Photooxidation of 8-Oxo-7,8-dihydro-2′-deoxyguanosine by Thermally Generated Triplet-Excited Ketones from 3-(Hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane and Comparison with Type I and Type II Photosensitizers

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Abstract: Calf thymus DNA and 8-oxo-7,8-dihydro-2′-deoxyguanosine (**8-oxodGuo**) were *photooxidized in the dark* by triplet-excited ketones generated in the thermal decomposition of 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane (**HTMD**). The oxidation of DNA led to **8-oxodGuo** and the type I photooxidation product 2,2-diamino[2-deoxy-*β*-D-*erythro*-pentofuranosyl)-4-amino]-5(2*H*)-oxazolone (**oxazolone**). While the yield of **oxazolone** progressively increased, **8-oxodGuo** was substantially consumed in DNA on successive exposure to **HTMD**. The oxidation of authentic **8-oxodGuo** by **HTMD** and established photosensitizers such as benzophenone (mainly type I) and Rose Bengal (predominantly type II) was studied in detail in regard to the concentration and time dependence and the influence of D_2O *versus* H_2O. The singlet-oxygen-derived 4*R** and 4*S** diastereomers of 4-hydroxy-8-oxo-4,8-dihydro-2′-deoxyguanosine (**4-HO-8-oxodGuo**) and **oxazolone** were the major products. A substantial kinetic D_2O effect (ca. 10-fold) in the Rose Bengal-photosensitized degradation of **8-oxodGuo** unequivocally established that in this case singlet oxygen (type II photooxidation) is involved. However, the efficient formation of **oxazolone** by benzophenone as a characteristic type I photooxidant, as well as in the **HTMD**-mediated oxidation (predominantly type I), and the fact that these processes exhibit a negligible D_2O effect provide cogent experimental evidence for an electron or hydrogen atom transfer mechanism (type I photooxidation) in the oxidative degradation of **8-oxodGuo** into **oxazolone**. The unprecedented observation that comparable product ratios of **4-HO-8-oxodGuo** and **oxazolone** were obtained in the **8-oxodGuo** oxidations, irrespective of whether Rose Bengal as a typical type II photooxidant or benzophenone as an established type I photooxidant was employed, is presumably due to electron-transfer chemistry of $^1O_2$ with the easily oxidized **8-oxodGuo** in view of its low oxidation potential. This nicely accounts for the fact that the primary oxidation product **8-oxodGuo**, which serves as important monitor of oxidative genotoxicity, may not accumulate appreciably in the photooxidation of DNA.

Introduction

8-Oxo-7,8-dihydro-2′-deoxyguanosine, for short **8-oxodGuo**, constitutes an important product of oxidative attack on nucleic acids.[1−4] This mutagenic DNA lesion[5−9] is generally agreed to be formed by at least three mechanisms. The first involves attack of hydroxyl radicals[10−12] at the C-8 position of the guanine and subsequent tautomerization to the more stable 8-keto form. The second mechanism entails [4+2] cycloaddition of singlet oxygen[13−17] to the C4−C5 and N7−C8 double bonds of guanine (Diels−Alder reaction) and rearrangement of the initially formed *endo*-peroxide to the C-8 hydroperoxide, which is expected to be readily reduced by oxidizable components in the system.[17] The third alternative is the photosensitized oxidation[18−20] to generate the guanine radical cation through electron transfer from guanine by the excited sensitizer (type I photooxidation[21]). Only in the DNA matrix is the radical cation persistent enough[10,22] to add water at the C-8 position and form **8-oxodGuo**. In aqueous solution at pH 7.0, however, the highly acidic intermediary guanosine radical cation predominantly deprotonates to afford a radical, which after addition of molecular oxygen at the C-5 position yields the characteristic type I photooxidation products of **dGuo.**[23,24] The latter is, as in the case of hydroxyl radical attack at guanine, the 2,2-

