Photochemistry and Photobiology, 2013, 89: 1456–1462

Type I Photosensitization of 2′-deoxyadenosine 5′-monophosphate (5′-dAMP) by Biopterin and its Photoproduct Formylpterin†

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Received 13 April 2013, accepted 3 July 2013, DOI: 10.1111/php.12134

ABSTRACT

Biopterin (Bip) and its photoproducts 6-formylpterin (Fop) and 6-carboxypterin (Cap) accumulate in the skin of patients suffering from vitiligo, a chronic depigmentation disorder where the protection against UV radiation fails because of the lack of melanin. These compounds absorb in the UV-A inducing a potential photosensitizing action that can cause damage to DNA and other biomolecules. In this work, we have investigated the capability of these pterin derivatives (Pt) to act as photosensitizers under UV-A irradiation for the degradation of 2′-deoxyadenosine 5′-monophosphate (5′-dAMP) in aqueous solutions, as model DNA target. Steady-state and time-resolved experiments were performed and the effect of pH was evaluated. The results showed that photosensitized degradation of 5′-dAMP was only observed under acidic conditions, and a mechanistic analysis revealed the participation of the triplet excited state of the pterin derivatives (^{3}Pt*) by electron transfer yielding the corresponding pair of radical ions ($Pt^{\bullet-}$ and $5'\text{-dAMP}^{\bullet+}$), with successive photosensitizer recovery by electron transfer from $Pt^{\bullet-}$ to O_2. Finally, $5'\text{-dAMP}^{\bullet+}$ participates in subsequent reactions to yield degradation products.

INTRODUCTION

Solar radiation induces modifications to genomic DNA and is implicated in the induction of human skin cancers (1,2). UV-B radiation (280–320 nm) damages DNA through the direct excitation of the nucleobases (3). On the other hand, UV-A (320–400 nm) and visible excitation of endogenous or exogenous photosensitizers can induce modifications in DNA including purine bases through different mechanisms (4–6). In particular, energy transfer from the triplet state of the photosensitizer to pyrimidine bases leads to the formation of cyclobutane pyrimidine dimers (7,8). Photosensitized reactions also contribute to DNA damage, either by processes involving the generation of radicals (type I mechanism), *e.g. via* electron transfer or hydrogen abstraction; and/or the production of singlet molecular oxygen ($^1O_2$) by energy transfer to triplet molecular oxygen (type II mechanism) (9). Therefore, the photochemical behavior and, in particular, the photosensitizing properties of compounds present in the skin are very important from a biomedical point of view.

Pterins are a family of heterocyclic compounds widespread in biological systems derived from 2-aminopteridin-4(3*H*)-one or pterin (Ptr). Unconjugated oxidized pterins (Scheme 1) are photochemically reactive by excitation with UV-A radiation in aqueous solution, where they can fluoresce, undergo photooxidation to produce different photoproducts, generate reactive oxygen species, such as singlet molecular oxygen ($^1O_2$), and photosensitize the oxidation of biomolecules (10,11). Under physiological conditions the unconjugated oxidized pterins are not present in mammals, but they accumulate in the skin of patients suffering from vitiligo (12,13), a depigmentation disorder where the protection of the skin against UV radiation fails because of the lack of melanin.

In particular, the oxidation of 7,8-dihydrobiopterin (H_2Bip), likely *via* a photochemical process (14), leads to the accumulation of biopterin (Bip, Scheme 1) in the skin under pathological conditions. Further UV-A excitation of Bip induces a cascade of self-oxidation processes to form 6-formylpterin (Fop) and 6-carboxypterin (Cap) as intermediate and final product, respectively, Scheme 1, (15,16). This latter compound has also been isolated from the affected tissues of vitiligo patients (13), indicating that excited states of pterins are photogenerated *in vivo*.

It was demonstrated for the first time in 1997 by Ito and Kawanishi (17) that UV-A excitation of pterins induces DNA damage. Later studies provided additional evidence on the photosensitizing capability of pterins to degrade DNA, but contradictory mechanisms were proposed (18,19). In previous studies performed with single nucleotides as substrates (20,21), we have demonstrated that pterin (Ptr), the parent unsubstituted compound of oxidized pterins (Scheme 1), can act as a photosensitizer through both type I and type II mechanisms. More recently, we have also demonstrated that the vitiligo-related pterin derivatives, *e.g.* Bip, Cap and Fop, are efficient photosensitizers to degrade 2′-deoxyguanosine 5′-monophosphate (5′-dGMP) nucleotide by competing type I and type II mechanisms (22). Stationary and transient photolysis experiments combined with product analysis indicated that, regardless of these pterins are relatively efficient $^1O_2$ photosensitizers, the dominant mechanism involved in the photodegradation of 5′-dGMP is initiated by an electron transfer reaction (22).

