SUPPORTING INFORMATION

Protonation state of trans axial water molecule switches the reactivity of high-valent manganese-oxo porphyrin

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Oxidation of dGpT by Mn-TMPyP/$KHSO_5$ at pH 6.5

Dinucleoside monophosphate dGpT (600 µM) was reacted in 20 mM triethylammonium acetate buffer pH 6.5, 100 mM NaCl at 0 °C with Mn-TMPyP (50 µM) and $KHSO_5$ added by 10 aliquots (concentration increments 100 µM) until 1.6 mol. equiv. with respect to dGpT was added. The reaction volume was 100 µL, the reaction lasted 5 min. The reaction was carried out in normal water and in $H_2{}^{18}O$. The reaction was analyzed by LC/ESI-MS (negative mode). **DGh** lesion was the major oxidation product. Figure S1 shows the LC trace and Figure S2 the corresponding in-line mass spectrum of **DGh** lesion.

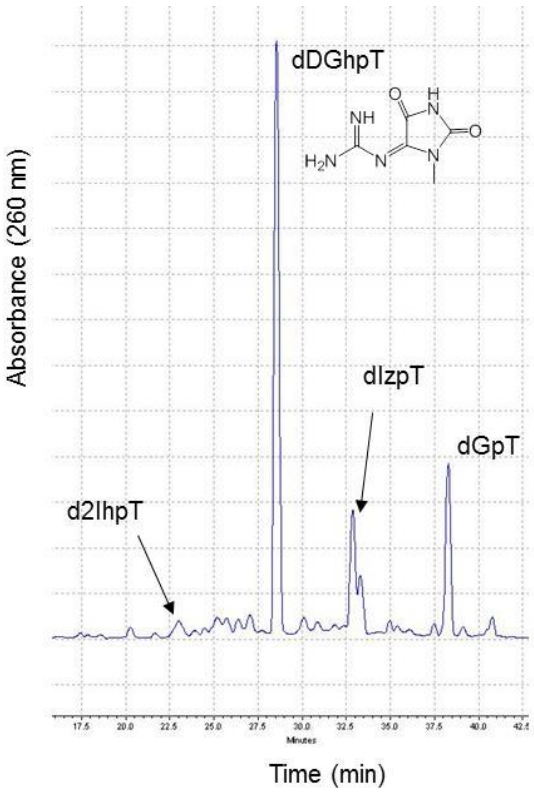


Figure S1. HPLC trace of the oxidation of dGpT by Mn-TMPyP/$KHSO_5$ at pH 6.5.

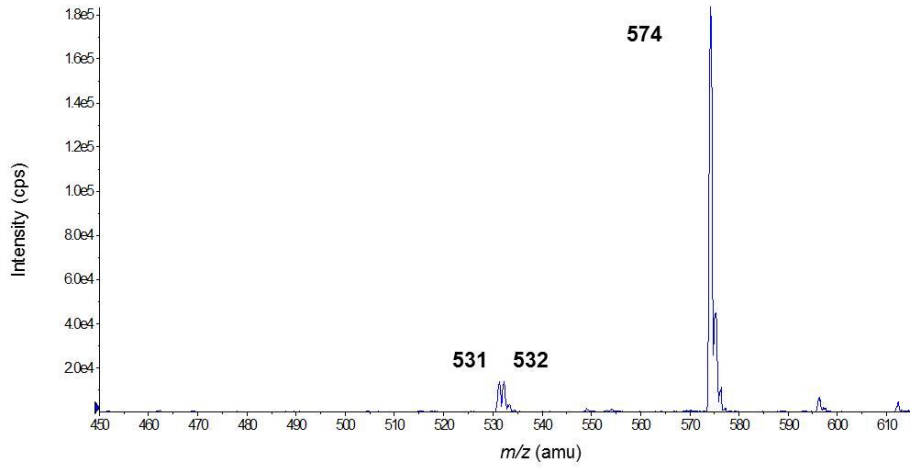
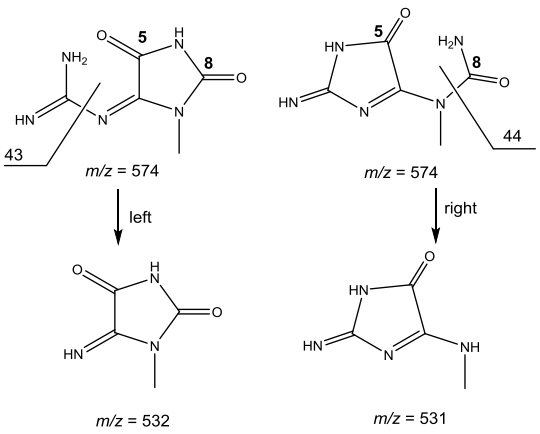


