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Published on Web 02/16/2008

Oxidation of 2′-Deoxyguanosine 5′-Monophosphate Photoinduced by Pterin: Type I versus Type II Mechanism

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Abstract: UV-A radiation (320−400 nm) induces damage to the DNA molecule and its components through different photosensitized reactions. Among these processes, photosensitized oxidations may occur through electron transfer or hydrogen abstraction (type I) and/or the production of singlet molecular oxygen ($^1O_2$) (type II). Pterins, heterocyclic compounds widespread in biological systems, participate in relevant biological processes and are able to act as photosensitizers. We have investigated the photosensitized oxidation of 2′-deoxyguanosine 5′-monophosphate (dGMP) by pterin (PT) in aqueous solution under UV-A irrradiation. Kinetic analysis was employed to evaluate the participation of both types of mechanism under different pH conditions. The rate constant of $^1O_2$ total quenching (k_t) by dGMP was determined by steady-state analysis of the $^1O_2$ NIR luminescence, whereas the rate constant of the chemical reaction between $^1O_2$ and dGMP (k_r) was evaluated from kinetic analysis of concentration profiles obtained by HPLC. The results show that the oxidation of dGMP photosensitized by PT occurs through two competing mechanisms that contribute in different proportions depending on the pH. The dominant mechanism in alkaline media involves the reaction of dGMP with $^1O_2$ produced by energy transfer from the PT triplet state to molecular oxygen (type II). In contrast, under acidic pH conditions, where PT and the guanine moiety of dGMP are not ionized, the main pathway for dGMP oxidation involves an initial electron transfer between dGMP and the PT triplet state (type I mechanism). The biological implications of the results obtained are also discussed.

1. Introduction

Solar radiation induces modifications to genomic DNA and is implicated in the induction of human skin cancers.[1,2] UV radiation is the most mutagenic and carcinogenic component of the solar radiation. UV−B radiation (280−320 nm) damages DNA through the direct excitation of the nucleobases.[3] On the other hand, although nucleobases absorb very weakly above 320 nm, UV-A radiation (320−400 nm) may damage DNA through photosensitized reactions.[3,4] This indirect action is mediated by a photosensitizer (endogenous or exogenous) which is excited by the UV-A radiation.

The chemical changes in DNA and its components resulting from photosensitized reactions can take place through different mechanisms. It has been demonstrated that energy transfer from the triplet state of the photosensitizer to pyrimidine bases leads to the formation of pyrimidine dimers.[4−6] Photosensitized oxidations also contribute to DNA damage induced by UV-A radiation. These processes involve the generation of radicals (type I), *e.g.*, *via* electron transfer or hydrogen abstraction, and/or the production of singlet molecular oxygen ($O_2(^1\Delta_g)$, denoted throughout as $^1O_2$) (type II).[7]

Singlet oxygen, the lowest electronic excited state of molecular oxygen, is an important oxidizing intermediate in chemical processes and one of the main chemical species responsible for the damaging effects of light on biological systems (photodynamic effects).[8] The main source of $^1O_2$ production *in vivo* is photosensitization.[9] In this process, $^1O_2$ is most often produced by energy transfer from the triplet excited state of a sensitizer

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10.1021/ja075367j CCC: $40.75

(^{3}Sens*) to dissolved molecular oxygen ($^3O_2$) (reactions 1 and 2).

$$^1\text{Sens} \xrightarrow{h\nu} {}^1\text{Sens}^* \xrightarrow{k_{ISC}} {}^3\text{Sens}^* \quad (1)$$

$$^3\text{Sens}^* + {}^3O_2 \xrightarrow{k_{et}} {}^1\text{Sens} + {}^1O_2 \quad (2)$$

Singlet oxygen ($^1O_2$) relaxes to its ground state ($^3O_2$) through solvent induced radiationless and radiative pathways (reactions 3 and 4). It may also be deactivated by a physical quencher (reaction 5) and/or oxidize an acceptor molecule (reaction 6).

$$^1O_2 \xrightarrow{k_d} {}^3O_2 \quad (3)$$

$$^1O_2 \xrightarrow{k_e} {}^3O_2 + h\nu' \quad (4)$$

$$Q + {}^1O_2 \xrightarrow{k_q} Q + {}^3O_2 \quad (5)$$

$$Q + {}^1O_2 \xrightarrow{k_r} QO_2 \quad (6)$$

The nucleobases are the preferential DNA substrates of type I oxidation.[3,10] Among the DNA bases, guanine that exhibits the lowest ionization potential is the preferential target for one-electron oxidation reactions, over adenine and pyrimidine bases.[11] Moreover, guanine is the only DNA constituent that significantly reacts with $^1O_2$.[11,12] However, although determination of the rate constants of $^1O_2$ physical and chemical quenching is an important tool to analyze the reactivity of a given compound toward $^1O_2$, to the best of our knowledge, there are no systematic studies on the kinetics of the reaction between $^1O_2$ and nucleotides.

The chemical nature of the products of the photosensitized oxidation of guanine, as well as their distribution, differs depending on the mechanism and on the molecule that bears the guanine moiety (nucleoside, nucleotide, single-stranded and double-stranded DNA, etc.).[11] However, some products, such as 8-oxo-7,8-dihydroguanine (8-oxoGua) is formed in both type I and type II mechanisms, although in different proportions. Moreover, 8-oxoGua is also generated in oxidation mediated by the hydroxyl radical (•OH).[11] Therefore, it is difficult to evaluate the mechanism involved in a given process by means of product analysis. In addition, many photosensitizers are able to act through both type I and type II mechanisms.

Pterins, heterocyclic compounds widespread in biological systems, are derived from 2-aminopteridin-4(1*H*)-one or pterin (PT) (Figure 1). Several pterin derivatives participate in relevant biological processes such as the synthesis of aminoacids[13] and nucleobases,[14] the nitric oxide metabolism,[15] and the activation of cell-mediated immune responses.[16] Pterins behave as weak acids in aqueous solution. In general, the dominant equilibrium

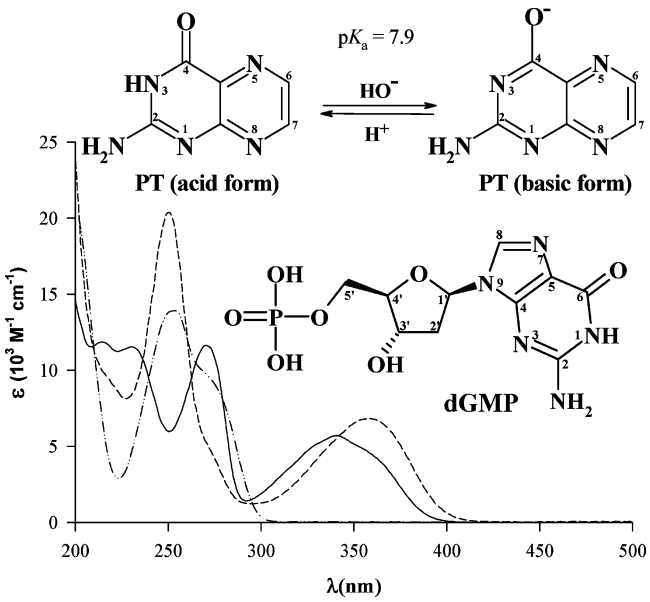

Figure 1. Molecular structure of PT and dGMP and the corresponding absorption spectra in air-equilibrated aqueous solutions. Solid line: acid form of PT (pH = 5.5). Dashed line: basic form of PT (pH = 10.5). Dashed−dotted line: dGMP (pH = 5.5).

at pH > 5 involves the lactam group (pyrimidine ring)[17](Figure 1). The pK_a of this equilibrium is 7.9 for PT.[18] Other functional groups of the pterin moiety (e.g., the 2-amino group or ring N-atoms) have pK_a values < 2.[17]

The participation of pterins in different photobiological processes has been suggested or demonstrated in the past decades, and interest in the photochemistry and photophysics of this group of compounds has subsequently increased. In addition, some pterin derivatives (e.g., biopterin, 6-formylpterin, 6-carboxypterin) accumulate in the skin of patients affected by vitiligo, a depigmentation disorder, where the protection against UV radiation fails due to the lack of melanin.[19,20] The photochemical behavior of pterins in aqueous solution has been summarized in a recent review.[21]

Ito and Kawanishi demonstrated for the first time in 1997[22] that, upon excitation with UV-A radiation, pterins are able to photoinduce DNA damage. It was reported that the main chemical modification of fragments of double-stranded DNA photoinduced by different pterin derivatives involves the hydroxylation of guanine yielding 8-oxoGua as a major product. This damage is sequence-specific, the oxidation taking place preferentially at the 5′ site of 5′-GG-3′ sequences. The damage in single-stranded DNA was reported to be less extended. Taking into account indirect evidence, the mechanism involved in this process was proposed to be an electron transfer with the subsequent formation of the guanine radical cation and a pterin radical anion. In a later study, photoinduced cleavage of plasmid pUC18 by PT was demonstrated.[23] Finally, in a more recent

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investigation, the chemical modification of DNA photoinduced by 6-carboxypterin (CPT) was studied.[24]

In a very recent work[25] we have proven that the acid form of PT photoinduces the oxidation of 2′-deoxyadenosine 5′-monophosphate (dAMP) in aqueous solution, *via* a type I mechanism. This result supports the hypothesis that pterins are able to damage DNA through an initial electron-transfer process.

