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Unveiling Photodegradation and Photosensitization Mechanisms of Unconjugated Pterins

Enrique M. Arpa* and Inés Corral*

Abstract: Unconjugated pterins are ubiquitous molecules that participate in countless enzymatic processes and are potentially involved in the photosensitization of singlet oxygen, amino acids, and nucleotides. Following electronic excitation with UV-A light, some of these pterins degrade, producing hydrogen peroxide as the main side product. This process, which is known to take place in vivo, contributes to oxidative stress and melanocyte destruction in vitiligo. In this work, we present for the first time mechanistic insight into the formation of transient triplet species that simultaneously trigger Type I and Type II photosensitizing processes and the initiation of degradation processes. Our calculations reveal that photodegradation of 6-biopterin, which accumulates in the skin of vitiligo patients, leads to 6-formylpterin through a retro-aldol reaction, and subsequently to 6-carboxypterin through a water-mediated aldehyde oxidation. Additionally, we show that the changes in the photosensitizing potential of these systems with pH come from the modulation of their excited-state redox potentials.

Introduction

Pterins are a class of biomolecules that are present in every kingdom of life, from the smallest bacteria to the most complex vertebrates. Structurally, pterins are based on a pteridine heterocycle, or pyrazino[2,3-d]pyrimidine, substituted with an amino group at C_2 and an endocyclic carbonyl moiety at C_4; this scaffold is actually called pterin (PT) itself (Figure 1). Pterin derivatives, which are biosynthesized using guanosine triphosphate as precursor,[1] can differ in the protonation state of the N_3 position, which has a pK_a around 8, the oxidation state of the pyrazine ring, and the nature of the substituents. Pterins carrying a p-aminobenzoyl glutamate at C_6 are called conjugated pterins, and vitamin B9 (folic acid, FA) belongs to this class. If the substituent is a small functional group or a short alkyl chain, these derivatives are denominated unconjugated pterins, and 6-biopterin (BPT), 6-formylpterin (FPT), and 6-carboxypterin (CPT) are prominent examples (Figure 1). Pterins are involved in a myriad of biological functions, working as pigments, nucleotide precursors, and cofactors for different enzymes.[2–7] Some pterin derivatives, such as methotrexate, are employed for medical purposes, being effective for the treatment of autoimmune diseases.[8] Pterins present interesting light-responsive properties, which are mildly affected by the substitution pattern (Table 1). Pterins are involved in a myriad of biological functions, working as pigments, nucleotide precursors, and cofactors for different enzymes. Some pterin derivatives, such as methotrexate, are employed for medical purposes, being effective for the treatment of autoimmune diseases. Pterins present interesting light-responsive properties, which are mildly affected by the substitution pattern (Table 1). Their maximum absorption lies in the UV-A region of the electromagnetic spectrum. Electronic excitation has been reported to trigger different photophysical processes. First, pterins are emissive species, which emit blue light.[9–11] In the

Figure 1. General structure of the pterin core (top left) and some examples of pterins. The atom numbering scheme will be used throughout the manuscript. PT: Pterin, BPT: 6-biopterin, FPT: 6-formylpterin, CPT: 6-carboxypterin, FA: Folic acid.
presence of $^3$O$_2$ pterins become singlet oxygen photosensitizers.$^{[12]}$ Pterins have also been reported as photosensitizers of an ample group of biomolecules,$^{[13–18]}$ such as aromatic amino acids$^{[15–18]}$ DNA plasmids,$^{[19]}$ and purine nucleotides.$^{[20–22]}$ Interestingly, the mechanism underlying biomolecule photo-oxidation by pterins is highly dependent on the pH of the environment. This way, acidic media, where the neutral form predominates, favor Type I reactions (single electron transfer), with little to no participation of singlet oxygen.$^{[15–22]}$ In alkaline media, where deprotonated forms (upon loss of the N$_2$H proton) predominate, there is either no photosensitization or it takes place through a Type II reaction (oxidation mediated by singlet oxygen)$^{[15,14,17,18,20,21]}$ Up to date, there is no explanation for the differential photoactivity upon variation of the pH.

