabeled water and (A′−C′) spectra to reactions performed in labeled water. ●, ○, and ◑ correspond to oxygen atom 100% ^{18}O, 100% ^{16}O, and partially labeled, respectively. dR stands for an intra-strand 2-deoxyribose unit so that the lesions described are observed within an ODN. Only the region of $[M - 2H]^{2-}$ species are shown.

Table 2. Selected HPLC-ESI/MS Data[a] of Oxidized Products of ODN I (5′-CAGCTG)[b]

m/z					m/z ($H_2{}^{18}O$)[e]
obsd[c]	calcd	ΔM[d]	Rt (min)	proposed structure	HPLC/ESI/MS
894.9	895.1		51.8	ODN I	894.9
				One Lesion	
885.5	885.6	−19	44.8	one dOxa	889.3
863.5	863.6	−63	44.8	one dOxa (loss of CO_2)	not obsd
841.9	842.1	−106	44.8	one dOxa (loss of C_2O_3NH)	843.0
875.6	875.6	−39	44.2	one dIz	not obsd
849.5	849.6	−91	44.2	one d-urea	850.4
828.4	828.6	−133	44.2	one abasic site	829.3
911.8	912.1	+34	42.2	one 5,8-di-OH-dG	913.8
884.3	884.3	−21	42.2	one dZ	886.4
862.3	862.6	−65	42.2	one dZ (loss of CO_2)	862.4
897.9	−	+6	41.2	?	898.9

[a] Only the $(M - nH)^{n-}$ peaks of the $n = 2$ series of ODN I are included in this Table. The charge state was deduced from increments of +0.5 in the isotopic distribution of mass peaks. [b] Analyses were performed after a 5 min reaction in ammonium acetate buffer (pH 6.5) at 0 °C followed by a heating step (90 °C, 15 min). See also Figure 3. [c] Mean value, standard deviation ±0.1 [d] ΔM corresponds to the mass of the oxidized ODN minus the mass of the unmodified ODN. The calculated mass of ODN I is 1792.2 (neutral form). [e] One experimental value. Only the major species are indicated (see text for details).

Table 3. Selectivity Between G_6 and G_3 Observed During the Oxidation of ODN I (5′-CAGCTG) by Mn-TMPyP/$KHSO_5$

	exp. conditions					
entry	T (°C)	reaction time (min)	intact G residues (%)[a]	oxidized G_6 (%)[a]	G_3	G_6/G_3
1	0	1	73	12	2	6/1
2	0	5	43	20	5	4/1
3	0	8	40	26	9	3/1
4	37[b]	1	55	10	7	1.4/1

[a] Quantification was done with standard curves for HPLC peaks corresponding to ODN I and its various cleavage products. [b] Lower concentration of Mn-TMPyP (5 μM).

Table 4. Selected HPLC-ESI/MS Data[a] of Oxidized Products of ODN II (5′-TGGTGCACCA)[b]

m/z				
obsd	calcd	ΔM[c]	Rt (min)	proposed structure
1008.2	1008.3		43.4	ODN II
995.3	995.3	−39	38−41	one dIz
1019.4	1019.7	+34	38−41	one 5,8-di-OH-dG
1009.5	1009.7	+4	38−41	one (G+4)
995.9	996.0	−37	38−41	one parabanic acid

[a] Only the $(M - nH)^{n-}$ peaks of the $n = 3$ series for ODN II are included in this Table. The charge state was deduced from the increments of +0.3 in the isotopic distribution of mass peaks. [b] Analysis performed after reaction at 0 °C for 5 min (no heating step). [c] ΔM correspond to the mass of the oxidized ODN minus the mass of the unmodified ODN. The calculated mass of ODN II is 3028.0 (neutral form).

5,8-di-OH-dG-, oxidized guanidinohydantoin-, parabanic acid-containing oligomers have been identified.