On the other hand, most oxidized pterin derivatives are very good $^1O_2$ sensitizers under UV-A radiation in aqueous solution.[21,26] Indeed pterins are able to photoinduce oxidation of substances that react with $^1O_2$, like DNA and other molecules bearing the guanine base in their chemical structures. Consequently both mechanisms could be involved in processes where pterins participate as photosensitizers. Moreover, in a very recent work, in contrast to the proposed type I mechanism, photosensitization *via* $^1O_2$ has been reported as the main mechanism responsible for the photoinduced cleavage of plasmid DNA (PBR 322) by CPT.[27]

In this work, we performed a kinetic study of the process of quenching of $^1O_2$ by 2′-deoxyguanosine 5′-monophosphate (dGMP), in order to assess the reactivity of this nucleotide toward $^1O_2$. Steady-state near-infrared detection methods were employed to determine the rate constant of $^1O_2$ total quenching (k_t) by dGMP, as well as the rate constant of the chemical reaction between $^1O_2$ and dGMP (k_r), under different pH conditions. We also describe the oxidation of dGMP photosensitized by PT in aqueous solution under UV-A radiation. The participation of type I (electron transfer) and type II ($^1O_2$) mechanisms in acidic and alkaline media were analyzed. In addition, the interactions of dGMP with different excited states (singlet and triplet) of PT were investigated. The biological implications of the results obtained are also discussed.

2. Experimental Section

2.1. General. Pterin (PT) (Shircks Laboratories), rose bengal (RB) (Aldrich), 2′-deoxyguanosine 5′-monophosphate (dGMP) (Sigma Chemical Co.), and superoxide dismutase (SOD) from bovine erythrocytes (Sigma Chemical Co.) were of the highest purity available (>98%) and were used without further purification. 1*H*-Phenalen-1-one (PHE) (perinaphthenone, Merck) was purified as indicated in ref 28.

The pH of aqueous solutions was adjusted by adding drops of 0.1−0.2 M aqueous NaOH or HCl solutions with a micropipette. The ionic strength was *ca.* 10^{-3} M in all experiments. In experiments using D_2O as solvent, D_2O (>99.9%; Euriso-top or Aldrich), DCl (99.5%; Aldrich) in D_2O, and NaOD (CEA) in D_2O were employed.

2.2. Steady-State Irradiation. 2.2.1. Irradiation Setup. Aqueous solutions containing PT and dGMP were irradiated in 1 cm path length quartz cells at room temperature with a Rayonet RPR lamp emitting at 350 nm (bandwidth ∼20 nm) (Southern N.E. Ultraviolet Co.). The experiments were performed in the presence and absence of air. Oxygen-free solutions were obtained by bubbling with Ar gas for 20 min.

2.2.2. Actinometry. Aberchrome 540 (Aberchromics Ltd.) was used as an actinometer for the measurements of the incident photon flux (P_0) at the excitation wavelength. Aberchrome 540 is the anhydride form of the (*E*)-α-(2,5-dimethyl-3-furylethylidene)(isopropylidene)-succinic acid which, under irradiation in the spectral range 316−366 nm, leads to a cyclized form. The reverse reaction to ring opening is induced by visible light. The method for the determination of P_0 has been described in detail elsewhere.[29] Values of the photon flux absorbed (P_a) were calculated from P_0 according to the Lambert−Beer law:

$$P_a = P_0 (1 - 10^{-A})$$

where A is the absorbance of the sensitizer at the excitation wavelength.

2.2.3. UV/vis Analysis. Electronic absorption spectra were recorded on a Varian Cary-3 spectrophotometer. Measurements were made in quartz cells of 1 cm optical path length. The absorption spectra of the solutions were recorded at regular intervals of irradiation time, and the signals were averaged and smoothed with the Varian software. Experimental-difference (ED) spectra were obtained by subtracting the spectrum at time $t = 0$ from the subsequent spectra recorded at different times t. Each ED spectrum was normalized yielding the normalized experimental-difference (NED) spectrum.

2.2.4. High-Performance Liquid Chromatography (HPLC). A System Gold HPLC setup (Beckman Instruments) was used to monitor and quantify the photosensitized reactions and photoproducts. A Pinnacle-II C18 column (250 mm × 4.6 mm, 5 μm; Restek) was used for product separation, the elution being achieved with a solution containing a mixture of 4.2% acetronitrile and 95.8% of a 20 mM potassium phosphate aqueous solution (pH = 5.5). HPLC runs were monitored by UV spectroscopy at 260 nm.

2.2.5. Determination of the Concentration of O_2. The O_2 consumption during irradiation was measured with an O_2-selective electrode (Orion 37-08-99). The solutions and the electrode were placed in a closed glass cell of 130 mL.

2.2.6. Detection and Quantification of H_2O_2. For the determination of H_2O_2, a Cholesterol Kit (Wiener Laboratorios S.A.I.C.) was used. H_2O_2 was quantified after reaction with 4-aminophenazone and phenol.[30,31] Briefly, 400 μL of irradiated solution were added to 1.8 mL of reagent. The absorbance at 505 nm of the resulting mixture was measured after 30 min at room temperature, using the reagent as a blank. Aqueous H_2O_2 solutions prepared from commercial standards were employed for obtaining the corresponding calibration curves.

2.2.7. Superoxide ($O_2^{\bullet-}$) Investigation. Solutions containing PT (50 μM) and dGMP (100 μM) were irradiated in the presence of SOD (260 U/mL) at pH 5.5. Results of UV/vis spectrophotometric analysis, HPLC, and H_2O_2 determination were compared with those obtained in the absence of SOD.

2.3. Singlet Oxygen ($^1O_2$) Studies. 2.3.1. Determination of the Rate Constants of $^1O_2$ Total Quenching (k_t) by dGMP. The rate constants of $^1O_2$ total quenching by dGMP at different pH values were determined by Stern−Volmer analysis of the $^1O_2$ luminescence quenching. The main features of the method have been described elsewhere.[26,32] Singlet oxygen was generated by photosensitization, using rose bengal (RB) ($\Phi_\Delta = 0.75$[33,34]) or 1*H*-phenalen-1-one (PHE) ($\Phi_\Delta = 0.97$[28,35]) as sensitizers. Because of the short $^1O_2$ lifetime (τ_Δ) in H_2O (3.8 μs), D_2O (where τ_Δ is much longer: 62 ± 3 μs)[36] was used in all luminescence experiments.

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Groups of experiments were carried out irradiating solutions of dGMP and the sensitizer at 547 or 367 nm, for RB and PHE, respectively; at these wavelengths, the investigated nucleotide does not absorb (Figure 1). The solutions were irradiated in a 1 cm × 1 cm spectroscopic cell, under magnetic stirring, using a xenon/mercury arc (1 kW) through a water filter, focusing optics, and a monochromator (ISA Jobin-Yvon B204, 6 nm bandwidth). The sensitizer concentration was kept constant, whereas the dGMP concentration was varied within a series of experiments. Under our experimental conditions, a linear relationship between the ratio of the signals in the absence (S_e^0) and in the presence (S_e) of quencher (Q: dGMP) and the quencher concentration was observed (eq 7),[26]

$$S_e^0/S_e = 1 + K_{SV}[Q] \quad (7)$$

where K_{SV} is the Stern−Volmer constant. If Q interacts only with $^1O_2$, the relation between K_{SV} and k_t is given by eq 8,

$$K_{SV} = k_t\tau_\Delta \quad (8)$$

where τ_Δ is the $^1O_2$ lifetime in the solvent used (D_2O) in the absence of Q. Therefore, knowing τ_Δ, k_t can be calculated from the slope of the Stern−Volmer plot.

