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Use of Short Duplexes for the Analysis of the Sequence-Dependent Cleavage of DNA by a Chemical Nuclease, a Manganese Porphyrin

Sophie Mourgues, Adam Kupan, Geneviève Pratviel,* and Bernard Meunier[a]

This article is dedicated to Professor W. Stec on the occasion of his 65th birthday.

*A manganese porphyrin, manganese(III)-bis(aqua)-*meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin, in the presence of $KHSO_5$ is able to perform deoxyribose or guanine oxidation depending on its mode of interaction with DNA. These two reactions involve an oxygen-atom transfer or an electron transfer, respectively. The oxidative reactivity of the manganese-oxo porphyrin was compared on short oligonucleotide duplexes of different sequences. The major mechanism of DNA damage is due to deoxyribose hydroxylation at a site of strong interaction, an $(A{\cdot}T)_3$ sequence. Guanine oxidation by electron transfer was found not to be competitive with this major mechanism. It was found that a single intrastrand guanine was three orders of magnitude less reactive than an $(A{\cdot}T)_3$ sequence. The reactivity of a 5′-GG sequence was found to be intermediate and was estimated to be two orders of magnitude less than that of an $(A{\cdot}T)_3$ site. Short oligonucleotide duplexes, as double-stranded-DNA models, proved to be convenient tools for the study of the comparative reactivity of this reagent toward different sequences of DNA. However, they showed a particular reactivity at their terminal base pairs (the "end effect") that biased their modeling capacity for double-helix-DNA models.*

Introduction

Oxidative DNA damage can be due to small and diffusing oxidative species, like hydroxyl radical or singlet oxygen species, generated by a reagent.[1–3] In these cases no special interaction of these reagents with DNA is necessary. The most striking example is Fe-ethylenediaminetetraacetate (Fe-EDTA), a hydroxyl-radical-generating system, which is a negatively charged complex and is used to damage DNA although it cannot approach the biological target due to the repulsion of the negative charges.[4] Even if the covalent attachment of this reagent onto a DNA binding moiety affords a more efficient oxidative entity,[5] the reactive moiety of the conjugated molecule still does not directly interact with DNA. The mechanism of DNA damage is identical, only its efficiency increased.

On the other hand, when the DNA-damaging agent is a DNA-binding molecule, the oxidative DNA damage depends on the interaction of the reagent with DNA itself. Reagents that can attack deoxyribose moieties of DNA must be molecules endowed with high-affinity binding sites with a well-defined interaction of the cleaver within DNA in order to locate the reactive center of the molecule in the vicinity of the deoxyribose groups. The efficiency, as well as the mechanism of oxidative DNA damage, depends on the interaction between the reactive entity of the reagent and DNA. This is the key point for the efficiency of the chemical nuclease Cu(1,10-phenanthroline)$_2$[6–8] or antitumor agents like Fe-bleomycin[9–11] and compounds of the enediynes family.[12–14] These DNA cleavers mediate DNA damage with different chemistries because they interact in different ways with DNA. When they are activated, Fe-bleomycin and Cu(1,10-phenanthroline)$_2$ are able to attack the C4′ or C1′ atoms of the deoxyribose moieties of DNA, respectively. One reagent may also interact in different ways depending on the sequence of DNA. Enediynes mediate oxidation of deoxyriboses at different carbon atoms when positioned at different sites of interaction.[14]

Oxidative DNA damage can also be due to electron abstraction from a guanine base.[2,3,15] In this case the oxidizing reagents must approach or contact DNA in such a way that the reaction is possible. Electron transfer between the oxidant and guanine bases may not require such a close interaction of the oxidative agent as deoxyribose oxidation.

In the present work we describe the case of a chemical nuclease able to perform deoxyribose and guanine oxidation and we compare the relative importance that these reaction pathways may play in DNA degradation. Three types of chemistry have been reported for this reagent: deoxyribose oxidation by hydroxylation at the C5′ or C1′ carbon atoms and guanine oxidation by electron transfer. Hydroxylation at the C5′ atom is due to a very precise and strong interaction of the chemical nuclease with a particular DNA sequence, whereas the interac-

[a] *S. Mourgues,[+] A. Kupan,[+] G. Pratviel, B. Meunier*
Laboratoire de Chimie de Coordination du CNRS
205, route de Narbonne, 31077 Toulouse Cedex 4 (France)
Fax: (+33) 561-553-003
E-mail: pratviel@lcc-toulouse.fr

[+] *These authors contributed equally to this work.*

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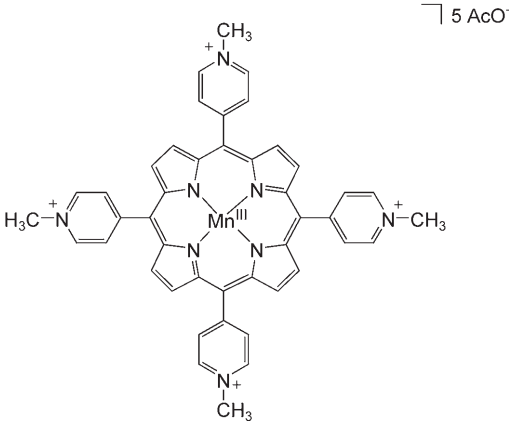
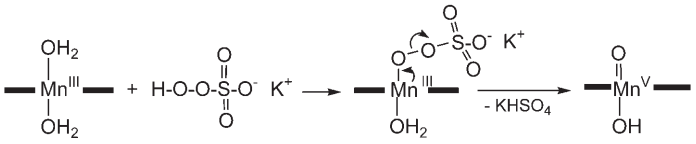
tion of the chemical nuclease responsible for oxidation of C1′ atoms and guanine bases is not known.

The cationic manganese porphyrin, manganese(III)-bis(aqua)-*meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin (Mn-TMPyP, Scheme 1), activated by $KHSO_5$ is able to oxidize DNA[16]



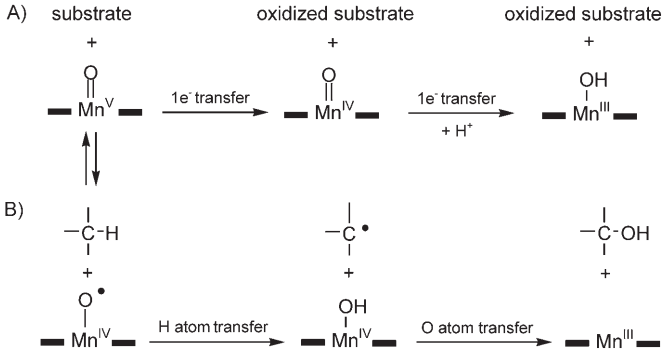
Scheme 1. Structure of the cationic manganese porphyrin Mn-TMPyP.

through a high-valency manganese-oxo species (Scheme 2).[17] The formally written Mn^{V}=O species is able to perform oxygen-atom transfer when the metal-oxo center is near to a carbon–hydrogen bond of a substrate (deoxyribose moiety in the case



Scheme 2. Activation of Mn-TMPyP into a metal-oxo species with $KHSO_5$. The porphyrin macrocycle is simplified for clarity.

of DNA; Scheme 3B) or to perform abstraction of electrons from an electron-rich substrate (guanine base; Scheme 3A). The first reaction, hydroxylation at the C5′ carbon atom, occurs in A/T-rich regions when the porphyrin is located in the minor groove of DNA, at a strong binding site consisting of a tri-



Scheme 3. The two types of chemistry performed by the manganese-oxo species, namely, electron transfer (A) and oxygen atom transfer (B).

