Evidence for the Photosensitized Formation of Singlet Oxygen by UVB Irradiation of 2′-Deoxyguanosine 5′-Monophosphate[1]

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Though there has been ongoing intense interest in the reactions of DNA and its bases with singlet oxygen,[2] only recently has the triplet photosensitized formation of singlet oxygen by DNA bases been observed,[3] despite the fact that emission from the triplet states of guanine and thymine in calf thymus DNA at low temperature (−193 °C) was reported some time ago.[4] In fact, three DNA bases were found to generate $^1O_2$ ($^1\Delta_g$) luminescence at 1270 nm;[3] *the exception was guanine (Gua).* The authors were unable to distinguish between two potential explanations for the absence of sensitized $^1O_2$ luminescence from dGuo, including its mono- and dinucleotides: (1) the inability of this base to generate $^1O_2$ and (2) a subsequent reaction between the base and $^1O_2$ so rapid as to prevent detectable emission. *We now report chemical evidence which supports the proposal that 2′-deoxyguanosine 5′-monophosphate (dGMP) photosensitizes the formation of $^1O_2$.* We also report that a portion of the $^1O_2$ so formed is created through the intermediacy of a dGMP/$^1O_2$ photoproduct which is itself an efficient $^1O_2$ sensitizer.

Our methodology initially utilized histidine (His) destruction as our $^1O_2$ probe, a previously employed technique[5] which exploits this amino acid's property as a highly reactive singlet oxygen quencher. Preliminary experiments utilized 2′-deoxyadenosine (dAdo) analogs since these compounds are themselves unreactive with singlet oxygen.[2,6] Irradiation of adenine (Ade), dAdo, and dAMP in water in the presence of His caused significant loss of the amino acid (14−46%; see entries 2 and 13−15 in Table 1). When an equimolar quantity of a competitive quencher, *i.e.*, sodium azide, was present, there was a 5-fold reduction in His loss (entries 16 and 17), in accord with the relative reactivities of these quenchers with singlet oxygen.[7] From entries 13−15 we can conclude that the relative efficiency for singlet oxygen sensitization is Ade > dAMP ≥ dAdo. Since we separately demonstrated that the loss of His was independent of His concentration down to 1 mM, we used 2 mM His to measure the loss caused by 308-nm excitation from a XeCl-charged excimer laser, and thus to calculate quantum efficiencies for singlet oxygen formation *in water*. Our values of 4.3×10^{-3} and 1.8×10^{-3} for Ade and dA, respectively, may be compared to a value of 30×10^{-3} reported for Ade *in acetonitrile*.[3]

With these data in hand we focused our attention on guanine and its analogs, for which, as noted above, no literature data are available. Initial studies with dGuo indicated that nucleoside photoproducts overlapped with the His peak (entry 11), though His loss was measurable for Gua (entry 12). We thus focused on the nucleotide dGMP, which was irradiated as 5 mM solutions in oxygen-saturated buffer in the presence of 2 mM His. Photolysis with corex-filtered light from a medium-pressure Hg lamp (λ > 270 nm) resulted in 3 and 14% losses[8] of His and dGMP, respectively (*cf.* entry 1 in Table 1).[10] The following observations confirm that the photoinitiated loss of the dGMP involves formation of $^1O_2$: (1) no loss of dGMP was observed when oxygen was excluded from the medium; (2) destruction of dGMP was completely quenched in the presence of sodium azide[12] (entries 3 and 4); (3) the loss of dGMP was accelerated *ca.* 7-fold in D_2O (a solvent known to slow down the nonradiative deactivation of $^1O_2$, thus prolonging its lifetime and enhancing its potential reactivity)[13] (entries 3 and 5); (4) the enhanced degradation in D_2O was also inhibited by sodium azide (entries 3−6); and (5) mannitol, a known