In order to check interaction between excited PT and dGMP (*vide infra*), experiments were performed using PT as a photosensitizer. For comparative purposes, these experiments were carried out under the same conditions as those performed with RB and PHE as $^1O_2$ sensitizers. Under conditions where a quencher (Q) interacts with both $^1O_2$ and the excited state(s) of the sensitizer, the quantum yield of $^1O_2$ production (Φ_Δ) decreases and the Stern−Volmer relation (eq 7) has to be modified as follows:

$$S_e^0/S_e = (\Phi_\Delta/\Phi_\Delta^{app})(1 + k_t\tau_\Delta[Q]) \quad (9)$$

where Φ_Δ^{app} is the apparent quantum yield of $^1O_2$ production by the sensitizer in the presence of Q.

2.3.2. Determination of the Rate Constant of the Chemical Reaction (k_r) between $^1O_2$ and dGMP. The rate of the sensitized photooxidation of dGMP was evaluated by following its disappearance by HPLC. The main features of the method have already been described in detail.[37] The rate of disappearance of a compound Q reacting with $^1O_2$ to yield an oxidized product (reaction 6) is given by eq 10.

$$-d[Q]/dt = k_r[^1O_2][Q] \quad (10)$$

If $^1O_2$ is produced by sensitization and applying the quasi-stationary hypothesis to the concentrations of excited states (reactions 1 to 6), eq 11 gives the steady-state concentration of $^1O_2$,

$$[^1O_2] = P_a\Phi_\Delta/(k_d + k_t^S[S] + k_t^Q[Q]) \quad (11)$$

where P_a (einstein L^{-1} s^{-1}) is the photon flux absorbed by the sensitizer, Φ_Δ is the quantum yield of $^1O_2$ production by the sensitizer, k_d is the nonradiative rate constant of $^1O_2$ deactivation (*vide supra*, reaction 3), [S] is the concentration of the sensitizer, k_t^S is the rate constant of $^1O_2$ total quenching by the sensitizer, and k_t^Q is the rate constant of $^1O_2$ total quenching by Q.

By combining eqs 10 and 11, and assuming that there is no interference by the oxidation product(s), eq 12 is obtained for the rate of disappearance of a compound Q reacting with $^1O_2$.

$$-\frac{d[Q]}{dt} = P_a\Phi_\Delta\frac{k_r[Q]}{k_d + k_t^S[S] + k_t^Q[Q]} \quad (12)$$

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Under our experimental conditions, the rate of $^1O_2$ total quenching by the sensitizer (S: RB, PHE, or PT) is negligible compared to deactivation by the solvent ($k_d \gg k_t^S[S]$). Therefore integration of eq 12 leads to eq 13,

$$f([Q]) = \ln([Q]/[Q]_o) - [(k_t^Q/k_d)([Q]_0 - [Q])] = -P_a\Phi_\Delta(k_r/k_d)t \quad (13)$$

and the plot of $f([Q])$ as a function of time should be linear. Therefore, knowing P_a, Φ_Δ, and k_d, k_r can be calculated from the slope of this plot.

For determining k_r, solutions in H_2O (3 cm^3) containing dGMP and RB or PHE as a sensitizer were irradiated. RB and PHE were excited at 547 and 367 nm, respectively. RB was used only in experiments performed in alkaline media because of its lack of stability in acidic media. On the other hand, PHE was used in both pH conditions. The concentration of dGMP was determined by HPLC at different irradiation times. The HPLC (Hewlett-Packard Series 1100) was equipped with an RP 18 LiChro CART 125-4 column. A solution containing a mixture of 2% acetronitrile and 98% 20 mM potassium phosphate aqueous solution (pH = 5.5) was used as eluent.

2.3.3. Comparison of Continuous Photolysis in H_2O and D_2O. Solutions of PT and dGMP were prepared in H_2O and D_2O. Couples of both types of solutions containing PT and dGMP at the same concentration were irradiated under identical experimental conditions. The effect of D_2O was evaluated by comparing results of UV−visible spectrophotometric analysis and HPLC analysis.

2.4. Laser Flash Photolysis. Laser flash photolysis experiments were performed using the frequency-tripled output (355 nm) of a Surelite II-10 Nd:YAG laser (Continuum Inc.) as an excitation source. Transient species were monitored at a right angle to the laser beam (5 ns duration pulses) using an LP900 Flash Photolysis System (Edinburgh Analytical Instruments). Data were collected using a Tektronix TDS 520A oscilloscope. Signal averaging was routinely performed to increase the signal-to-noise ratio. Samples, contained in 1 cm path length quartz cells, were bubbled with argon prior to irradiation.

2.5. Fluorescence Measurements. Steady-state fluorescence measurements were performed using a Perkin-Elmer LS 50B spectrofluorometer. Fluorescence spectra of air-equilibrated aqueous solutions in quartz cells (1 cm path length), obtained by excitation at 350 nm, were recorded between 360 and 650 nm. The spectra were corrected for differences in instrumental response and light scattering. For determining the quenching of fluorescence of PT by dGMP, emission spectra of PT solutions (25 μM) were recorded in the absence and in the presence of dGMP (0 to 20 mM), at pH 5.5 and 10.5. The fluorescence intensity (I) was obtained by integration of the spectra.

3. Results

3.1. Irradiation of Solutions Containing PT and dGMP. Air-equilibrated aqueous solutions containing pterin (PT) (50 μM) and 2′-deoxyguanosine 5′-monophosphate (dGMP) (100−1000 μM) were irradiated at 350 nm during different periods of time (up to 240 min). As can be inferred from the corresponding absorption spectra (Figure 1), under these experimental conditions, PT was excited, whereas dGMP did not absorb radiation. In order to avoid interferences between the acid and the basic forms of PT, the experiments were performed in the pH ranges 5.0−5.8, where PT is present at more than 99% in its acid form, and 10.2−10.7, where PT is present at more than 99% in the basic form. The photochemical reactions were followed by UV−visible spectrophotometry and HPLC.

Thermal reactions between PT and dGMP were discarded after control experiments performed by keeping solutions

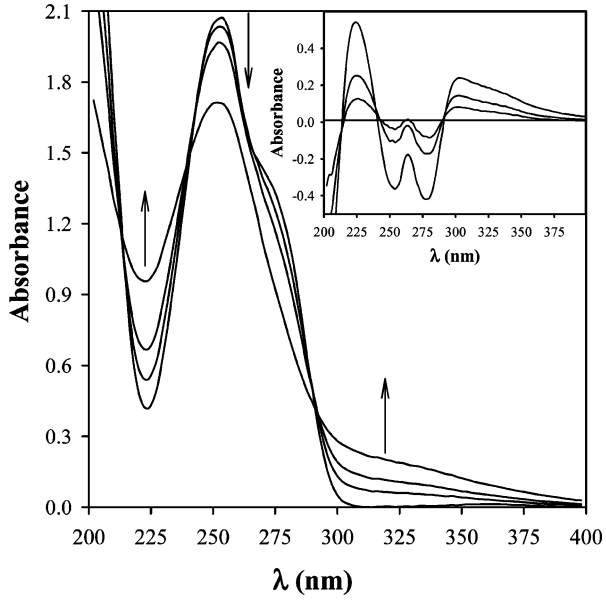

Figure 2. Evolution of the absorption spectra of air-equilibrated solutions of dGMP (500 μM) in the presence of pterin (50 μM), as a function of the irradiation time (0, 5, 10, and 30 min). In this figure, the spectrum of a pterin solution was subtracted from the experimental spectra recorded at different times (pH = 5.5; arrows indicate the changes observed at different wavelengths, optical path length = 2 mm). Inset: Experimental-difference spectra.

containing both compounds in the dark. These experiments were carried out under different experimental conditions (concentration, pH, and time). In another set of control experiments, dGMP solutions were irradiated in the absence of PT and no chemical modification of the nucleotide was detected, thus discarding, as expected, direct effects of the radiation used on the dGMP molecule.

In both acidic and alkaline media, significant changes in the absorption spectra of the solutions containing PT and dGMP were observed after irradiation. The spectral changes registered at pH 5.5 (Figure 2) reveal that the typical band belonging to dGMP decreased, whereas product(s) absorbing at wavelengths longer than 300 nm were formed. Results obtained in experiments performed at pH 10.5 also showed considerable spectral changes, with a strong decrease of the absorbance in the 250−280 nm wavelength range (see Supporting Information). Comparison of the absorption spectra suggests that the products formed were different depending on the pH.

Under the same pH conditions, the concentration profiles of PT and dGMP were determined by HPLC (Figure 3). A decrease of the dGMP concentration was observed as a function of irradiation time, whereas the PT concentration did not change in the analyzed time window. In addition, several products were detected by HPLC analysis, all of them having retention times lower than those corresponding to both dGMP and PT. Therefore, these products should be very polar substances, most probably because of the incorporation of oxygen into their structures.