The low quantum yields reported for FA suggest that very efficient internal conversion to the ground state is facilitated by the p-aminobenzoyl glutamate.$^{[23]}$ In contrast, unconjugated pterins were found to undergo photodegradation with UV-A light. Irradiation of BPT leads to the formation of FPT, along with one equivalent of acetaldehyde and one of H$_2$O$_2$.$^{[24,25]}$ In 1977, Knappe and co-workers proposed decomposition of BPT to occur through Norrish type II photocleavage,$^{[26]}$ by hydrogen atom transfer from the terminal methyl group to the $N_2$ position followed by cleavage of the alkyl chain, producing FPT (cf Figure 1). These works report that the reactions proceed from the triplet state of these pterins. Moreover, if the irradiation of BPT and FPT takes place in total absence of O$_2$, then labile reddish intermediates are formed, which are rapidly oxidized if O$_2$ is introduced in the medium. In these experiments, H$_2$O$_2$ was always generated after O$_2$ incorporation.$^{[27]}$

NMR spectroscopy experiments suggested that the reddish intermediates correspond to dihydropterins.$^{[27]}$ Further irradiation of FPT leads to its oxidation to CPT and the formation of another equivalent of H$_2$O$_2$.$^{[28]}$ Finally, while CPT also undergoes photodegradation, to PT and other non-pteridinic products,$^{[29]}$ the extremely low quantum yield for this process, even lower than that of FA, suggests that it is a photostable system.

Several succeeded in unravelling the early stages of the excited-state dynamics that lead to photodegradation and photosensitization reactions. In 1981, Santos and co-workers, by combining microsecond transient absorption (TAS) and phosphorescence spectroscopies, identified a long-lived triplet state,$^{[30]}$ consistently with the high singlet oxygen quantum yields experimentally found ($\Phi_{\Delta \Omega}$, Table 1). From the triplet state, a semireduced species is formed upon hydrogen atom abstraction from a solvent molecule, with a quantum yield of 0.06 (Table 1). This semireduced species gives back the hydrogen to repopulate the ground state, describing the hydrogen atom a sort of U-turn motion. This relaxation mechanism supports the proposed first step in the photodegradation of BPT.$^{[28]}$ By employing femtosecond TAS, Crespo-Hernández and co-workers demonstrated that intersystem crossing to the triplet manifold occurs in the sub-picosecond time scale.$^{[29]}$ They also proposed that the populated $\pi\pi^*$ states decay to the ground state via hydrogen atom U-turn mechanism or singlet oxygen sensitization.

The electronic relaxation pathways along the singlet states were studied by Cao and co-workers using multiconfigurational simulations.$^{[30]}$ According to the authors, UV-A irradiation of PT in its anionic (deprotonated) form, would lead to the population of a planar $\pi\pi^*$ minimum. Deactivation from this minimum along the singlet manifold would be hampered by the high energy barrier (29.9 kcal mol$^{-1}$, 1.30 eV) necessary to access the S$_1$/S$_0$ conical intersection, pumeked at the $C_i$ position. Despite the decay of the system along the singlet $\pi\pi^*$ has been explored, a complete description of the potential singlet and triplet competing deactivation routes is lacking.

The photochemistry of these unconjugated pterins plays a crucial role in vitiligo, an autoimmune disorder characterized by the apparition of white skin areas due to melanin down-regulation. This depigmentation leads to a disturbance in the protection mechanisms against UV light exposure.$^{[31]}$ The biologically active form of BPT is 5,6,7,8-tetrahydrodibopterin (BPTH$_2$), which is found as cofactor for multiple enzymes.$^{[32]}$ In vitiligo-damaged tissues, an abnormally high concentration of H$_2$O$_2$ causes the oxidation of BPTH$_2$ to BPT, which is toxic for melanocytes.$^{[33]}$ As a consequence, all BPTH$_2$-dependent enzymes are shut down. Deactivation of phenylalanine hydroxylase hampers the formation of new melanin, as the first step in melanin synthesis is the formation of tyrosine from phenylalanine. With lower levels of melanin, BPT can absorb higher doses of UV-A light, causing further damage, either by forming singlet oxygen or by self-degrading. The photodegradation of BPT to FPT and CPT has been demonstrated to occur in vivo,$^{[34]}$ amplifying the oxidative stress due to the concomitant formation of H$_2$O$_2$.

