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Fe(II) phthalocyanine catalyzed oxidation of dGMP by molecular oxygen

Aleksandra A. Kuznetsova [a,b], Lyudmila I. Solovyeva [c], Oleg L. Kaliya [c], Evgeniy A. Lukyanets [c], Dmitri G. Knorre [b], Olga S. Fedorova [a,b,*]

[a] Novosibirsk State University, 630090 Novosibirsk, Russia
[b] Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of Russian Academy of Sciences, 630090 Novosibirsk, Russia
[c] State Research Centre 'Organic Intermediates and Dyes Institute' (SRC 'NIOPIK'), 103787 Moscow, Russia

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ABSTRACT

We show that iron(II)-phthalocyanines are able to catalyze guanosine oxidation by molecular oxygen in the presence of reducing agents such as ascorbic acid and 2-mercaptoethanol. The products of 5′-monophosphate-2′-deoxyguanosine (dGMP) oxidation were directly analyzed using the HPLC-ESI/MS method. The main oxidation products were 5′-phospho-2′-deoxy-8-oxo-7,8-dihydroguanine and the 1,N2-glyoxal adduct of the 5′-monophosphate-2′-deoxyguanosine.

Many efficient DNA-damaging agents have been developed over the last several decades.[1] Interest in these agents exists due to the search for new anticancer and antiviral drugs. Among these agents, complexes of transition metals are considered to be promising reagents for oxidative DNA cleavage.

The antitumor activity of transition metal complexes in the presence of O_2 and easily oxidizable substrates was first found by Kimoto et al.,[2] who used a copper glycyl-glycyl-histidine complex with sodium ascorbate as a conjugated reducing agent. Currently, phthalocyanine complexes of Co(II) and Fe(II) are being investigated as drugs for the catalytic therapy of cancer.[3]

Among the possible oxidation sites on DNA, guanosine is the most oxidizable nucleoside.[4] There is currently no information about guanosine-oxidation products induced by molecular oxygen in the presence of phthalocyanines Co(II) or Fe(II).

Recently, we have studied sequence-directed oxidative cleavage of DNA with O_2 and H_2O_2 in the presence of conjugates of cobalt(II) and iron(II) phthalocyanines ($CoPc_{neg}$ and $FePc_{neg}$, respectively) attached to oligonucleotides.[5a,b] It was shown that single-stranded DNA is efficiently damaged in complexes with these conjugates. In addition, the site-directed modification of single-stranded DNA by O_2 and H_2O_2 in the presence of heterogeneous dimeric complexes of negatively and positively charged Fe(II) and Co(II) phthalocyanines ($FePc_{pos}$·$FePc_{neg}$ and $CoPc_{pos}$·$CoPc_{neg}$, respectively) was investigated.[5c] These complexes were formed directly on single-stranded DNA through interaction between the negatively charged phthalocyanine in the oligonucleotide-conjugate and the positively charged phthalocyanine in the solution. The resulting phthalocyanine complexes showed a significant increase in catalytic activity compared to monomeric forms of phthalocyanines Fe(II) and Co(II)[5c] and led to direct DNA strand cleavage. It was determined that oxidation of DNA by molecular oxygen catalyzed by a complex of Fe(II)-phthalocyanines proceeds with a higher rate than that catalyzed by Co(II)-phthalocyanines; however, the latter led to a greater extent of target DNA modification. In all cases the guanine residues located close to the source of the oxidizing species were the most susceptible to modification.

In the present work, we investigated the oxidation of 2′-deoxyguanosine-5′-monophosphate (dGMP) as a model compound to provide molecular insight into the structure of guanine lesions generated by metallophthalocyanine-oligonucleotide conjugates. We used $FePc_{neg}$ and its dimeric complex $FePc_{neg}$·$FePc_{pos}$ immobilized on SiO_2 as catalysts (Fig. 1), and reducing substrates (L-ascorbic acid, H_2A or 2-mercaptoethanol, RSH) for dGMP oxidation by molecular oxygen.