In order to find out the role of O_2 in the studied photoinduced process, solutions containing PT (50 μM) and dGMP (110 and 500 μM), previously purged with Ar, were irradiated. Significant changes in the absorption spectra of solutions after more than 60 min of irradiation were observed neither in acidic (pH 5.0−5.5) nor in alkaline (pH 10.2−10.6) media. HPLC measurements

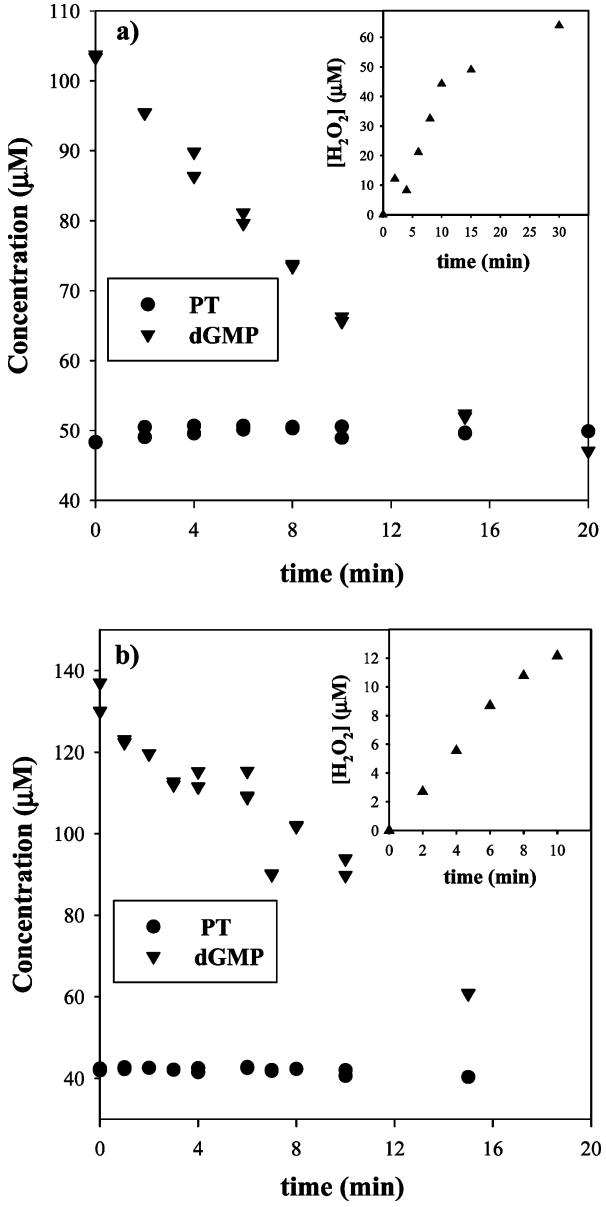

Figure 3. Evolution of the dGMP and PT concentrations in air-equilibrated aqueous solutions under UV-A irradiation (350 nm) as a function of time (concentrations were determined by HPLC analysis): (a) pH = 5.5; (b) pH = 10.5. Insets: Evolution of H_2O_2 concentration.

showed that, under these experimental conditions, the dGMP concentration did not decrease. Accordingly, no new product was detected.

The evolution of the O_2 concentration during the irradiation of air-equilibrated solutions containing dGMP (380 μM) and PT (93 μM) was monitored using an oxygen electrode in a closed cell. In both acidic and alkaline media, the O_2 concentration decreased as a function of irradiation time (Figure 4). Control experiments in the absence of dGMP were performed in order to check the consumption of O_2 resulting from the photolysis of PT itself.[38] Under both pH conditions, the decrease of the O_2 concentration was negligible in comparison with that observed in the presence of dGMP (Figure 4). These results together with those presented in the previous paragraph strongly

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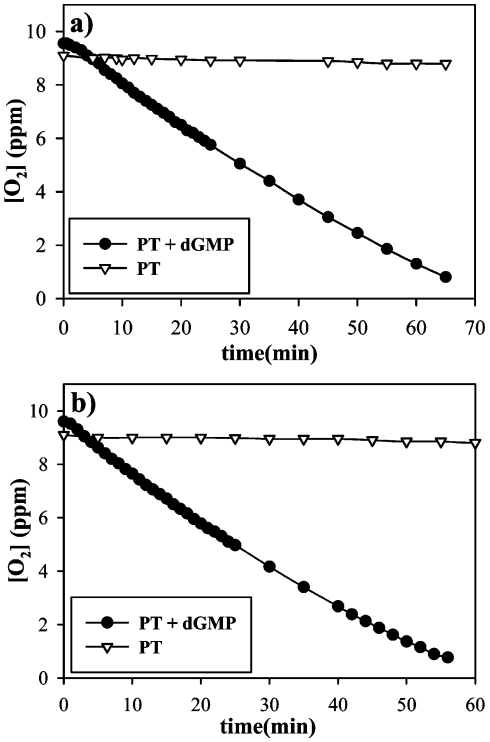

Figure 4. Evolution of the O_2 concentration in irradiated solutions containing PT (93 μM) and dGMP (380 μM) as a function of irradiation time: (a) pH = 5.5; (b) pH = 10.4. Control experiments (▽): Photolysis of PT (93 μM) in the absence of dGMP.

suggest that the process observed in air-equilibrated solutions is a photoinduced oxidation.

The generation of hydrogen peroxide (H_2O_2) as a product of the photosensitized oxidation of dGMP by PT was also investigated. H_2O_2 was detected and quantified in both acidic and alkaline solutions, irradiated under aerobic conditions. Series of experiments were performed at different initial concentrations of dGMP, whereas the PT concentration was kept constant (50 μM). In all cases, the H_2O_2 concentration increased with irradiation time (Figure 3) and the rate of H_2O_2 production was of the same order of magnitude as the rate of dGMP consumption.

Results presented so far (consumption of dGMP and O_2, production of H_2O_2, and constant concentration of PT during the reaction) clearly demonstrate that PT photosensitizes the oxidation of dGMP under UV-A irradiation at pH 5.5 and 10.5. However, the results are compatible with both type I and type II mechanisms and do not allow assessment of the contribution of each mechanism under acidic or alkaline conditions.

In particular, H_2O_2 is a product which is often observed in both cases. In the type II mechanism, H_2O_2 is a common product of the decomposition of hydroperoxides, which are frequently formed by the reaction of $^1O_2$ with unsaturated organic compounds.[39] In type I photosensitized oxidations, formation of H_2O_2 could be the result of spontaneous disproportionation of the superoxide anion ($O_2^{\bullet-}$), especially in aqueous solutions.[40] Therefore, in order to investigate the participation of $O_2^{\bullet-}$ in the mechanism, experiments were carried out, under both pH conditions, in the presence of superoxide dismutase (SOD). This

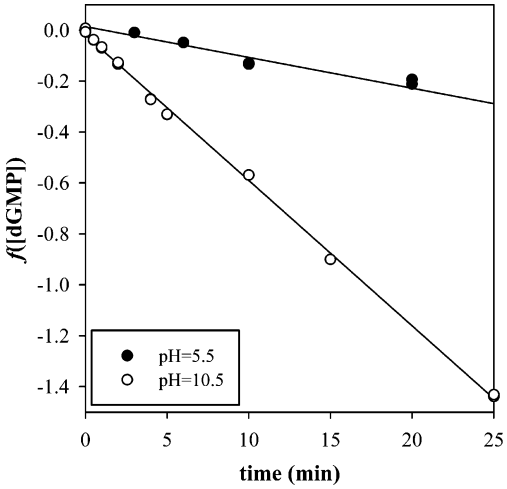

Figure 5. Photosensitized oxidation of dGMP in air-equilibrated aqueous solution: plot of $f([Q]) = \ln([Q]/[Q]_0) - [(k_t^Q/k_d)([Q]_0 - [Q])]$ as a function of the irradiation time (eq 13, Experimental section). Sensitizers: PHE at pH = 5.5 (λ_{exc} = 367 nm), RB at pH 10.5 (λ_{exc} = 547 nm), concentrations of dGMP determined by HPLC analysis. Note that the slopes of these plots ($(-P_a\Phi_\Delta k_r)/k_d$) do not reflect directly the values of k_r, as experiments were carried out using different incident photon fluxes.

enzyme catalyzes the conversion of $O_2^{\bullet-}$ into H_2O_2 and O_2. At pH 5.5 a significant increase in the consumption of dGMP was observed when SOD was present in the solution. This result suggests that elimination of $O_2^{\bullet-}$ inhibits a step that prevents the photoinduced oxidation of dGMP. In contrast, in alkaline media no changes in the concentration profile of dGMP were registered. The implications of these observations are discussed in the Conclusions.