Continuing our survey to establish general mechanisms for the degradation of biomolecules by photosensitization of biomedical rele-

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†This article is part of the Special Issue dedicated to the memory of Elsa Abuin.
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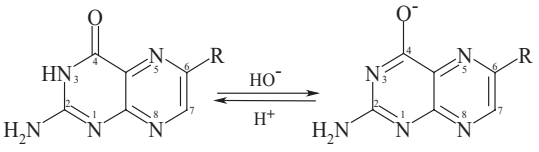
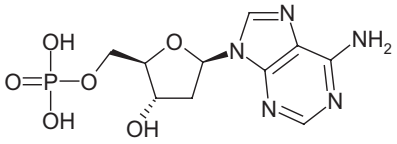


-R	Compound
-H	pterin **(Ptr)**
$-(CHOH)_2-CH_3$	biopterin **(Bip)**
-CHO	6-formylpterin **(Fop)**
-COOH	6-carboxypterin **(Cap)**



2'-deoxyadenosine 5'-monophosphate **(5'-dAMP)**

Scheme 1. Molecular structure of the acid and basic forms of the unconjugated oxidized pterins, and of the neutral form of the nucleotide 5′-dAMP.

vant pterins, in this work we present a study using the vitiligo-related pterins as photosensitizers for the degradation of 2′-deoxyadenosine 5′-monophosphate (5′-dAMP) in aqueous acidic (pH 5.5) and basic (pH 10.5) solutions under UV-A radiation. The choice of 5′-dAMP as nucleotide target has several advantages such as its high water solubility, easy quantification by chromatographic methods (23), and almost not reaction toward $^1O_2$ (20,24), allowing a neat analysis of the photosensitized electron transfer processes involved. The steady-state and time-resolved photolysis studies together with HPLC analysis of both 5′-dAMP and pterin derivatives, indicated that the molecular degradation mechanism of the 5′-dAMP photosensitized by the vitiligo-related pterin derivatives is initiated by electron transfer (Type I) from the nucleobase to the triplet excited state of by the pterin derivatives (^{3}Pt*), yielding the corresponding pair of radical ions ($Pt^{\bullet-}$ and 5′-$dAMP^{\bullet+}$), with successive secondary dark reactions involving ground state molecular oxygen for the pterin derivative recovery and the formation of 5′-dAMP degradation products.

MATERIALS AND METHODS

Chemicals. Pterin derivatives Ptr, Bip, Fop and Cap were purchased from Schircks Laboratories (Jona, Switzerland) and used without further purification. KI (purity >99%), 5′-dAMP, formic acid, superoxide dismutase (SOD) from bovine erythrocytes and other chemicals were provided by Sigma-Aldrich Argentina (Buenos Aires, Argentina), and used as received.

General. pH measurements were performed with a pH-meter PHM220 (Radiometer Copenhagen) combined with a pH electrode pHC2011-8 (Radiometer Analytical). The pH of the aqueous solutions was adjusted by adding very small aliquots (few μL) of concentrated (0.1–2.0 M) HCl or NaOH solutions using a micropipette.

Photolysis experiments with air-equilibrated solutions were performed in open quartz cells with magnetic stirring. Taking into account these experimental conditions, and that the consumption of O_2 was in all cases relatively slow, the O_2 concentration can be considered constant during the experiments performed with aerated solutions. Other experiments were carried out with argon and oxygen-saturated solutions, which were obtained by bubbling for 20 min with these gases, previously water saturated (purity >99.998%; Linde Argentina, Buenos Aires, Argentina).

UV/Vis spectrophotometry. Electronic absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer (Shimadzu Corp., Tokyo, Japan), using quartz cells of 0.4 cm optical pathlength. The absorption spectra of the solutions were recorded at regular intervals of irradiation time.