Discussion

Mechanism of Guanine Oxidation in ds DNA by Mn-TMPyP/$KHSO_5$. The high-valent Mn(V)═O species generated by the reaction of $KHSO_5$ with Mn-TMPyP is able to abstract two electrons and one proton from a guanine residue leading to a guanine cation. The positive charge seems to be located at C5. The guanine cation can be trapped by a water molecule or a monopersulfate ion reacting at C5. In the present report both reactivities were observed. The trapping of the guanine cation by the peroxide was previously reported at the nucleoside level and led to imidazolone in a quantitative way.[30] The oxygen atom of imidazolone did not exchange with $H_2{}^{18}O$ water and resulted from the incorporation of a peroxidic oxygen at C5. As an important feature of the present work, we found from the labeling studies in $H_2{}^{18}O$ that the oxygen atom of imidazolone came mainly from water. The different products resulting from the quenching of the guanine cation by a water molecule are reported in Scheme 4. After the addition of a water molecule to the guanine cation at C5, a second molecule of water would add at the C8 carbon leading to the +34 amu intermediate, proposed to be the 5,8-dihydroxy-7,8-dihydroguanine (5,8-di-OH-dG). This was confirmed by the incorporation of two oxygen atoms from $H_2{}^{18}O$. The oxygen atom of the hydroxyl function at C8 is probably exchanged during liquid chromatography studies. An easy ring-chain tautomerism in the five-membered ring would lead to an aldehyde group at C8 which readily exchanges its oxygen atom with water. No intermediate corresponding to an ODN I whose mass would only be increased by 16 amu was seen (one 8-oxo-7,8-dihydroguanine residue). The addition of a third molecule of water at C6 would induce the six-membered ring cleavage and the loss of CO_2.[39] Oxidation of the C8 carbinol function should give the guanidinohydantoin, the intermediate referred to as the G+6 compound. Under our experimental conditions it was not detected but was probably rapidly converted to the oxidized guanidinohydantoin (the G+4 derivative in Scheme 4). The ^{18}O-labeling of the oxidized guanidinohydantoin (G+4) showed consistently two ^{18}O atoms incorporation from labeled water. (G+4) is the precursor of imidazolone as previously described.[30]

The formation of parabanic acid corresponds to an oxidation of the former C4 of guanine. We propose that an hydroxylation at C4 of the guanidinohydantoin intermediate (G+6 compound) by the Mn(V)═O species followed by the release of the guanidinium moiety could be responsible for the formation of parabanic acid. The ^{18}O-labeling of this product as well as the mechanism of its formation is discussed below.

In summary, four main guanine oxidation products were identified, 5,8-di-OH-dG, the oxidized guanidinohydantoin (G+4), imidazolone, and parabanic acid. None of these products was stable upon heating at 90 °C for 15 min at neutral pH. All of these compounds were also alkali-labile. Among the products formed by heating, several are well-known for their alkali-lability, the abasic site, imidazolone, oxazolone, and the urea derivative.[40,41] The parabanic acid was transformed into oxaluric acid that is probably alkali-labile as well (Scheme 3). Thus, it is not necessary to involve the oxidation of the sugars of DNA to explain the formation of alkali-labile products.[16]

Until now, the 5,8-dihydroxy-7,8-dihydroguanine derivative (5,8-di-OH-dG) has not been reported in the literature as a guanine oxidation intermediate. It corresponds to the first intermediate obtained from a two-electron oxidation of guanine. One-electron oxidation of guanine leads to a 5-peroxyl intermediate after the trapping of a guanine neutral radical by molecular oxygen.[21] A similar 5-hydroxyl compound, 5-hydroxy-8-oxo-7,8-dihydroguanine(5-OH-8-oxo-dG), was pro-

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(40) Poje, M.; Sokolic-Maravic, L. *Tetrahedron* **1988**, *44*, 6723−6728.
(41) Darby, G. K.; Jones, A. S.; Tittensor, J. R.; Walker, R. T. *Nature* **1967**, *216*, 793−794.