$FePc_{neg}$–SiO_2 was synthesized through the formation of an amide bond between 3-aminopropyl modified SiO_2 and an *N*-succinimide ester of $FePc_{neg}$ (see Supplementary data). The dimeric complex $FePc_{pos}$·$FePc_{neg}$–SiO_2 was prepared by treatment of $FePc_{neg}$–SiO_2 with an aqueous solution of $FePc_{pos}$.[6]

The dGMP oxidation products were directly analyzed using the HPLC-ESI/MS method.[6] The oxidation of dGMP by molecular oxygen in the presence of $FePc_{neg}$–SiO_2 or $FePc_{pos}$·$FePc_{neg}$–SiO_2 and reducing substrate resulted in several products. The RP-HPLC traces of these products are shown in Figure 2. Six products were identified:

* Corresponding author. Tel.: +7 383 334 6274; fax: +7 383 333 3677.
E-mail address: fedorova@niboch.nsc.ru (O.S. Fedorova).

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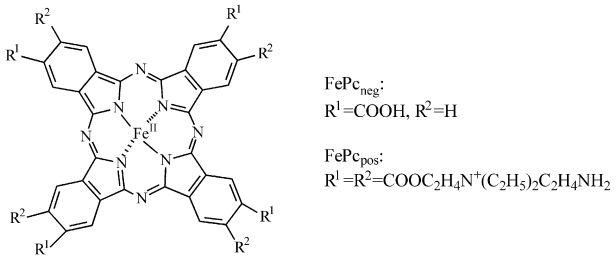

Figure 1. The structures of $FePc_{neg}$ and $FePc_{pos}$ used in this work.

guanine (Gua), 7,8-dihydro-8-oxo-guanine (8-oxoGua), 1,N2-glyoxal-5′-monophosphate-2′-deoxyguanosine (1,N2-glyoxal-dGMP), 5′-monophosphate-2′-deoxy-7,8-dihydro-8-oxo-guanosine (8-oxo-dGMP), 5′-aldehyde-2′-deoxyguanosine (5′-aldehyde-dG) and 2′-deoxy-7,8-dihydro-8-oxo-guanosine (8-oxo-dG). Identification of the dGMP oxidation products was done using ESI-MS and by comparison of the retention times and UV absorbance spectra with those of a commercial standard and described in the literature[7] (Supplementary data, Table S1).

The main difference in oxidation product formation in the presence of $FePc_{neg}$–SiO_2 or $FePc_{pos}$·$FePc_{neg}$–SiO_2 was the yield. In general, $FePc_{pos}$·$FePc_{neg}$–SiO_2 led to a higher yield of 8-oxo-dGMP in the presence of H_2A and 1,N2-glyoxal-dGMP in the presence of RSH. This can be correlated with the higher catalytic activity of dimeric complexes or with the electrostatic interaction between the positive charged Pc and the negative charged phosphate group of dGMP. As a result, higher degradation of dGMP occurred. Potentially, both processes could contribute to oxidation.

The main difference in oxidation product formation in the presence of H_2A or RSH was found in the yields of 8-oxo-dGMP and 1,N2-glyoxal-dGMP. The presence of H_2A in the reaction solution led primarily to formation of 8-oxo-dGMP. As a result, the free 8-oxoGua base was also detected in these systems. The presence of RSH led preferentially to the formation of 1,N2-glyoxal-dGMP. In addition, the formation of 5′-aldehyde-dGua was observed.

The compounds ultimately characterized are discussed in the order of their retention times below. The UV and MS spectra of the identified oxidation products of dGMP are shown in Figure 3.

Free *guanine* (peak 1 in Figs. 2 and 3a) was formed in all cases of dGMP oxidation (*m/z* = 150.1). The formation of *7,8-dihydro-8-oxo-guanine* (peak 2 in Fig. 2 and 3b) was observed only in the presence of H_2A (*m/z* = 166.0). Release of free bases is thought to be an indication of sugar damage.[8] A 2′-deoxyribose residue in the backbone of a DNA strand has seven hydrogen atoms attached to carbon, which in principle, are available for abstraction by an oxidizing agent or free radicals. It is well-known that the hydroxyl radical is able to abstract hydrogen from each 2′-deoxyribose carbon of DNA.[8] Release of free base in aerobic conditions is possible when abstraction of hydrogen atoms from C1′-, C2′- and C5′-sites of 2′-deoxyribose occurs.[8] It should be noted, that release of free bases is also possible under DNA oxidation by high-valent metal-oxo species such as Fe^{V}=O in activated bleomycin,[9a] Mn^{V}=O in manganese(III) porphyrin activated by $KHSO_5$,[9b] and Cu(III)=O in 1,10-phenanthroline complex.[9c] Thus, the formation of free bases can be evidenced either by OH· or by high-valent metal-oxo species generation in our oxidation systems.