Table 1. Aerobic Photolyses of Gua, dGuo, dGMP, Ade, dAdo, and dAMP in Aqueous Solution[a]

entry[b]	reactants	solvent	% loss of histidine	% loss of nucleotide
1	dGMP/His	PB/H_2O	2.8 (±23.2%)	13.8 (±1.4%)
2	dAMP/His	PB/H_2O	21.6 (±3.9%)	<1.0
3	dGMP	PB/H_2O		4.5 (±5.9%)[c]
4	dGMP/NaN_3[d]	PB/H_2O		0.0[c]
5	dGMP	PB/D_2O		32.0 (±5.6%)[c]
6	dGMP/NaN_3[d]	PB/D_2O		0.0[c]
7	dGMP	PB/H_2O		2.7 (±5.6%)
8	dGMP/Mannitol	PB/H_2O		2.8 (±0.0%)
9	dGMP	PB/D_2O		31.0 (±3.5%)
10	dGMP/Mannitol	PB/D_2O		29.4 (±8.2%)
11	dGuo/His	PB/H_2O	*e*	f
12	Gua[g]/His	PB/H_2O	27.2 (± 3.9%)	*f*
13	Ade/His	H_2O	45.7[h]	*f*
14	dAdo/His	H_2O	14.4[h]	*f*
15	dAMP/His	H_2O	18.7[h]	*f*
16	Ade/His	PB/H_2O	36.4 (±13.6%)	*f*
17	Ade/His/NaN_3[d]	PB/H_2O	7.7 (±7.8%)	*f*

[a] Photolyses in duplicate unless otherwise indicated. Nucleosides or nucleotides made up in distilled water or 50 mM sodium phosphate buffer (PB) pH/pD 7 (5 mM) in the absence and/or presence of a singlet oxygen or hydroxyl radical quencher. Solutions were placed in matched quartz photolysis tubes which were bubbled with oxygen for 10 min and then stoppled and parafilm-sealed. Irradiations used a corex-filtered medium-pressure Hg lamp (λ > 270 nm) at *ca.* 15 °C for 4 h in a turntable. Analyses by RP-HPLC on analytical Alltech C18 or Hamilton semiprep PRP-1 columns monitored at 225 and/or 254 nm. [b] Entries are grouped by experiment. [c] Average results of three different experiments. [d] Addition of 20 mM sodium azide did not alter the pH of the solution. [e] Overlap of the His peak with the dGuo products prevented measurement of His loss. [f] Purine loss not determined. [g] A saturated solution of Gua was used in this experiment. [h] Result of a single experiment; unsensitized irradiation of His under these conditions gave *ca.* 3.0% His loss.

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(8) Treatment of dGMP with the His/singlet oxygen products formed by rose bengal photosensitization (26−44% conversions) in the dark for up to 24 h gave no evidence for oxidation of the nucleotide. We thank one of the referees for suggesting this experiment to rule out dGMP loss by these potential oxidants.[9]

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(10) One would normally expect the relative values of these losses to be reversed, since there is a 10-fold greater rate of reactivity of histidine *vs* dGuo with singlet oxygen in neutral media.[7,11] We presume that the ratio observed here reflects the fact that singlet oxygen is being formed in the proximity of dGMP as well as the possible contribution of photoionization processes in the nucleotide's degradation (see below).

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hydroxyl radical scavenger,[14] had no effect on the dGMP-sensitized chemistry (entries 7−10).[15−17]

In the course of these studies we noticed an acceleration of dGMP decomposition as the irradiation progressed. We considered that this induction period for dGMP decomposition might be caused by an accumulation of hydrogen peroxide (H_2O_2) from the dismutation of superoxide anion ($O_2^{\bullet-}$), with the latter having been generated by the photooxidation of dGMP.[18] There is a recent report[20] that H_2O_2 and $O_2^{\bullet-}$ react to form $^1O_2$ through a Haber−Weiss reaction. Such an involvement of superoxide was tested by irradiating dGMP in the presence of 100 μg/mL superoxide dismutase, a concentration of the enzyme which could be expected to completely quench any superoxide anion.[21] A small (10−15%) but observable inhibition[22] of the self-sensitized photodecomposition of the dGMP was observed, suggestive that a minor portion of the singlet oxygen may indeed be generated through initial superoxide formation.[24]