In this work, we provide a thorough description of the photochemistry of unconjugated pterins using high level computational simulations. In particular, we present the photo-physics of the PT chromophore including singlet and triplet states, several plausible pathways for the photodegradation reactions, and the thermodynamic feasibility of photosensitization reactions at different pH conditions.

### Results and Discussion

As most of the experimental measurements for the photo-physics of the chromophore and the photodegradation reactions have been recorded at pH above 8.0, to ensure that the $N_2$ position is mostly deprotonated and that the chromophore is in its anionic state, we have carried out all the simulations

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**Table 1. Absorption ($\lambda_a$) and fluorescence ($\lambda_f$) wavelengths in nm, and quantum yields of fluorescence ($\Phi_F$), singlet oxygen photosensitization ($\Phi_{\Delta \Omega}$) and photodegradation ($\Phi_{\Delta \Omega}$) of different pterins at alkaline pH.$^{[15–22]}$**

<table>
<thead>
<tr>
<th>Pterin</th>
<th>$\lambda_a$</th>
<th>$\lambda_f$</th>
<th>$\Phi_F$</th>
<th>$\Phi_{\Delta \Omega}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>358</td>
<td>456</td>
<td>0.27</td>
<td>0.30</td>
</tr>
<tr>
<td>BPT</td>
<td>363</td>
<td>455</td>
<td>0.29</td>
<td>0.40</td>
</tr>
<tr>
<td>FPT</td>
<td>370</td>
<td>454</td>
<td>0.07</td>
<td>0.47</td>
</tr>
<tr>
<td>CPT</td>
<td>364</td>
<td>451</td>
<td>0.18</td>
<td>0.31</td>
</tr>
<tr>
<td>FA</td>
<td>365</td>
<td>452</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

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focusing on the deprotonated forms. The terms “anionic” and “deprotonated” will be used indistinctly for the identification of such species. Experimental spectroscopic measurements have determined that anionic PT shows a strong absorption in the UV-A region of the spectrum, with a single absorption band centered around 360 nm.11,29 This is in satisfactory agreement with our MS-CASPT2 simulations (Table 2), which revealed that this absorption band corresponds to a \( S_1 \rightarrow \pi\pi^* \) transition.

Figure 2 shows the topography of the CASPT2//CASSCF potential energy surfaces of the states which are accessible upon irradiation with UV-A light. Further computational details can be found at the end of this manuscript and in Section 1 of the Supporting Information. Consistently with the calculations of Cao and co-workers,32 the excited population will initially decay from the FC region (3.48 eV)11 to a \( \pi\pi^* \) planar minimum, labeled \( S_1A \), at 3.29 eV. From this minimum, two possible pathways for ground-state repopulation were considered. Radiative decay would produce fluorescence vertical emission at 2.95 eV (420 nm; oscillator strength of 0.062), in good agreement with the experimental fluorescence wavelength of 456 nm.11

Alternatively, non-radiative decay could take place through a \( \pi\pi^*/S_0 \) conical intersection at 4.49 eV, labeled C2P. This crossing lies, however, 1 eV above the initial \( S_1 \) FC energy hampering internal conversion through this funnel. These results are in excellent agreement with the findings of Cao and co-workers for the dynamics of the \( \pi\pi^* \) state.11

In contrast to previous works, a region of the \( S_1 \) state of \( \pi\pi^* \) character was successfully located. A TS located in the vicinity of the \( S_1/S_2 \) crossing point at 3.47 eV allows population transfer from \( S_1A \) to a new minimum, labeled \( S_2B \) on Figure 2. This new minimum also presents a planar structure, but with an elongated \( C=O \) bond (1.34 Å) compared to that of \( S_1A \) (1.25 Å).