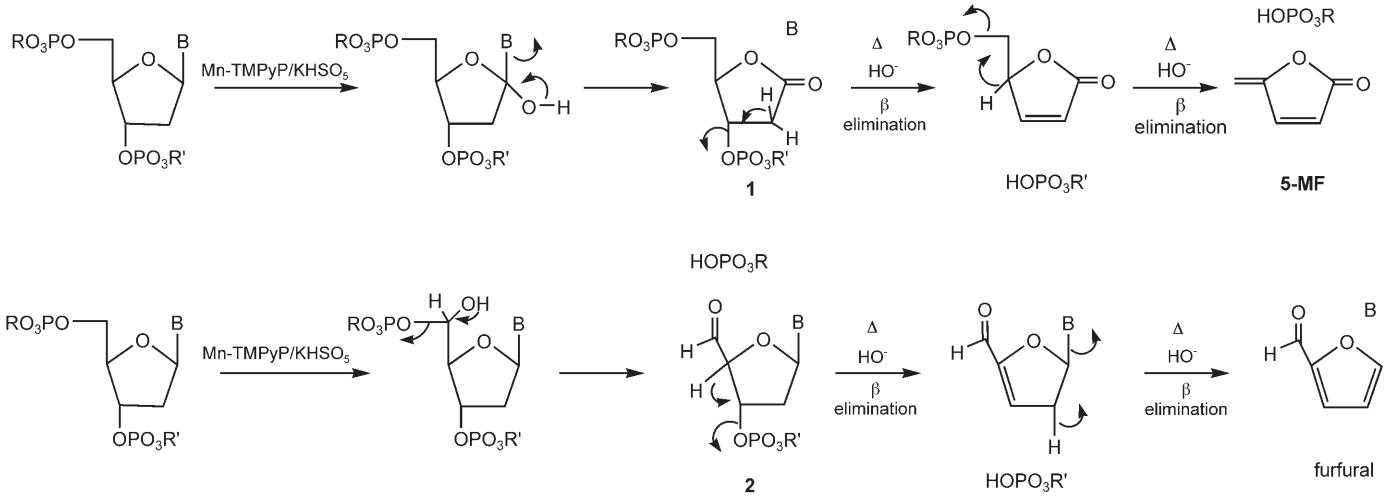
nucleotide sequence containing only A·T base pairs, an $(A{\cdot}T)_3$ site.[18–22] The binding of the manganese porphyrin in the minor groove of an $(A{\cdot}T)_3$ site puts the Mn^{V}=O center in the right position to perform hydroxylation at the C5′ carbon atom of the deoxyribose unit located on the 3′ side of the $(A{\cdot}T)_3$ sequence. This reaction is extremely efficient due to the high affinity of the manganese porphyrin for that site[23,24] and to the perfect match between the Mn^{V}=O position and the target C–H bond. The interaction of Mn-TMPyP in the minor groove is no longer possible when the trinucleotide sequence contains two G·C base pairs, namely, in G/C-rich sequences. The presence of axial ligands on the manganese, that is, two water molecules for Mn-TMPyP[25] or the metal-oxo group for the Mn^{V}=O species, precludes the intercalation of the manganese porphyrin between base pairs of DNA. In G/C-rich sequences, the second reactivity is observed, that is, oxygen-atom transfer at the C1′ carbon atom of the deoxyribose unit.[26,27] The way that the Mn^{V}=O species reaches a C1′–H bond of a deoxyribose moiety is not clear since this bond does not seem to be directly accessible due to its deep location in the minor groove. The hydroxylation mechanisms at the C1′ and C5′ atoms of the deoxyribose units are shown in Scheme 4. The third type of chemistry mediated by Mn-TMPyP/$KHSO_5$ consists of electron abstraction from a guanine base[28–30] and the mechanism of guanine oxidation has been studied in detail.[31–36] The resulting guanine oxidation products are shown in Scheme 5.

The three different DNA damage mechanisms of Mn-TMPyP/$KHSO_5$ were previously studied in a separate fashion without evaluation of the different reaction pathways. The quantitative comparison between these different reaction pathways is the aim of the present study. For this purpose, we used short oligonucleotide DNA duplexes of various sequences as DNA substrates and compared their sensitivity toward oxidation by Mn-TMPyP/$KHSO_5$. We showed that guanine oxidation was not competitive with deoxyribose oxidation at the C5′ atom. Guanine oxidation was only found to be significant in cases with accessible guanine residues, such as terminal guanine residues on short oligonucleotides.

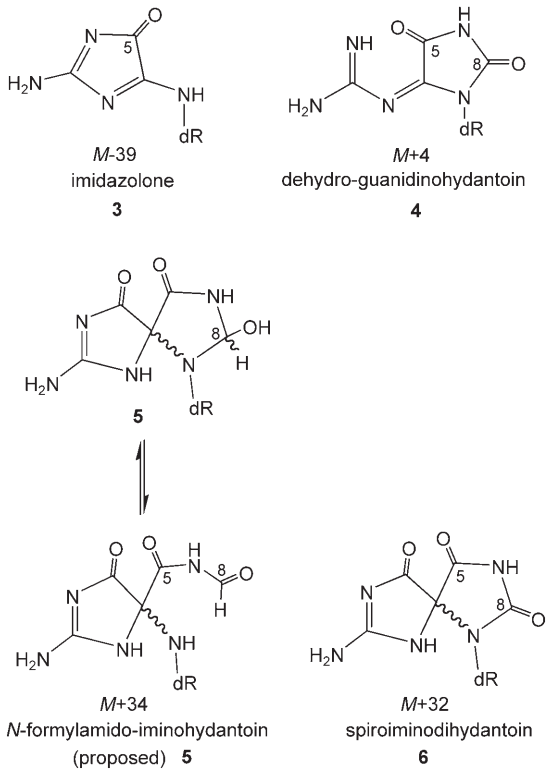
Results and Discussion

DNA damage by the cationic manganese porphyrin Mn-TMPyP in the presence of $KHSO_5$ was investigated on short oligonucleotide duplexes in order to compare the relative efficiency of oxygen-atom transfer at deoxyribose units with that of electron abstraction from guanine bases with relation to the sequence of DNA (Schemes 3–5). Different single- and double-stranded DNA sequences were included in the series of experiments.

The activation of the Mn^{III} porphyrin into a Mn^{V}=O species is due to the added oxidant, $KHSO_5$ (Scheme 2). In the comparative assays, the concentrations of the Mn-TMPyP and DNA were kept constant whereas the concentration of $KHSO_5$ was varied. The $KHSO_5$ concentration necessary to observe oxida-



Scheme 4. Oxygen-atom transfer mediated by Mn-TMPyP/$KHSO_5$ results in the hydroxylation of C5′ and C1′ carbon atoms of the deoxyribose units. Δ refers to a heating step at 90 °C for 15 min at pH 8. B = base, 5-MF = 5-methylene-2-furanone.



Scheme 5. Guanine oxidation products by electron transfer observed with Mn-TMPyP/$KHSO_5$. The numbering of carbon atoms refers to that of the initial guanine residue. The mass of each product is indicated with respect to that of the initial guanine-containing oligonucleotide, referred to as *M*. dR = deoxyribose included in a DNA strand.

tive DNA damage was compared with the different DNA substrates. It was correlated with the affinity of the metalloporphyrin for the DNA target and the accessibility of the oxidation site on DNA. Activation of the manganese porphyrin in the absence of DNA resulted in bleaching of the complex (not shown). When the manganese porphyrin interacted strongly with the DNA target and was activated in the vicinity of the target, oxidation of DNA occurred before bleaching of the catalyst. When DNA oxidation could be observed at low concentrations of $KHSO_5$, the probability of the proper location of the manganese porphyrin in the vicinity of the DNA was high and the activation was efficient. Thus, the relative concentration of $KHSO_5$ necessary to observe oxidative DNA damage by the metalloporphyrin correlated with the efficiency of the oxidation of DNA by the reagent at that sequence. Furthermore, one could also compare the mechanisms of oxidative damage depending on the sequence.

Due to the small size of the DNA duplexes (6- or 8-mers), the reactions were performed at 0 °C so that the two strands were annealed. At the end of the reaction time, the reaction media were analyzed directly by liquid chromatography coupled to electrospray mass analysis (negative ionization; HPLC/ESI-MS). Under the chromatographic conditions, the DNA strands eluted separately. The cleaved or chemically modified strands were eluted at different retention times (t_R) compared to the undamaged oligonucleotides. UV detection (260 nm) gave the relative amount of each DNA species. We considered that the area of the HPLC peaks of an oligonucleotide carrying one modified guanine residue was not significantly different from the area of the initial oligonucleotide strand. The loss of absorbance due to one modified guanine residue per oligonucleotide was considered as being below 10%. In the case of cleaved DNA strands, the extinction coefficient (ε) values of the shorter oligonucleotides were calculated as for the initial full-length strand. This allowed us to calculate the yield of the chemical reactions. The products of the reaction were identified by their mass compared to the mass of the initial material, with the latter being referred to as *M*. The major signal on the mass spectra corresponded to the doubly charged species $[M-2H]^{2-}$.