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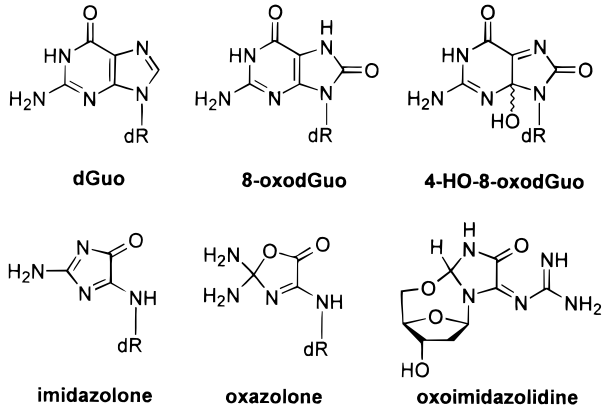
diamino[(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-amino]-5(2*H*)-oxazolone (**oxazolone**), which is the hydrolysis product of the corresponding **imidazolone** precursor. Additionally, the cyclic



nucleoside 2(*S*)-2,5′-anhydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-guanidinylidene-2-hydroxy-4-oxoimidazolidine[25] (**oxoimidazolidine**) is formed through intramolecular attack of the 5′-hydroxyl functionality on the C-8 position of the intermediary guanine radical.

As alternatives to the well-established triplet photosensitizers, 1,2-dioxetanes may be used, since they serve as efficient sources of triplet-excited carbonyl products on thermal activation.[26−28] This unique class of four-membered ring peroxides is of biological interest, since they have been implicated as labile intermediates in oxidative stress, for the induction of spontaneous mutations and in enzymatic oxidations.[1,27,29−31] The triplet-state ketones generated in the thermal decomposition of 1,2-dioxetanes give rise to photochemical and photobiological transformations *in the dark*. They exhibit strong genotoxic activity in isolated as well as in bacterial and cellular DNA,[32−34] with the predominant formation of oxidative base modifications in isolated DNA.[35,36]

Our recently published studies[37] on the oxidation of calf thymus DNA by 1,2-dioxetanes revealed that **8-oxodGuo** is efficiently formed, unequivocally through the action of thermally generated triplet-excited ketones. With a large excess of 3-(hydroxymethyl)-3,4,4-trimethyl-1,2-dioxetane (**HTMD**) the yield of **8-oxodGuo** decreased significantly. In the reaction of **dGuo** with **HTMD**,[38] besides a significant yield of **8-oxodGuo** (up to 1%), also the characteristic singlet oxygen product 4-hydroxy-8-oxo-4,8-dihydro-2′-deoxyguanosine,[39,40] for short **4-HO-8-oxodGuo**, was detected, in addition to the type I photooxidation products **oxazolone** and **oxoimidazolidine**. It was established that **HTMD** generates $^1O_2$ on thermal decomposition[46] and singlet oxygen is involved in the formation of **8-oxodGuo** from **dGuo** (substantial D_2O effect). Nevertheless, comparison with the well-established type I (benzophenone,[41] riboflavin[20]) and predominant type II (Rose Bengal,[42] methylene blue[43]) photosensitizers revealed that both type I and type II photooxidation modes operate with comparable efficiency in the **HTMD**-induced photooxidation of **dGuo**.

For several years it has been suggested that **8-oxodGuo** is readily attacked by singlet oxygen to produce the two 4*R** and 4*S** diastereomers of **4-HO-8-oxodGuo**.[40] This was recently confirmed by Sheu and Foote,[44] by employing a derivative of **8-oxodGuo** soluble in organic solvents, which was photooxidized with tetraphenylporphine (TPP) as sensitizer in the presence of O_2. Low-temperature NMR studies in acetone-d_6 revealed that, indeed, singlet oxygen adds to the C4−C5 double bond of **8-oxodGuo** through [2+2] cycloaddition to yield a set of intermediary diastereomeric dioxetanes, which are transformed *in situ* to **4-HO-8-oxodGuo** through the corresponding 4-hydroperoxides.

Recently, the photosensitized degradation of **8-oxodGuo** by methylene blue was reported,[45] in which **4-HO-8-oxodGuo** and **imidazolone** (the precursor of **oxazolone**) were efficiently generated. On the basis of a substantial D_2O effect on the conversion rate of **8-oxodGuo** and the pronounced quenching with NaN_3, singlet oxygen was proposed to be responsible for the formation of both observed oxidation products.

Additional to singlet oxygen chemistry, photoexcited methylene blue is also capable of electron transfer chemistry with nucleic acids (45% type I and 55% type II photooxidation products with acetylated 2′-deoxyguanosine).[19,42] Therefore, to avoid such type I activity, we employed the quite selective singlet-oxygen-generating photosensitizer Rose Bengal (95% type II products[19,42]) to clarify definitively the mechanism of the photooxidative fate of **8-oxodGuo**. For comparison, we investigated the photooxidation of authentic **8-oxodGuo** by the predominant type I photosensitizer benzophenone,[42] which mainly reacts through electron and/or hydrogen transfer chemistry, and by **HTMD** as a thermal source of triplet-excited ketones in the dark. Our present results confirm the efficient photooxidative reactivity of Rose Bengal, benzophenone, and HTMD toward **8-oxodGuo** and suggest that, in addition to the previously proposed type II photooxidation ($^1O_2$), type I photooxidation is also involved in the degradation of **8-oxodGuo** to afford **oxazolone**.