Table 2. Vertical excitation energies and oscillator strengths for the two lowest-energy singlet states of anionic PT at the MS-CASPT2//ANO-L-VTZ level of theory in gas-phase. See Section 1 of the Supporting Information for orbital labels.

<table>
<thead>
<tr>
<th>State</th>
<th>( \Delta E ) [eV] (nm)</th>
<th>( f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S_1(n\pi^*) )</td>
<td>3.40 (364)</td>
<td>0.068</td>
</tr>
<tr>
<td>( S_2(n\pi^*) )</td>
<td>3.61 (343)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

No fluorescence is expected from the \( S_2B \) minimum, considering the \( S_1 \rightarrow S_0 \) oscillator strength (ca. 10 \(^{-4} \)). The energy of such transition (2.37 eV, 524 nm) does not match the experimentally observed value either. Non-radiative decay from the \( \pi\pi^* \) minimum to the ground state is also prevented, considering the high energy of the \( \pi\pi^*/S_0 \) conical intersection. This funnel, labeled C4CO, is characterized by a drastic reduction of the \( N–C=O \) angle (from 114.6° in \( S_2B \) to 83.5°).

Considering that ground-state repopulation from the singlet manifold is heavily hampered, aside from the \( \pi\pi^* \) fluorescence, possible pathways for intersystem crossing to triplet states were explored. At the position of the \( S_1A \) minimum, the \( \pi\pi^* \) state is vertically located at 3.81 eV and the spin-orbit coupling (SOC) amounts to 32 cm\(^{-1} \). Nonetheless, a \( S_1/T_1 \) crossing point at 3.56 eV was located, in which the energy gap between both states vanishes, and the SOC slightly increases to 34 cm\(^{-1} \). The population reaching the \( \pi\pi^* \) is expected to rapidly decay to a \( \pi\pi^*/S_0 \) minimum \( (T_1, 2.74 \text{ eV}) \), considering the proximity of the \( T_2/T_1 \) crossing point to the \( T_0 \) minimum. There is an alternative pathway for the population to reach the \( \pi\pi^* \) state, starting from the \( \pi\pi^* \). At the position of the \( S_2B \) minimum, the energy difference between the \( \pi\pi^* \) and \( \pi\pi^*/S_0 \) states is 0.20 eV, with the \( \pi\pi^* \) lying below. However, a remarkable SOC between those states of 57 cm\(^{-1} \) was found at this region of the PES. Direct \( \pi\pi^* \rightarrow \pi\pi^* \) intersystem crossing at \( S_2B \) to finally reach the most stable \( \pi\pi^* \) minimum is thus feasible. Therefore, two relaxation pathways that would drive the population to the \( \pi\pi^* \) state are proposed:

1. \( S_1FC \rightarrow \pi\pi^*/(S,A) \rightarrow \pi\pi^*/n\pi^*/(S,T_1) \rightarrow \pi\pi^*/(T_1,T_1) \rightarrow \pi\pi^*/(T_1,T_0) \rightarrow \pi\pi^*/(S,T_0) \rightarrow \pi\pi^*/S_0 \)
2. \( S_1FC \rightarrow \pi\pi^*/(S,A) \rightarrow \pi\pi^*/(C_1,C_2) \rightarrow \pi\pi^*/(S,B) \rightarrow \pi\pi^*/(S,T_1) \rightarrow \pi\pi^*/(T_1,T_1) \)

Interestingly, all these points of the excited potential energy surface have a planar geometry, and only mild deformations are observed. Thus, the relaxation to the \( \pi\pi^* \) state is not geometrically driven, and thus compatible with a fast process.