From previous work it was known that guanine was the only base damaged with Mn-TMPyP/$KHSO_5$[32] and the masses of the oligonucleotides carrying one guanine lesion are reported

in Scheme 5. Direct DNA cleavage due to a C5′ hydroxylation mechanism can be unambiguously identified by the mass of the corresponding DNA fragments.[19,20,37,38] A C1′ hydroxylation mechanism would generate an oligonucleotide carrying an oxidized abasic site with a ribonolactone structure (**1**, Scheme 4), corresponding to a mass of $M-119$, $M-110$, $M-95$, and $M-135$ amu for the reactions occurring at the A, T, C, and G residues, respectively. With this method of analysis it is possible to monitor the formation of all products of the reaction in one sample.

The degradation of DNA was dependent on the presence of both reactants, Mn-TMPyP and $KHSO_5$. Control experiments in the absence of one of them did not show any reaction under comparable reaction conditions.

Table 1. HPLC/ESI-MS data on the oxidation of 5′-CCAAAGG/3′-GGTTTCC by Mn-TMPyP/$KHSO_5$. [$KHSO_5$] = 10 μM.

m/z ($z=2$) $[M-2H]^{2-}$ observed	ΔM[a]	t_R [min]	Area of HPLC peaks [%][b]	Proposed structure[c]
1056.2		43.5	85	undamaged A strand
1042.7		45	90	undamaged T strand
1044.9	+4	40.7	1	G_{ox} (T strand)
1059.9	+34	40		G_{ox} (T strand)
753.6		39.3	1	5′-CCTTT-p
1057.7	+4	31.6	1.5	G_{ox} (A strand)
1073.7	+34	30.9		G_{ox} (A strand)
767.0		29.5	3.5	5′-CCAAA-p
		22.7	4.5	3′-CHOGG

[a] ΔM: Mass of the modified oligonucleotide minus the mass of the initial oligonucleotide. M is the molecular mass of the neutral form. [b] Area of the HPLC peak as compared to the area of the starting oligonucleotide (taken as 100%). The total is less than 100% due to the lower absorbance of oxidized products at 260 nm as compared to the absorbance of guanine and to the loss of some material. In this particular case the area% was corrected by an ε ratio to estimate the yield of products. [c] G_{ox} = oxidation of guanine residue.

Comparison of oxidative damage of short duplexes of different sequences

Double-stranded oligonucleotides containing an $(A\cdot T)_3$ site: The strong interaction of the manganese porphyrin in the minor groove in the so-called $(A\cdot T)_3$ site is known to give rise to high reactivity and high selectivity for the DNA-degradation reaction. The oxidation chemistry consists of the selective oxidation of the C5′ carbon atom of the deoxyribose unit located on the 3′ side of each $(A\cdot T)_3$ site on both strands of DNA.[19,38] The oligonucleotide 5′-GCAAAAGC/3′-CGTTTTCG, containing two $(A\cdot T)_3$ sites, was tested in a previous work. It was found that a 10 μM concentration of $KHSO_5$ induced selective cleavage of the duplex at 20% of the $(A\cdot T)_3$ sites.[38] However, since the experimental conditions were not exactly the same as those of the present work (the duplex was at a concentration of 5 μM), we included a new duplex in the series of experiments. This duplex, 5′-CCAAAGG/3′-GGTTTCC, was designed to contain only one $(A\cdot T)_3$ site. Incubation of Mn-TMPyP (10 μM) with this DNA substrate (10 μM of each strand) showed that the Mn^{V}=O species was able to perform the cleavage of the duplex by the C5′ hydroxylation mechanism in the $(A\cdot T)_3$ site in the presence of a 10 μM concentration of $KHSO_5$. The analysis of the reaction is shown in Table 1 and Figure S1 A in the Supporting Information. Figure S1 A shows the chromatogram of the reaction with a $KHSO_5$ concentration of 100 μM instead of 10 μM for clarity.

The cleavage of the A strand led to the 5′-CCAAA-3′-phosphate (5′-CCAAA-3′-p) (t_R = 29.5 min, m/z = 767.0 amu, z = 2) and 5′-CHOGG (**2**, Scheme 4; t_R = 22.7 min) fragments, whereas the same 5′-CHOGG dinucleotide derivative and the 5′-CCTTT-3′-p (t_R = 39.3, m/z = 753.6 amu, z = 2) fragments were formed from the cleavage of the T strand. Products of guanine oxidation were also observed (40 and 40.7 min for the T strand and 30.9 and 31.6 min for the A strand) and accounted for ≈2–3% of the products of the reaction, whereas the products of the direct cleavage of the oligonucleotide at the $(A\cdot T)_3$ site accounted for ≈5%. The peak areas corresponding to the undamaged oligonucleotides at the end of the reaction showed a decreased value of ≈10% as compared to the initial area. The yield of the reaction, under these experimental conditions, was ≈10% of DNA degradation (Table 1).

Guanine oxidation accounted for one third of the reaction. This was surprisingly high compared to previously tested sequences containing $(A\cdot T)_3$ sites,[19,20,38] for which guanine oxidation was absent. This point will be interpreted as the "end effect" (see below).

The very low concentration of $KHSO_5$ (10 μM) that triggered the reaction confirmed that the porphyrin was activated within the minor groove of the $(A\cdot T)_3$ site and reacted immediately with DNA.

Double-stranded oligonucleotides containing no $(A\cdot T)_3$ site

In comparison with the previous duplex, the oxidation of the self-complementary oligonucleotide, 5′-ATCGCGAT proved to be very difficult. A 100 μM concentration of $KHSO_5$ did not give rise to DNA damage. The concentration had to be increased to 10 mM to observe some DNA oxidation (Table 2, Figure S1 B in the Supporting Information). The yield of the reaction, even at this very high concentration of $KHSO_5$, was low (≈10%). The major product of degradation (≈5%) was an oligonucleotide with a mass corresponding to the loss of 119 amu as compared to the starting oligonucleotide ($M-119$; t_R = 36.5 min, m/z = 1143.95 amu, z = 2). The m/z signal of the initial oligonucleotide was observed at t_R = 44.1 min with m/z = 1203.35 amu and z = 2. The mass of the $M-119$ modified oligonucleotide may be attributed to deoxyribose oxidation with the formation of a ribonolactone derivative, **1**, due to the C1′ oxidation mechanism described in Scheme 4 and associated with the loss of one adenine base. However, no other product corresponding to deoxyribose oxidation at C, T, or G nucleotide units was detected. Since one deoxyadenosine nucleoside was located at the 5′ end of the sequence, this position was postulated to be more reactive than the intrastrand adenosine nu-

Table 2. HPLC/ESI-MS data on the oxidation of 5′-ATCGCGAT by Mn-TMPyP/$KHSO_5$. [$KHSO_5$] = 10 mM.

m/z ($z=2$) $[M-2H]^{2-}$ observed	ΔM[a]	t_R [min]	Area of HPLC peaks [%][b]	Proposed structure[c]
1203.35		44.1	90	undamaged strand
1183.8	−39	42.5		**3**
1143.95	−119	36.5	5	**7** or **8**

[a] ΔM: Mass of the modified oligonucleotide minus the mass of the initial oligonucleotide. M is the molecular mass of the neutral form. [b] Area of the HPLC peak as compared to the area of the starting strand (taken as 100%). Percentage estimated on the integration of the UV-detected HPLC peaks at 260 nm. The total is less than 100% due to the lower absorbance of oxidized products at 260 nm as compared to the absorbance of guanine and to the loss of some material.