3.2. Rate Constant of the Chemical Reaction between $^1O_2$ and dGMP. The rate constant of the chemical reaction between $^1O_2$ and dGMP (k_r) in H_2O was determined from the HPLC analysis of the disappearance of dGMP during the photosensitized oxidation, using either of two standard $^1O_2$ sensitizers, rose bengal (RB) or 1*H*-phenalen-1-one (PHE). In agreement with a kinetic analysis based on eq 13 (see Experimental Section), the plot of $f([Q])$, for Q = dGMP, as a function of irradiation time was linear (Figure 5). Values of $(1.7 \pm 0.3) \times 10^7$ M^{-1} s^{-1} and $(9.6 \pm 0.8) \times 10^7$ M^{-1} s^{-1} were obtained for k_r at pH 5.5 and 10.5, using PHE and RB as photosensitizers, respectively. As a control, k_r was also measured in alkaline media using PHE as a sensitizer, and a value of $(1.1 \pm 0.2) \times 10^8$ M^{-1} s^{-1} was obtained (data not shown).

One of the main guanine oxidation products, 8-oxoGua, is a very efficient $^1O_2$ quencher.[41] Therefore, in order to avoid interferences, the above-mentioned experiments were carried out at relatively low initial concentrations of dGMP (<150 μM). Under these experimental conditions, the quenching of $^1O_2$ by the reactant itself, albeit taken into account (eqs 11−13), is very low in comparison with the nonradiative deactivation of $^1O_2$ in H_2O (Reaction 3) ($k_d > 20\ k_t^Q[Q]$, Q = dGMP). Consequently, significant changes in the steady-state concentration of $^1O_2$ (eq 11) can be discarded, at least at low conversion when the concentration of product(s) is small enough. Finally, as additional control experiments, the initial rates of dGMP consumption ($(-d[Q]/dt)_0$) were determined, within a period of time where the decrease in dGMP concentration was lower than 10%,

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(41) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 474−477.

and k_r values were calculated from eq 10. In both acidic and alkaline media, the values determined by this procedure were equal to those obtained from eq 13, within experimental error.

It is noteworthy that the chemical reactivity of dGMP toward $^1O_2$ is higher in alkaline media (corresponding rate constant approximately 5.6 times larger than that in acidic media). This fact can be explained on the basis of charge effects as a consequence of the different acid−base equilibria of the nucleotides in H_2O. dGMP has ionizable groups in both the phosphate and the purine base moieties. The corresponding pK_a values for dGMP have already been reported (p$K_{a1}(PO_3H_2) \approx$ 0.65, p$K_{a2}(PO_3H_2) = 6.29$, pK_a(N1) = 9.56).[42,43] Taking into account these values, the predominant acid−base forms of dGMP at different pH values were established. At pH 5.5, the dGMP molecule has a charge of −1, with the charge on the phosphate group and the purine base in its neutral form. At pH 10.5, the total charge of the dGMP molecule is −3 and the guanine moiety is deprotonated due to the lactam group (Figure 1). Since the attack of $^1O_2$ takes place onto the guanine moiety, its deprotonation must be responsible for the high increase of k_r value with the pH. This kind of behavior has already been described in the literature. For instance, ionization of OH groups in dihydroxynaphthalenes leads to an increase in k_r values.[44] Similarly, the reactivity of the basic form of 6-methylpterin toward $^1O_2$ is much higher than that of the acid form.[45]

3.3. Role of $^1O_2$ in the Photosensitized Oxidation of dGMP by PT. Taking into account the values of k_r (*vide supra*), the role of $^1O_2$ in the oxidation of dGMP photosensitized by PT can be evaluated. Equation 14 expresses the rate of the chemical reaction between $^1O_2$ and dGMP:

$$(d[dGMP]/dt)_1 = -k_r[^1O_2][dGMP] \quad (14)$$

The steady-state concentration of $^1O_2$ during irradiation of a solution containing PT and dGMP is given by eq 15,

$$[^1O_2] = P_a \Phi_\Delta/(k_d + k_t^{PT}[PT] + k_t^{dGMP}[dGMP]) \quad (15)$$

where P_a and Φ_Δ are, in this case, the photon flux absorbed by PT and its quantum yield of $^1O_2$ production, respectively; k_t^{PT} and k_t^{dGMP} are the rate constants of $^1O_2$ total quenching by PT and dGMP, respectively. Considering the k_t^{PT} value previously determined in D_2O,[26] the quenching of $^1O_2$ by PT is negligible ($k_t^{PT}[PT] \ll k_d$). Values of k_t^{dGMP} were measured in D_2O at pH 5.5 and 10.5 (*vide infra*). Therefore, the value of the initial rate of the reaction between $^1O_2$ and dGMP, in a given experiment, can be calculated from eq 14, using the corresponding k_r value, the $[^1O_2]$ calculated from eq 15, and the initial [dGMP].

An aqueous solution containing PT (150 μM) and dGMP (150 μM) at pH = 5.5 was irradiated at 367 nm, under the same experimental conditions as those used for the determination of k_r . The experimental rate of dGMP consumption was (2.2 ± 0.1) μM/min, whereas $(d[dGMP]/dt)_1$ calculated from eqs 14 and 15 was (0.17 ± 0.4) μM/min. Similar kinetic analyses were performed for other experiments, carried out with different initial concentrations of PT and dGMP and using radiation at 350 nm (Rayonet lamp), and the same result was obtained: calculated $(d[dGMP]/dt)_1$ was much lower than the experimental rate of dGMP consumption. Therefore, these results show that the chemical reaction between dGMP and $^1O_2$ does not contribute significantly to the photosensitized oxidation of dGMP by the acid form of PT at pH 5.5. The predominant mechanism under these pH conditions involves most probably an electron transfer between the guanine moiety and the triplet excited state of PT (*vide infra*).

On the other hand, the same kinetic analysis was performed for experiments carried out in alkaline media. For instance, an aqueous solution containing PT (100 μM) and dGMP (132 μM) at pH = 10.5 was irradiated at 367 nm. In this case, the experimental initial rate of dGMP consumption was (2.8 ± 0.1) μM/min, whereas the rate of the reaction of dGMP with $^1O_2$, calculated using eqs 14 and 15, was (1.6 ± 0.2) μM/min, almost 10 times higher than that calculated at pH 5.5. This is due to higher values of k_r and Φ_Δ in alkaline media ($\Phi_\Delta = 0.18$ at pH = 5.5, $\Phi_\Delta = 0.30$ at pH = 10.5).[26] Therefore, it is clear that, in contrast to acidic media, photosensitization *via* $^1O_2$ is the dominant pathway for the photosensitized oxidation by the basic form of PT.

In order to confirm the hypotheses proposed in the previous paragraphs, comparative photolysis experiments were performed in H_2O and D_2O. Taking into account the longer $^1O_2$ lifetime in D_2O than in H_2O,[36] the photosensitized oxidation of dGMP should be faster in the deuterated solvent if $^1O_2$ would contribute significantly to the process. Air-equilibrated solutions containing PT (50 μM) and dGMP (100 μM) in H_2O and D_2O at pH and pD 5.5 and 5.9, respectively, were irradiated under otherwise identical conditions. The evolutions of the absorption spectra and of the concentrations of PT and dGMP as a function of the irradiation time show that the studied process is not faster in D_2O than in H_2O. On the contrary, a slightly lower rate of dGMP disappearance was observed in D_2O ((2.8 ± 0.1) μM min^{-1}) than in H_2O ((3.4 ± 0.1) μM min^{-1}). These results confirm that $^1O_2$, albeit present in the reaction mixture, does not participate in the mechanism of photosensitization in acidic media. The decrease in the rate of the process observed in D_2O cannot be easily explained and requires further investigation. However, this result could indicate that the solvent participates in one of the steps of the photosensitized process and would be consistent with the hypothesis that oxidation of 2′-deoxyguanosine in acidic media occurs mainly *via* an electron-transfer mechanism where H_2O is involved.[46]

A similar set of experiments was performed in alkaline media (pH = 10.6, pD = 10.8). In contrast to the results observed in acidic media, the rate of dGMP disappearance was 1 order of magnitude higher in D_2O ((34 ± 4) μM min^{-1}) than in H_2O ((3.2 ± 0.2) μM min^{-1}). In agreement with calculations shown above, these results confirm that oxidation *via* $^1O_2$ is the main mechanism of the photosensitized oxidation of dGMP by PT in alkaline media.