High-performance liquid chromatography. A high-performance liquid chromatography (HPLC) Prominence apparatus from Shimadzu (solvent delivery module LC-20AT, on-line degasser DGU-20A5, communications bus module CBM-20, auto sampler SIL-20A HT, column oven CTO-10AS VP and photodiode array detector SPD-M20A) was employed for monitoring the reaction. A Synergi Polar-RP column (ether-linked phenyl phase with polar endcapping, 150 × 4.6 mm, 4 μm, Phenomenex) was used for product separation. An aqueous solution of formic acid (25 mM, pH = 3.2) was used as mobile phase.

Steady-state photolysis set-up. Aqueous solutions containing a given pterin derivative and 5′-dAMP 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., Branford, CT). The experiments were performed in the presence and absence of dissolved O_2 into the solutions.

Laser flash photolysis experiments. Our laser flash photolysis (LFP) apparatus was described elsewhere (22). Briefly, excitation of the pterin derivatives was performed with the third harmonic at 355 nm of a Nd: YAG Minilite II laser (7 ns FWHM, 5 mJ per pulse) of Continuum (Santa Clara, CA). The transient absorption spectra of aqueous solutions of pterins (≈90 μM) previously saturated by bubbling of Ar, air or O_2 were recorded with the m-LFP 112 laser flash photolysis apparatus (Luzchem Research Inc, Ottawa, Canada) linked to a 300 MHz Tektronik TDS 3032B digital oscilloscope (Wilsonville, OR) for signal acquisition. The signal analysis was done with the OriginPro 8.0 software from OriginLab Corporation (Northampton, MA).

RESULTS AND DISCUSSION

Evaluation of the photosensitizing activity of Bip and its photoproducts

As all pterins show $pK_a \approx 8$, to avoid interference between the acid and the basic forms of the pterins, the photosensitization experiments were performed at extremes pH of 5.5 and 10.5, where the pterin derivatives are present at more than 99% in their acid and basic forms, respectively (Scheme 1). Figure 1 shows the absorption spectra of the acid (pH 5.5) and basic (pH 10.5) forms

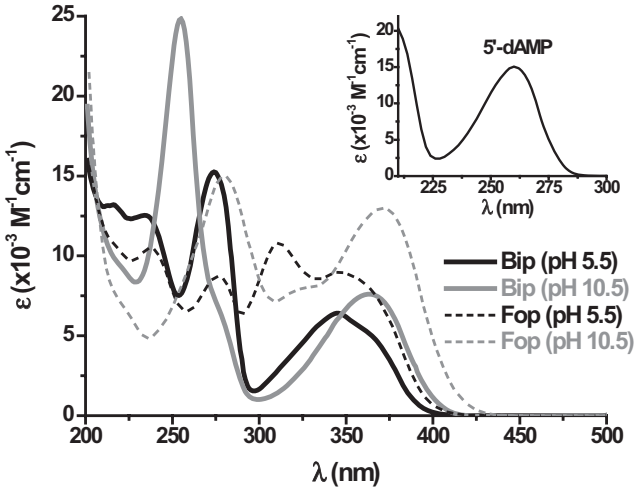


Figure 1. Absorption spectra of Bip (solid lines) and Fop (dashed lines) in air-equilibrated aqueous solution at pH 5.5 (black lines) and at pH 10.5 (gray lines). Absorption spectra of Ptr and Cap in acidic and alkaline solutions are almost identical to those shown for Bip. Inset: absorption spectrum of 5′-dAMP in aqueous solutions ($5.5 \leq pH \leq 10.5$).

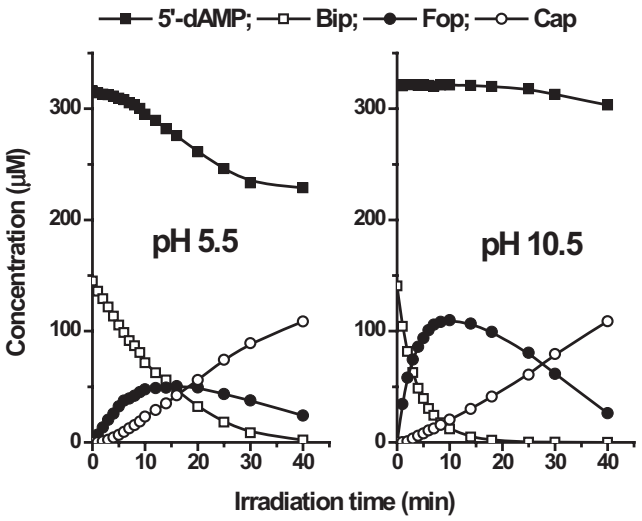


Figure 2. Evolution of the 5′-dAMP, Bip, Fop and Cap concentrations determined by high-performance liquid chromatography analysis in air-equilibrated aqueous solutions of Bip (≈150 μM) and 5′-dAMP (≈320 μM) exposed to UV-A radiation (350 ± 10 nm) as a function of the elapsed irradiation time at pH 5.5 and 10.5.