Figure S2. In-line mass spectrum of **DGh** lesion (dDGhpT) at retention time 28.5 min when the reaction was carried out in normal water.

The molecular signal of dDGhpT lesion is observed at *m/z* = 574 (*z* = 1) associated to two fragmentation ions at *m/z* = 531 and 532 (*z* = 1) that correspond to the loss of 43 and 42 amu with respect to the parent ion at 574, respectively. The origin of these fragments is shown in Scheme S1. The fragmentation giving the 531 fragment corresponds to the loss of the former C8 of G, which is a site of O-atom incorporation during oxidation.



Scheme S1. Fragmentation of dDGhpT leading to fragments with *m/z* = 531 (left fragmentation) and 532 amu (right fragmentation) (*z* = 1). Numbering correspond to former guanine carbons.

Labeling of DGh in $H_2{}^{18}O$ at pH 6.5

Before analysis of the labeling of **DGh** lesion during the oxidation reaction in $H_2{}^{18}O$ the control of the non-exchange of O-atoms of **DGh** with solvent was performed. A sample of modified dinucleoside dDGhpT prepared in normal water. It was isolated by HPLC chromatography and the fraction was

lyophilized. The product was incubated in labeled water for 1h. LC/ESI-MS analysis exhibited a single *m/z* signal at 574 amu, showing that the two newly incorporated O-atoms of **DGh** lesion do not exchange with solvent.

Oxidation of dGpT by Mn-TMPyP/$KHSO_5$ was then carried out in labeled water and the in-line ESI-MS spectrum is shown in Figure S3.

