Oxaluric Acid as the Major Product of Singlet Oxygen-Mediated Oxidation of 8-Oxo-7,8-dihydroguanine in DNA

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Abstract: Oxidative reactions of DNA commonly result in base modifications. Among the four DNA bases, guanine is the most susceptible to oxidation, and one of its main oxidized compounds, namely 8-oxo-7,8-dihydroguanine (8-oxoGua), has been extensively studied in terms of formation, repair, and mutagenicity. However, the latter modified purine base is readily subjected to further oxidation reactions which have recently become a matter of interest. Emphasis was placed in this work on the identification of the final singlet oxygen oxidation products of 8-oxoGua in single-stranded DNA. Oxaluric acid was found to be the predominant product of the reaction. Insights in the mechanistic pattern of oxaluric acid formation were gained from isotopic labeling experiments in association with mass spectrometry measurements. It was found that oxaluric acid is formed via an oxidized guanidinohydantoin intermediate, arising from the likely degradation of a transient 5-hydroperoxide. Two subsequent hydrolytic steps that are accompanied by the release of guanidine are likely to be involved in the formation of oxaluric acid.

Introduction

Oxidative degradation of DNA is likely to be involved in aging processes and several human diseases.[1−4] Reactive oxygen and nitrogen species, including OH radical, H_2O_2, singlet oxygen ($^1O_2$), and peroxynitrite are implicated in the generation of various forms of damage to DNA. Up to now, a large number of investigations have been conducted to identify the main oxidative lesions within DNA and to assess their biological consequences.[5−8] Among the four DNA bases, guanine is easily oxidized and is the most sensitive to one-electron oxidation.[6,9] The related modified purine, 8-oxo-7,8-dihydroguanine (8-oxoGua), has received considerable attention since it is a relevant biomarker of oxidative stress under various conditions that include ionizing radiation, reactive oxygen species, chemical oxidation, and photoionization.[5,8,10−13] Interestingly, several studies have shown that 8-oxoGua is highly susceptible to various oxidizing agents,[13−20] suggesting that the latter modified base may also be sensitive to in vivo oxidation. Thus, the resulting products may play a role in the mutagenic process associated with oxidative damage to DNA. Thus, it is not surprising that the study of the secondary oxidation of guanine has become a matter of interest. Photooxidation studies of 8-oxo-7,8-dihydroguanine derivatives involving $^1O_2$ led to a set of different products. Raoul and Cadet found cyanuric acid as the major product of $^1O_2$-mediated decomposition of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo).[17] Sheu and Foote also reported the formation of cyanuric acid in addition to parabanic acid and a seven-membered-ring product for the $^1O_2$-oxidation of a silylated derivative of 8-oxo-7,8-dihydroguanosine.[19] Both of the latter compounds, in addition to oxaluric acid, were also identified in the peroxynitrite-mediated oxidation of 3′,5′-di-acetylated 8-oxo-7,8-dihydro-2′-deoxyguanosine.[15] On the other hand, electrochemical one-electron oxidation of 8-oxodGuo was found to give rise to the corresponding guanidinohydantoin nucleoside.[18] More recently, Luo et al. reported the formation of a spiroiminodihydantoin nucleoside as the major product for the one-electron oxidation of 8-oxodGuo by $IrCl_6^{2-}$ in neutral

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aqueous solution.[21] While the further oxidation of 8-oxodGuo has been extensively explored, only a few studies were conducted on DNA. Such investigations appear to be important since the chemistry involved in the oxidation of 8-oxoGua may differ from the monomer to the DNA polymer. As an example, one-electron oxidation of 8-oxoGua containing oligonucleotide (ODN) with $IrCl_6^{2-}$ led to the formation of guanidinohydantoin as the major decomposition product and not to the spiroiminodihydantoin compound observed with the nucleoside.[22] In contrast, the peroxynitrite-mediated oxidation of 8-oxoGua in defined oligonucleotide sequences was shown to generate cyanuric acid and oxaluric acid as described for 8-oxodGuo, the ratio between both oxidized products being dependent on the peroxinitrite concentration.[23,24]

To our knowledge, only little information is available on the $^1O_2$-induced oxidation of 8-oxoGua within DNA. The need for such a study is of particular interest since $^1O_2$ has been shown to be produced in biological systems under various conditions.[25] These include enzymatic processes catalyzed by peroxidases or oxygenases and photosensitized reactions (type II mechanism).

In the present study, we identified the final oxidized product of 8-oxoGua under singlet oxygen generation conditions. This was achieved by using the endoperoxide $DHPNO_2$[26] as a clean chemical source of singlet oxygen together with oligomers having a unique 8-oxoGua residue. Oxaluric acid was found to be the final stable decomposition product resulting from the $^1O_2$ oxidation of 8-oxoGua. Mechanistic insights were gained from isotopic labeling experiments coupled to mass spectrometry measurements. As a result, a reaction pathway for the $^1O_2$-promoted oxidation of 8-oxoGua is proposed. We found that oxaluric acid is formed via an oxidized guanidinohydantoin intermediate, arising probably from the degradation of a 5-hydroperoxide. Then, two hydrolytic steps involving the latter oxidized guanidinohydantoin with the release of guanidine are likely to give rise to oxaluric acid.