posed in the case of the oxidation of 8-oxo-dG. It corresponded to an increase of +32 amu compared to the molecular mass of guanine because the substrate (8-oxo-dG ODN) was initially oxidized at the C8 position of guanine (Scheme 1).[19] The compound (G+32) was not observed in this work (its corresponding $[M - 2H]^{2-}$ signal at *m*/*z* 911 was not detected in the mass spectra of ODN I). This implies that the oxidation of the secondary alcohol at the C8 of guanine may not be so fast and may occur only after decarboxylation on the open structures. These two 5-hydroxyl derivatives of guanine, 5,8-di-OH-dG and 5-OH-8-oxo-dG, represent markers of the attack of water on an oxidized intermediate of guanine or 8-oxo-7,8-dihydroguanine, exactly as in the case of the oxidation of urate.[39,42]

In the oxidation of an 8-oxo-dG-modified ODN by Ni complexes[19] or by electrochemical oxidation of 8-oxo-dG,[43] the 5-OH-8-oxo-dG is transformed to guanidinohydantoin. The latter compound can be detected if the oxidizing systems are unable to further oxidize it. This is not the case in this work due to the strong oxidizing properties of Mn(V)═O species which can behave as an hydroxylating agent or a two-electron oxidant. Consequently, (G+6) could be quickly converted to oxidized guanidinohydantoin (G+4) or parabanic acid.

The cyclization involving the nucleophilic attack of the guanidinium NH_2 on the C5 carbonyl group of guanidinohydantoin (G+6) was not observed under the used experimental conditions.[44] The resulting dihydro-imidazolone would have exactly the same molecular mass as that of the parabanic acid but would incorporate only one ^{18}O atom in $H_2{}^{18}O$.

The G+4 compound was found to be unexpectedly stable in this work. This derivative was observed as a transient species during the oxidation of the guanine nucleoside.[30] Thus, the fast intramolecular nucleophilic attack of the guanidine NH_2 on the C5 carbonyl group, which occurred readily on the 4-imino-5-keto form of the nucleoside derivative (intermediate G+4), was considerably slowed in double-stranded DNA (Scheme 4). The reduction of its nucleophilic character is probably due to hydrogen bonding with the complementary cytosine. However, a heating step or even a lyophilization procedure promoted its degradation. The G+4 compound could be formed either from a 5-peroxyl intermediate[30] or a 5-hydroxyl intermediate resulting from the quenching of the guanine cation by $KHSO_5$ or water, respectively. The labeling of the former C5 of guanine should reflect, in the G+4 derivative, the one found on dIz-containing ODNs, i.e. 40% of ^{16}O. Since its $[M - 2H]^{2-}$ signal in labeled water experiments showed a high percentage of incorporation of two ^{18}O atoms (about 80%), the former C8 carbon of guanine must be essentially composed of ^{18}O. Thus, oxidation of the C8 occurred without oxygen atom transfer from the Mn(V)═O species (Scheme 4).

Parabanic acid has been described in the literature. It was shown to arise from the reaction of singlet oxygen onto silylated 8-oxo-7,8-dihydroguanosine.[34] The previously proposed mechanism for its formation involved a peroxide intermediate at C4 of guanine due to a [2 + 4] cycloaddition of $^1O_2$ on C8 and C4.[45] The C4-peroxyl intermediate was then reduced to a C4-hydroxyl before the formation of parabanic acid.[34] To produce parabanic acid, the C4 must be oxidized. Under our experimental conditions, the mechanism of parabanic acid formation cannot involve the attack of a water molecule on the former C4 of guanine because the overall isotopic distribution of the $[M - 2H]^{2-}$ signal of this product in $H_2{}^{18}O$ had a maximum peak corresponding to the incorporation of two ^{18}O atoms (*m*/*z* 878.4 compared 876.4, Table 1). If the hydrolysis of the N3−C4 bond was responsible for its formation, its isotopic distribution should have shown a major *m*/*z* peak corresponding to the incorporation of three ^{18}O atoms with a peak at *m*/*z* 879.4, which is not the case. We propose that the reduction of the label on the hydroxylation product arose from the oxo−hydroxo tautomerism on the Mn(V)═O species.[46,47] When an hydroxylation of a C−H bond is performed by a Mn(V)═O entity in $H_2{}^{18}O$ water, the oxygen atom that is inserted in the C−H bond is only partly labeled. The mechanism of formation of parabanic acid probably includes one hydroxylation step, which occurred at the C4. Consequently, the isotopic distribution of parabanic acid should show a $[M - 2H]^{2-}$ signal with two peaks: one at *m*/*z* 878.4 for two ^{18}O atoms incorporated and one at *m*/*z* 879.4 for three ^{18}O atoms incorporated. The ratio of the two species depends on the oxo−hydroxo tautomerism equilibrium in the experimental conditions. In Figure 2 C, the ratio of the two species 878.4/879.4 is about 70/30.