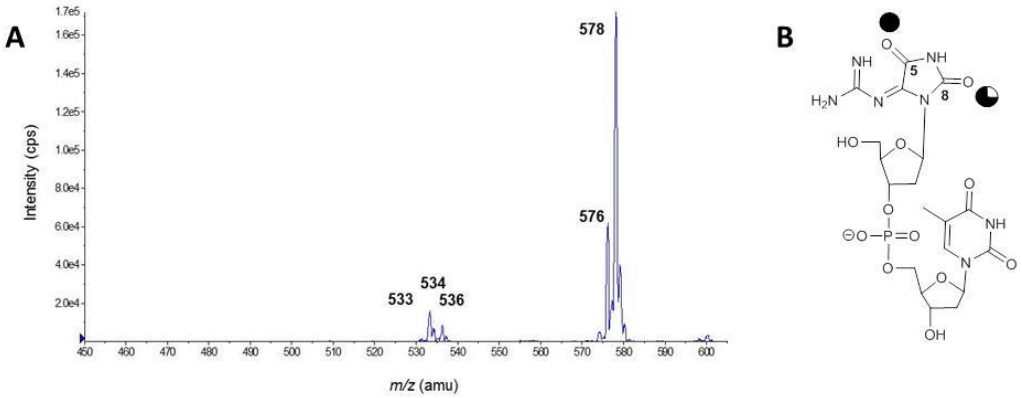


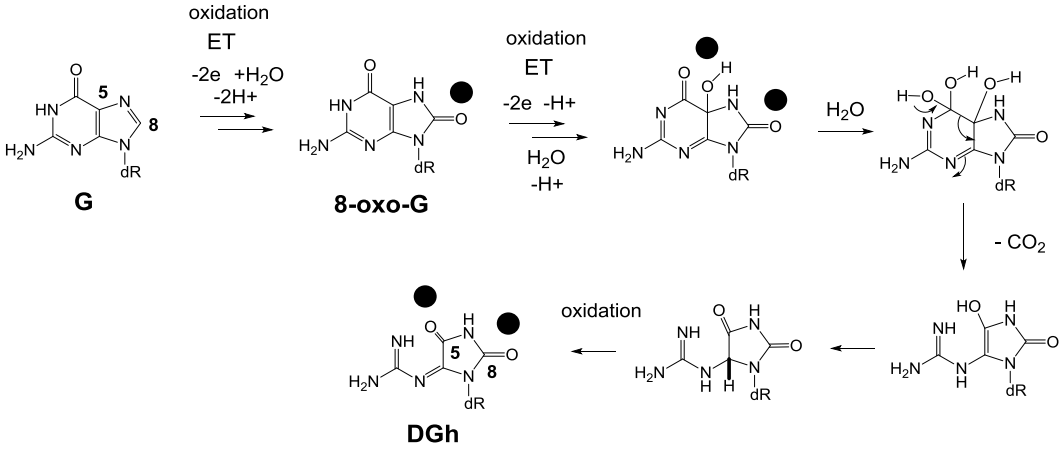
Figure S3. A: In-line mass spectrum of **DGh** lesion (dDGhpT) at retention time 28.5 min when reaction was carried out in $H_2{}^{18}O$. B: Separate labeling of O-atoms at former C5 and C8 atoms of G are shown for **DGh** lesion, with black and white color symbol indicating the percentage of ^{18}O- and ^{16}O-atom, respectively.

The compound did not show the previous *m/z* signal at 574 amu (containing 2 ^{16}O-atoms). It appeared as a mixture of 576 and 578 ions (*z* = 1) in 25:75 ratio. The labeling of dDGhpT corresponded to the incorporation of one ^{18}O-atom (*m/z* = 576) and two ^{18}O-atoms (*m/z* = 578). Importantly, the previous two fragments at *m/z* = 531 and 532 were absent. This is a clear demonstration that the oxygen atom at C5 is 100% labeled with ^{18}O from $H_2{}^{18}O$ and that the site of mixed labeling of **DGh** lesion was at C8. The fragmentation signals were replaced by new ones *m/z* = 533, 534 and 536 amu. From the parent peak at *m/z* = 578 amu that contains two ^{18}O-atoms (at C5 and C8) one expects two fragments with *m/z* = 536 and 533 for left and right fragmentation, respectively (Scheme S1). The *m/z* = 576 amu labeled parent ion, gave rise to one fragment with *m/z* = 534 for the left fragmentation and a fragmentation at *m/z* = 533 for the right fragmentation with an ^{18}O-atom at C5 and associated to the loss of the $CONH_2$ fragment carrying an ^{16}O-atom at C8.

This labeling study allowed us to conclude that the formation of **DGh** lesion under the used experimental conditions (pH 6.5) was due to an electron transfer oxidation leading to a guanine cation $(G\text{-}H)^+$, which reacted with a molecule of water at C5. The labeling of C8-atom of **DGh**