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Table 1. Product Studies of the Guanine Photooxidation in the Repetitive Thermal Treatment of Calf Thymus DNA with **HTMD**

batch	conditions[a]	conversion of guanine (%)	yield[b] **8-oxoGua**	yield[b] **oxazolone**	product balance[c] (%)
1	5 mM **HTMD**	8	4.3	2.6	86
2	+ 5 mM **HTMD**	22	4.8	4.7	43
3	+ 5 mM **HTMD**	34	3.8	6.2	29
4	+ 5 mM **HTMD**	58	2.4	7.4	17
5	+ 5 mM **HTMD**	79	1.9	8.5	14

[a] 0.1 mg/mL calf thymus DNA in 5 mM phosphate buffer (pH 7.0), at 37 °C, repetitively treated with 5 mM **HTMD** in aqueous acetonitrile (90:10) each for 24 h. [b] Yield relative to available guanine in DNA (625 pmol/μg DNA), mean value of three independent runs, standard deviation ±5% of the stated value. [c] Detected products relative to converted guanine.

Results and Discussion

As listed in Table 1, the thermal treatment of calf thymus DNA at 37 °C for 24 h in the presence of 5 mM **HTMD** afforded, besides substantial amounts of **8-oxoGua** (4.3% relative to guanine in DNA), the **oxazolone** in 2.6% yield. For the latter photooxidation product the well-established fluorescence-labeling HPLC assay with 1,2-naphthoquinone-4-sulfonic acid, introduced by Ravanat *et al.*,[40] was employed. To assess the persistence of these guanine oxidation products toward **HTMD**, we repetitively treated the oxidized DNA solution with new **HTMD** batches at 37 °C. The determination of the guanine conversion in DNA allowed the evaluation of the product balance, which we define as the yield of detected guanine oxidation products relative to the amount of converted guanine. The second treatment of an already oxidized DNA solution with 5 mM **HTMD** resulted in 22% conversion of the guanine in DNA, and the yields of **8-oxoGua** and **oxazolone** both increased to almost 5% (entry 2, Table 1). Further treatments of the already oxidized DNA with additional **HTMD** batches selectively consumed up to 80% of the available guanine, whereas the other DNA bases remained intact, as confirmed by HPLC/UV detection. After the fifth batch of **HTMD**, the maximum yield (4.8%, entry 2) of the already formed **8-oxoGua** was substantially reduced to 1.9% (entry 5) and the yield of **oxazolone** increased continuously to 8.5%. The product balance dropped from ca. 80% after the first to 14% after the fifth **HTMD** treatment.

The results of the repetitive treatment of DNA with a large excess of **HTMD** imply that **8-oxodGuo** is readily further oxidized by **HTMD** and that **oxazolone** is a likely product of this degradation. However, since the product balance for guanine decreased significantly in the repetitive treatment of DNA with **HTMD** (Table 1), the formation of **oxazolone** cannot account exclusively for the consumption of guanine and/or **8-oxodGuo** in DNA. This suggests that other still unknown oxidation products are generated. Recently, 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)cyanuric acid and its relatively unstable precursor have been identified as major singlet oxygen products in the oxidation of **8-oxodGuo**.[54] In fact, the almost linear increase in the yield of **oxazolone** on repetitive **HTMD** treatment suggests that, at least in DNA, the subsequent oxidation of **8-oxodGuo** contributes only a fraction of the formed **oxazolone**.

To clarify the fate of **8-oxodGuo** in the **HTMD**-induced oxidation of DNA and to gain mechanistic insight, we examined the oxidation of authentic **8-oxodGuo** with **HTMD**. For comparison, characteristic type I (benzophenone) and predominant type II (Rose Bengal) photosensitizers were employed.