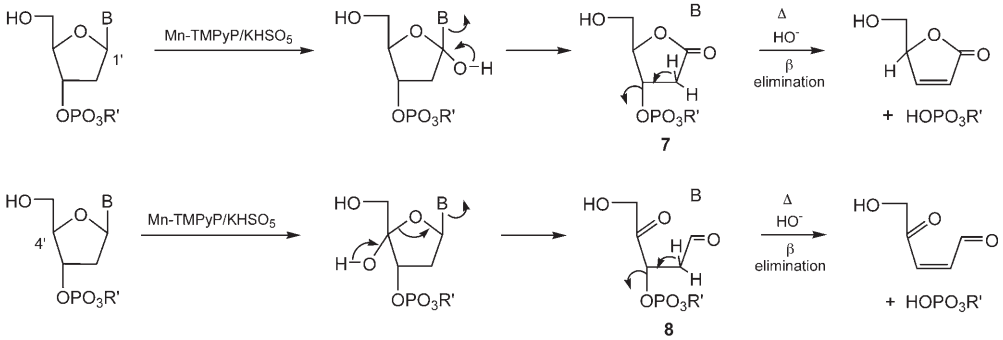
cleoside unit. Alternatively, deoxyribose oxidation at the 5′-nucleoside unit may also be due to an unprecedented chemistry for Mn-TMPyP/$KHSO_5$, namely, C4′ oxidation as shown in Scheme 6; this would lead to a product of the same molecular mass, $M-119$. The C1′ or C4′ oxidation mechanism at the 5′ ends of the short duplexes would result in the formation of products **7** or **8**, respectively (Scheme 6). The other smaller peaks on the chromatogram (Figure S1B in the Supporting Information) probably corresponded to the formation of guanine oxidation products. We did find a DNA strand with a mass compatible with one guanine base transformed into imidazolone (**3**, Scheme 5; t_R = 42.5 min, m/z = 1183.4 amu).[39,40] However, most of the other products were not analyzed due to their low abundance. From this data, we concluded that the manganese porphyrin was not reactive toward this sequence of DNA and that the oxidation of an isolated intrastrand guanine was 1000 times less reactive than the $(A\cdot T)_3$ site with Mn-TMPyP/$KHSO_5$. The ratio of the two concentrations of $KHSO_5$ necessary to observe a reaction under identical experimental conditions was 1000.

Since the redox potential of a 5′-G in a 5′-GG sequence is known to be lower than that of a single guanine residue,[41] a GG-containing duplex oligonucleotide should be more sensitive to oxidation. Indeed, the 5′-TTGGTA/3′-AAACCATA duplex was more easily degradated than the previous 5′-ATCGCGAT duplex. It was still less reactive than an $(A\cdot T)_3$ site. A $KHSO_5$ concentration of 100 μM did not give rise to observable DNA damage whereas 50% of conversion for the GG strand was observed with a $KHSO_5$ concentration of 1 mM. The results are shown in Table 3 and Figure S1C in the Supporting Information. The initial GG strand eluted at a retention time of t_R = 40 min with a m/z = 909.75 amu and $z=2$ (Table 3). The products of guanine oxidation were the imidazolone (**3**, Scheme 5; t_R = 38 min, m/z = 890.35 amu),[39,40] the dehydroguanidinohydantoin (**4**; t_R = 38 min, m/z = 911.75 amu),[36,42,43] and a proposed *N*-formylamido-iminohydantoin (**5**; t_R = 32.8 min, m/z = 926.55 amu)[32,33] derivative. On this sequence, which was selected to present an oxidation-sensitive GG site, the guanine

Table 3. HPLC/ESI-MS data on the oxidation of 5′-TTGGTA/3′AAACCATA by Mn-TMPyP/$KHSO_5$. [$KHSO_5$] = 1 mM.

m/z ($z=2$) $[M-2H]^{2-}$ observed	ΔM[a]	t_R [min]	Area of HPLC peaks [%][b]	Proposed structure
1192.33 (calcd)[c]		40	80	undamaged CC strand
909.75		39.2	50	undamaged GG strand
890.35	−39	38	5	**3**
911.75	+4	38		**4**
892.25	−35 (−39+4)	36.9	5	Two lesions (**3**+**4**)
892.25	−35 (−39+4)	36.5	10	Two lesions (**3**+**4**)
913.75	8 (4+4)	36.5		or (2×**4**)
854.75	−110	35.8	5	**7** or **8** at 5′-T of the GG strand
926.55	+34	32.8	1	**5**

[a] ΔM: Mass of the modified oligonucleotide minus the mass of the initial oligonucleotide. M is the molecular mass of the neutral form. [b] Area of the HPLC peak as compared to the area of the starting strand (taken as 100%). Percentage estimated on the integration of the UV-detected HPLC peaks at 260 nm. The total is less than 100% due to the lower absorbance of oxidized products at 260 nm as compared to the absorbance of guanine and to the loss of some material. [c] The selected mass range for analysis did not allow the analysis the C strand.



Scheme 6. The "end effect". Formation of an oxidized abasic site by hydroxylation of C–H bonds of deoxyribose (C1′–H or C4′–H) at the 5′ end of an oligonucleotide mediated by Mn-TMPyP/$KHSO_5$. This scheme should be compared with Scheme 4.

oxidation chemistry was observed. Deoxyribose oxidation at the 5′ end of the GG-strand was also detected in a minor product. The observed $M-110$ species ($t_R = 35.8$ min, $m/z = 854.75$ amu) corresponding to the loss of a thymine base associated with the formation of an oxidized abasic site (**7** or **8**) was again indicative of the fact that this chemistry probably took place at the end of the duplex because of the accessibility of the C1′–H or C4′–H bonds. No oxidation of deoxyribose corresponding to the loss of bases other than thymine was detected on the GG strand.

From this data we concluded that a GG sequence inside a duplex was 100 times less reactive than a $(A{\cdot}T)_3$ site for Mn-TMPyp/$KHSO_5$ degradation and 10 times more reactive than a single guanine base in double-stranded DNA. This result is compatible with the relative affinity of Mn-TMPyP for A/T regions of DNA compared to G/C regions, with affinity constants of 12×10^4 and $0.2\times10^4\,M^{-1}$, respectively[23], and with the fact that guanine oxidation was not observed on long DNA sequences; only the reaction at $(A{\cdot}T)_3$ sites was observed.[44] Consequently, guanine oxidation is concluded not to be competitive with C5′ oxidation at the $(A{\cdot}T)_3$ site with Mn-TMPyP/$KHSO_5$ in long DNA substrates. The guanine oxidation observed in the case of the first tested duplex, 5′-CCAAAGG/3′-GGTTTCC, is interpreted in terms of a particular reactivity of the ends of these small duplexes and this is developed in the next paragraph.

Oxidation at the last base pair of the duplexes: the "end effect"

In the use of short oligonucleotides for comparing the sensitivity of different DNA sequences toward one reagent, an increased reactivity of the last base pairs of the duplex was observed. This reactivity at the end of the duplex is no longer representative of double-stranded DNA. The "end effect" is illustrated with the oxidation of double-stranded oligonucleotides with a terminal guanine residue.

Oxidation of the self-complementary oligonucleotide, 5′-GTCGAC, with Mn-TMPyP/$KHSO_5$ under the same experimental conditions showed an unexpectedly high yield in comparison to the other sequences with no $(A{\cdot}T)_3$ site. With a $KHSO_5$ concentration of 100 μM, 75% conversion could be observed (Table 4, Figure S1 D in the Supporting Information). With a $KHSO_5$ concentration of 10 μM, 10% degradation was also observed. This efficiency was in the same range as that of the manganese porphyrin within an $(A{\cdot}T)_3$ site. The high sensitivity of the external guanine residue at the end of the duplex was the origin of this high reactivity. The yield of DNA damage reached that of a single-stranded DNA, 5′-TACGAC, which was also tested as a control, since the oxidized guanine bases can be considered as equally accessible in both cases (not shown). The single-stranded DNA at a concentration of 10 μM was 50% oxidized by 10 μM Mn-TMPyP and 100 μM $KHSO_5$ under the same experimental conditions as those used for the double-stranded DNA.

All the oxidation products proved to be alkali-labile lesions.[32,40,45] Piperidine treatment of the oligonucleotide, after the oxidation reaction, confirmed that the terminal guanine residue was the major site of oxidative damage (80%, not shown). The "end effect" was not only observable in the quantification of the oxidation products but also in the nature of the oxidation products.