of the pterins derivatives Bip and Fop in aqueous solutions. The absorption spectra of Cap are almost identical to those observed for Bip at the same pH values. In all cases, the pterins derivatives are good UV-A absorbers with molar absorption coefficients $\varepsilon \approx 5\text{–}15 \times 10^3\ M^{-1}\ cm^{-1}$. Under these experimental conditions, only the pterin derivatives were excited with continuous UV-A radiation (350 ± 10 nm), as 5′-dAMP does not absorb in this spectral region in the studied pH range (Fig. 1).

To find out if the studied pterin derivatives (Bip, Fop and Cap) are able to photoinduce the degradation of 5′-dAMP, air-equilibrated solutions containing a given photosensitizer and the nucleotide were exposed to UV-A radiation for different periods of time. Figure 2 shows the kinetic profile of the pterins derivatives and 5′-dAMP determined by HPLC obtained by photosensitization of ≈150 μM Bip in aerated aqueous solutions at pH 5.5 and 10.5, respectively.

The concentration profiles of the pterin derivatives indicated that after the first min of irradiation, the oxidation of Bip is significant with sequential formation of Fop and Cap. However, despite of the faster phototransformation of Bip into Fop and Cap in basic than in acid conditions, no significant photosensitized degradation of the nucleotide 5′-dAMP was observed at pH 10.5.

In acid media, the consumption of 5′-dAMP is accelerated with the formation of the photooxidation products of Bip, in particular Fop, indicating the effective participation of these pterins derivatives in the nucleotide degradation. To confirm this assumption, separated experiments at pH 5.5 were performed using either Fop or Cap as initial photosensitizer. In both cases, HPLC analysis showed a decrease in the 5′-dAMP concentration as a function of irradiation time (data not shown). It is worth mentioning that the concentration profiles observed for Bip and its photoproducts were expected taking into account the previous studies on the mechanisms involved in the UV-A-induced photodegradation of Bip (15,16,25).

No thermal reactions between the pterins and 5′-dAMP were observed by keeping solutions containing both compounds in the dark. In another set of control experiments, air-equilibrated 5′-dAMP (~100 μM) solutions were irradiated at 350 nm in the absence of photosensitizers and no degradation of the nucleotide was detected, thus excluding spurious effects of direct light absorption by 5′-dAMP. Thus, these control experiments confirm the photosensitization action of Bip and its photoderivatives.

The HPLC analysis of experiments performed with Ar-saturated solutions containing Bip (117 μM) and 5′-dAMP (320 μM) at pH 5.5 irradiated for 30 min, indicated no degradation of 5′-dAMP. The same results were observed for Fop or Cap as initial photosensitizers under the same experimental conditions (data not shown). These results confirm the participation of dissolved O_2 in the photoinduced degradation of 5′-dAMP in the presence of Bip, Fop or Cap.

Therefore, the above results so far clearly demonstrate that Bip and its photoproducts are able to photosensitize the degradation of 5′-dAMP under UV-A irradiation only in aerobic acidic media.

Role of the excited triplet state of Bip and Fop

Steady-state photolysis. To investigate if the photosensitized processes are initiated by the triplet excited states of Bip and its photoproducts, photolysis experiments in the presence of 300 μM of potassium iodide (KI) and in O_2-saturated solutions were performed. It has been previously demonstrated that I^- at micromolar concentrations quenches efficiently only the triplet excited states of Bip and other oxidized pterins (26,27). The same effect is expected for dissolved O_2 at saturation concentration in water (≈1 mM), as O_2 does not quench singlet excited states of pterins (28), but it is an efficient quencher of their triplet states (22,29,30).