The thermal decomposition of **HTMD** in the presence of **8-oxodGuo** was carried out at different concentrations and

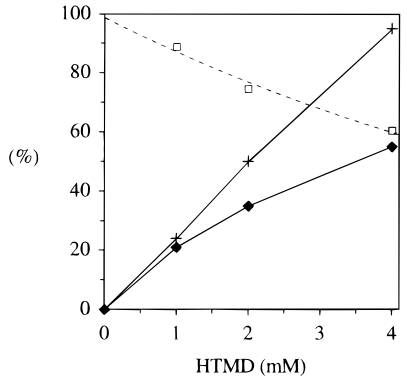


Figure 1. Concentration profile for **HTMD**-induced oxidation of **8-oxodGuo**: carried out in the dark at 50 °C for 12 h with 100 μM **8-oxodGuo** in 5 mM sodium cacodylate buffer (pH 7.0) and 10% acetonitrile as cosolvent. The values on the ordinate are mean values of at least three independent runs, error ±10% of the stated value: conversion of **8-oxodGuo** (+), absolute yield (◆) of **4-HO-8-oxodGuo**, and relative one, based on consumed **8-oxodGuo** (□).

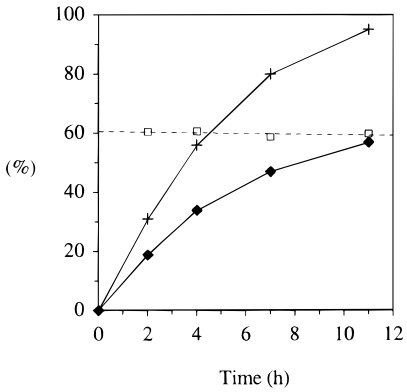


Figure 2. Time profile for the **HTMD**-induced oxidation of **8-oxodGuo**: carried out in the dark at 50 °C for 12 h with 100 μM **8-oxodGuo** in 5 mM sodium cacodylate buffer (pH 7.0) and 4 mM HTMD, dissolved in acetonitrile (10 vol %). The values on the ordinate are mean values of at least three independent runs, error ±10% of the stated value: conversion of **8-oxodGuo** (+), absolute yield (◆) of **4-HO-8-oxodGuo**, and relative one, based on consumed **8-oxodGuo** (□).

reaction times to assess the respective concentration and time profiles for this dioxetane-induced oxidation of **8-oxodGuo** (Figures 1 and 2). Control experiments without **HTMD** revealed that authentic **8-oxodGuo** and **4-HO-8-oxodGuo** persisted (less than 2% consumption, as determined by HPLC analysis) under the reaction conditions (50 °C, 15 h, pH 7.0). In agreement with the proposed [44] singlet oxygen mechanism for the oxidation of **8-oxodGuo**, the characteristic type II photooxygenation products of **dGuo**,[39,40] namely, the 4*R** and 4*S** diastereomers of **4-HO-8-oxodGuo**, were detected in high yields. At an excess of 40 equiv (4 mM) of **HTMD**, the **8-oxodGuo** was nearly quantitatively (91%) consumed after 9 h at 50 °C (Figure 1) to afford **4-HO-8-oxodGuo** in 50% yield. Remarkable is the observation that the relative yield of **4-HO-8-oxodGuo** (based on converted **8-oxodGuo**) was significantly increased to ca. 90% when a lower **HTMD** excess (10 equiv.) was employed (Figure 1). Thus, the substantial decrease of the relative **4-HO-8-oxodGuo** yield at high **HTMD** excess suggests the formation of additional oxidation products, such as 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-cyanuric acid[54] or yet unknown products.

In contrast to the concentration profile (Figure 1), the time profile (Figure 2) reveals that the relative yield of **4-HO-8-oxodGuo** is independent of time for 2−12 h, even when lower **HTMD** concentrations (1 or 2 mM) are applied (data not shown

in Figure 2). Additionally, the concentration profile exhibits a linear increase of **8-oxodGuo** consumption with increasing **HTMD** concentration (Figure 1), whereas in the time profile (Figure 2) a nonlinear dependence is observed. Both profiles establish that the conversion of **8-oxodGuo** is proportional to the yield of the triplet-excited ketones, which are released from **HTMD** by its thermal decomposition. In this context it should be kept in mind that contrary to the photosensitized oxidations, which operate catalytically by energy transfer to generate time-invariant concentrations of triplet-excited states, the dioxetane-derived triplet states are stoichiometrically produced; i.e., the dioxetane decomposition is irreversible and the thermally produced triplet states are not recycled. Therefore, the momentary concentration of triplet-excited ketones depends on the initial concentration of the dioxetane and its decomposition rate. The concentration-dependent (Figure 1) and time-independent (Figure 2) relative yields of **4-HO-8-oxodGuo** suggest that the formation of this photooxidation product is mainly governed by the steady-state concentration of triplet-excited states generated from the **HTMD** *versus* the concentration of available substrate (**8-oxodGuo** or O_2).