Table 4. HPLC/ESI-MS data on the oxidation of the self-complementary oligonucleotide 5′-GTCGAC by Mn-TMPyP/$KHSO_5$. [$KHSO_5$] = 100 μM.

m/z ($z=2$) $[M-2H]^{2-}$ observed	ΔM[a]	t_R [min]	Area of HPLC peaks [%][b]	Proposed structure[c]
894.85		34.4	25	undamaged strand
827.2	−135	29.7	5	**7** or **8**
875.25	−39	29.2	1	**3**
896.75	+4	25.9	15	**4**
910.85	+32	24.9	2	**6**
902.85	+16	22.5–24	10	M+16[c]

[a] ΔM: Mass of the modified oligonucleotide minus the mass of the initial oligonucleotide. M is the molecular mass of the neutral form. [b] Area of the HPLC peak as compared to the area of the initial oligonucleotide before reaction (taken as 100%). Percentage estimated on the integration of the UV-detected HPLC peaks at 260 nm. The total is less than 100% due to the lower absorbance of oxidized products at 260 nm as compared to the absorbance of guanine and to the loss of some material. [c] M+16 is observed at pH 7 but **5** is mainly observed at pH 8 (t_R = 23–25 min, m/z = 911.75).

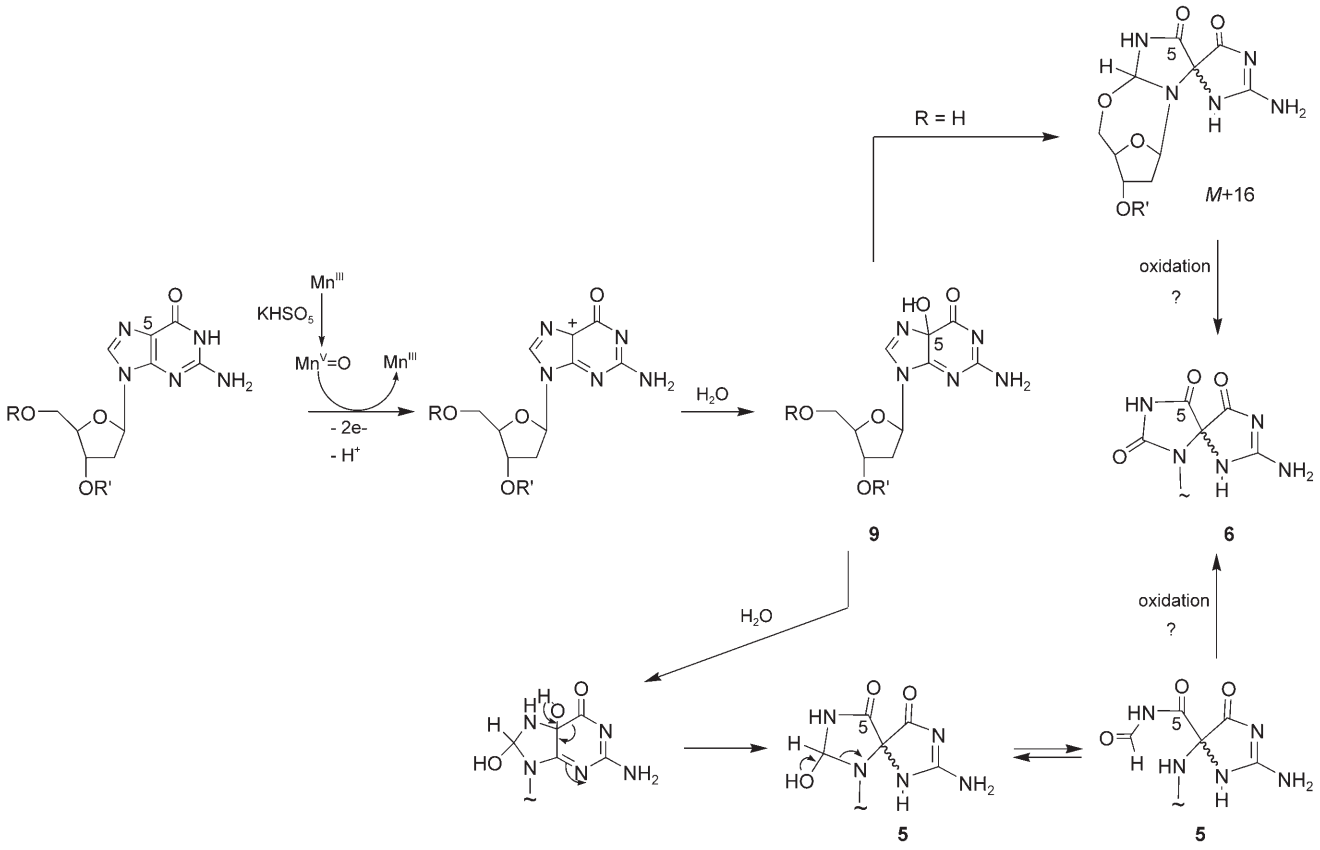
The products of guanine oxidation for the 5′-GTCGAC duplex were **3** ($t_R = 29.1$ min, $m/z = 875.3$ amu), **4** ($t_R = 25.9$ min, $m/z = 896.75$ amu), **6** ($t_R = 24.8$ min, $m/z = 910.7$ amu),[45–50] and a product whose mass corresponded to an increase of 16 amu as compared to the mass of the initial oligonucleotide (M+16; $t_R = 22.5$–23.5 min, $m/z = 902.85$ amu; Table 4). After having studied a series of short duplex sequences, we noted that the requirement for the formation of **6** and the M+16 derivative was the presence of a guanine at the 5′ end of the short duplex. Clearly, **6** and the M+16 product were due to the "end effect".

Although compound **6** is a common product of guanine oxidation,[45–50] it was observed for the first time with Mn-TMPyP/$KHSO_5$ in this work. Due to the absence of **5** under the experimental conditions of this work, **6** could be easily observed as a single HPLC peak (Figure S1 D in the Supporting Information). Under the experimental conditions of previous work (tested oligonucleotides with no 5′-G residue, different pH value), **6** coeluted with derivative **5** and, since **6** occurred in a minor amount compared to **5**, it was not detected. The mass analysis shown in Figure S2 in the Supporting Information illustrates the difficulty in discriminating between these two compounds with very close molecular masses and retention times when both of them are formed in the reaction medium. Oxidation of 5′-GTCGAC at pH 8 led to the formation of the two modified oligonucleotides **5** (t_R = 23–25 min) and **6** ($t_R = 25$ min). The two compounds were observed, under the same HPLC peak at 25 min, with m/z signals at 910. 6 and 911.6 amu, respectively, for doubly charged species. Only a high-resolution mass analysis allowed us to detect the two species (Figure S2 in the Supporting Information). Under the experimental conditions of this work, it seemed that **5** was replaced by the M+16 product. We noted that the M+16 derivative was the major product at

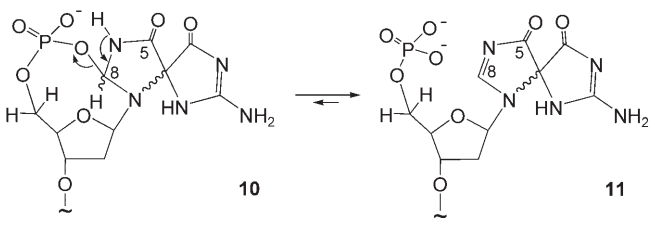
pH 7 and **5** was the major one at pH 8. The partitioning between the reaction pathways leading to the *M*+16 derivative and **5** depends on the pH value of the reaction medium.