Figure 3 shows the kinetic profiles of 5′-dAMP concentration obtained by HPLC during UV-A photolysis in air-equilibrated solutions at pH 5.5 with either Bip or Fop as photosensitizer under different conditions. It can be observed that for both pterins the presence of 300 μM KI almost avoids the nucleotide degradation, while also in O_2-saturated solutions the rate of

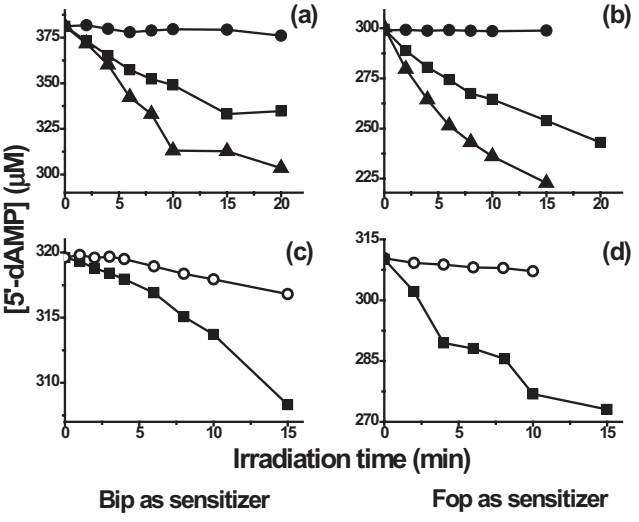


Figure 3. High-performance liquid chromatography profiles of the Bip (113 μM) or Fop (74 μM) photoinduced degradation of 5′-dAMP in aqueous solutions (pH 5.5) under UV-A irradiation (350 nm). Symbols: (■) Air-equilibrated solutions (controls); Air-equilibrated solutions with: (●) 300 μM of KI and (▲) 50 U/mL of superoxide dismutase; and (○) O_2-saturated solutions.

5′-dAMP disappearance is much lower than in air-saturated conditions. Therefore, both experiments strongly suggest the participation of the excited triplet states of the pterin derivatives in the photosensitized degradation of 5′-dAMP.

According to the evidence shown thus far, the photosensitized degradation of 5′-dAMP should start with an electron transfer from the nucleotide to the triplet excited state of the pterin derivative, as 5′-dAMP is not oxidized by $^1O_2$ (20,24). The feasibility of this electron transfer process can be evaluated by estimation of free energy change (ΔG) of the reaction by using the Eq. (1) (31):

$$\Delta G(\text{eV}) = \left[E_{(\text{dAMP}^{\bullet+}/\text{dAMP})} - E_{(\text{Pt}/\text{Pt}^{\bullet-})} - \frac{e_0^2}{\varepsilon R_{\text{D}^+\text{A}^-}}\right] - \Delta E^{\text{T}}_{0,0} \quad (1)$$

where $E_{(\text{dAMP}^{\bullet+}/\text{dAMP})}$ and $E_{(\text{Pt}/\text{Pt}^{\bullet-})}$ are the standard electron potentials of electron donor and acceptor, respectively. These values have already been reported for 5′-dAMP ($E_{(\text{dAMP}^{\bullet+}/\text{dAMP})} = 1.44$V *vs* NHE) (32) and for several pterin derivatives ($E_{(\text{Pt}/\text{Pt}^{\bullet-})} \approx -0.55$ V *vs* NHE) (33,34). The energy difference between the triplet excited and the ground states of the pterins $\Delta E^{\text{T}}_{0,0} \approx 2.52$ eV has been estimated from its phosphorescence spectra (33). The term $e_o^2/\varepsilon R_{\text{D}^+\text{A}^-}$ is the solvation energy of an ion pair D^+A^- and it can be ignored in the case of strong polar solvents. Therefore, an exergonic $\Delta G \approx -0.53$ eV ($\equiv -51.1$ kJ mol^{-1}) was calculated supporting that electron transfer from dAMP to the triplet excited state of the pterins can spontaneously occur.

It is well established that, in a typical type I process, ground state O_2 will readily quench an organic radical anion to produce the superoxide anion ($O_2^{\bullet-}$) (35,36). Accordingly, to investigate the participation of $O_2^{\bullet-}$ in the mechanism, experiments at pH 5.5 using either Bip or Fop as initial photosensitizer were carried out in the presence of SOD, an enzyme that catalyzes the conversion of $O_2^{\bullet-}$ into H_2O_2 and O_2 (37). The data showed a significant increase in the rate of 5′-dAMP consumption when SOD was present in the solution (triangle symbols in Fig. 3a and b). This fact suggests that elimination of $O_2^{\bullet-}$ inhibits a step that prevents the photoinduced degradation of 5′-dAMP, providing further evidence of an electron transfer-mediated process. One plausible explanation for the effect of SOD might involve the $O_2^{\bullet-}$-mediated reduction in an oxidizing adenine radical that is likely to be the 6-aminyl radical resulting from the deprotonation of the adenine radical cation (38). In that respect it has previously shown that $O_2^{\bullet-}$ reduces the highly oxidizing guanine radical that arises from the deprotonation of the guanine radical cation (39).