Experimental Section

Materials. NAP-25 Sephadex columns were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). $ZipTipC_{18}$ columns were from Millipore (Milford, MA). Methylene blue was from Merck (Darmstadt, Germany). Riboflavin was obtained from BDH Biochemicals (Poole, U.K.). Acetonitrile (HPLC grade) was purchased from Carlo-Erba (Milan, Italy). Buffers for HPLC were prepared using water purified with a Milli-Q system from Millipore (Milford, MA).

HPLC, ESI-MS/MS, and MALDI-TOF-MS Analyses. Oligonucleotide mixtures resulting from oxidative reactions performed on 8-oxoGua containing oligonucleotides were analyzed by HPLC on a Hypersil (Interchim, Montluçon, France) ODS column (5 μm, 250 × 4.6 mm). This was achieved using a gradient of acetonitrile (from 0 to 10% either in 30 or 90 min for the 3mer or the 15mer, respectively) in 25 mM ammonium formiate (pH 7) at a flow rate of 1 mL/min. Detection of the oligonucleotides was performed with a UV−visible spectrophotometer set at 260 nm. Electrospray ionization (ESI)-MS and tandem MS (ESI-MS/MS) experiments were carried out on an LC-Q instrument (Finnigan, San Jose, CA). The spectra were obtained in the negative ion mode using a spraying solution of 50:50 water/methanol. Oligonucleotides subjected to MALDI-TOF mass spectrometry analysis were first desalted by using $ZipTipC_{18}$ columns according to the protocol described by the manufacturer. The mass spectra were obtained with a commercial time-of-flight mass spectrometer (Voyager-DE; Perseptive Biosystems, Farmingham, MA) equipped with a 337 nm nitrogen laser and a pulsed delay source extraction. The spectra were recorded from 256 laser shots with an accelerating voltage of 25 kV in the linear and positive modes. For the matrix, a mixture of 3-hydroxypicolinic acid and picolinic acid in a 4:1 (w/w) ratio were dissolved in a 50% acetonitrile aqueous solution that contained 0.1% TFA and a small amount of Dowex-50W 50 × 8-200 (Sigma) cation-exchange resin. Then, 1 μL of a 0.1% TFA aqueous solution of the sample was added to 1 μL of the matrix and the resulting solution was stirred. The sample was subsequently placed on the top of the target plate and then allowed to dry by itself. The spectra were calibrated using synthetic oligonucleotides of known molecular weight.

Oligonucleotides	Sequences (5' to 3')
1	T(8OG)T
2	T(INT)T
3	T(OXA)T
4	CTC CTC T(8OG)T CAC TCC
5	CTC CTC T(INT)T CAC TCC
6	CTC CTC T(OXA)T CAC TCC

8OG : 8-oxoGua; INT : intermediate; OXA : oxaluric acid

Figure 1. Oligonucleotide sequences.

Oligonucleotide Synthesis. Oligonucleotides (Figure 1) were synthesized by standard phosphoramidite chemistry using an Applied Biosystems Inc. 392 DNA synthesizer. The 8-oxoGua containing oligonucleotides **1** and **4** were prepared using a commercially available phosphoramidite monomer of 8-oxodGuo (Glen Research, Sterling, VA). Oligonucleotides **1** and **4** were deprotected in a concentrated ammonia solution for 15 h at 55 °C. The latter solution contained 0.25 M β-mercaptoethanol to prevent further oxidation of 8-oxoGua during the deprotection step. Oligonucleotide **4** was purified by PAGE using a 20% polyacrylamide/7 M urea gel and then desalted on a NAP-25 Sephadex column. The crude 5′-tritylated oligomer **1** was purified and detritylated online by reverse-phase HPLC following the previously reported method.[27] The integrity of **1** and **4** was assessed by MALDI-TOF mass spectrometry.

Oxidation of 8-oxoGua-Containing Oligonucleotides. The synthesis of the endoperoxide $DHPNO_2$, a chemical source of $^1O_2$, has been described.[26] Oxidation of 8-oxoGua containing oligonucleotide **1** with $DHPNO_2$ was performed by incubating a 100 μM solution of the oligomer with an aqueous solution of 13 mM $DHPNO_2$. The resulting solution was heated for 1 h at 37 °C to allow decomposition of the endoperoxide. This leads to the generation of $^1O_2$ in a 50% yield. The sample was kept at 37 °C for 4 h and purified by HPLC as described above. The collected fractions were then analyzed by ESI-MS.

Photosensitization experiments involving methylene blue were performed with visible light generated by a 75 W tungsten lamp. The solution to be irradiated was placed in a glass vial 10 cm apart from the light source. Typically, 200 μL of 100 μM solutions of **1** and **4** were incubated in the presence of methylene blue at a concentration of 1 OD/mL. Then, the solutions were irradiated for 30 min and kept 4 h (3mer) and 5 or 24 h (15mer) at 37 °C. Subsequently, the reaction mixture was subjected to HPLC, ESI-MS, or MALDI-TOF-MS analysis as described above.