Unlike **6**, the guanine oxidation product with an increase of 16 amu is not a common product of guanine oxidation. An increase of mass of 16 amu might be attributed to the formation of 8-oxo-7,8-dihydroguanine derivative (8-oxoG). However, we excluded this hypothesis on the basis of its alkali-labile character. The *M*+16 modified oligonucleotides were sensitive to piperidine treatment whereas the 8-oxoG lesion is not.[51] Furthermore, the *M*+16 product eluted as a broad peak, which is indicative of several isomers, at a retention time in the same range as that of the spiro derivatives (**5** and **6**). Oligonucleotides carrying lesion **5**, which is in the form of four diastereoisomers, also appear as a broad peak on the chromatograms. A mechanism for the formation of the new *M*+16 oligonucleotide may be proposed. It is reminiscent of the one described for the formation of **5** and **6** (Scheme 7). It is based on the nucleophilic attack of the 5′-OH group at the 5′ end of the oligonucleotide on the C8 atom of guanine. The new *M*+16 product was stable upon incubation during 30 min at 0 °C; consequently, it did not correspond to compound **9**, an unstable common precursor of **5** and **6**, that would also have a mass corresponding to an increase of 16 amu compared to the mass of guanine (Scheme 7).[52] Reaction of the 5′-OH group as a nucleophile on the C8 carbon atom of guanine after oxidation of the latter has been reported previously for the guanine oxidation pathway leading to cyclic analogues of **4**.[53,54] Furthermore, this reaction is favored by the *anti* conformation of the base in the double-stranded duplex. To test this hypothesis, we incubated a duplex with two 5′-phosphorylated strands with Mn-TMPyP/$KHSO_5$. Surprisingly, a *M*+16 derivative also formed during the oxidation of the self-complementary 5′-p-GTCGAC. The HPLC/ESI-MS analysis showed a signal at $m/z=$ 934.75 amu ($z=2$) for 5′-p-GTCGAC at $t_R=32$ min and a *M*+16 compound with $m/z=942.75$ amu ($z=2$) together with the corresponding derivative **5** at $m/z=951.75$ amu for $z=2$ at $t_R=29$ min (not shown). The structure of this other *M*+16 derivative could be **10** (Scheme 8). However, since the phosphate group is a good leaving group, **10** should not be stable. The release of the phosphate group, after the formation of the spiro structure at the former C4 atom of guanine, would give compound **11**. We propose that the former C8 atom of guanine is not as electrophilic in structure **11** as it was in structure **9**. Consequently, structure **11** may be stable and may not transform into structure **5**. Another possibility would be that the presence of a negatively charged and bulky 5′-phosphate group at the 5′ end of the duplex precluded the attack of a water molecule at the C8 atom of the oxidized guanine, so that the oxidized guanine derivative remained as **11** (the spiro rearrangement of **9**) instead of transforming into the more classically observed derivative **5**.

The "end effect" also included the formation of another new product of oxidation, whose mass corresponded to a decrease



Scheme 7. Proposed mechanism of formation of **5**, **6**, and the *M*+16 derivatives from the oxidation of guanine by electron transfer with Mn-TMPyP/$KHSO_5$. The C5 carbon atom of the guanine residue is marked.



Scheme 8. Possible structure of an *M*+16 derivative formed from a 5′-phosphate oligonucleotide. The numbering of the carbon atoms refers to those in the initial guanine residue.

of 135 amu compared with the initial oligonucleotide (*M*−135; Figure S1 D in the Supporting Information). On 5′-GTCGAC the *M*−135 product was one of the major oxidation products. Its mass could be interpreted by deoxyribose oxidation occurring at the last 5′-end deoxyribose unit, associated with the loss of the guanine base and the concomitant formation of the 1′- or 4′-oxidized abasic site (**7** or **8** in Scheme 6). These C1′–H and C4′–H bonds are accessible from the outside of the duplex by the Mn^{V}=O porphyrin whereas this is not the case for those of the 3′-end nucleotide unit.

We tried to characterize the *M*−135 compound. We performed a heating step (incubation at 90 °C for 15 min, pH 8) that led to the release of the 5′-p-TCGAC oligonucleotide as expected for **7** or **8** (Scheme 6). The HPLC/ESI-MS analysis of the reaction medium, after this heating step, showed the transformation of the peak corresponding to **7** or **8** at t_R = 29.7 min into a peak at t_R = 21 min and *m/z* = 770.8 amu for the 5′-phosphate 5-mer. We also performed a labeling experiment. The reaction was carried out in $H_2{}^{18}O$ labeled water. The oxygen atom incorporated in *M*−135 contained 50% ^{18}O and 50% ^{16}O, in accordance with a mechanism of direct sugar hydroxylation by Mn-TMPyP/$KHSO_5$.[27] The *m/z* value for *M*−135 in H_2O was observed at 827.25 amu and appeared as a mixture of two *m/z* signals at 827.25 and 828.22 amu when the reaction was in $H_2{}^{18}O$ (Figure 1). The labeling experiment was compatible with structures **7** or **8**. We added the reducing agent $NaBH_4$ to the reaction medium and observed that the *M*−135 oligonucleotide was reduced. Its molecular mass increased by 4 amu. Although it was reported that ribonolactone **1** (or **7**) was not sensitive to $NaBH_4$,[55] the reduction of ribonolactones seems to be possible.[56] The $NaBH_4$ reduction did not allow us to discriminate between structures **7** and **8** for the *M*−135 compound. The last reagent that was tested on the *M*−135 oligonucleotide was an oxylamine derivative, carboxymethoxylamine. The *M*−135 product was not sensitive to this last reagent. This data is in favor of structure **7**.[37,57,58]

When Mn-TMPyP/$KHSO_5$ was treated with other double-stranded DNA sequences like self-complementary 5′-CGTACG or 5′-GTCGTA/3′-CAGCAT, the products *M*−95 (due to the loss of cytosine with sugar oxidation at the 5′ end) or *M*−135 for the loss of guanine and *M*−110 for the loss of thymine were observed for each tested duplex, respectively. However, the 5′-deoxyribose oxidation at a 5′-C or a 5′-T residue did not reach the level observed with a 5′-G nucleoside unit.

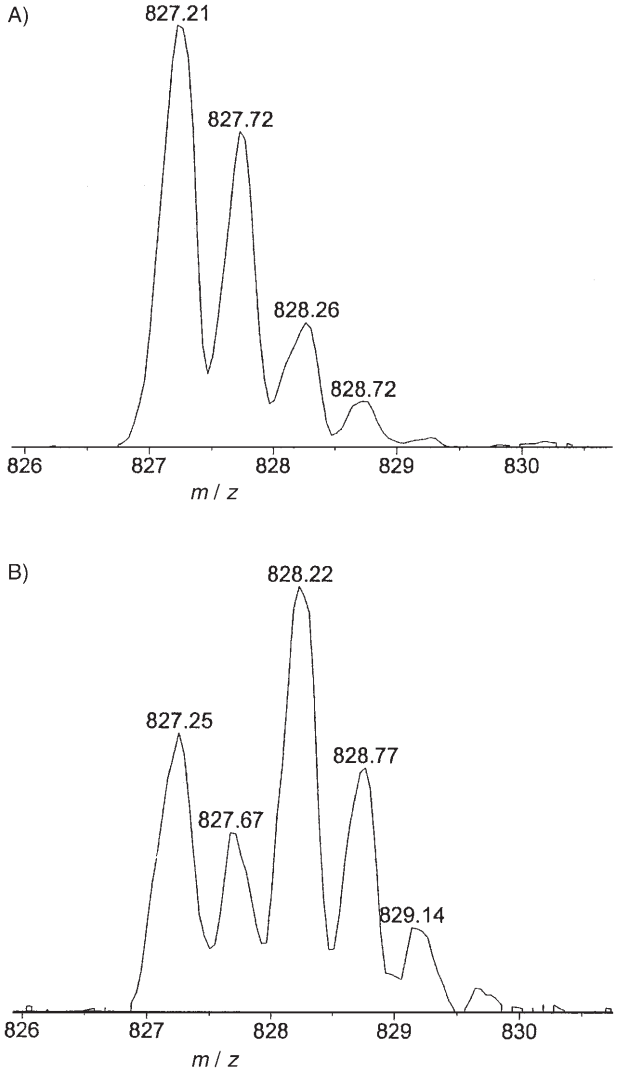


Figure 1. HPLC/ESI-MS mass spectrum of the *M*−135 compound when the reaction was performed in A) $H_2{}^{16}O$ or B) $H_2{}^{18}O$. The observed signals correspond to doubly charged species and show the incorporation of one ^{18}O atom in 50% of the product in (B).

The last base pair of the duplexes also included a 3′-nucleoside, which might be sensitive to oxidation. The C2′′–H bond of the deoxyribose of the 3′ end could be accessible for hydroxylation by the Mn^{V}=O species. This reaction would have led to an arabinose derivative of that last sugar whose mass would have corresponded to an increase of 16 amu as compared to the initial oligonucleotide. However, as discussed above, the *M*+16 oligonucleotide was instead attributed to a product derived from guanine oxidation since this *M*+16 product was only observed in the case of a 5′-guanine-terminated and not in the case of a 3′-guanine-terminated oligonucleotide.