The primary source of the induction period is therefore most reasonably attributable to the formation of dGMP photoproducts. It has been presumed that the purines are relatively stable to UV irradiation,[2] though UV spectral changes have been observed during the irradiation of several guanine derivatives at −110 °C.[28] In fact we observe an increase in absorption by the dGMP photolysate at 308 nm as our photolyses proceed (*cf*. Figure 1). This absorption remained unchanged upon allowing the solution to sit in the dark for 5 h at room temperature.[29] Evidence that dGMP photoproducts responsible for this absorption sensitize the destruction of the nucleotide was provided by experiments

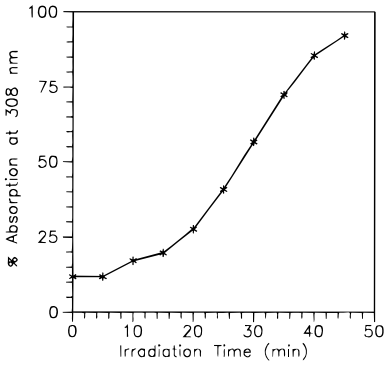

Figure 1. Absorption increase (monitored at 308 nm) during the irradiation at room temperature of an oxygen-saturated solution (3 mL) of dGMP (5 mM) in 50 mM phosphate buffer, pD 7, with 308-nm radiation from a Lumonics EX-700 Pulsemaster XeCl-charged excimer laser. The fluence rate and pulse energy were 0.23 J cm^{-2} s^{-1} and 12.7 mJ, respectively.

initially designed to measure the quantum efficiency for destruction of the base and thus an estimate of the quantum efficiency of singlet oxygen formation (Φ_Δ). Surprisingly, photolysis of dGMP with 266-nm light from a Nd:YAG laser gave no detectable destruction of the nucleotide after 30 min of irradiation. With our HPLC analytical conditions we can set an upper limit for $\Phi_\Delta \leq 0.003$, consistent with the upper limit (0.005) for Φ_Δ for Gua and dGMP estimated by Bishop et al.[3] However, irradiation with 308-nm light led to a loss of dGMP of *ca*. 20%. This level of dGMP destruction is much greater than one would expect from the *ca*. 2.5-fold higher average absorbed power of the 308-nm light vis-à-vis the 266-nm irradiation. *We conclude that the irradiation of dGMP forms photoproducts which absorb in the UVB region and are potent sensitizers of dGMP destruction via the formation of singlet oxygen.* We believe that photolysis with monochromatic 266-nm light also produced such products but that competitive absorption of 266 nm light by excess dGMP prevented their subsequent excitation. Support for this conjecture comes from a prolonged photolysis of dGMP with 254-nm light, during the course of which we noted the buildup of UVB-absorbing products (data not shown).

There are strong indications that the photoproducts not only are singlet oxygen sensitizers but are themselves formed from dGMP through the intervention of singlet oxygen. Thus, formation of the dGMP photoproducts (1) does not occur when the irradiation is conducted under argon, (2) is enhanced by photolysis in D_2O relative to that observed in H_2O, and (3) is completely quenched by NaN_3. It is possible that the photoproducts are formed in aqueous solution through the intermediacy of species analogous to those isolated and identified from the reactions of singlet oxygen with dGuo.[26] 8-Oxoguanosine, produced by reaction of dGuo with $^1O_2$, is an even better substrate for singlet oxygen,[26,30] but its products are relatively transparent in the UV.[26,27]

Further studies to isolate and characterize the dGMP photoproducts are in progress. It remains to be seen as to whether our finding of autophotooxidative damage of dGMP represents an additional source of UVB-induced lesions in the genome. It is noteworthy that dGuo is the most strongly absorbing of UVB light of the DNA bases.

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