The formation of *1,N2-glyoxal-dGMP* (peak 3 in Figs. 2 and 3c) was observed in all cases of dGMP oxidation. However, in the $FePc_{pos}$·$FePc_{neg}$–SiO_2/RSH oxidation system it was the main oxidation product. The ESI-MS spectrum of 1,N2-glyoxal-dGMP showed *m/z* peaks corresponding to molecular ions $[M-H]^-$ at 404.1 and 346.2 (loss of glyoxal moiety). The formation of the glyoxal adducts of dG was first shown under oxidation of DNA by O_2 in the presence of the Fe(II)–EDTA complex,[10a] and its formation could be a consequence of sugar moiety oxidation. Besides, it was shown that 2-phosphoglycolaldehyde reacted with dG to form the 1,N2-glyoxal-dG adducts[10b] (Supplementary data, Fig. S1). The phosphoglycolaldehyde residue can form by oxidation of the C3′-atom of 2′-deoxyribose.[8] Since in our conditions glyoxal-dGMP was observed in all cases, it can be assumed that the Fe(II)–phthalocyanine complex is capable of oxidizing the C3′-atom of 2′-deoxyribose. The formation of the phosphoglycolaldehyde under 2′-deoxyribose oxidation by hydroxyl radicals should be accompanied by formation of base propenoic acid.[8] Unfortunately, we could not detect the formation of base propenoic acid.

Peak 4 in Figure 2 was identified as unreacted *dGMP*.

The formation of *8-oxo-dGMP* (peak 5 in Figs. 2 and 3d) was observed in the presence of H_2A and also with low yield in the

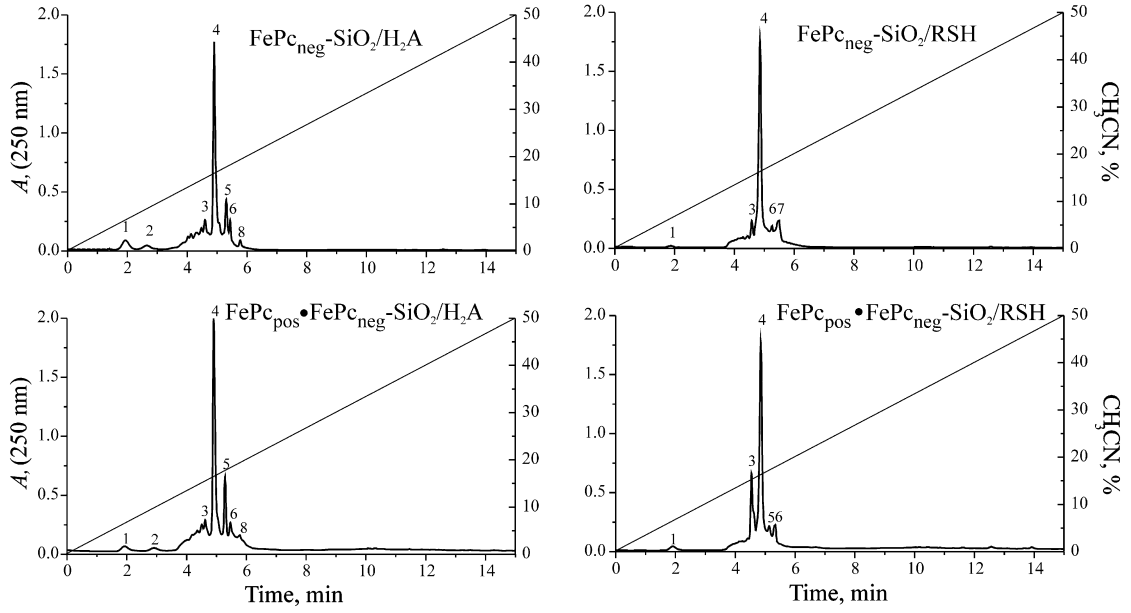

Figure 2. HPLC profiles of reaction mixtures: oxidation of dGMP by O_2 in the presence of $FePc_{neg}$–SiO_2 or $FePc_{pos}$ × $FePc_{neg}$–SiO_2 and H_2A or RSH.