To corroborate our proposal, we simulated the transient absorption spectra, at the position of the different singlet and triplet minima (Figure 3 and Section 1.3 in the Supporting Information), and compared it with the experimentally recorded results.39 These experiments show, at very short time delays (< 500 fs), an initial absorption at about 550 nm and stimulated emission at about 450 nm. The positive absorption signal is...
consistent with the population of 1ππ* (S, A). After some time (5 ps onwards), the 550 nm absorption band is slightly blue-shifted and loses intensity, stimulated emission vanishes, and a new absorption band centered around 400 nm arises. These observations can be related to the depopulation of the 1ππ* state in favor of 0ππ* (absorbing at 450 nm) and 3ππ* (T, 1), with an absorption centered at 400 nm. Therefore, these results heavily support the second proposed deactivation pathway.

The results presented herein are consistent with the experimental observations of formation of long-lived triplet states. First, the calculated vertical T1→S0 emission (2.48 eV, 500 nm) coincides with the experimental phosphorescence wavelengths (480 nm for PT, 505 nm for 3-methylpterin). Also, intersystem crossing to S0 would be energetically prevented since the 3ππ*/S0 crossing points coincide with the C2P and C4CO funnels, which lie up to 2 eV higher in energy relative to the T1 minimum. Therefore, it is safe to assume that all subsequent photochemical processes will occur from the 3ππ* state. This applies not only to PT, but also to all unconjugated pterins (such as BPT, FPT and CPT), which all share the PT chromophore and the spectroscopic features (cf Table 1).

In the following, we will rationalize the photosensitizing properties of unconjugated pterins which are known to be dependent on the ambient conditions, in particular on the pH of the solution. Both Type I (photooxidation of the substrate) and Type II (generation of singlet oxygen) reactions have been documented for these systems. However, as stated in the introduction, while in acidic media both reactions can take place, in alkaline solutions only Type II reactions are observed, if any. The feasibility of Type II reactions can be estimated from the triplet energy of the sensitizer. For all the pterins considered, and independently of their protonation state, the triplet energies, both vertical and adiabatic, are at least 2.5 times larger than 0.98 eV, the 0O2←O2 energy difference (Table 3). Thus, we conclude that unconjugated pterins can act as 1O2 photosensitizers at all pH conditions. To assess the viability of Type I reactions it becomes mandatory to calculate the excited-state redox potentials for the photoreduction of these pterins. Using the ground-state redox potentials and the adiabatic excited-state energies, we calculated the excited-state redox potentials as described elsewhere. Here, deprotonation leads to a drastic decrease of the redox potentials, with differences with respect to those of the neutral forms up to 0.7 V. This can be understood in terms of pure electronic repulsion. Upon deprotonation, unconjugated pterins naturally gain a negative charge which can be delocalized along the π system. A reduction reaction would imply adding an extra electron to an anionic system and displacing the excited-state redox potential below the redox potential for the oxidation of most organic substrates, preventing the reaction. Additionally, it is worth highlighting that the redox potential of CPT is larger than that of BPT. Therefore, the in vivo photodegradation of BPT into CPT in vitiligo patients contributes to a larger oxidative stress not only due to concomitant formation of H2O2, but also to the formation of stronger photosensitizing pterins.

Next, we will revisit and/or examine the photodegradation mechanism of the unconjugated pterins of the interest of this work, triggered from the long-lived triplet state T1. As commented before, UV-A irradiation leads to the breaking of the alkylation chain in BPT, releasing acetaldehyde and dihydrofumarylpterin. The latter is then oxidized furnishing FPT. According to Knappe et al. (Figure 4, red pathway), the first transformation occurs in two steps. First, a Norrish type II photocleavage of a C–H bond in the terminal methyl group of BPT induces an intramolecular hydrogen migration, forming the 1,4-biradical 11a. Subsequent C–C0 dissociation releases acetaldehyde and 12, or 5,0-dihydrofumarylpterin. Oxidation of this product by O2 yields FPT and H2O2. Analysis of the 1BPT→12 potential energy profile showed two main features. First, according to spin density analysis on the [12+CH2CHO] complex in its lowest-energy triplet state, acetaldehyde is released as a closed-shell singlet and 12 as an open-shell triplet. And second, the process is stepwise with quite large activation barriers (0.88 eV for 1BPT→TS1a, 0.90 for 11a→TS2a).