Laser flash photolysis. Direct evidence of the interaction of the excited triplet states of the pterin derivatives with 5′-dAMP and O_2 was provided by transient absorption spectroscopy. For Bip, it has been reported that upon excitation, ultrafast intramolecular proton transfer produces two tautomeric triplet state species, which decay simultaneously with fast and slow decay components assigned to the lactim and lactam tautomers, respectively (40,41). Recently, by ns-LFP experiments, we have determined lifetimes values of 0.34 (±0.04) μs and 2.5 (±0.5) μs for the two triplet excited states of Bip at pH 5.5 (22). In the present work, we have carried out a similar study for Bip at pH 10.5, and also biexponential decay behavior was observed for the excited triplet species with lifetimes of 0.5 (±0.1) μs and 2.6 (±0.5) μs, respectively.

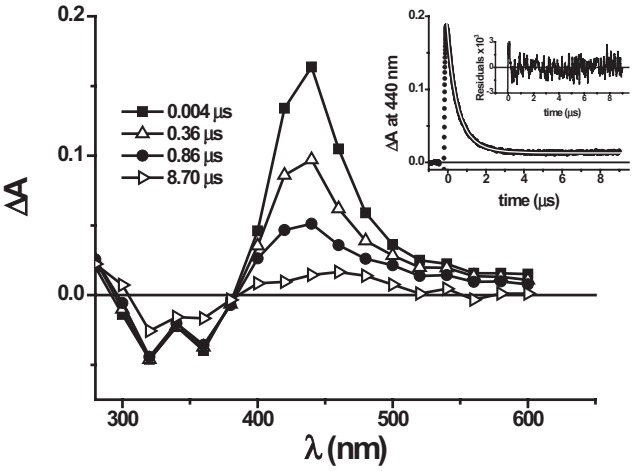


Figure 4. Transient absorption spectrum of Ar-saturated aqueous solutions of Fop (67 μM, pH 5.5) in the absence of 5′-dAMP obtained by laser flash photolysis experiments at different times after laser excitation at 355 nm (4 mJ/pulse, 8 ns FWHM). Inset: time dependence of the absorbance at 440 nm, with biexponential fitting (white line) and residuals analysis.

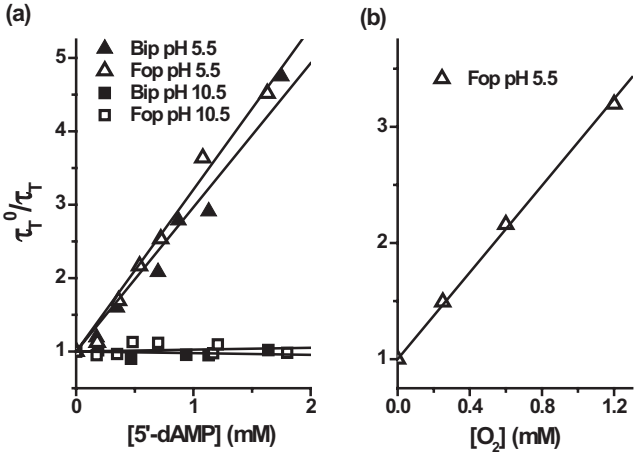


Figure 5. Stern–Volmer plots of the quenching of the long-lived triplet excited states of: (a) Bip or Fop by 5′-dAMP as a function of pH; (b) Fop by dissolved O_2 at pH 5.5. The triplet lifetime values were calculated analyzing the transient decay at 420 nm. [Bip] = 95 μM, [Fop] = 50 μM.