The results presented in this section show that PT is able to photoinduce the oxidation of dGMP by both type I and type II mechanisms, the contribution of each strongly depending on the pH of the solution. It is clear that photosensitization *via*

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$^1O_2$ is strikingly enhanced in alkaline media, but it is not easy to evaluate how the electron-transfer process itself is affected by the pH. However, considering that photosensitized oxidation of 2′-deoxyadenosine 5′-monophosphate (dAMP) by PT *via* electron transfer is significant at pH 5.5 and does not occur at all at pH 10.5,[25] at least a decrease in the rate of electron transfer in alkaline media should be expected.

It is interesting to assess the biological implications of these results. The pH of biological systems is about 7 but slightly varies depending on the particular fluid, e.g., 7.4 for human plasma. The exact proportion of each acid−base form of a pterin derivative or nucleotide will be given by its pK_a value and by the pH of the medium. Since pK_a of pterins is approximately 8,[21] both acid−base forms are present at physiological pH, but the acid (neutral) form will be the predominant one in most cases. On the other hand, the predominant acid−base form of the guanine moiety will be always the neutral form, since its pK_a is much higher (pK_a(N1) = 9.56).[42] Therefore type I photooxidation should be the main mechanism responsible for oxidation of purine nucleotides photosensitized by PT in biological systems. However, occurrence of PT-mediated one-electron oxidation of the guanine residues in cellular DNA would require a close proximity between the photosensitizer and the guanine target, and this point has not been investigated yet. If this is not the case, a possible contribution of $^1O_2$ that may diffuse from the site where it is generated cannot be completely discarded.

3.4. Rate Constants of $^1O_2$ Total Quenching by dGMP. The values of the rate constants of $^1O_2$ total quenching (k_t) by dGMP were determined in D_2O. The Stern−Volmer plots of the quenching of the near-infrared $^1O_2$ luminescence (eq 7) were linear within the range of concentrations used (Figure 6). The values of k_t were calculated from the slopes of these plots, and values of $(1.7 \pm 0.1) \times 10^7$ M^{-1} s^{-1} and $(8.5 \pm 0.6) \times 10^7$ M^{-1} s^{-1} were obtained in acidic media (pD = 5.5, PHE as a $^1O_2$ photosensitizer) and in alkaline media (pD = 10.5, RB as a sensitizer), respectively (Figure 6). The value of k_t was also determined at pD 10.5 using PHE, and a value of $(8 \pm 1) \times 10^7$ M^{-1} s^{-1} was obtained (data not shown).

In both acidic and alkaline media, these k_t values are close, within experimental error, to the corresponding k_r values, thus indicating that the deactivation of $^1O_2$ by dGMP is mainly a chemical process (reaction 6). It should be noted that the k_t and k_r values for another purine nucleotide, dAMP, reported in a recent study in D_2O (pD = 5.5),[25] were 4.1×10^5 M^{-1} s^{-1} and 8.0×10^3 M^{-1} s^{-1}, respectively. These results show that the deactivation of $^1O_2$ by dAMP is almost only physical (reaction 5) ($k_t^{dAMP} \approx k_q^{dAMP}$). Therefore, assuming that k_q^{dGMP} is of the same order of magnitude as k_q^{dAMP}, the physical quenching of $^1O_2$ by dGMP should be negligible in comparison to the corresponding chemical reaction, with $k_t^{dGMP} \approx k_r^{dGMP}$ as observed.

Another set of experiments of quenching of the near-infrared $^1O_2$ luminescence by dGMP was performed using PT as a $^1O_2$ sensitizer, instead of PHE or RB, under otherwise identical experimental conditions. In both acidic and alkaline media, the corresponding Stern−Volmer plots were linear within the range of concentrations used (Figure 6). However, the K_{SV} value determined with PT ($(8.8 \pm 0.3) \times 10^3$ M^{-1}) at pD 10.5 was significantly higher than that obtained in experiments carried out with RB ($(5.2 \pm 0.2) \times 10^3$ M^{-1}). The difference was even larger at pD 5.5 with a K_{SV} value 4 times higher ($(3.8 \pm 0.1) \times 10^3$ M^{-1}) than that obtained in experiments carried out with PHE ($(0.98 \pm 0.04) \times 10^3$ M^{-1}).

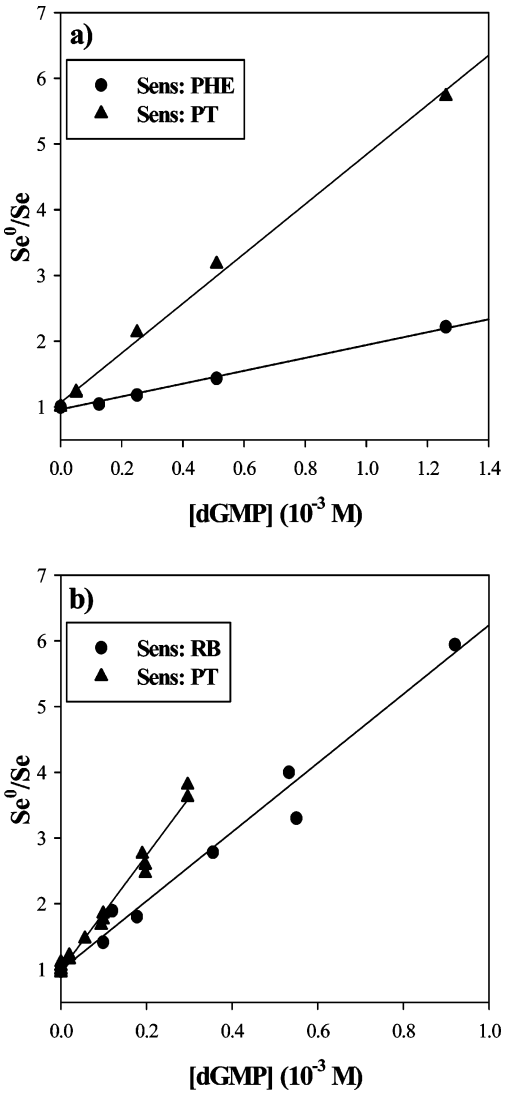

Figure 6. Stern−Volmer plots of the quenching of the $^1O_2$ near-infrared luminescence by dGMP in D_2O (Experimental section, PHE, RB, and PT were used as sensitizers): (a) pD = 5.5; (b) pD = 10.5.

These results suggest that, in experiments where PT is the $^1O_2$ sensitizer, the deactivation of $^1O_2$ by dGMP is not the only process responsible for the decrease of the $^1O_2$ emission as a function of the dGMP concentration. In order to explain such a behavior, quenching of the excited states of PT by dGMP may be proposed. If this is the case, the $^1O_2$ luminescence signal decreases not only because of the physical and chemical quenching of $^1O_2$ by dGMP but also because of a lower production of $^1O_2$, thus leading to a larger apparent K_{SV}. (A detailed kinetic analysis shows that, under conditions where dGMP interacts both with singlet oxygen and the triplet excited state of PT, the corresponding Stern−Volmer plot should be quadratic in dGMP. However, depending on the values of the different quenching rate constants and on the concentration range of dGMP, the curvature may be quite small and the Stern−Volmer plot could appear to be linear within experimental error.) Therefore, these experimental results show evidence of the interaction of dGMP with excited PT, especially in acidic media.

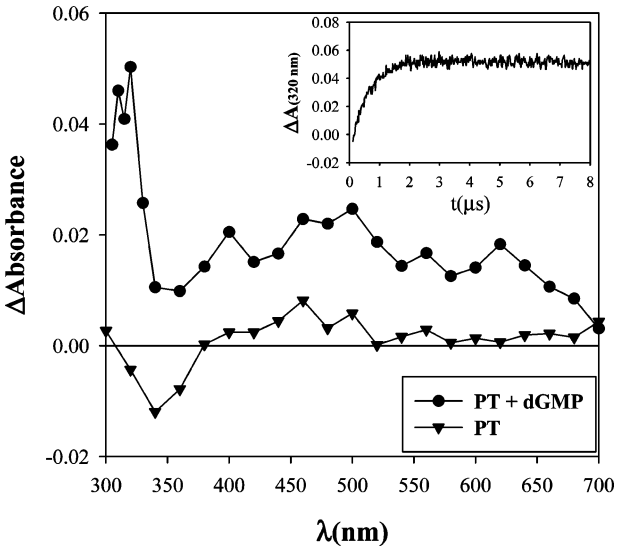

Figure 7. Differential transient absorption spectra recorded 7.7 μs after the 355-nm laser pulse in oxygen-free aqueous solutions of PT (90 μM), in the absence (▼) and in the presence (●) of dGMP (500 μM) (pH = 5.5). Inset: Time dependence of the absorbance at 320 nm.