The Mn-TMPyP/$KHSO_5$ chemical nuclease showed a particular reactivity toward the terminal base pair at the end of short DNA duplexes. A terminal guanine was found to be about 1000 times more reactive than an intrastrand guanine. Oxidation of the C1′ or C4′ atoms of the deoxyribose of the last 5′-nucleoside unit was also observed at the end of the duplex

and not at intrastrand units. This end effect may be explained by 1) a better accessibility of the target with respect to the oxidative reagent or 2) a partial stacking of the manganese porphyrin with the last base pair of the short duplex. This partial stacking with the last G•C base pair may be at the origin of the guanine oxidation observed with 5′-CCAAAGG/3′-GGTTTCC. In this particular case the end GG sequence may have been more reactive toward oxidation than the previously tested sequences that did not contain a GG and an $(A•T)_3$ site together. We propose that guanine oxidation was not due to the manganese porphyrin being located in the minor groove of the duplex (and responsible for the major degradation pathway for the duplex, namely the C5′ oxidation) but was due to a manganese porphyrin interacting with the last base pair. This result is consistent with the fact that when the manganese porphyrin interacts within the minor groove of A/T-rich DNA, no guanine oxidation was detected.[19,20,38,44] Guanine oxidation is clearly due to a different interaction of the Mn^{V}=O entity with DNA. On longer DNA sequences, oxidation of intrastrand guanine is possible but it represents a minor process.

The fact that the C1′ oxidation was not observed at intrastrand deoxyriboses indicated that this target was not directly accessible to the Mn^{V}=O entity in the double helix of DNA. This is not surprising due to the hidden location of the C1′–H bond within the minor groove. Furthermore, this mechanism of random attack of intrastrand deoxyribose units, if it took place, was probably below the limit of detection under the used experimental conditions. We propose that this oxidation mechanism, previously observed on high-molecular-weight DNA,[26,59] takes place only after partial melting or cleavage of DNA due to the initial degradation events of guanine oxidation or C5′ oxidation at $(A•T)_3$ sites, respectively.

Conclusion

Oxidative DNA damage with the manganese porphyrin Mn-TMPyP/$KHSO_5$ was assayed on short DNA duplexes to compare the reactivity of different sequences toward the chemical nuclease. Three oxidative degradation mechanisms were possible: 1) hydroxylation of the C5′ atom of the deoxyribose unit by oxygen-atom transfer taking place at high-affinity binding sites consisting of an $(A•T)_3$ sequence, 2) hydroxylation of the C1′ or C4′ atoms of the deoxyribose unit by oxygen-atom transfer at the 5′ end of oligonucleotides, or 3) guanine oxidation by electron transfer.

The relative quantification of the oxidative damage of different sequences showed that C5′ hydroxylation at three contiguous A or T bases was 1000 times more efficient than guanine oxidation by electron transfer at an isolated guanine residue. The oxidation of an intrastrand GG sequence appeared to be 10 times more reactive than at a single guanine residue but still 100 times less reactive than at the $(A•T)_3$ site. However, when a guanine residue was located at the terminal base pair of the short duplex, its reactivity reached that of the $(A•T)_3$ site. The oxidation of deoxyribose units appeared to be possible only when the C–H bond was accessible to the active Mn^{V}=O species, that is, it was observed only at the deoxyribose unit located at the 5′ end of the duplexes.

Deoxyribose oxidation by oxygen-atom transfer needs the metal-oxo species to be in close vicinity to the C–H bonds of the sugar; guanine oxidation by electron transfer is also dependent on the distance between the metal-oxo entity and the nucleobase. This tight interaction is possible within an $(A•T)_3$ site (C5′ hydroxylation) and this is the key to the manganese porphyrin efficiency in DNA cleavage. Guanine oxidation and intrastrand C1′ deoxyribose oxidation appear to be secondary mechanisms of DNA degradation by this chemical nuclease. Short oligonucleotide DNA duplexes were convenient tools for the study of the comparative reactivity of the damaging reagent with various sequences of DNA.

Experimental Section

Materials: Potassium monopersulfate, $KHSO_5$ (triple salt $2KHSO_5•K_2SO_4•KHSO_4$, Curox) was from Interox. Carboxymethoxylamine, $NaBH_4$, adenosine triphosphate (ATP), and polynucleotide kinase were from Sigma–Aldrich. Mn-TMPyP was prepared according to a previously reported protocol.[60] The oligonucleotides were synthesized by standard solid-phase β-cyanoethyl phosphoramidite chemistry. They were purified by HPLC with a reversed-phase column (Nucleosil C18, 10 μm, 250×4.2 mm; Interchrom, Montluçon, France; eluents: A = 0.1 M triethylammonium acetate (pH 6.5), B = CH_3CN; linear gradient: 5→15% B over 60 min; flow rate: 1 mL min^{-1}; λ = 260 nm). $H_2{}^{18}O$ (95.1%) was purchased from Eurisotop, France.

Oxidation of oligonucleotides by Mn-TMPyP/$KHSO_5$: Oligonucleotide (duplex; 10 μM) was incubated with Mn-TMPyP (10 μM) in phosphate buffer (50 mM; pH 7.2) containing NaCl (100 mM) at 0 °C for 10 min. The reaction was initiated by addition of $KHSO_5$ at a final concentration varying from 0.01–10 mM. After 5 min at 0 °C, the reaction was stopped by the addition of 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid buffer (10 mM; pH 8) and the whole reaction medium (100 μL) was directly injected for HPLC or HPLC/ESI-MS analysis. Concentrations given were final concentrations.

When a reduction step was performed on some samples, $NaBH_4$ (final concentration 0.1 M), was added into the reaction medium. After 30 min at room temperature, acetone was added to quench the reductant. The reaction medium was lyophilized before HPLC analysis.

Piperidine treatment consisted of incubation of the sample in 1 M piperidine solution for 1 h at 90 °C followed by several successive lyophilizations.

The reaction with carboxymethoxylamine was performed directly on the reaction medium after 5 min of reaction. Carboxymethoxylamine was added at 40 mM concentration and was allowed to react for 1 h at room temperature.

For labeling experiments, the same reaction was also performed in labeled water ($H_2{}^{18}O$) after drying of the reaction medium (before the addition of $KHSO_5$) and dissolution in labeled water. The addition of $KHSO_5$ corresponded to 1% of non-labeled water in the final reaction volume.

HPLC/ESI-MS analyses: The reaction mixtures (100 μL) were analyzed by HPLC with a reversed-phase column (Nucleosil C18, 5 or 10 μm, 250×4.6 mm; Interchrom) eluted with a linear gradient

(eluents: A = 10 mM triethylammonium acetate buffer (pH 6.5), B = acetonitrile; 5→15% B over 60 min; flow rate: 1 mL min^{-1}). A diode array detector (Waters) allowed detection of the products at $\lambda = 260$ nm. For HPLC/ESI-MS analysis the same column was coupled to an electrospray mass spectrometer, a Perkin–Elmer SCIEX API 365 equipped with a turbo ion-spray source. The temperature of the gas (N_2) stream was set at 450 °C. The flow eluted from the column (50% volume) was introduced into the electrospray source. The analyses were carried out in the negative mode. The use of the turbo ion-spray source allowed us to perform HPLC/ESI-MS analyses from diluted duplex oligonucleotides solutions (100 μL corresponded to 1 nmol of injected duplex DNA). The range of mass acquisition was varied depending on the oligonucleotide and on the sensitivity or resolution that was necessary.

Phosphorylation of 5′-GTCGAC at the 5′-end: Single-stranded oligonucleotide (20 nmol) was treated with 5 Units of T4 polynucleotide kinase in the presence of ATP for 1 h at 37 °C. Purification of the 5′-phosphate oligonucleotide was performed by HPLC.