Results

Attempts were made in this work to isolate and identify the major products obtained upon $^1O_2$-mediated oxidation of 8-

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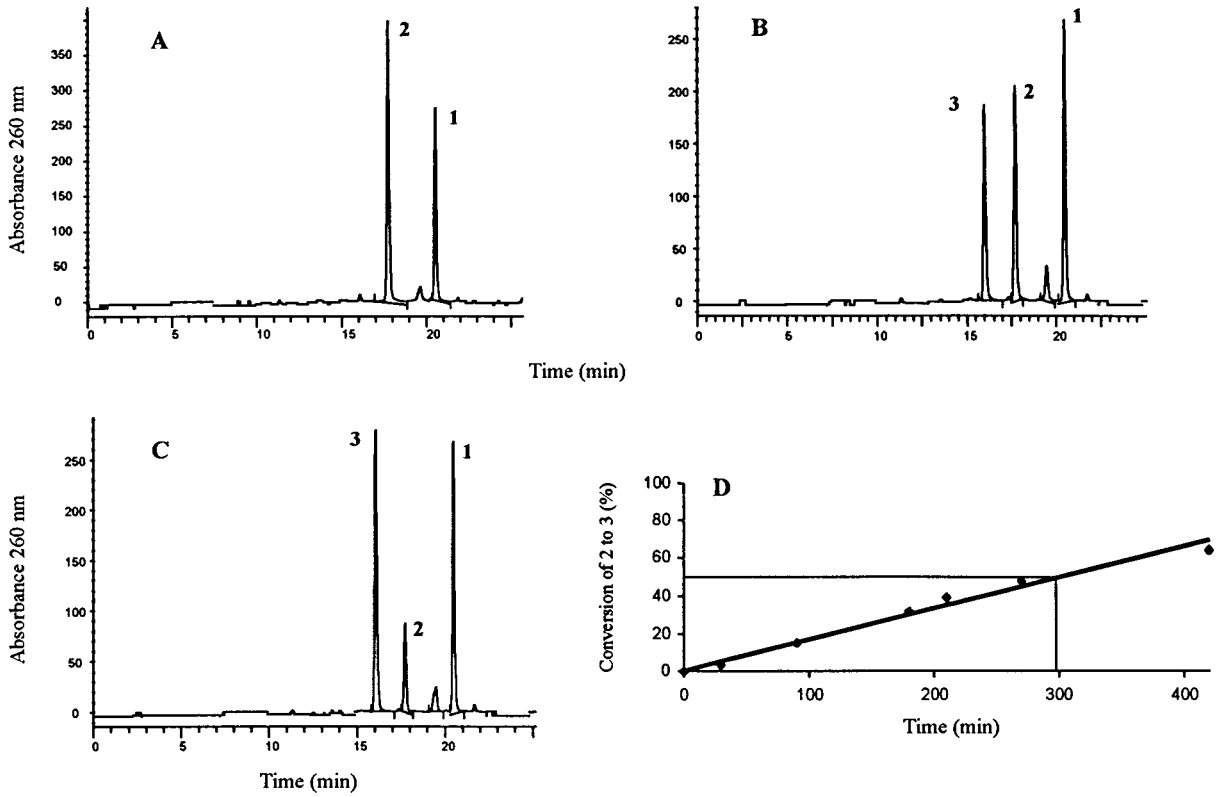


Figure 2. HPLC analysis of $^1O_2$-mediated oxidation of ODN **1** with $DHPNO_2$ (A) and after incubation at 37 °C for 4 (B) and 9 h (C). Time-course of the conversion of ODN **2** to ODN **3** (D).

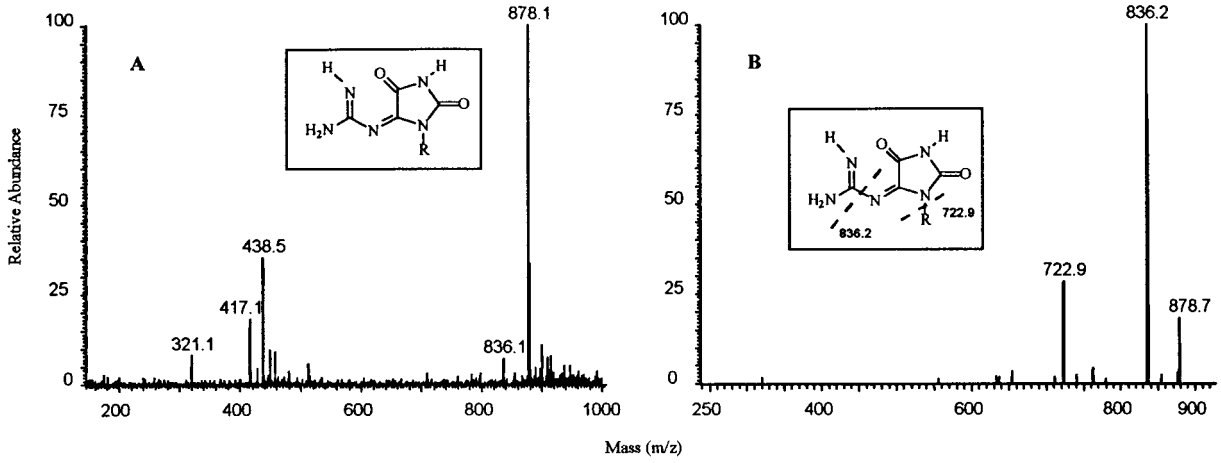


Figure 3. ESI-MS spectra of ODN **2** (A) and ESI-MS/MS spectra of ODN **2** (B). Oxidative reaction conducted with $DHPNO_2$. R stands for an intrastrand 2-deoxyribose unit.