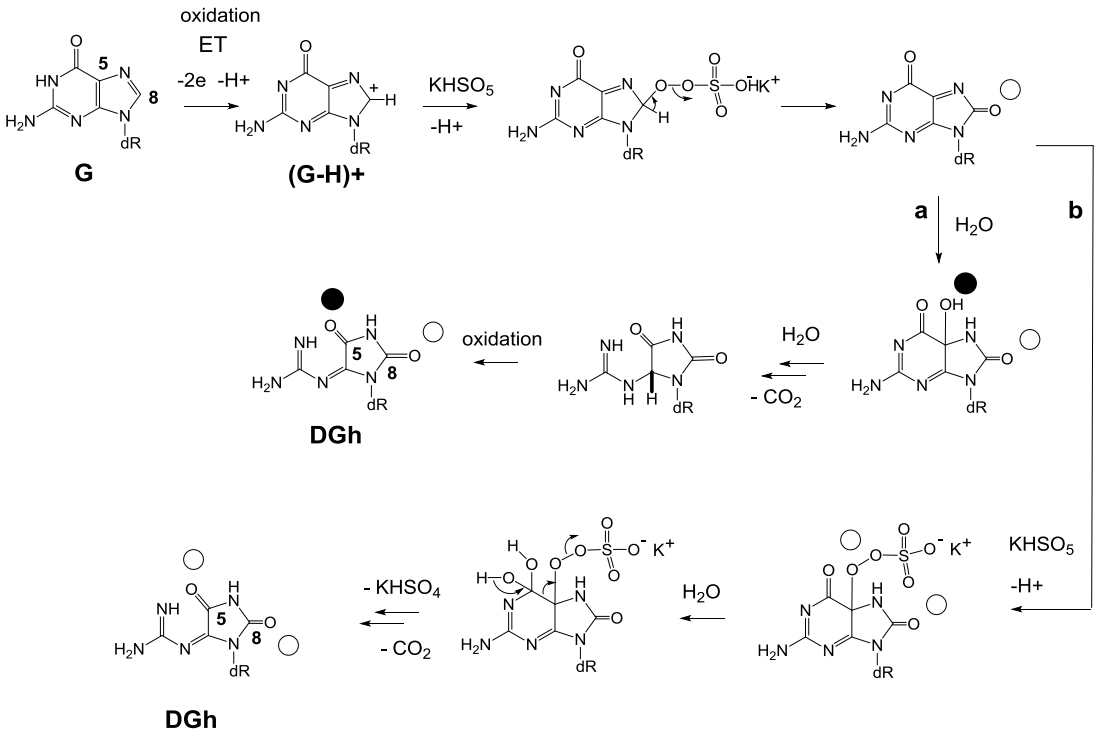
consisted of ^{16}O- and ^{18}O-atom in 25:75 ratio consistent with the nucleophilic attack of a water molecule followed by hydroxylation by oxygen atom transfer by manganese-oxo porphyrin at C8 (Scheme 2).

In the present work, **DGh** did not arise from secondary oxidation of 8-oxo-7,8-dihydroguanine (8-oxo-G). If this was the case **DGh** should have exhibited a 100% ^{18}O-atom at C8 in $H_2{}^{18}O$ (the oxygen atom at C8 of 8-oxo-G does not exchange) (Scheme S2).



Scheme S2. Possible mechanism of **DGh** formation from oxidation of intermediate 8-oxo-G. The numbering of the carbon atoms refers to that of the initial guanine. ET and dR stand electron transfer and deoxyribose, respectively. The origin of oxygen-atoms is shown. Black circle: 100% ^{18}O-atom from labeled water $H_2{}^{18}O$. This mechanism is not observed in the present work.

Alternatively, the data may be explained by a mixed contribution of two different mechanisms such as the one depicted in Scheme S2 that would be at the origin of the incorporation of 2 ^{18}O-atom in **DGh** (75%) and another one leading to 100% ^{16}O-atom incorporation at C8 and 100% ^{18}O-atom at C5 (25%) (Scheme S3, pathway a). In order to obtain a 100% ^{16}O labeling at C8, one may propose the attack of $KHSO_5$ at a transient guanine cationic intermediate centered at C8 instead of C5 followed by the nucleophilic attack of a water molecule at C5 (Scheme S3). However, this mechanism is not likely because it is difficult to propose $KHSO_5$ acting as a nucleophile exclusively at C8 and not at C5 (Scheme S3, pathway b). Therefore, this alternative mechanism was not considered.



Scheme S3. Possible mechanism of **DGh** formation from nucleophilic attack of $KHSO_5$ at C8 of $(G\text{-}H)^+$ cationic species. The numbering of the carbon atoms refers to that of the initial guanine. ET and dR stand electron transfer and deoxyribose, respectively. The origin of oxygen-atoms is shown. Black circle: 100% ^{18}O-atom from labeled water $H_2{}^{18}O$, white circle: 100% ^{16}O. This mechanism is not considered in the present work.