The estimate[47] that ca. 90% of the **HTMD**-derived triplet states react with **8-oxodGuo**, whereas only ca. 40% react with **dGuo**,[38] emphasizes that **8-oxodGuo** is more efficiently oxidized by the thermally generated triplet ketones than **dGuo**. This is expected since the oxidation potential of **8-oxodGuo** (0.85 V *vs* AgCl) is significantly lower than for **dGuo** (1.28 V *vs* AgCl).[17] Therefore, it is not surprising that the yield of **8-oxodGuo** in the **HTMD**-induced oxidation of DNA decreases significantly at a high excess of dioxetane. Consequently, **8-oxodGuo** does not accumulate appreciably in the reaction with DNA and **dGuo** because its further oxidation is preferred over that of unmodified guanine.

For mechanistic elucidation, the photosensitized oxidation of **8-oxodGuo** by well-established type I and type II photosensitizers was investigated. As a characteristic type I sensitizer benzophenone, which mainly acts by hydrogen abstraction,[41] was chosen, and Rose Bengal[42] was employed as an efficient type II ($^1O_2$) sensitizer. With both sensitizers, quantitative conversion of **8-oxodGuo** was achieved.[48] The relative yield of **4-HO-8-oxodGuo** for both sensitizers did not exceed 35 ± 5% (referred to consumed **8-oxodGuo**), within the experimental error independent of the sensitizer concentration (5−500 μM benzophenone, 0.2−2 μM Rose Bengal) and the irradiation time (10−70 min).

The moderate **4-HO-8-oxodGuo** yield (ca. 35% relative to converted **8-oxodGuo**), especially for both photosensitizers benzophenone (type I) and Rose Bengal (type II), implies that, besides **4-HO-8-oxodGuo**, other products, such as 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)cyanuric acid,[54] are formed in the photosensitized or the **HTMD**-induced oxidation of **8-oxodGuo**. As a significant oxidation product in the methylene blue-sensitized photooxidation of **8-oxodGuo**,[45] **oxazolone** was detected, which has been rigorously characterized spectrally (^{1}H NMR, UV, FAB-MS). To assess the formation of **oxazolone** in our **HTMD**-induced or photosensitized (benzophenone, Rose Bengal) oxidation of **8-oxodGuo**, we employed the established[40] fluorescence-labeling HPLC assay with 1,2-naphthoquinone-4-sulfonic acid (NQS), which quantitatively monitors the formation of guanidine (the latter is released after alkaline treatment from **oxazolone**). This assay, unfortunately, cannot distinguish the origin of guanidine,[49] i.e., whether it stems from **oxazolone** or free guanidine, the latter formed as a side product in the oxidation of **8-oxodGuo**.[44] Nonetheless, in analogy with the previously reported[2,40,45] photooxidations of nucleic acid derivatives, we assume that the guanidine in our oxidations of **8-oxodGuo** also originates mainly from the **oxazolone**. In fact, control experiments revealed that no detectable amounts of guanidine were observed without alkaline treatment of the reaction mixture nor after thermal treatment of authentic **8-oxodGuo** or **4-HO-8-oxodGuo** for 15 h at 50 °C. The yield of the guanidine-releasing **oxazolone** in the oxidation of **8-oxodGuo** on **HTMD** treatment and by the photosensitizers benzophenone and Rose Bengal are listed in Table 2. Thus, with a large excess of **HTMD** (entry 1, Table 2), as well as in the photosensitized oxidation of **8-oxodGuo** by benzophenone or rose bengal (entries 2 and 3, Table 2), high yields (41 and 47% referred to consumed **8-oxodGuo**) of **oxazolone** were observed, which even exceed the yield (25−29%, Table 2) of **4-HO-8-oxodGuo**. The product balance could be greatly improved (from ca. 26% to ca. 70%) through the quantitative determination of **oxazolone** by the HPLC/fluorescence assay (Table 2). Interesting and unexpected is the result that the ratio of oxidation products **4-HO-8-oxodGuo** and **oxazolone** is independent of the employed sensitizer within the experimental error.