Other two alternative mechanisms for the degradation of BPT were explored. Considering the much higher acidity of OH groups with respect to CH3, intramolecular hydrogen migration from C–OH (Figure 4, green pathway) and C–OH (Figure 4, blue pathway) were also evaluated. For the former, the initial

<table>
<thead>
<tr>
<th>Pterin</th>
<th>ΔE0↑ (eV)</th>
<th>ΔE0↓ (eV)</th>
<th>E* (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>3.04</td>
<td>2.72</td>
<td>1.66</td>
</tr>
<tr>
<td>BPT</td>
<td>2.73</td>
<td>2.57</td>
<td>2.19</td>
</tr>
<tr>
<td>FPT</td>
<td>2.83</td>
<td>2.59</td>
<td>2.14</td>
</tr>
<tr>
<td>APT</td>
<td>2.75</td>
<td>2.66</td>
<td>2.40</td>
</tr>
<tr>
<td>CPT</td>
<td>2.78</td>
<td>2.60</td>
<td>2.48</td>
</tr>
</tbody>
</table>
hydrogen migration is considerably more favorable, with an activation barrier of 0.50 eV. Nonetheless, the resulting 1,3-biradical \( ^11b \) cannot undergo \( C_7-C_8 \) bond breaking. 1,2-H shift from \( C_7 \) to \( C_6 \) would lead to the formation of 6-lactoylpterin (also referred to as sepiapterin, SPT). As SPT was not detected in photodegradation experiments, this pathway must be hampered by the high energy of \( ^3\text{TS}_2c \), the transition state for the 1,2-H shift.

The hydrogen abstraction from \( C_7-OH \), blue path, forms a new 1,4-biradical, \( ^11c \), with an activation barrier (0.48 eV) comparable to that of \( C_7-OH \). However, dissociation of the \( C_7-C_8 \) bond in \( ^11c \) is a barrierless process. The \( ^1\text{BPT} \rightarrow ^1\text{I}^1c \rightarrow ^1\text{I}^2c \) sequence can be identified as a retro-aldol reaction, as it starts from a \( \beta \)-hydroxyiminium (N=\( C_7-C_8-C_9-OH \) fragment in \( ^1\text{BPT} \) which is transformed into an aldehyde (acetaldehyde) and an enamine (H—N=C—C=C—N=C in \( ^1\text{I}^2c \)). Moreover, the reddish coloration observed in anaerobic conditions, in which the final \( ^1\text{I}^2c \rightarrow ^1\text{FPT} \) oxidation cannot take place, is ascribed to the spectroscopic features of some tautomeric forms of \( ^1\text{I}^2c \) (see Section 4 of the Supporting Information), which show absorption in the blue region of the spectrum. Therefore, our results suggest that the photodegradation of BPT to FPT occurs via retro-aldol reaction followed by dihydroformylpterin air oxidation.

Despite the short lifetime expected for the triplet \( ^3\pi\pi^* \) given the proximity of the \( T_2/T_1 \) crossing (cf Figure 2), the retro-aldol pathway starting from the \( ^3\pi\pi^* \) state was also explored (Section 5.1 of the Supporting Information). Strikingly, while both pathways converge to the formation of \( ^1\text{I}^1c \), the activation barrier for the first step is considerably larger for the \( ^3\pi\pi^* \) state (0.66 eV) than for the \( ^1\pi\pi^* \) (0.46 eV). This contradicts the previously proposed relaxation pathway for PT, which assumed that \( ^1\pi\pi^* \) states are more prone to undergo hydrogen atom abstraction.\(^{[29,30]}\) It has been recently proposed that the relief of antiaromaticity is the main driving force for excited-state (intramolecular) proton transfer in aromatic molecules.\(^{[39,40]}\) It is thus plausible that excited-state (anti)aromaticity modulates the height of the activation barriers of the retro-aldol reaction in \( ^1\text{I}^1c \). In fact, NICS-scans\(^{[41,42]}\) (nucleus-independent chemical shift) on the pyrazine subunit of the pterin chromophore register a decrease of antiaromaticity along the \( ^1\text{BPT} \rightarrow ^1\text{I}^2c \) retro-aldol reaction coordinate (Section 5.2 of the Supporting Information). These results confirm that the loss of excited-state antiaromaticity facilitates the retro-aldol pathway by lowering the activation barriers in the \( ^3\pi\pi^* \) state.