Labeling of 2Ih in $H_2{}^{18}O$ at pH 6.5

A small amount of **2Ih** lesion formed during oxidation of dGpT at pH 6.5. The retention time was 23 min (Figure S1). The in-line mass spectrum showed a signal at *m/z* = 604 amu associated with a fragmentation ion with *m/z* = 576 (z = 1) corresponding to the loss of CO from the former C8 of G (not shown). This lesion could also be analyzed by LC/ESI-MS when the oxidation was performed in labeled water (Figure S4).

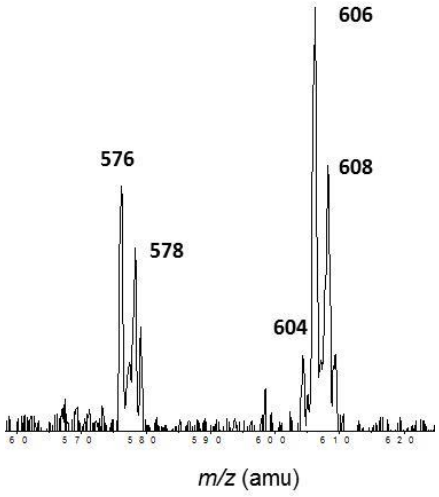
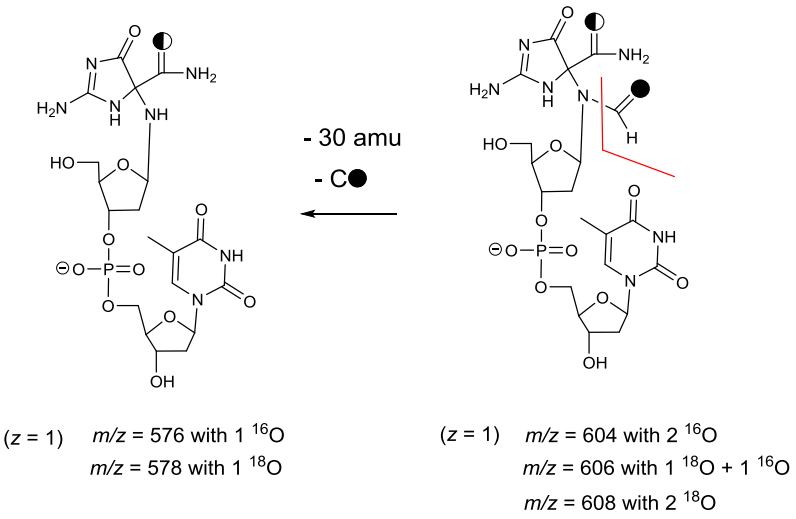


Figure S4. In-line mass spectrum of **2Ih** lesion (d2IhpT) at retention time 23 min (see Fig.S1) when reaction was carried out in $H_2{}^{18}O$.

The in-line spectrum showed that **2Ih** lesion incorporated one (*m/z* = 606, *z* = 1) and two (*m/z* = 608, *z* = 1) ^{18}O-atoms from $H_2{}^{18}O$. The fragmentation of the parent ions occurred with the loss of a 30 amu fragment, which corresponded to the loss of labeled CO from former C8 of G, as shown in Scheme S4.



Scheme S4. Fragmentation of d2IhpT leading to fragments with *m/z* = 576 (from the parent signal at 606) and 578 amu (from 608) (*z* = 1). Numbering correspond to former guanine carbons. The O-atom is shown in black for ^{18}O- and ^{16}O-atom, respectively.

The constant fragmentation involving the loss of labeled CO indicated that the oxygen atom incorporated at that position came exclusively from $H_2{}^{18}O$. The oxygen atom incorporated at C5 had a mixed label. This data is accordance with the mechanism of **2Ih** formation shown in Scheme 3.