Table 2. Product Studies of the Oxidation of **8-oxodGuo** by Thermal Treatment with **HTMD** or Photosensitized by Benzophenone or Rose Bengal[a]

oxidant	conversion of **8-oxodGuo** (%)	yield[e] (%) **4-HO-8-oxodGuo**	yield[e] (%) **oxazolone**[f]	product balance[g] (%)
HTMD/ΔT[b]	51	15 (29)	21 (41)	70
benzophenone/$h\nu$[c]	85	22 (26)	40 (47)	73
Rose Bengal (2 μM)/$h\nu$[d]	50	13 (25)	24 (47)	72

[a] 100 μM **8-oxodGuo**, 5 mM sodium cacodylate buffer, pH 7.0. [b] 7 mM HTMD, aqueous acetonitrile (90:10), 50 °C, 180 min. [c] 50 μM benzophenone, aqueous acetonitrile (98:2), RPR (350 nm), irradiated at a distance of ca. 5 cm, 20 min, 4 °C. [d] 2 μM Rose Bengal, 150-W sodium lamp, irradiated at a distance of ca. 20 cm, 30 min, 4 °C. [e] Yields relative to conversion of **8-oxodGuo** given in parantheses. [f] Detected by the fluorescence-labeling assay with 1,2-naphthoquinone-4-sulfonic acid (NQS) after alkaline treatment (1 N NaOH) at 65 °C for 9 min. [g] Detected products relative to converted **8-oxodGuo**.

To probe for the participation of singlet oxygen in the **HTMD**-induced and photosensitized oxidation of **8-oxodGuo**, the effect of deuterium oxide on the conversion of **8-oxodGuo** and the formation of **4-HO-8-oxodGuo** was investigated (Table 3). The thermal treatment of **8-oxodGuo** with **HTMD** (entries 1−3, Table 3) exhibits a negligible D_2O effect in regard to the conversion of **8-oxodGuo** (ca. 20% decrease in D_2O) and the relative yield of **4-HO-8-oxodGuo** (ca. 10% decrease in D_2O). Similarly, only a slight but opposite D_2O effect was observed for the benzophenone-sensitized oxidation of **8-oxodGuo** (entry 4, Table 3). In contrast, a pronounced kinetic D_2O effect was found in the Rose-Bengal-sensitized oxidation since **8-oxodGuo** was degraded about 10-fold faster in D_2O than in H_2O (entry 5 with 6, Table 3). The relative yield of **4-HO-8-oxodGuo**, however, remained constant at 31% in both solvents.

(47) A triplet yield of ca. 5% for **HTMD** in aqueous solution[33] and a half-life of $t_{1/2}$ = 6 h at 50 °C (measured by chemiluminescence) were employed for this estimate.

(48) Reaction conditions for the quantitative conversion of **8-oxodGuo**: 100 μM **8-oxodGuo** in 5 mM phosphate buffer, pH 7.0, 4 °C, 500 μM benzophenone, 70-min irradiation with a 125-W blacklight lamp at a 10-cm irradiation distance, and 2 μM Rose Bengal after 60-min irradiation with a 150-W sodium lamp at a distance of 20 cm.

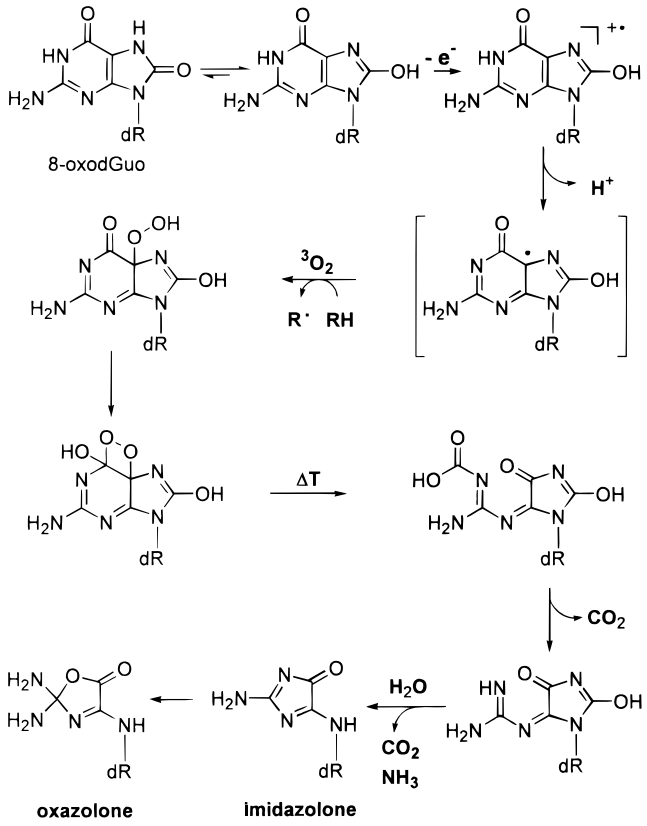
(49) At present, no direct assay is available to monitor the formation of **oxazolone** specifically and quantitatively accurate enough. The employed indirect fluorescence-labeling HPLC assay requires alkaline treatment not only for the release of guanidine from **oxazolone** but also for its condensation with NQS. This prevents a clear distinction to be made between free and released guanidine.