Finally, the potential pathways behind the formation of CPT upon UV-A irradiation of FPT are discussed. In aqueous solution, aldehydes can coexist with their hydrated acetal-like forms. Thus, the pathways with 6-dihydroxymethylpterin (APT) as starting point were also evaluated. Figure 5 shows the calculated photodegradation pathways for FPT (left) and APT (right). Starting with APT and after population of the \( ^3\pi\pi^* \) state, reaction with \( O_2 \) produces CPT in a single step, upon \( O-H \) and \( C_7-H \) hydrogen abstraction. This direct oxygen cannot proceed in FPT. However, ground-state aldehydes can undergo \( O_2 \) insertion into the \( C-H \) bond to form a peracid in a spin-allowed process.\(^{[43]}\) Thus, the \( T_1 \) of FPT can react with \( ^1\text{O}_2 \) with the same outcome. Indeed, reaction of \( ^1\text{FPT} \) with \( ^1\text{O}_2 \) produces 6-peroxyacylpterin, I4, in a two-step process. First, \( ^1\text{O}_2 \) insertion into the \( C_7-H \) bond forms the zwitterionic intermediate I3 in a barrierless way. Then, migration of the 6-acetylpterin group to the terminal oxygen yields the peracid. There are two main pathways from which I4 can lead to the formation of CPT. The first one is hydrolysis.\(^{[44]}\) Addition of a solvent water molecule would form intermediate I5a, concomitant to the loss of hydrogen.

Figure 4. Proposed degradation pathways of BPT upon UV-A excitation and population of the lowest-lying \( ^3\pi\pi^* \) state. Gibbs free energies are given in eV relative to the ground state of BPT (superscripts denote the spin multiplicity). Red profiles correspond to the mechanism proposed by Knapp et al.,\(^{[20]}\) while blue and green profiles correspond to alternative mechanisms proposed in this work.

\[ \text{BPT} \rightarrow \text{I}^1c \rightarrow \text{I}^2c \rightarrow \text{I}^3c \rightarrow \text{I}^4c \rightarrow \text{I}^5c \rightarrow \text{I}^6c \]
peroxide. The second I4—CPT pathway is by Baeyer-Villiger oxidation,[45] which is discussed in Section 5.3 of the Supporting Information. No H₂O₂, a byproduct experimentally detected,[26] is expected in the Baeyer-Villiger pathway suggesting that the predominant mechanism is the peracid hydrolysis.

The presented photodegradation pathways for FPT and APT show two critical differences with respect to the retro-aldol degradation of BPT. First, for FPT/APT, oxygen is incorporated at the first step of the degradation of both molecules, while for BPT it participates in the last one. And second, also for FPT/APT, none of the intermediates correspond to a dihydrocarboxypterin (CPTH₂). However, anaerobic irradiation of FPT leads to the formation of a labile reddish intermediate, which forms CPT and H₂O₂ on admission of O₂.[23] Therefore, there has to be an alternative mechanism for the photodegradation of FPT and APT that can operate when oxygen is removed from the reaction mixture and that forms a CPTH₂-like intermediate. Figure 6 shows the calculated pathways for the anaerobic

Figure 5. Proposed degradation pathways of FPT upon UV-A excitation and population of the lowest-lying 1ππ* state in the presence of molecular oxygen. Gibbs free energies are given in eV relative to the ground state of FPT (superscripts denote the spin multiplicity).