The characterization of the G+34 lesion as well as the study of its stability and possible further oxidation is in progress.

Conclusions

Oxidation of guanine residues by the Mn-TMPyP/$KHSO_5$ system in double-stranded DNA led to the proposal of a new guanine oxidation intermediate, namely, 5,8-dihydroxy-7,8-dihydroguanine. It transforms either into guanidinohydantoin, imidazolone, or parabanic acid, depending on the oxidizing conditions. When Mn(V)═O species was used, guanidinohydantoin did not accumulate. The observation of the transient oxidized guanidinohydantoin on ds DNA (G+4) is also reported for the first time. Upon heating at 90 °C for 15 min at neutral pH, 5,8-dihydroxy-7,8-dihydroguanine, imidazolone, or parabanic acid proved to be unstable and led to alkali-labile sites at guanine residues. The alkali-lability may be explained by their transformation into urea derivatives and abasic sites. The observed guanine oxidation products on a ds DNA substrate could thus explain the alkali-labile breaks previously observed in the oxidation of a G-rich DNA with a manganese−porphyrin−oligonucleotide conjugate.[31,32]

The high resolution of ESI/MS spectra allowed us to obtain the exact molecular mass of the damaged DNA strands before strand cleavage and to determine the origin and the number of oxygen atoms on DNA oxidation products, when performing reactions in ^{18}O-labeled water.

In conclusion, the distribution of the different oxidation products of guanine residues in double-stranded DNA is highly influenced by the nature of the oxidant (one- versus two-electron oxidants, hydroxylating- versus non-hydroxylating agents) and also differs from the distribution observed in studies performed with nucleoside models, since the pairing of guanine and the polyanionic nature of ds DNA are important factors able to affect the kinetics of the transformation of G-oxidized intermediates.

Experimental Section

Materials. Potassium monopersulfate, $KHSO_5$ (triple salt 2 $KHSO_5{\cdot}K_2SO_4{\cdot}KHSO_4$, Curox) was from Interox, piperidine from Fluka. Oligonucleotides were synthesized by standard solid-phase

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β-cyanoethyl phosphoramidite chemistry. They were purified by HPLC using a reverse-phase column (Nucleosil C18, 10 μm from Interchrom; eluents, A = 0.1 M triethylammonium acetate (TEAA) (pH 6.5), B = CH_3CN; linear gradient, 1−20% B in the case of ODN I and 1−30% in the case of ODN II over 60 min; flow rate, 1.5 mL/min; λ = 260 nm). Mn-TMPyP was prepared as previously described.[48] Labeled water (96.5 atom % ^{18}O) was from Euriso-top (Saclay, France). Snake venom phosphodiesterase (*Crotalus adamanteus*), P_1 nuclease (*Penicillium citrium*), and bacterial alkaline phosphatase were purchased from Sigma.