oxoGua in single-stranded DNA fragments. Oxidative reactions were carried out using $DHPNO_2$, a chemical source of $^1O_2$. The experiments were first conducted on a 3mer ODN to allow detailed identification studies and then extended to a 15mer. Treatment of the 8-oxoGua containing 3mer **1** with $DHPNO_2$ followed by immediate HPLC analysis reveals the formation of an additional oligonucleotide **2** (Figure 2A). HPLC analysis after incubation of the mixture at 37 °C for 4 or 9 h shows that ODN **2** converts to ODN **3** (Figure 2B,C). The ratio between **2** and **3** depends on the incubation time at 37 °C, ODN **3** being the final product of the reaction. Isolation of ODNs **2** and **3** was efficiently performed on a Hypersil ODS column. Figure 2D shows the time-course conversion of ODN **2** to ODN **3**. Total conversion of **2** is observed within approximately 10 h at 37 °C. This result, suggesting that **2** is an intermediate in the formation of **3**, received further confirmation (vide infra). In the following, the modified oligomers **2** and **3** were studied in detail and identified by using mass spectrometry methods together with $H_2{}^{18}O$ and $^{18}[^1O_2]$ labeling experiments.

Characterization of ODN 2. Due to the relative instability of ODN **2**, ESI-MS analyses were performed immediately after HPLC purification of the ODNs mixture (Figure 2B). The ESI-MS spectrum in the negative mode reported in Figure 3A shows a predominant peak at *m*/*z* 878.1. The other peak at *m*/*z* 438.5 is accounted for by the doubly charged oligonucleotide. ESI-MS/MS experiments revealed that **2** is fragmented, yielding a major product at *m*/*z* 836.2, indicating a loss of 41.9 amu with respect to the starting ODN **2** (Figure 3B). The peak at *m*/*z* 722.9 results from the cleavage of the *N*-glycosidic bond at the modified base site. Further insights into the structure of **2** were gained by performing oxidation experiments with a chemical source of labeled ^{18}O singlet oxygen. This was achieved using

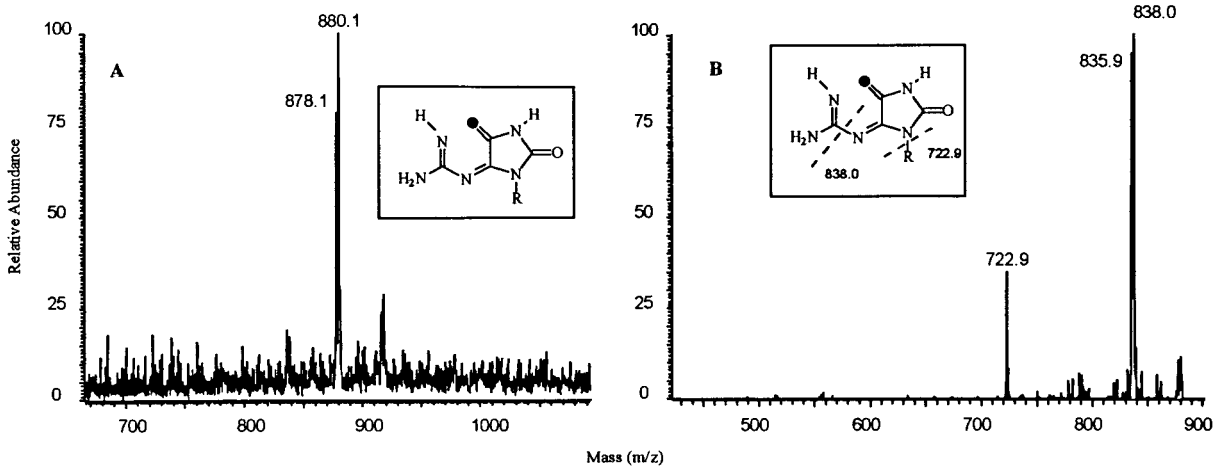
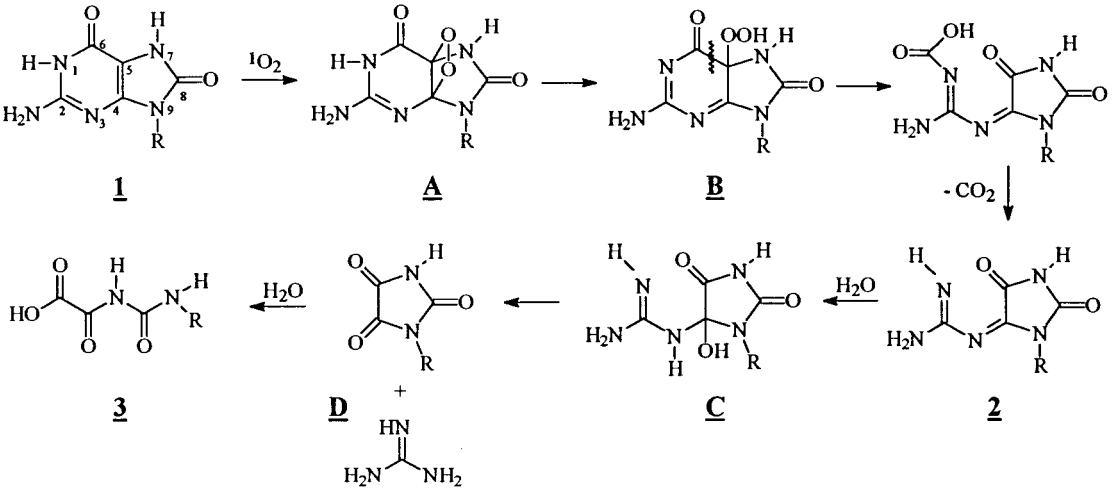


Figure 4. ESI-MS spectra of ODN **2** (A) and ESI-MS/MS spectra of ODN **2** (B). Oxidative reaction performed with DHPN$^{18}O_2$. A solid capital ● corresponds to an ^{18}O oxygen atom. R stands for an intrastrand 2-deoxyribose unit.