To the best of our knowledge, information about the triplet excited states of Fop has not been yet published. Therefore, we characterized the transient absorption spectrum of Ar-saturated Fop solutions at pH 5.5 and 10.5 with laser-pulsed excitation at 355 nm. The transient spectrum of Fop was very similar to that observed for Bip under similar conditions (22), as shown in Fig. 4 at pH 5.5, showing a strong absorption between 400 and 550 nm. The transient also shows biexponential decay behavior, with lifetimes of 0.8 (±0.2) μs and 2.9 (±0.6) μs at pH 5.5 and of 3.1 (±0.7) μs and 14 (±2) μs at pH 10.5, respectively. The observed transients could be assigned to the triplet excited states of Fop based on the following facts: (1) increase in the corresponding decay rates in the presence of O_2, (2) lifetimes are comparable to those reported for the triplet states of other pterin derivatives (42,43).

Quenching of Pt triplet excited states by 5′-dAMP and O_2. The interaction between the excited triplet states of Bip or Fop with 5′-dAMP or O_2 at both pH 5.5 and 10.5, was analyzed by the calculation of the corresponding rate constants of quenching of each transient (k_q^Q) using the Stern–Volmer equation [Eq. (2)], where τ_T^0 and τ_T are the lifetimes of the triplet states in the absence and presence of quencher Q (*i.e.* 5′-dAMP or O_2).

$$\frac{\tau_T^0}{\tau_T} = 1 + \tau_T^0 k_q^Q [Q] \qquad (2)$$

as it was observed before for the quenching of Bip by 2′-deoxyguanosine 5′-monophosphate (5′-dGMP) (22), only the longer lived excited triplet state corresponding to the lactam tautomer of the pterin derivatives was efficiently quenched by 5′-dAMP or O_2, indicating that mainly this species participates in the photosensitized process.

The corresponding Stern–Volmer plots with 5′-dAMP as quencher are shown in Fig. 5a, and it can be observed that 5′-dAMP only quenches the triplet excited states of Bip and Fop in acidic conditions, in fully agreement with the results of steady-state photolysis (see above), where 5′-dAMP was only degraded by both photoexcited Pt in acidic media, Fig. 2. At pH 5.5, both Bip and Fop triplet excited states are quenched by 5′-dAMP with similar values of k_q^{dAMP} of $1.1(\pm 0.2) \times 10^9$ M^{-1} s^{-1} and 1.3 $(\pm 0.2) \times 10^9$ M^{-1} s^{-1}, respectively. These results provide direct evidence for the participation of both triplet excited states of the pterin derivatives in the degradation of 5′-dAMP in acidic media.

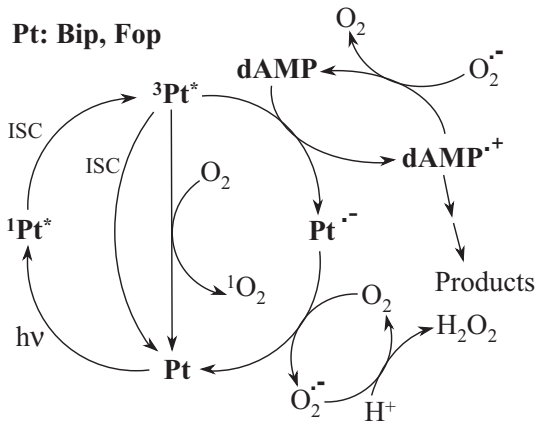
The strong pH effect on the variation in k_q^{dAMP} and therefore reactivity toward 5′-dAMP for both pterins could be explained in terms of electrostatic effects, as the acid and basic forms of pterins have a total of electrical charges of 0 and −1, respectively (Scheme 1). Therefore, coulombic repulsion between the alkaline form of pterins and the negative 5′-dAMP, very likely hinders the molecular interaction necessary to allow the electron transfer quenching process to proceed, avoiding the nucleotide photodegradation.

To explore in more detail, the effect of O_2 concentration on the degradation efficiency of 5′-dAMP by photosensitization with the pterin derivatives in acidic media, the quenching of the triplet excited states of pterins by O_2 must be evaluated and compared to the quenching by the nucleotide. The rate constant of the quenching of the long-lived triplet excited state of Bip by O_2 ($k_q^{O_2}$) has already been determined at pH 5.5 as 8 $(\pm 2) \times 10^8$ M^{-1} s^{-1} (22). In this work, also a $k_q^{O_2} = 8$ $(\pm 1) \times 10^8$ M^{-1} s^{-1} for the quenching of the long-lived triplet excited state of Fop by O_2 at pH 5.5 was calculated with Eq. (2) (Fig. 5b).