Table 3. Effect of D_2O on the Oxidation of **8-oxodGuo** in the Thermal Treatment with **HTMD** and in the Photosensitization by Benzophenone and Rose Bengal[a]

		H_2O		D_2O	
oxidant	time (min)	conversion[e] of **8-oxodGuo** (%)	yield[e,f] of **4-HO-8-oxodGuo** (%)	conversion[e] of **8-oxodGuo** (%)	yield[e,f] of **4-HO-8-oxodGuo** (%)
HTMD/ΔT[b]	90	22	7.7 (35)	21	6.4 (31)
HTMD/ΔT[b]	360	61	20 (33)	48	15 (31)
HTMD/ΔT[b]	780	73	27 (37)	55	17 (31)
benzophenone/$h\nu$[c]	30	58	24 (41)	42	16 (38)
Rose Bengal/$h\nu$[d]	60	81	25 (31)		
Rose Bengal/$h\nu$[d]	6			86	27 (31)

[a] 100 μM **8-oxodGuo**, 10 mM phosphate buffer (pH, pD 7.0). [b] 5 mM HTMD, aqueous acetonitrile (90:10), 50 °C. [c] 400 μM benzophenone, aqueous acetonitrile (98:2), 125-W blacklight, irradiated at a distance of 10 cm, 4°C. [d] 2 μM Rose Bengal, 150-W sodium lamp, irradiated at a distance of 20 cm, 4 °C. [e] Mean value of triplicate determinations, standard deviation ±5% of the stated value. [f] Yield of **4-HO-8-oxodGuo** relative to conversion of **8-oxodGuo** given in parantheses.

Scheme 1. Proposed Type I Photooxidation (Electron Transfer) Mechanism for the Formation of **Oxazolone** from **8-oxodGuo**



The fair (ca. 70%) balance and sensitizer-independent ratio of oxidation products and the D_2O effect allow us to draw some pertinent mechanistic conclusions on these **HTMD**-induced and photosensitized **8-oxodGuo** oxidations. The present observations that **8-oxodGuo** is efficiently degraded by benzophenone as photosensitizer, which is a characteristic type I photooxidant, and the negligible D_2O effect in the **HTMD**-induced and benzophenone-photosensitized oxidations (Table 3) are in contrast with a solely singlet-oxygen-mediated mechanism[45] for the oxidative degradation of **8-oxodGuo**. On the basis of our results, we conclude that at least in the benzophenone-photosensitized and the thermal **HTMD**-induced oxidations of **8-oxodGuo**, the type I process (electron transfer, hydrogen abstraction) plays a decisive role, additional to the established singlet oxygen photooxidation (type II) of **8-oxodGuo** to **4-HO-8-oxodGuo**. We offer the mechanism in Scheme 1 to account for the present type I photooxidation of **8-oxodGuo** to yield **oxazolone**.

Initial electron transfer from **8-oxodGuo** in its enolic form to the excited sensitizer leads after deprotonation to the reactive C-5 radical species, which in the presence of molecular oxygen yields the intermediary C-5 hydroperoxide. This mechanism is analogous to the one reported for the formation of **oxazolone** in the type I photooxidation of **dGuo**.[24] That electron transfer from **8-oxodGuo** to the excited triplet state occurs more easily than from **dGuo** is corroborated by the favorable oxidation potential for the 2′,3′,5′-tris[(*tert*-butyldimethylsilyl)oxy] derivative of **8-oxodGuo** (0.85 V against AgCl) *versus* that of **dGuo** (1.28 V).[17] Consequently, **8-oxodGuo** is readily photooxidized not only by type II sensitizers (singlet oxygen) to yield **4-HO-8-oxodGuo**, but by type I sensitizers (electron transfer, hydrogen abstraction) as well to afford **oxazolone** as a secondary oxidation product of **dGuo**.