Scheme 1. Proposed Mechanism of $^1O_2$-Mediated Oxidation of 8-oxoGua within Single-Stranded DNA[a]



[a] R stands for an intrastrand 2-deoxyribose unit.

the endoperoxide DHPNO_2, photochemically prepared with labeled singlet oxygen $^{18}[^1O_2]$.[28] ESI-MS analysis of oligomer **2** following oxidation of ODN **1** through the release of $^{18}[^1O_2]$ from DHPN$^{18}O_2$ is shown in Figure 4A. The spectrum exhibits a major peak at *m*/*z* 880.1. This indicates an increase of 2 amu, corresponding to the incorporation of one ^{18}O atom. Moreover, a major fragment at *m*/*z* 838.0 indicating a loss of 42.1 amu with respect to ODN **2** was observed in the ESI-MS/MS spectrum (Figure 4B). On the other hand, the cleavage of the *N*-glycosidic bond at N1 gives rise to a nonlabeled fragment at 722.9. At this stage, the suggested structure of the unstable compound **2** as an oxidized guanidinohydantoin (compound **2**, Scheme 1) fits well with our first results. Thus, it is reasonable to propose, as summarized in Scheme 1, that $^1O_2$ reacts with 8-oxoGua to generate an unstable dioxetane (**A**). The latter intermediate is converted into the 5-hydroperoxy-8-oxoGua derivative (**B**) which gives rise to compound **2** after opening of the pyrimidine ring at the C5−C6 bond and subsequent decarboxylation. The experiments with labeled $^{18}[^1O_2]$ provide further support for this decomposition pathway since only one ^{18}O is incorporated into ODN **2**. Furthermore, the structure of **2** is in agreement with the loss of 42 amu ($H_2N-C=NH$) during ESI-MS/MS experiments. While the purity of the labeled DHPN$^{18}O_2$ used in this study was near 95%, nonlabeled products were generated in about 40 to 50% yield. The reason for that observation is unclear; however, we cannot exclude a possible oxygen exchange with a water molecule during the decomposition of the α-keto-peroxide derivative (compound **B**, Scheme 1) leading to the oxidized guanidinohydantoin (compound **2**, Scheme 1).

Characterization of ODN 3. The ESI-MS spectrum of oligonucleotide **3** reported in Figure 5A exhibits a pseudomolecular peak at *m*/*z* 855.0. When the oxidation experiments were performed with ^{18}O-labeled singlet oxygen, the ESI-MS spectrum of **3** shows a major peak at *m*/*z* 857.0. This is indicative of an increase of 2 amu with respect to the unlabeled oligomer (Figure 5B). This suggests that the ^{18}O atom incorporated into the unstable compound **2** is not released during the conversion of the latter compound into **3** (Scheme 1). ESI-MS/MS analysis of ^{18}O-labeled ODN **3** gave rise to two major fragments at *m*/*z* 782.9 and 739.8, respectively. These may be rationalized in terms of a loss of 74.1 and 117.2 amu, respectively, with respect to the starting labeled ODN **3** at 857.0 amu (Figure 5C). Interestingly, two similar fragments at *m*/*z* 783.1 and 740.0 were observed in the ESI-MS/MS experiment involving the unlabeled ODN **3** with *m*/*z* 855.0 (Figure 5D). This suggests that the labeled oxygen is present in the cleaved fragments lost during ESI-MS/MS experiments (Figure 5 and Scheme 1). Further insights into the structure of **3** were gained from $H_2{}^{18}O$-labeling experiments aimed at following the