Figure 6. Proposed anaerobic degradation pathways of FPT upon UV-A excitation and population of the lowest-lying 3ππ* state. Reaction of molecular oxygen occurs in the last stage. Gibbs free energies are given in eV relative to the ground state of FPT (superscripts denote the spin multiplicity).
photodegradation of FPT (left) and APT (right). For FPT, a water-mediated Norrish type I photo cleavage triggers an intramolecular hydrogen migration from C1 to N3, forming the 1,2-biradical \( \text{I6a} \). The effect of catalytic water molecules in several activation barriers is discussed in detail in Section 5.4 of the Supporting Information. Water addition forms \( \text{I7} \), or 5,6-dihydrocarboxypterin. This product is also observed in the photodegradation of APT, after intramolecular hydrogen migration from one OH to N3 to form the 1,3-biradical \( \text{I6a} \) followed by 1,2-H shift. As for I2, some tautomers of I7 would absorb in the blue region of the spectrum and produce a reddish coloration, and reaction with \( \text{O}_2 \) produces both CPT and \( \text{H}_2\text{O}_2 \).

A comparison of the aerobic and anaerobic photodegradation pathways for FPT in Figures 5 and 6 reveals that the first steps are barrierless in the former. The aerobic pathways presented in Figure 5, however, cannot proceed at any degree if \( \text{O}_2 \) is not present in the environment. Thus, the presence or absence of \( \text{O}_2 \) is key to determine the preferred photodegradation pathway. If it is absent, only the anaerobic pathway is viable. On the other hand, if present, the more favorable aerobic pathway will be followed. To corroborate this hypothesis, the identification of the peracid I4 is required. This can be an arduous task, considering that the activation barriers for its hydrolysis are low, and that its spectral features do not significantly differ from those of FPT or CPT (Section 4 of the Supporting Information).

**Conclusion**

All in all, this work scrutinizes the photochemistry of unconjugated pterins at the molecular level, by using multiconfigurational and TD-DFT calculations. Specifically, we propose a mechanism for the ultrafast population of the most stable triplet \( \pi\pi^* \) state for their common chromophore, pterin; this is supported by previous transient absorption spectra. This state is the one responsible for the photosensitizing properties of these systems and the starting point for subsequent photodegradation processes. Here we provide a rationalization for the pH-dependent photosensitizing properties of pterins. In acidic solutions, the excited-state redox potentials are sufficiently large to allow Type I photosensitization reactions. However, upon increase of the pH, these potentials are significantly lowered, and only Type II reactions through singlet oxygen sensitization are possible. Our calculations also reveal that the degradation of bioppterin to formylpterin follows a retro-aldol pathway that leads to a dihydroformylpterin intermediate with a reddish hue that can be rapidly oxidized to formylpterin.

For the degradation of formylpterin and its hydrated form, two different mechanisms were identified; they operate depending on the presence or absence of oxygen. These results seem to be universal for all unconjugated pterins, and could be of high relevance in understanding the photochemical evolution of some skin diseases, such as vitiligo.

**Computational Methods**

*Photophysics of the pterin chromophore*: Potential energy minima and conical intersections were optimized at the XMS4-CASP72/cc-pVQZ level of theory using a SA4-CASSCF(12,11) wave function as reference. Final energies were calculated at the MS4-CASP72/ANO-L-VTZ level. Photosensitization and photodegradation: Geometries of minima and transition states were optimized at the M06-2X/cc-pVTZ level of theory in gas-phase. More accurate electronic energies, nucleus-independent chemical shifts, and excitation energies were calculated on top of the optimized geometries at the M06-2X/aug-cc-pVTZ level including implicit water solvation with the SMD model.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords:** ab initio calculations · DFT calculations · photodegradation · pterin · vitiligo


[34] Minimal discrepancies with respect to Table arise from the different number of states and size of the active spaces.

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