This latter fact is in agreement with the hypothesis that the photosensitized oxidation of dGMP by PT at pH 5.5 takes place primarily *via* a mechanism in which $^1O_2$ does not participate. The interaction of dGMP with excited PT, revealed by the results shown in this section, could play a major role in such a mechanism.

3.5. Laser-Flash Photolysis Experiments. The radicals of (deoxy)guanosine and (d)GMP have been obtained and studied in aqueous solution by pulse radiolysis[47] and 193-nm laser flash photolysis.[48] Radical cations formed after one-electron-oxidation of guanine nucleosides or nucleotides are relatively strong acids and undergo fast deprotonation at pH > 5.[47,49] The resulting neutral radicals have characteristic spectral features and long lifetimes (>100 μs), allowing their detection in different systems.

In order to obtain direct evidence of electron transfer between dGMP and the excited state(s) of PT, laser flash photolysis experiments were performed at pH 5.5. Oxygen-free aqueous solutions of PT (90 μM) were excited at 355 nm in the absence and the presence of dGMP (500 μM). In the former case, differential transient absorption spectra recorded in the 300−700 nm wavelength range were in agreement with previous studies performed for PT and other pterin derivatives in H_2O[50] and DMSO.[51] The transients reported in these publications for PT were proposed to be triplet states with lifetimes shorter than 3 μs. Accordingly, the intensity of the spectral changes registered in our experiments after 6 μs were very small (Figure 7). In contrast, the experiments performed in the presence of dGMP showed a transient that did not decay during more than 10 μs. In addition, the differential transient absorption spectra obtained (Figure 7) have the characteristic narrow absorption band ($\lambda_{max} \approx 320$ nm) of neutral and cation radicals of 2′-deoxyguanosine.[48] Therefore these results are direct evidence that when PT, in aqueous solution at pH 5.5, is excited by UV-A radiation in the presence of dGMP, the nucleotide looses an electron.

The formation of the dGMP radical cation, very likely in its deprotonated form (dGMP(−H)$^\bullet$), can be monitored by the time evolution of the absorbance at 320 nm after the laser pulse (Inset in Figure 7). The lifetime of the singlet excited state of PT (S_1), determined by fluorescence time-resolved measurements[52] (*vide infra*), was reported to be only a few nanoseconds. Therefore, considering the time window of the absorbance changes recorded at 320 nm, the radical cannot be generated by electron transfer from S_1. This fact strongly suggests that the dGMP radical is produced by reaction with the PT triplet state (Reaction 16).

$$^3PT^* + dGMP \rightarrow PT^{\bullet -} + dGMP^{\bullet +} \qquad (16)$$

3.6. Quenching of the Fluorescence of PT by dGMP. Emission spectra of PT were recorded in the presence and in the absence of dGMP. Groups of experiments were carried out with aqueous solutions of PT (25 μM) and different concentrations of dGMP (0 to 20 mM) at pH 5.5 and 10.5. Under both pH conditions, a strong decrease in the fluorescence intensity was observed, the wavelength of the emission maximum remaining unchanged (Figure 8). The decrease in the integrated fluorescence intensity as a function of the concentration of dGMP followed Stern−Volmer behavior in both media (Figure 8). The corresponding values of the Stern−Volmer constants (K_{SV}) at pH 5.5 and 10.5 are (51 ± 3) M^{-1} and (22 ± 3) M^{-1}, respectively.

Quenching of fluorescence is direct evidence of an interaction between the first excited singlet state (S_1) of PT and dGMP. However, in the range of dGMP concentrations used in experiments of $^1O_2$ quenching (0−1.3 mM) (Figure 6), the quenching of PT fluorescence was negligible; *e.g.*, less than 10% of S_1 was quenched at [dGMP] = 1 mM. This fact shows that the strong decrease in the production of $^1O_2$ by PT (*vide supra*), detected in the experiments of the quenching of the near-infrared $^1O_2$ luminescence, cannot be primarily due to the deactivation of the S_1 state of PT by dGMP. Therefore, the quenching of the triplet excited state of PT (T_1) should be mainly responsible for the observed decrease.

Assuming that the S_1 quenching by dGMP is dynamic, the bimolecular rate constant of fluorescence quenching (k_q^F) can be calculated from the K_{SV} value, using eq 17:

$$K_{SV} = \tau_F k_q^F \qquad (17)$$

where τ_F is the fluorescence lifetime of PT (7.6 ± 0.4 ns and 5.0 ± 0.4 ns at pH 5.5 and 10.5, respectively).[52] If the electron transfer would take place from S_1, the maximum value of the rate constant for the reaction between excited PT and dGMP (k_{et}^{S1}) would be k_q^F ((6.7 ± 0.7) × 10^9 and (4 ± 1) × 10^9 M^{-1} s^{-1} at pH 5.5 and 10.5, respectively). Therefore, assuming this mechanism, the maximum rate of dGMP consumption would be given by eq 18 and can be calculated for a given experiment.

$$(d[dGMP]/dt)_2 = -k_{et}^{S1}[^1PT^*][dGMP] \qquad (18)$$

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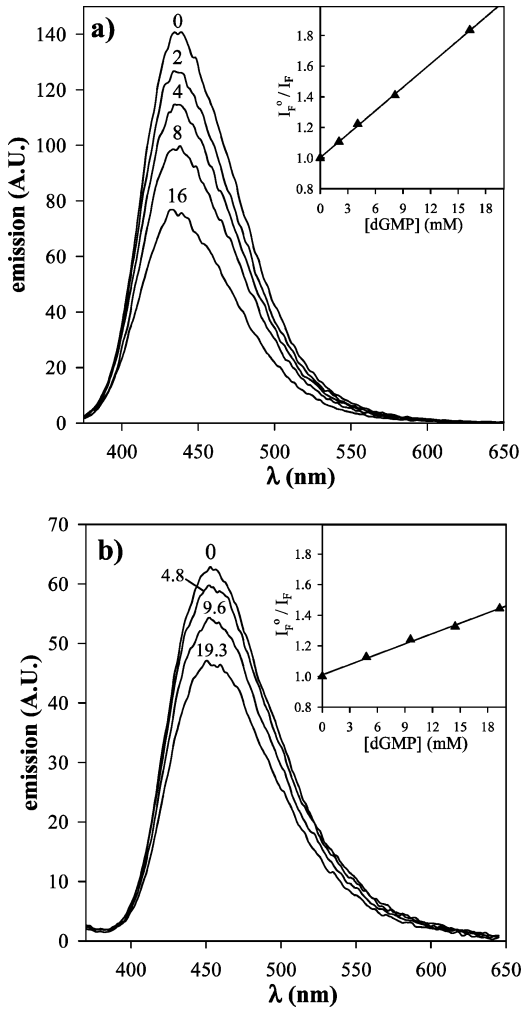

Figure 8. Quenching of the fluorescence of PT by dGMP: Corrected fluorescence spectra (excitation at 350 nm) of solutions of pterin (25 μM) in the absence and in the presence of different concentrations of dGMP; the dGMP concentration (mM) appears above each spectrum: (a) pH = 5.5; (b) pH = 10.5. Insets: Stern−Volmer plots of the integrated fluorescence intensities (I_F).

where [^{1}PT*] is the steady-state concentration of PT in the S_1 state and can be calculated from eq 19:

$$[^1\text{PT}^*] = P_a/(1/\tau_F + k_{et}^{S1}[\text{dGMP}]) \qquad (19)$$

This estimated rate (eq 18) was calculated for several experiments performed under different conditions. In all cases, the theoretical rate was lower than the experimental one. For instance, for the same experiment used for evaluating the reaction with $^1O_2$ (*vide supra*), the calculated rate was 0.83 μM min^{-1}, whereas the experimental rate was 2.2 ± 0.1 μM min^{-1}. These results are in agreement with a minor or negligible contribution of an electron transfer involving the S_1 state of PT.

4. Conclusions

The capability of pterins to photoinduce damage to DNA has been demonstrated, the main target on the DNA molecule being guanine.[22] However, there is no agreement concerning the mechanism involved: type I (electron transfer) or type II (reaction with singlet molecular oxygen ($^1O_2$)). Moreover, although many studies have demonstrated that guanine is easily oxidized by $^1O_2$, to the best of our knowledge, there are no systematic studies on the reactivity of dGMP with $^1O_2$.