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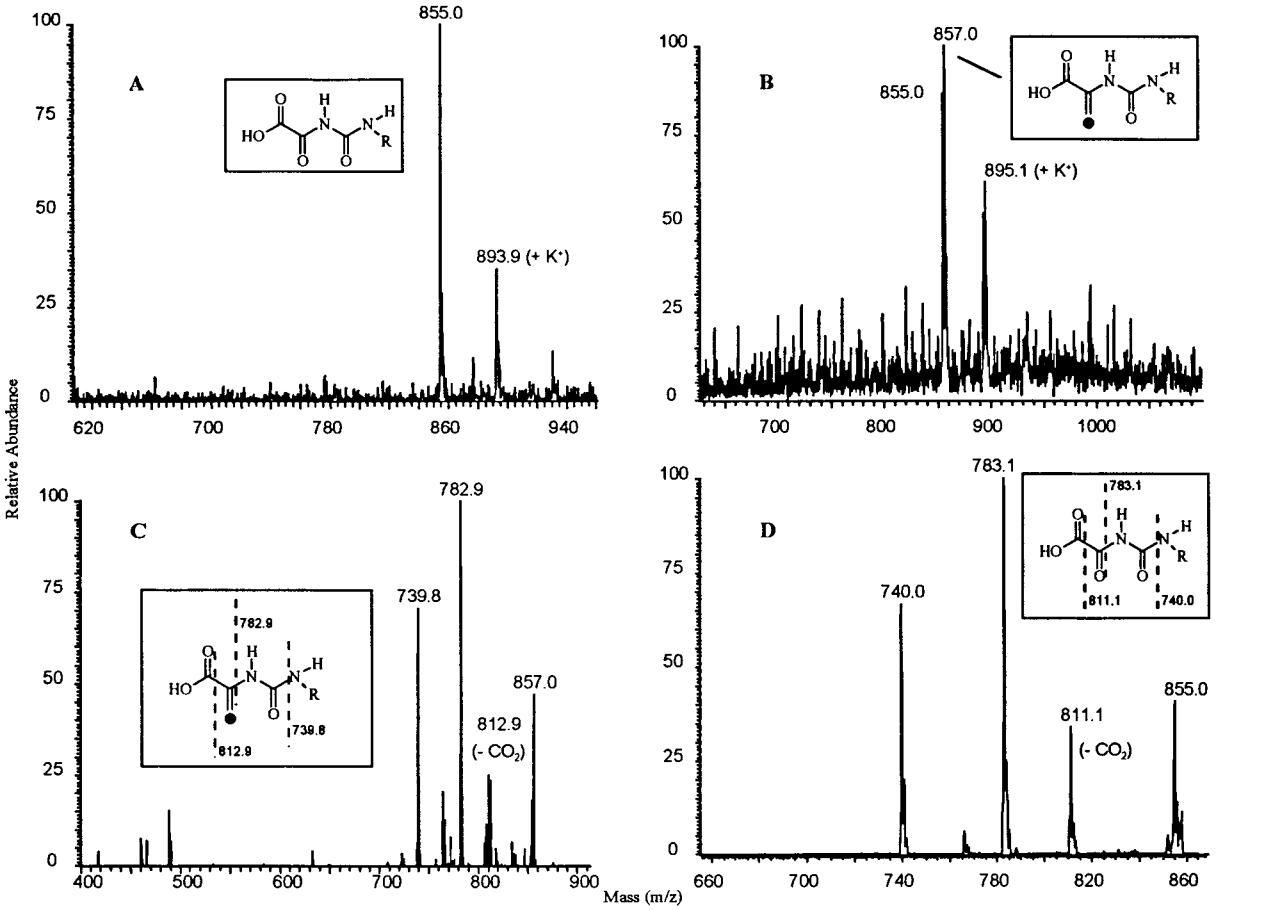

Figure 5. ESI-MS spectra of ODN **3** (A, B) and ESI-MS/MS spectra of ODN **3** (C, D). Oxidation performed with either $DHPNO_2$ (A, D) or $DHPN^{18}O_2$ (B, C). A solid capital ● corresponds to an ^{18}O oxygen atom. R stands for an intrastrand 2-deoxyribose unit.

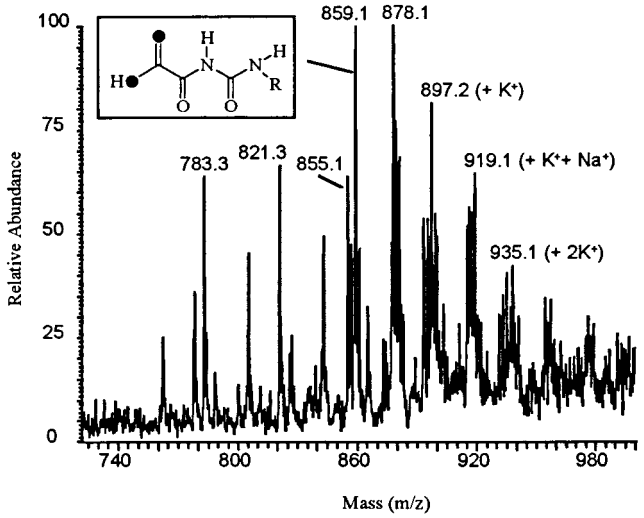

Figure 6. ESI-MS spectra of ODN **3**. Conversion of ODN **2** to ODN **3** in $H^{18}O_2$.A solid capital ● corresponds to an ^{18}O oxygen atom. R stands for an intrastrand 2-deoxyribose unit.

conversion of **2** into **3** (Figure 6). Incubation of **2** at 37 °C for 2 h in $H_2{}^{18}O$ gave rise to a product that exibits a pseudomolecular peak at *m*/*z* 859.1 in addition to the starting ODN **2** at *m*/*z* 878.1 in the ESI mass spectrum. This indicates an increase of 4 amu compared to the unlabeled ODN **3** (*m*/*z* at 855.0) when hydrolysis of **2** was performed in $H_2{}^{16}O$. This result strongly suggests that the latter oligomer is converted into **3** by two successive hydrolytic steps, leading to the incorporation of two atoms of labeled oxygen. Thus, it is reasonable to assume that ODN **3** contains the oxaluric acid modified base (**3**, Scheme 1). As proposed in Scheme 1, hydration of the Schiff base intermediate **2** generates compound **C**, which is converted upon release of guanidine into parabanic acid (**D**, Scheme 1). However, we were unable to isolate the parabanic acid containing oligomer (see discussion below). The latter ODN is then likely to undergo further hydrolysis to generate the oxaluric acid containing oligonucleotide **3**. Interestingly, the measurement of released guanidine upon alkaline treatment[29] of intermediate **2** further confirms the decomposition pathway from ODN **2** to ODN **3** (Supporting Information). The structure of oxaluric acid also provides evidence for the fragments observed during ESI-MS/MS experiments at *m*/*z* 783.1 and 740.0, corresponding to a loss of −CO−COOH (71.9 amu) and −CO−NH−CO−COOH (115 amu), respectively, from the starting ODN **3** (*m*/*z* 855.0).

