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# One-Electron Oxidation of the Guanine Moiety of 2′-Deoxyguanosine: Influence of 8-Oxo-7,8-dihydro-2′-deoxyguanosine

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One-electron oxidation of the guanine moiety generates the related radical cation which, in aqueous aerated solution, undergoes transformation by two competitive reactions (Figure 1), that is, hydration and deprotonation.[1]

Hydration of the guanine radical cation (Figure 1), which is efficient within double stranded DNA, is known to give rise predominantly to 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo)[1] together with a small amount of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua).[2] Deprotonation of the guanine radical cation of dGuo leads to the overwhelming formation of 2,2-diamino-5-[2-deoxy-$\beta$-D-*erythro*-pentofuranosyl)amino]-5(2*H*)-oxazolone (dZ) subsequent to the hydrolysis of its unstable precursor 2-amino-5-[(2-deoxy-$\beta$-D-*erythro*-pentofuranosyl)amino]-*4H*-imidazol-4-one (dIz). The p$K_a$ of the guanine radical cation was determined to be 3.9;[1] thus, at neutral pH, the predominant deprotonation of the guanine radical cation could explain the absence of 8-oxodGuo upon one-electron oxidation of dGuo. It should be also mentioned that recent works have highlighted the susceptibility for 8-oxodGuo to be further oxidized[4] by either one-electron oxidants[5] or singlet oxygen.[6] Several modified nucleosides, including dIz and dZ and a diastereomeric mixture of spiroiminodihydantoin nucleoside (dSp),[7] have been identified as 8-oxodGuo degradation products.

The purpose of the present work was to gain further insight into the mechanisms of one-electron oxidation of the guanine and 8-oxo-7,8-dihydroguanine moieties of the corresponding free nucleosides dGuo and 8-oxodGuo. First, the time course of degradation of both dGuo and 8-oxodGuo upon a riboflavin-mediated type I photosensitization reaction has been determined (Figure 2).[8] Under our experimental conditions (1 mM nucleoside, 40 $\mu$M riboflavin), the degradation of both nucleosides was almost linear with the irradiation time. The decomposition of 8-oxodGuo (15% per min) was found to be about 4 times faster than that of dGuo (4% per min). Such a result is in agreement with previous findings showing that 8-oxodGuo could undergo further oxidation (vide supra).

However, an interesting observation was made when a [1:1] mixture of dGuo and 8-oxodGuo was irradiated in the presence of riboflavin (Figure 2C). Under these conditions, no detectable degradation of dGuo could be observed until complete decomposition of 8-oxodGuo has occurred. The data indicate that 8-oxodGuo, even when present in a relative ratio of 0.1% (vide infra), is able to efficiently protect dGuo against one-electron oxidation.

The efficient dGuo protection exerted by 8-oxodGuo may be partly explained by a competitive reaction between the two compounds for the type I photosensitization reaction. In fact, 8-oxodGuo is implicated more efficiently in an electron transfer reaction with the guanine radical cation and/or its corresponding deprotonated neutral radical which predominantly exists at neutral pH.[9] Such a reaction leads to the degradation of 8-oxodGuo and restores the integrity of the guanine moiety. This observation is in

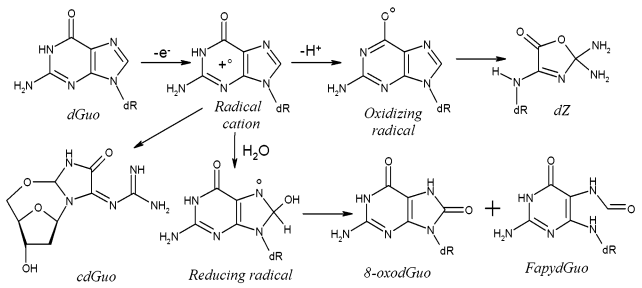


***Figure 1.*** Proposed mechanism for the reactions involved in the one-electron degradation pathway of the guanine moiety of dGuo (dR = 2-deoxyribose) in free nucleoside and DNA.

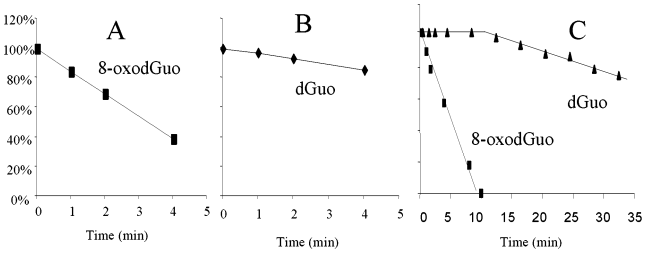


***Figure 2.*** Time course degradation of either 1 mM dGuo (A), 1 mM 8-oxodGuo (B), or a [1:1] mixture of 1 mM dGuo and 1 mM 8-oxodGuo (C) upon UVA-mediated photosensitization in the presence of riboflavin.

agreement with a recent work[9] in which the rate constant for the oxidation of 8-oxodGuo by the guanine oxidizing radical has been determined to be $4.6 \times 10^8$ $M^{-1}$ $s^{-1}$.