Considering the corresponding values of $k_q^{O_2}$ and k_q^{dAMP}, the competition between the quenching of the triplet excited state of a given pterin derivative (i.e. $^3Pt^* = {}^3Bip^*$ or $^3Fop^*$) by 5′-dAMP and by O_2 can be analyzed for the conditions used in the steady-state experiments. The fraction of $^3Pt^*$ quenched by 5′-dAMP in presence of O_2 (f_q^{dAMP}) is given by Eq. (3):

$$f_q^{dAMP} = \frac{k_q^{dAMP}[dAMP]}{(k_d + k_q^{O_2}[O_2] + k_q^{dAMP}[dAMP])} \qquad (3)$$

where k_d is the rate for the unimolecular decay of $^3Pt^*$ in the absence of quencher. For the 5′-dAMP concentration conditions used in the experiments of Fig. 3c and d, Eq. (3) yields f_q^{dAMP} values of 0.36 and 0.42 for Bip and Fop in air-equilibrated solutions, respectively, and values of 0.20 and 0.23 for Bip and Fop in O_2-saturated solutions, respectively. Therefore, the increment of O_2 concentration reduces significantly the quenching efficiency of the excited triplet states of both pterins derivatives by the nucleotide, in complete agreement with the decrease in the rate of 5′-dAMP consumption registered when the steady irradiation was carried out in O_2-saturated solutions.



Scheme 2. Proposed reaction mechanism of the oxidation of 5′-dAMP photosensitized by Bip and Fop.

CONCLUSIONS

We have proved that the acid forms of biopterin (Bip) and its photoproduct, 6-formylpterin (Fop), both compounds that accumulate in the skin of patients suffering from vitiligo, are efficient type I photosensitizers in the oxidation of 2′-deoxyadenosine 5′-monophosphate (5′-dAMP). In contrast, no evidence of a photochemical reaction induced by the basic forms of both pterins was provided. Therefore, considering a $pK_a \approx 8$ for the pterins, at physiological pH only the acid form fraction (≈ 0.5) could be responsible of the photosensitized degradation of the nucleobase.

As 5′-dAMP does not react with singlet molecular oxygen produced efficiently by UV-A excitation of both Bip and Fop, the mechanistic analysis indicates that the pterin-sensitized degradation of 5′-dAMP starts with the electron transfer from the 5′-dAMP molecule to the pterin derivative triplet excited state ($^3Pt^*$), leading to the formation of the corresponding ion radicals ($Pt^{\bullet -}$ and 5′-$dAMP^{\bullet +}$), Scheme 2. In the following step, the radical ions may recombine or react with O_2 to yield degradation products. The electron transfer from $Pt^{\bullet -}$ to O_2 regenerates the ground state of Pt and forms the superoxide anion $O_2^{\bullet -}$. Finally, this species may disproportionate to its conjugated acid ($HO_2^{\bullet}$) to form H_2O_2 or react with 5′-$dAMP^{\bullet +}$ to regenerate 5′-dAMP. The degradation product pattern of 5′-dAMP obtained by photosensitization of these vitiligo-related pterins, could be expected similar to that found by means of electrospray ionization mass spectra using the parent pterin Ptr as photosensitizer (20). In this case, 8-oxo-7,8-dihydro-2-deoxyadenosine 5-monophosphate (8-oxodAMP) and a tetracyclic compound (8-P-dAMP) were characterized as the two major nucleotide degradation products. 8-OxodAMP has been shown to arise from 8-hydroxy-7,8-dihydroadenyl radical upon one-electron oxidation (38,44). The same

degradation products were found for the oxidation of 5′-dAMP photosensitized by lumazine (23), compound structurally related to pterins. Forthcoming analytical experiments are planned to confirm or not if the breakdown pattern of 5′-dAMP is independent on the nature of excited triplet state of the pterin derivative.

However, in this study we have shown kinetic and mechanistic evidences that pterin derivatives presented in the affected skin where the protection against UV radiation fails, can damage DNA components by UV-A photosensitization, being these results relevant from a biomedical point of view.

Acknowledgements—This work has been supported in part by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-grant PIPs 0425/12 and 0374/12), Agencia de Promoción Científica y Tecnológica (ANPCyT-grant PICTs 2005-33919, 2006-01482, and 2012-2666), Universidad Nacional de La Plata (UNLP-grant X586), and Universidad Nacional de Santiago del Estero (UNSE-grant 23A/162). M.P.S. thanks CONICET for doctoral research fellowship. C.D.B. and A.H.T. are research members of CONICET.

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