Similar results that involve the formation of an oxidized guanidinohydantoin as an intermediate in the formation of oxaluric acid were obtained in the methylene blue-mediated photooxidation of the 8-oxoGua containing 3mer **1** through the type II photosensitization mechanism (Supporting Information). It has to be remembered that the latter photosensitizer induces mainly the formation of $^1O_2$. Photooxidative reactions with methylene blue (MB) were also conducted on a 15mer that contained a 8-oxoGua residue **4** (ODN 4, Figure 1), to confirm the results described above within a short oligomer. The HPLC-elution profile and the MALDI-MS analyses obtained upon MB-mediated photosensitization of **4** are reported in Figure 7. Two

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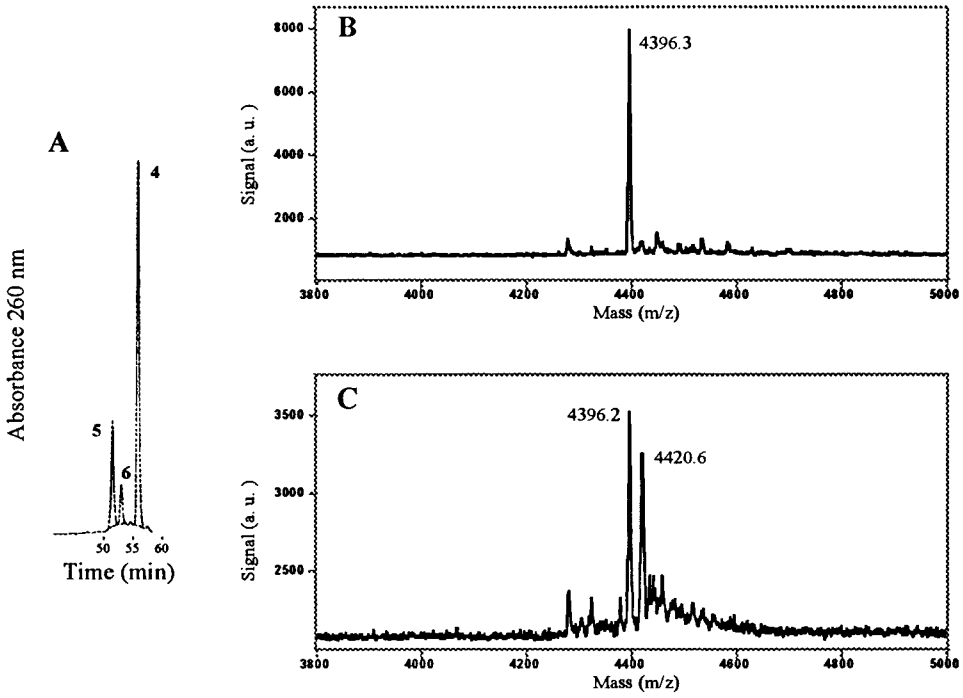

Figure 7. HPLC analysis of methylene blue-mediated photooxidation of ODN **4** after 5 h at 37 °C (A), MALDI-TOF-MS analysis of ODN **6** (B), and MALDI-TOF mass spectra for the conversion of ODN **5** to ODN **6** after 10 h at 37 °C (C).

major products were observed 5 h after the irradiation (Figure 7A, products **5** and **6**). MALDI-MS analysis of the collected fraction **6** (Figure 7B) shows a pseudo-molecular peak at *m*/*z* 4396.3 which is accounted for by the oxaluric acid-containing oligomer (calculated M + H^+ 4397). MALDI-MS analysis of oligonucleotide **5**, after a 10 h incubation period at 37 °C, is indicative of the partial decomposition of the DNA fragment (Figure 7C). Two oligonucleotides were observed at *m*/*z* 4420.6 and 4396.2, respectively, suggesting that **5** is an intermediate in the formation of **6** which is possibly accounted for by the oxidized guanidinohydantoin-containing 15mer (calculated M + H^+ 4420.2). It has to be noted that in contrast to the 3mer sequence, the 15mer oxidized guanidinohydantoin intermediate is eluted first. Moreover the conversion of the latter intermediate into the final oxaluric acid oligomer is slower for the longer sequence, where complete conversion requires approximately 24 h (10 h for the 3mer).