HPLC−ESI/MS. ODN (200 μM duplex) was incubated in Tris/HCl buffer pH 7 (50 mM), NaCl (100 mM) at 0 °C or in ammonium acetate buffer pH 6.5 (200 mM) with Mn-TMPyP (200 μM). After preincubation (15 min), $KHSO_5$ (2 mM) was added. Final volume was 40 μL. After 5 min of reaction at 0 °C, the reaction medium was either directly injected or stopped by the addition of Hepes buffer pH 8 (50 mM), heated for 15 min at 90 °C, and then injected. An analytical reverse phase column (Nucleosil C18, 10 μm) was used. The gradient was linear (eluents, A = 10 mM TEAA pH 6.5, B = CH_3CN; 3−10% B over 60 min in the case of ODN I and 3−15% B in the case of II; flow rate, 1 mL/min; λ = 260 nm). The column was coupled to an electrospray mass spectrometer. Only 4% of the flow eluted from the column was introduced into the electrospray source. The ESI/MS spectrometer was a Perkin-Elmer SCIEX API 365 used in negative mode.

Labeling Experiments. The reaction was performed as described above, except that the reactants were dissolved in $H_2{}^{18}O$. The reaction mixture was directly injected for HPLC−ESI/MS analysis.

Oxidation of ODN I and ODN II by Mn-TMPyP/$KHSO_5$. For the following analysis which did not require an HPLC−ESI/MS, a typical experiment consisted of preincubation of ODN (10 μM duplex) with Mn-TMPyP (10 μM) in Tris/HCl buffer pH 7 (50 mM), NaCl (100 mM) at 0 °C or in ammonium acetate buffer pH 6.5 (100 mM). The reaction was initiated by the addition of $KHSO_5$ at a final concentration of 500 μM when the reaction was performed in Tris/NaCl buffer or 100 μM when in ammonium acetate buffer (same conversion than in Tris/NaCl). Total volume was 100 μL. After 5 min at 0 °C, the reaction was stopped by addition of Hepes buffer pH 8 (10 mM). Concentrations in brackets are final concentrations.

HLPC Analyses. The reaction media were analyzed by HPLC, with an analytical reverse phase column (Nucleosil C18, 10 μm, 250 × 4.6 mm) eluted with a linear gradient (eluents, A = 0.1 M TEAA pH 6.5, B = CH_3CN; 1−20% B over 60 min in the case of ODN I and 1−30% B over 60 min in the case of ODN II; flow rate, 1.5 mL/min; λ = 260 nm). A diode array detector (Kontron Instrument) allowed detection of the products at 260 nm and monitoring of UV−vis spectra.

Piperidine Treatment. The reaction medium (final volume 100 μL, in Tris/NaCl buffer) was placed in piperidine (0.3 M) and then heated at 90 °C during 30 min. Subsequent dephosphorylation with alkaline phosphatase (1 unit, 30 min, 37 °C) produced fragments which were quantified on HPLC by comparison with standards.

Enzymatic Digestion. The reaction was performed on a 2-fold scale (compared to the typical experiment) in Tris/NaCl or in ammonium acetate buffer. The samples were digested with snake venom phosphodisterase (0.16 unit), P_1 nuclease (3 units) and alkaline phosphatase (1.4 unit) at 0 °C for 15 h. The nucleosides were eluted isocratically on an Uptisphere reverse phase column, with MeOH/H_2O, 1/99 for 13 min, 7/93 for 21 min and finally 20/80 for 26 min at 1 mL/min. Detection was performed at 260 nm. The retention times for imidazolone, 2′-deoxycytidine, 2′-deoxyguanosine, thymidine and 2′-deoxyadenosine were respectively 14.1, 23.5, 41.6, 44.1, and 53.1 min.

Quantification of Oxidative Degradation of Deoxyribose Units. After typical oxidation (made in a 4-scale fold), the reaction medium was heated for 15 min at 90 °C and chilled in liquid nitrogen to stop the heated reaction. Bases and 5-MF were eluted in the same chromatographic conditions as that for the nucleosides (see above). Quantification of 5-MF was done by comparison of peak area versus standard injection of authentic samples.

Abbreviations

amu, atomic mass units; ODN, oligodeoxyribonucleotide; ds or ss, double- or single-stranded respectively; Mn-TMPyP, see ref 26; dIz and dZ, see ref 20; 5,8-di-OH-dG, 5,8-dihydroxy-7,8-dihydroguanine; dOxa, 3-amino-4-carbonyl-5-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-2-oxoacetic acid; d-urea, urea derivative.

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