In this work, we have shown that the deactivation of $^1O_2$ by dGMP is mainly a chemical process (reaction 6). Indeed, within experimental error, the rate constants of $^1O_2$ total quenching by dGMP ($k_t = (1.7 \pm 0.1) \times 10^7$ M^{-1} s^{-1} and $(8.5 \pm 0.6) \times 10^7$ M^{-1} s^{-1} in acidic and alkaline media, respectively) are similar to the rate constants of the chemical reaction between $^1O_2$ and dGMP in the corresponding media ($k_r = (1.7 \pm 0.3) \times 10^7$ M^{-1} s^{-1} and $(9.6 \pm 0.8) \times 10^7$ M^{-1} s^{-1}, respectively). It is noteworthy that the reactivity of dGMP toward $^1O_2$ is much higher in alkaline media. This fact can be explained on the basis of charge effects as a consequence of the different acid−base equilibria of the nucleotides in H_2O. At pH 10.5, the guanine moiety is deprotonated at its lactam group (Figure 1). Since the attack of $^1O_2$ takes place onto the guanine moiety, its deprotonation must be responsible for the high increase of k_r value with the pH. This kind of behavior has already been described in the literature for other compounds.[44,45]

In this work, we have also investigated the photosensitized oxidation of 2′-deoxyguanosine 5′-monophosphate (dGMP) by pterin (PT) in aqueous solution under UV-A irradiation. We have shown that when aerated solutions containing dGMP and PT at pH 5.5 or 10.5 are exposed to UV-A radiation, dGMP is consumed, whereas the photosensitizer (PT) concentration does not change significantly. During this process, O_2 is consumed and H_2O_2 is generated.

The role of $^1O_2$ in the oxidation of dGMP photosensitized by PT was evaluated from kinetic calculations taking into account the values obtained for k_r and from comparative photolysis experiments in H_2O and D_2O. Results show that the chemical reaction between dGMP and $^1O_2$ does not contribute significantly to the photosensitized oxidation of dGMP by the acid form of PT (pH 5.5), thus suggesting the participation of a type I mechanism under these pH conditions. In contrast, photosensitized oxidation by the basic form of PT (pH 10.5) occurs mainly *via* $^1O_2$. Therefore, significant photosensitization of DNA components by pterin derivatives *via* a type II mechanism should be discarded at a pH lower than 9.

Experiments of quenching of the near-infrared $^1O_2$ luminescence by dGMP, performed using PT as a $^1O_2$ sensitizer, suggest interactions of dGMP with the excited states of PT. Kinetic comparison of these results with those obtained in experiments of quenching of PT fluorescence by dGMP shows that quenching of the S_1 state of PT by dGMP was negligible in the range of dGMP concentrations used for the study of the photosensitized process. Therefore significant quenching of the PT triplet state by dGMP should occur. Direct evidence of electron transfer between dGMP and excited PT was obtained in acidic media by laser flash photolysis experiments showing the presence of the dGMP radical after excitation of PT at 355 nm. The formation of such a radical occurs within a time window of a few microseconds, which is in favor of the participation of the PT triplet state in the process.

Finally, taking into account all the results presented in this work, a mechanistic scheme may be proposed for the photosensitized oxidation of dGMP by PT in the presence of oxygen. After excitation of PT and formation of its triplet excited state (^{3}PT*),

$$PT \xrightarrow{h\nu} {}^1PT^* \xrightarrow{k_{ISC}} {}^3PT^* \quad (20)$$

two reaction pathways compete for the deactivation of the latter: (a) *Energy transfer from $^3PT^*$ to molecular oxygen* ($^3O_2$) leads to the regeneration of PT and the formation of singlet oxygen ($^1O_2$) and to the subsequent oxidation of dGMP (type II mechanism, reactions a and b):

$$^3PT^* + {}^3O_2 \rightarrow PT + {}^1O_2 \quad (a)$$

$$dGMP + {}^1O_2 \rightarrow dGMP_{(ox)} \quad (b)$$

(b) *Electron transfer between dGMP and $^3PT^*$* leads to the formation of the corresponding radical ions, $PT^{\bullet-}$ and $dGMP^{\bullet+}$ (type I mechanism, reaction c).

$$^3PT^* + dGMP \rightarrow PT^{\bullet-} + dGMP^{\bullet+} \quad (c)$$

These radical ions may recombine (reaction d) but also initiate a series of consecutive and parallel reactions where different reactive species interact in a more complex pattern than that in the type II mechanism. Following reaction c, the electron transfer from $PT^{\bullet-}$ to O_2 regenerates PT and forms the superoxide anion ($O_2^{\bullet-}$) (reaction e). The latter may disproportionate with its conjugated acid ($HO_2^{\bullet}$) to form H_2O_2 (reaction f), a process in competition with reaction h (*vide infra*).

$$PT^{\bullet-} + dGMP^{\bullet+} \rightarrow PT + dGMP \quad (d)$$

$$PT^{\bullet-} + O_2 \rightarrow PT + O_2^{\bullet-} \quad (e)$$

$$O_2^{\bullet-} \rightarrow \rightarrow \rightarrow H_2O_2 \quad (f)$$

The radical cation $dGMP^{\bullet+}$ undergoes fast deprotonation[47,49] to the corresponding neutral radical $dGMP(-H)^{\bullet}$ (reaction g). The latter radical and $O_2^{\bullet-}$ react very fast[53] through two competitive pathways:[54] (i) electron transfer with the regeneration of dGMP and O_2 (reaction h) and (ii) radical recombination followed by the formation of end products (reaction i). Oxidation of $dGMP(-H)^{\bullet}$ by O_2 is very slow[53,55] and should not contribute significantly to the formation of dGMP oxidation products.

$$dGMP^{\bullet+} \rightleftharpoons dGMP(-H)^{\bullet} + H^+ \quad (g)$$

$$dGMP(-H)^{\bullet} + O_2^{\bullet-} + H^+ \rightarrow dGMP + O_2 \quad (h)$$

$$dGMP(-H)^{\bullet} + O_2^{\bullet-} \rightarrow dGMP(-H)O_2^- \rightarrow dGMP_{(ox)} \quad (i)$$

In the presence of SOD that catalyzes the disproportionation of $O_2^{\bullet-}$ (reaction f), the only pathway that regenerates dGMP (reaction h) is prevented and an enhancement of the photosensitized degradation of dGMP is observed experimentally. Other slower processes may play a role, in particular in the absence of $O_2^{\bullet-}$ and other reactive species. For example, $dGMP^{\bullet+}$ may undergo hydration followed by the formation of 8-oxo-7,8-dihydroguanosine 5′-monophosphate (8-oxo-dGMP) and other oxidized products[56] (reaction j). Other minor pathways might also contribute to the consumption of dGMP (dimerizations or reactions of radicals with H_2O_2).

$$dGMP^{\bullet+} + H_2O \rightarrow \text{8-oxo-dGMP} \rightarrow \rightarrow dGMP_{(ox)} \quad (j)$$

Type I and type II mechanisms[7] of the oxidation of dGMP photosensitized by PT are competitive and contribute in different proportions depending on the pH. In alkaline media, where the quantum yield of $^1O_2$ production (Φ_Δ) and the rate constant of the chemical reaction between dGMP and $^1O_2$ (k_r) are higher than those in acidic media, the main mechanism involves $^1O_2$ as the reactive intermediate. On the other hand, under acidic conditions, where the reaction with $^1O_2$ is much slower and the initial electron transfer (reaction c) is likely to be more efficient, the type I mechanism is the main pathway. Since this is the situation at physiological pH, it can be inferred that electron transfer should be the main mechanism responsible for oxidation of nucleotides photosensitized by PT in biological systems.

Acknowledgment. The present work was partially supported by Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET-PIP 02470/00 and 6301/05), Agencia de Promoción Científica y Tecnológica (ANPCyT Grant PICT 06-12610), and Universidad Nacional de La Plata (UNLP). A.L.C. and E.O. thank Secretaría de Ciencia, Tecnología e Innovación Productiva (SECyT, Argentina) and Bundesministerium für Forschung und Bildung (BMFB, Germany) for financial support of their project EVI/013. M.L.D. and G.P. thank CONICET for graduate research fellowships. A.H.T., C.L., and M.L.D. thank the Deutscher Akademischer Austauschdienst (DAAD) for research fellowships. A.L.C., F.M.C., A.H.T., and C.L. are research members of CONICET. The authors thank Dr. Narayanapillai Manoj for his crucial contributions in laser flash photolysis experiments and Mariana Vignoni for her help in steady-state experiments.

Supporting Information Available: Evolution of the absorption spectra of air-equilibrated solutions of dGMP (80 μM) in the presence of pterin (50 μM), as a function of the irradiation time (pH = 10.5). This material is available free of charge via the Internet at http://pubs.acs.org.

JA075367J

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