Discussion

Singlet oxygen reacts with nucleic acids almost exclusively at the guanine residues where the five-membered imidazole ring is the reactive site. Studies on free nucleosides have shown that 8-oxodGuo is much more reactive toward singlet oxygen than the parent 2′-deoxyguanosine nucleoside.[20] As previously reported, [2 + 2] cycloaddition of $^1O_2$ to the C4−C5 bond of 8-oxodGuo leads to the transient formation of a 4,5-dioxetane which subsequently rearranges into hydroperoxide derivatives at C4 and C5.[19] Structural information on the latter products has been inferred from NMR analyses performed at low temperature. However, upon warming, they generate a variety of compounds including cyanuric acid and parabanic acid nucleosides. Formation of cyanuric acid via the dioxetane route has also been described for the photooxidation of 8-oxodGuo in the presence of methylene blue.[17] In addition, significant amounts of oxazolone, arising probably from the decomposition of the 5-hydroperoxide, were also found. The present work, aimed at determining the major $^1O_2$-mediated oxidation products of 8-oxoGua in single-stranded DNA, shows that the formation of the unstable dioxetane and its subsequent conversion into the related 5-hydroperoxide are likely to be the first events involved in the $^1O_2$-oxidation of the 8-oxoGua moiety. The latter α-keto-peroxide further evolves and leads after opening of the pyrimidine ring at the C5−C6 bond and subsequent decarboxylation to a relatively stable oxidized guanidinohydantoin (**2**, Scheme 1). In contrast to what was observed with the nucleoside 8-oxodGuo, there is no evidence in this work for the formation of 4-OH-8-oxoGua upon singlet oxygen-mediated oxidation of 8-oxoGua within single-stranded DNA. In fact, as previously described, $^1O_2$-oxidation of 8-oxodGuo generates both 4*R* and 4*S* diastereoisomers of 4-OH-8-oxodGuo via the formation of the 4,5-dioxetane and its subsequent rearragement into the related 4-hydroperoxide.[17,19] On the basis of the present results it appears that the formation of the 5-hydroperoxide via the opening of the 4,5-dioxetane is favored at least in single-stranded DNA and probably in duplex DNA since 4-OH-8oxoGua has never been detected upon oxidation of DNA with singlet oxygen. Moreover, 1,2-cleavage of the 4,5-dioxetane leading to cyanuric acid via the formation of an unstable nine-membered ring was found to be the major decomposition pathway of 8-oxodGuo upon reaction with $^1O_2$.[17,19] Interestingly, the present results indicate that cyanuric acid is not formed following $^1O_2$-oxidation of 8-oxoGua in single-stranded DNA. In contrast to the results obtained with the free 8-oxodGuo, none of the latter pathways leading either to 4-OH-8-oxodGuo or cyanuric acid appears to be involved, at least in detectable yield, in the singlet oxygen-mediated oxidation of 8-oxoGua within single-stranded DNA. However, up to now the reasons for the higher specificity of the oxidation reaction of 8-oxoGua within short oligonucleotides are still unclear. As recently reported by Vialas et al., the oxidized guanidinohydantoin intermediate is supposed to be a precursor of imidazolone and oxazolone upon two-electron oxidation of guanine either at the nucleoside level or in duplex DNA.[30,31] Furthermore, the oxidized guanidinohydantoin compound was also proposed to be formed during the photooxidation

of 8-oxodGuo either by a type I or a type II mechanism as the intermediate in the formation of imidazolone and oxazolone.[16] In the present study, there is no evidence for the formation of the latter modified bases. Under our experimental conditions, the oxidized guanidinohydantoin residue is likely to undergo preferential hydration at the Schiff base, thus leading after guanidine release and further hydrolysis to oxaluric acid.[32] At this point it is reasonable to propose that once the N3−C4 double bond is hydrated, the release of guanidine results in the formation of a diketo product respectively at C5 and C6 to form compound D (Scheme 1), namely the parabanic acid. In these experiments, we were unable to isolate the latter product, probably due to its instability and susceptibility to further hydrolysis with the subsequent formation of oxaluric acid. $H_2{}^{18}O$-labeling experiments showed that two ^{18}O atoms resulting from the hydration and the hydrolytic steps were incorporated into oxaluric acid following the generation of the oxidized guanidinohydantoin intermediate. This result supports the decomposition pathway giving rise to oxaluric acid as the final product. Recently, Niles et al. described a decomposition pathway involving parabanic acid as a precursor in the formation of oxaluric acid upon oxidation of 8-oxodGuo by peroxynitrite.[15] These authors also reported the formation of oxaluric acid in single-stranded oligonucleotides upon specific oxidation of a unique 8-oxoGua residue with peroxynitrite.[23,24] The latter results, together with those of the present work, pointed out the importance of oxaluric acid as a major oxidation product of 8-oxoGua. Thus, this suggests that oxaluric acid may play a role in the biological consequences such as mutagenesis associated with damaged DNA bases. Studies aimed at determining the biological properties in terms of DNA repair and mutagenicity of oxaluric acid-containing DNA fragments are in progress. Preliminary results have shown that the latter modified base may be a good candidate to explain the occurrence of transversions in the mutation spectra observed during DNA replication following exposure to oxidizing agents. Such investigations appear to be relevant since we recently gained evidence for the formation of oxaluric acid in isolated double stranded. It will now be important to assess if the latter DNA damage is formed at the cellular level upon exposure of cells to different oxidizing agents.

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Supporting Information Available: HPLC analysis (fluorimetric detection) of released guanidine upon basic treatment of ODN 2, and HPLC analysis of methylene blue-mediated oxidation of ODN 1 after incubation at 37 °C for 1 h (A) or 3 h (B) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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