In light of these results, we have further analyzed the degradation products of dGuo upon one-electron oxidation of the free nucleoside.[10] Emphasis was placed on the determination of the possible transient formation of 8-oxodGuo. As previously described, no detectable formation of 8-oxodGuo was observed upon riboflavin and UVA-mediated dGuo photooxidation.[11] However, such a result could be explained either by the absence of formation of 8-oxodGuo or by an efficient oxidation of initially produced 8-oxodGuo. To distinguish between the two possible mechanisms, the photosensitization reaction of 1mM dGuo was performed in $H_2{}^{18}O$. Following irradiation, the solution was analyzed by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS), and 8-oxodGuo as well as [$^{18}$O]-labeled 8-oxodGuo were monitored.[12] Upon irradiation, the level of 8-oxodGuo remains very low, but $^{18}$O-labeled 8-oxodGuo was detected.[13] The presence of the labeled nucleoside indicates that hydration of the guanine radical cation, or its deprotonated form, has occurred. Therefore, the absence of 8-oxodGuo is due to its selective decomposition that compensates its formation through hydration of the guanine radical cation.

Because 8-oxodGuo is rapidly and selectively decomposed, we may ask the question of the origin of the different photooxidation

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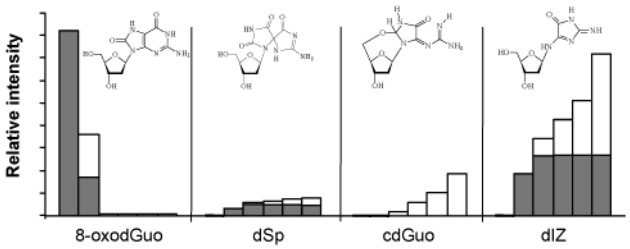


***Figure 3.*** Time course formation of the main products of riboflavin-mediated photosensitization of an aqueous solution of 1mM dGuo and 1 $\mu$M [$^{15}N_5$]-8-oxodGuo. From left to right, columns represent the relative amount of the different products for 0, 15, 30, 45, 60, and 90 s of irradiation. The dark column indicates the proportion of labeled nucleosides.

products. For instance, dZ and dIz have been shown to be formed from 8-oxodGuo,[14] and, therefore, their formation under the type I photooxidation reaction of an aqueous solution of dGuo might be explained by the transient formation of 8-oxodGuo that rapidly further decomposes. To determine the origin of the different photooxidation products, an aqueous solution of 1 mM dGuo was spiked with [$^{15}N_5$]-8-oxodGuo to a 1 $\mu$M final concentration. Such a solution was irradiated in the presence of riboflavin, and the time course of the formation of the different products was determined by HPLC-MS/MS.[15] Such an approach allows us to measure dSp,[16] dIz, and cdGuo, a cyclonucleoside that has been identified as a type I photooxidation product of dGuo.[17] In addition, for each molecule, the unlabeled as well as the possible [$^{15}$N]-labeled nucleoside were measured (Figure 3).

As already observed, 8-oxodGuo was rapidly decomposed, and the relative labeling of 8-oxodGuo decreases with the time of irradiation, due to the formation of unlabeled 8-oxodGuo. In addition, dIz was the main photooxidation product, and small amounts of dSp and cdGuo were detected. Using HPLC-MS/MS, we have shown that dIz and dSp are labeled with [$^{15}$N]-atoms, at least for short irradiation times (left columns), indicating that they arise mostly from the decomposition of 8-oxodGuo. In contrast, cdGuo is the only product which is not labeled and therefore could be unambiguously assigned as a dGuo photooxidation product that does not involve the transient formation of 8-oxodGuo.

It is important to note that the relative rate of formation of dSp with respect to dIz decreases when the level of 8-oxodGuo becomes very low. In addition, dSp was shown to be predominantly produced upon riboflavin-mediated photosensitization of 8-oxodGuo in aerated aqueous solutions (see Supporting Information). Such a result suggests that dIZ could be produced by two different mechanisms, either directly from dGuo or through the transient formation of 8-oxodGuo.[18]

The most important finding of the present study is that the presence of traces of 8-oxodGuo is sufficient to prevent the conversion of the highly oxidizing guanine radical into decomposition products. This is explained by the occurrence of an efficient one-electron transfer between the latter oxyl radical and 8-oxodGuo ($k = 4.6 \times 10^8$ $M^{-1}$ $s^{-1}$). Such a process, favored by the low reactivity of the guanine oxidizing radical with oxygen[19] ($k < 10^2$ $M^{-1}$ $s^{-1}$), has been already observed in isolated DNA[20] but was not expected at the nucleoside level.

Further work is required to better understand the mechanism of formation of the different oxidation products within dGuo and isolated DNA. In addition, it should be pointed out that, at the nucleoside level, the absence of detection 8-oxodGuo upon one-electron oxidation of dGuo should not be directly correlated to a lack of its formation.

**Supporting Information Available:** HPLC conditions used for monitoring the degradation of either dGuo or 8-oxodGuo, as well as typical chromatograms. HPLC-MS/MS chromatograms showing the detection of $^{18}$O-labeled 8-oxodGuo and unlabeled and [$^{15}$N]-labeled nucleosides and the relative formation of dIZ and dSp upon riboflavin-mediated photosensitization of either dGuo or 8-oxodGuo (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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