At first glance, the sensitizer-independent product distribution in the photooxidation of **8-oxodGuo** (Table 2) is puzzling in view of the established predominant reaction modes of the employed sensitizers. In particular, the characteristic type II ($^1O_2$) photooxidant Rose Bengal also affords a high yield of the type I product **oxazolone**. This may be explained by the fact that singlet oxygen, in addition to the established [2+2] or [4+2] cycloaddition, is also prone to engage in electron transfer with electron-rich substrates, especially in polar aqueous media.[50] Because of the low oxidation potential of **8-oxodGuo**, we suggest that singlet oxygen accepts an electron from the substrate with formation of the superoxide radical anion ($O_2^{\bullet-}$) and the intermediary radical cation of **8-oxodGuo**. Although this electron transfer is by ca. 10 kcal/mol endergonic,[51] if the process is irreversible and initiates a radical chain oxidation of **8-oxodGuo** to **oxazolone**, it is a viable transformation. Indeed, it was reported that an electron transfer process takes place to the extent of 15% between singlet oxygen and sodium azide, although the latter has an even higher oxidation potential (1.35 V against NHE)[52] than **8-oxodGuo** (0.85 V against AgCl). Consequently, the singlet oxygen derived from Rose Bengal photosensitization reacts with **8-oxodGuo** through electron transfer to afford substantial amounts of **oxazolone** and thereby mimics type I photoactivity. This unprecedented reaction mode of $^1O_2$ accounts for the ca. 10-fold faster oxidation but similar relative yields of **4-HO-8-oxodGuo** in the Rose Bengal-

(50) Saito, I.; Matsuura, T.; Inoue, K. *J. Am. Chem. Soc.* **1983**, *105*, 3200−3206.

(51) $\Delta G = 23.06[E(D/D^{+*}) - E(A^{-*}/A)] - e^2/\epsilon\alpha - \Delta E_{0,0}$; ($E$(**8-oxodGuo**/**8-oxodGuo**$^{+*}$) = 0.85 V *vs* AgCl,[17] $E(O_2^{-*}/O_2)$ = 0.57 V *vs* SCE,[53] $e^2/\epsilon\alpha$ = 0.53 kcal/mol, $\Delta E_{0,0}$ = 22.5 kcal/mol, ΔG = 9.2 kcal/mol). Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259−271.

(52) Alfassi, Z. B.; Harriman, A.; Huie, R. E.; Mosseri, S.; Neta, P. *J. Phys. Chem.* **1987**, *91*, 2120−2122.

(53) Sawyer, D. T.; Seo, E. T. *Inorg. Chem.* **1977**, *16*, 499−501.

(54) Raoul, S.; Cadet, J. *J. Am. Chem. Soc.* **1996**, *118*, 1892−1898.

sensitized oxidations of **8-oxodGuo** when D_2O instead of H_2O is used as the reaction medium.

Conclusion

Our results clearly establish that triplet-excited ketones, generated thermally from the dioxetane **HTMD** in the dark, provide a powerful mechanistic tool to study photobiological problems, as demonstrated here for the photosensitized oxidation of **8-oxodGuo**. On thermal activation, **HTMD** efficiently oxidizes **8-oxodGuo** and affords up to ca. 90% relative yield of the singlet oxygen product **4-HO-8-oxodGuo**. The established photosensitizers benzophenone and Rose Bengal, which were examined for comparison, both yield ca. 35% **4-HO-8-oxodGuo**. Additionally, guanidine, which presumably is released from **oxazolone** through alkaline hydrolysis, is detected in the **HTMD**-induced and directly photosensitized oxidations. We propose that, in addition to the already established singlet-oxygen-mediated degradation of **8-oxodGuo**,[40] a type I photooxidation process (electron transfer or hydrogen abstraction from the substrate to the triplet excited state) operates, which leads to **oxazolone**. For the unexpected high yields of **oxazolone** (type I photooxidation product) in the Rose-Bengal-photosensitized oxidation of **8-oxodGuo**, singlet oxygen is made responsible, through electron transfer from the readily oxidizable **8-oxodGuo**.

Since **8-oxodGuo** is extensively used as a characteristic marker for oxidative DNA damage, caution must be exercised when the oxidative reactivity of mutagens is assessed by solely monitoring the formation of **8-oxodGuo**. Furthermore, the proposed type I mediated oxidation of **8-oxodGuo** suggests that a clear distinction between the type I and type II photooxidation modes requires a detailed product study to ascertain the predominant mode of photooxidation for a given photosensitizer.

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Supporting Information Available: Text describing the experimental procedures (5 pages). See any current masthead page for ordering and Internet access instructions.

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