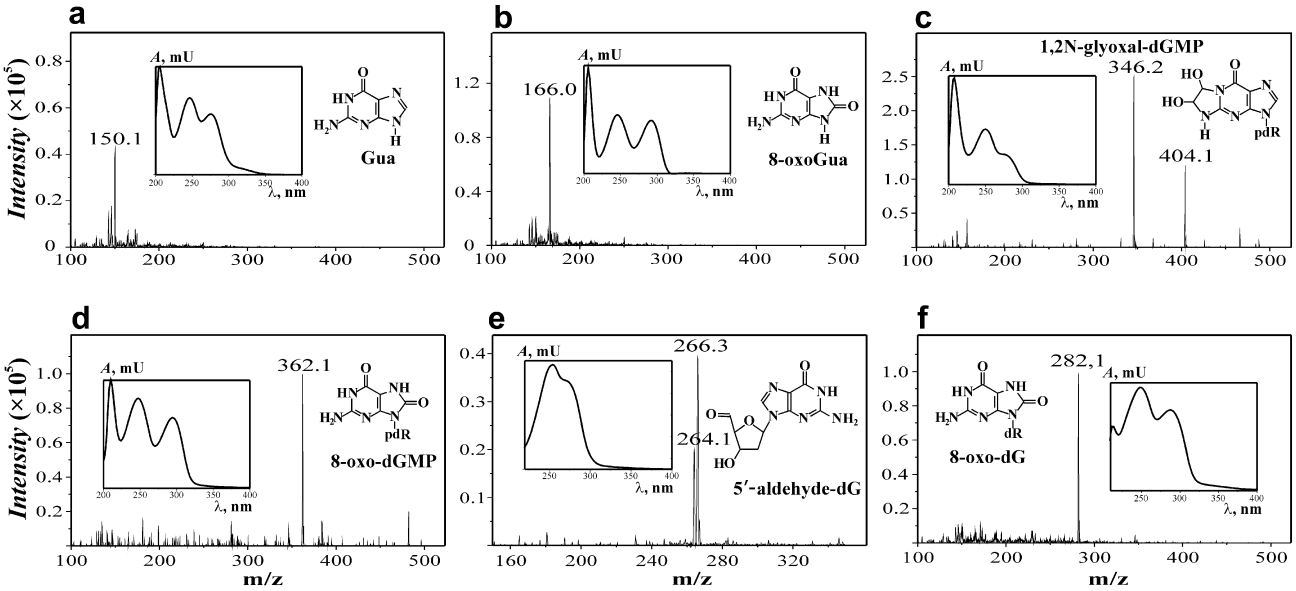

Figure 3. UV–vis and ESI-MS spectra of the dGMP oxidation products (dR is 2′-deoxyribose and pdR is 5′-monophosphate-2′-deoxyribose).