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Keywords: DNA damage · DNA · guanine · metalloporphyrins · oxidation

[1] C. von Sonntag *The Chemical Basis of Radiation Biology*, Taylor and Francis, London, **1987**.
[2] J. Cadet, M. Berger, T. Douki, J.-L. Ravanat, *Rev. Physiol. Biochem. Pharmacol.* **1997**, *131*, 1–87.
[3] B. Armitage, *Chem. Rev.* **1998**, *98*, 1171–1200.
[4] W. J. Dixon, J. J. Hayes, J. R. Levin, M. F. Weidner, B. A. Dombrowski, T. D. Tullius, *Methods Enzymol.* **1991**, *208*, 380–413.
[5] R. P. Hertzberg, P. B. Dervan, *J. Am. Chem. Soc.* **1982**, *104*, 313–315.
[6] D. S. Sigman, *Acc. Chem. Res.* **1986**, *19*, 180–186.
[7] D. S. Sigman, A. Mazunder, D. M. Perrin, *Chem. Rev.* **1993**, *93*, 2295–2316.
[8] C. B. Chen, L. Milne, R. Landgraf, D. M. Perrin, D. S. Sigman, *ChemBioChem* **2001**, *2*, 735–740.
[9] R. M. Burger, *Chem. Rev.* **1998**, *98*, 1153–1169.
[10] S. M. Hecht, *J. Nat. Prod.* **2000**, *63*, 158–198.
[11] J. Chen, J. Stubbe, *Nat. Rev. Cancer* **2005**, *5*, 102–112.
[12] K. C. Nicolaou, W.-M. Day, *Angew. Chem.* **1991**, *103*, 1453–1481; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1387–1530.
[13] I. H. Goldberg, *Acc. Chem. Res.* **1991**, *24*, 191–198.
[14] Z. Xi, I. H. Goldberg in *Comprehensive Natural Products Chemistry, Vol.* 7 (Eds: D. H. R. Barton, K. Nakanishi), Elsevier, Oxford, **1999**, pp. 553–592.
[15] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109–1151.
[16] G. Pratviel, J. Bernadou, B. Meunier, *Adv. Inorg. Chem.* **1998**, *45*, 251–312.
[17] B. Meunier, S. P. de Visser, S. Shaik, *Chem. Rev.* **2004**, *104*, 3947–3980.
[18] B. Ward, A. Skorobogaty, J. C. Dabrowiak, *Biochemistry* **1986**, *25*, 6875–6883.
[19] M. Pitié, G. Pratviel, J. Bernadou, B. Meunier, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3967–3971.
[20] G. Pratviel, V. Duarte, J. Bernadou, B. Meunier, *J. Am. Chem. Soc.* **1993**, *115*, 7939–7943.
[21] G. Pratviel, M. Pitié, C. Périgaud, G. Gosselin, J. Bernadou, B. Meunier, *J. Chem. Soc. Chem. Commun.* **1993**, 149–151.
[22] P. Arnaud, K. Zakrzewska, B. Meunier, *J. Comput. Chem.* **2003**, *24*, 797–805.
[23] L. Ding, J. Bernadou, B. Meunier, *Bioconjugate Chem.* **1991**, *2*, 201–206.
[24] J. C. Dabrowiak, B. Ward, J. Goodisman, *Biochemistry* **1989**, *28*, 3314–3322.
[25] S. Prince, F. Körber, P. R. Cooke, J. R. Lindsay Smith, M. A. Mazid, *Acta Crystallogr. Sect. C* **1993**, *49*, 1158–1160.
[26] G. Pratviel, M. Pitié, J. Bernadou, B. Meunier, *Angew. Chem.* **1991**, *103*, 718–720; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 702–704.
[27] M. Pitié, J. Bernadou, B. Meunier, *J. Am. Chem. Soc.* **1995**, *117*, 2935–2936.
[28] B. Mestre, G. Pratviel, B. Meunier, *Bioconjugate Chem.* **1995**, *6*, 466–472.
[29] B. Mestre, A. Jakobs, G. Pratviel, B. Meunier, *Biochemistry* **1996**, *35*, 9140–9149.
[30] B. Mestre, M. Pitié, C. Loup, C. Claparols, G. Pratviel, B. Meunier, *Nucleic Acids Res.* **1997**, *25*, 1022–1027.
[31] C. Vialas, G. Pratviel, C. Claparols, B. Meunier, *J. Am. Chem. Soc.* **1998**, *120*, 11548–11553.
[32] C. Vialas, C. Claparols, G. Pratviel, B. Meunier, *J. Am. Chem. Soc.* **2000**, *122*, 2157–2167.
[33] A. Lapi, G. Pratviel, B. Meunier, *Met.-Based Drugs* **2001**, *8*, 47–56.
[34] A. Chworos, Y. Coppel, I. Dubey, G. Pratviel, B. Meunier, *J. Am. Chem. Soc.* **2001**, *123*, 5867–5877.
[35] C. Seguy, G. Pratviel, B. Meunier, *Chem. Commun.* **2001**, 2116–2117.
[36] A. Chworos, C. Seguy, G. Pratviel, B. Meunier, *Chem. Res. Toxicol.* **2002**, *15*, 1643–1651.
[37] A. Angeloff, I. Dubey, G. Pratviel, J. Bernadou, B. Meunier, *Chem. Res. Toxicol.* **2001**, *14*, 1413–1420.
[38] A. Chworos, P. Arnaud, K. Zakrzewska, P. Guga, G. Pratviel, W. Stec, B. Meunier, *J. Biol. Inorg. Chem.* **2004**, *9*, 374–384.
[39] J. Cadet, M. Berger, G. W. Buchko, P. C. Joshi, S. Raoul, J.-L. Ravanat, *J. Am. Chem. Soc.* **1994**, *116*, 7403–7404.
[40] S. Raoul, M. Berger, G. W. Buchko, P. C. Joshi, B. Morin, M. Weinfeld, J. Cadet, *J. Chem. Soc. Perkin Trans. 2* **1996**, *2*, 371–381.
[41] I. Saito, M. Takayama, H. Sugiyama, K. Nakatani, *J. Am. Chem. Soc.* **1995**, *117*, 6406–6407.
[42] V. Duarte, D. Gasparutto, L. F. Yamaguchi, J.-L. Ravanat, G. R. Martinez, M. H. G. Medeiros, P. Di Mascio, J. Cadet, *J. Am. Chem. Soc.* **2000**, *122*, 12622–12628.
[43] W. Luo, J. G. Muller, E. M. Rachlin, C. J. Burrows, *Chem. Res. Toxicol.* **2001**, *14*, 927–938.
[44] K. Wietzerbin, J. G. Muller, R. A. Jameton, G. Pratviel, J. Bernadou, B. Meunier, C. J. Burrows, *Inorg. Chem.* **1999**, *38*, 4123–4127.
[45] W. Luo, J. G. Muller, E. M. Rachlin, C. J. Burrows, *Org. Lett.* **2000**, *2*, 613–616.
[46] W. Luo, J. G. Muller, C. J. Burrows, *Org. Lett.* **2001**, *3*, 2801–2804.
[47] J. C. Niles, J. S. Wishnok, S. R. Tannenbaum, *Org. Lett.* **2001**, *3*, 963–966.
[48] T. Suzuki, M. Masuda, M. D. Friesen, H. Ohshima, *Chem. Res. Toxicol.* **2001**, *14*, 1163–1169.
[49] W. Adam, M. A. Arnold, M. Grüne, W. M. Nau, U. Pischel, C. R. Saha-Möller, *Org. Lett.* **2002**, *4*, 537–540.
[50] A. Joffe, N. E. Geacintov, V. Shafirovich, *Chem. Res. Toxicol.* **2003**, *16*, 1528–1538.
[51] J. G. Muller, V. Duarte, R. P. Hickerson, C. J. Burrows, *Nucleic Acids Res.* **1998**, *26*, 2247–2249.
[52] J. E. B. McCallum, C. Y. Kuniyoshi, C. S. Foote, *J. Am. Chem. Soc.* **2004**, *126*, 16777–16782.
[53] G. W. Buchko, J. Cadet, J.-L. Ravanat, P. Labataille, *Int. J. Radiat. Biol.* **1993**, *63*, 669–676.
[54] B. Morin, J. Cadet, *J. Am. Chem. Soc.* **1995**, *117*, 12408–12415.
[55] L. S. Kappen, C. Chen, I. H. Goldberg, *Biochemistry* **1988**, *27*, 4331–4340.
[56] M. L. Wolfrom, H. B. Wood, *J. Am. Chem. Soc.* **1951**, *73*, 2933–2934.
[57] D. Boturyn, E. Defrancq, V. Ducros, C. Fontaine, J. Lhomme, *Nucleosides Nucleotides*, **1997**, *16*, 2069–2077.
[58] K. Sato, M. M. Greenberg, *J. Am. Chem. Soc.* **2005**, *127*, 2806–2807.
[59] G. Pratviel, M. Pitié, J. Bernadou, B. Meunier, *Nucleic Acids Res.* **1991**, *19*, 6283–6288.
[60] J. Bernadou, G. Pratviel, F. Bennis, M. Girardet, B. Meunier, *Biochemistry* **1989**, *28*, 7268–7275.

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