$FePc_{pos}$·$FePc_{neg}$–SiO_2/RSH oxidation system. It should be noted that in the $FePc_{pos}$·$FePc_{neg}$–SiO_2/H_2A oxidation system 8-oxo-dGMP was the main oxidation product. The formation of 8-oxo-dGMP could also result from OH· generation in oxidation systems.[4] A hydroxyl radical adds to purines, giving rise to C4–OH-, C5–OH-, and C8–OH-adduct radicals.[11a] C4–OH- and C5–OH-adduct radicals undergo dehydration and form the oxidizing radicals (G–H)·, which reconstitute guanine upon reduction. The interaction of (G–H)· with $O_2^{\cdot-}$ results in the formation of imidazolone and dihydroguanidinohydantoin derivatives.[11b] One-electron oxidation and one-electron reduction of C8–OH-adduct radicals lead to the formation of 7,8-dihydro-8-oxo-guanine and 2,6-diamino-4-hydroxy-5-form-amidopyrimidine (FAPyG), respectively.[11a] We did not detect the formation of FAPyG. This is in agreement with observations made for an isolated DNA upon exposure to HO· radicals in aqueous solution. Indeed, 8-oxo-guanine was the major degradation product when DNA was exposed to γ-rays under aerobic conditions.[11c] However, a drastic increase in the yield of FAPyG at the expense of 8-oxo-guanine was observed when O_2 is absent.[11d] We also did not detect the formation of imidazolone or oxazolone derivatives. The latter is formed from imidazolone under hydrolytic conversion.[12a] It is known[12b] that γ-irradiation of an aerated aqueous solution of dG led predominantly to the formation of oxazolone, whereas FAPyG and 8-oxo-guanine were produced with low yield. However, when either cysteine or ascorbate was added, even at a low concentration, a drastic decrease in the yield of oxazolone was observed.[12c] Thus, our results are in agreement with these observations.

Peak 6 in Figure 2 is *dG*. In control experiments it was shown that dG was present in parent dGMP.

The formation of *5′-aldehyde-dGua* (peak 7 in Figs. 2 and 3e) was observed only in the $FePc_{neg}$–SiO_2/RSH oxidation system. The ESI-MS spectrum of 5′-aldehyde-dGua showed *m/z* peaks corresponding to the molecular weight $[M-H]^-$ at 264.1 and an *m/z* peak corresponding to the molecular weight $[M-H]^-$ of dG at 266.3. The presence of a signal from dG could be due to the overlapping peaks in the eluting conditions. The formation of 5′-aldehyde-2′-deoxyguanosine is possible when oxidation of the C5′-atom of 2′-deoxyribose occurs. The oxidizing species causing such damage can be both hydroxyl radicals[8] and high-valent metal-oxo species.[9b]

The formation of *8-oxo-dG* (peak 8 in Figs. 2 and 3f) was observed in the presence of H_2A and not observed in the presence of RSH. It should be noted that $FePc_{neg}$–SiO_2 led to a higher yield of 8-oxo-dG compared to $FePc_{pos}$·$FePc_{neg}$–SiO_2. The 8-oxo-dG could be formed from dG in the same way as the 8-oxo-dGMP was formed from dGMP.

In conclusion, the dGMP oxidation by molecular oxygen catalyzed by Fe-phthalocyanine complexes was investigated. The main oxidation products were 8-oxo-dGMP and 1,N2-glyoxal-dGMP. The formation of 8-oxoguanine derivatives, as well as the products of sugar oxidation (free guanine bases and 1,N2-glyoxal adduct), could be evidence of several oxidizing species in the reaction mixture: hydroxyl radicals and high valent iron-oxo species.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.088.

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6. All reactions were carried out according to the following standard procedure: the reaction mixture (250 μL) contained 50 mM phosphate buffer (pH 7.5), 1 mM dGMP, 5 mM H_2A or 50 mM RSH, and 5 mg $FePc_{neg}$–SiO_2; the studies were performed at 25 °C; the reducing agent was introduced in 4 additions every 2 h and the first addition initiated the reaction. The $FePc_{neg}$–SiO_2 was preliminarily treated with an aqueous solution of $FePc_{pos}$ (3 mg/ml) in the case of dGMP oxidation in the presence of dimer complex. After 20 h the solution of oxidated dGMP was separated from the Silica Gel by centrifugation and analyzed by HPLC (chromatograph Agilent Technologies 1200 series with diode array detector) on an analytical reverse phase column (Nucleosil C18, 10 μm, 250 × 4.6 mm) in a linear gradient of 0–50% CH_3CN in 25 mM TEA-AcOH buffer (pH 7.5). Detection was at 250 nm. Chromatogram peaks corresponding to different oxidation product of dGMP were collected and separately analyzed on an ESI/MS spectrometer (Agilent 6410 Triple Quad LC/MS, Agilent Technologies) in negative mode.
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