Journal of Photochemistry and Photobiology 7 (2021) 100045

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology

journal homepage: www.elsevier.com/locate/jpap

A model to understand type I oxidations of biomolecules photosensitized by pterins

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ARTICLE INFO

Keywords:
Pterins photosensitization singlet oxygen radicals photooxidation

ABSTRACT

Photosensitized oxidations, in part responsible for the harmful effect of UV and visible radiation on biological systems, can take place through type I (generation of radicals) and type II (singlet oxygen) mechanisms. Pterins are heterocyclic compounds, widespread in living systems and involved in relevant biological functions. Pterins present a profuse and amazing photochemistry and are endogenous photosensitizers that act mainly *via* type I mechanism. This survey is aimed to contribute to a better understanding on the complex set of competitive pathways involved in type I photosensitization. Based on studies performed in model systems with pterins as photosensitizers, this review explores the mechanisms involved in the type I photooxidations of proteins, DNA and lipids sensitized by pterins. The generation of radicals and their subsequent reactions are described together with the analysis of the role of O_2 and reactive oxygen species. The chemical modification of pterins seeking for better properties and the effect of the photochemical processes at a cellular level are also analyzed.

1. Introduction

Electromagnetic radiation induces modifications in biomolecules. The direct damage is initiated by the absorption of photons by nucleobases, aromatic amino acids and other chromophores present in living systems, generating excited electronic states, which leads to many photochemical reactions. Alternatively, the indirect damage takes place through photosensitized reactions, which are defined as photochemical alterations occurring in one molecular entity as a result of the initial absorption of radiation by another molecular entity called photosensitizer (or sensitizer) [1]. Most of the solar ultraviolet (UV) energy incidence on Earth's surface corresponds to UVA radiation (320–400 nm), which, as visible (Vis) light, is barely absorbed by the main biological chromophores, but can induce damage through photosensitized reactions [2–4]. UVA radiation has been recognized as a class I carcinogen [5–8]. On the other hand, photosensitized reactions, promoted by UVA/Vis radiation, have been used in important applications in disinfection [9–12] and photodynamic therapy (PDT) [13–15]. Many natural heterocyclic compounds, such as porphyrins, flavins, pterins and lumazines, can act as endogenous photosensitizers [16]. These compounds can physiologically be present in the tissues at low concentrations, can be normal metabolites that accumulate under certain pathological circumstances or degradation products of common components, such is the case of some tryptophan (Trp) oxidation products [17,18].

Photosensitized oxidations can take place through different mechanisms [19,20]. In type I mechanisms, an electron transfer (or hydrogen abstraction) from the substrate or target molecule (S) to the sensitizer in an excited state, typically a triplet excited state (^{3}Sens*), takes place (Reaction 1). Then, the corresponding formed radicals can be involved in a large number of competitive reactions to finally yield oxidized stable photoproducts. In type II mechanisms, an energy transfer from the triplet excited state of the sensitizer to dissolved O_2 occurs, yielding singlet molecular oxygen [$O_2(^1\Delta_g)$, denoted throughout as $^1O_2$] (Reaction 2), which, in turn, reacts with the substrate (Reaction 3). A given sensitizer can act through type I and/or type II mechanisms depending, among several factors, on the redox potential of its excited state and the ability to transfer energy to dissolved O_2, respectively. On the other hand, the susceptibility of a given biomolecule to undergo type I and/or type II photooxidations depends, among other factors, on the ionization potential and the capability to react with $^1O_2$.

$$^3\mathrm{Sens}^* + \mathrm{S} \rightarrow \mathrm{Sens}^{\cdot-}/\mathrm{SensH}^{\cdot} + \mathrm{S}^{\cdot+}/\mathrm{S(-H)}^{\cdot} \quad (1)$$

$$^3\mathrm{Sens}^* + \mathrm{O_2} \rightarrow \mathrm{Sens} + {}^1\mathrm{O_2} \quad (2)$$

$$\mathrm{S} + {}^1\mathrm{O_2} \rightarrow \mathrm{S_{(ox)}} \quad (3)$$

Pterins are widespread in biological system and participate in a broad range of functions, including, pigments and enzyme cofactors

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https://doi.org/10.1016/j.jpap.2021.100045
Received 4 April 2021; Received in revised form 30 May 2021; Accepted 2 June 2021
Available online 15 June 2021
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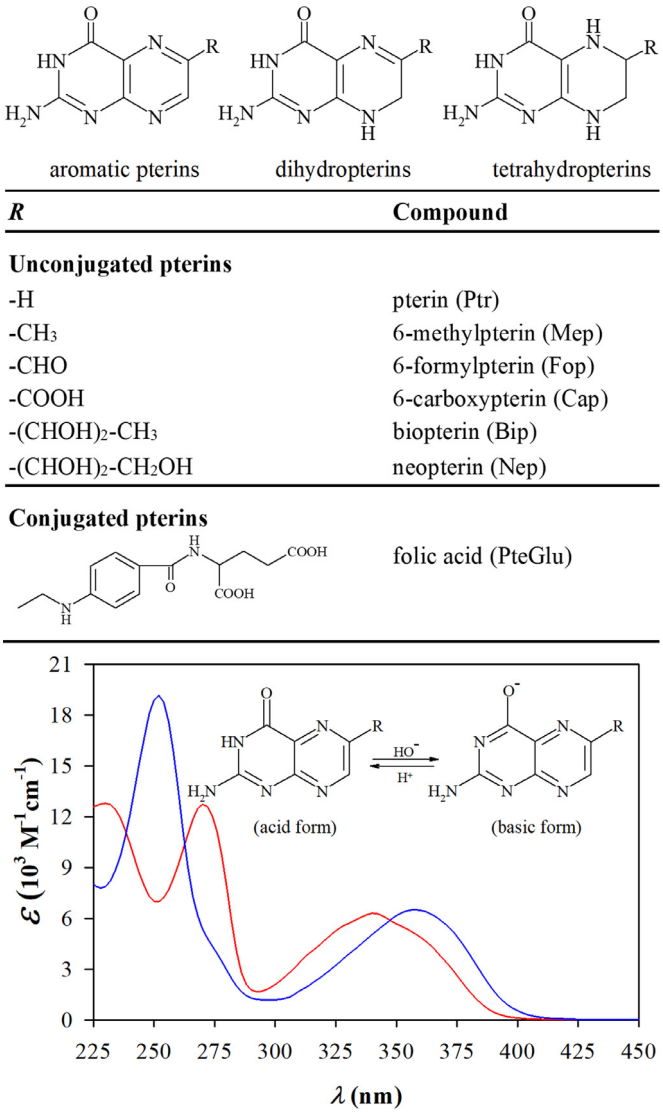

Fig. 1. Molecular structures of pterin derivatives, main acid–base equilibrium of pterin derivatives and absorption spectra of the acid (red) and basic (blue) forms of pterin (Ptr), which are typical for many unconjugated oxidized pterins.

[21–24]. These compounds, derived from 2-aminopteridin-4(1*H*)-one or pterin (Ptr), can exist in living systems in different redox states and be classified into three classes according to this property: oxidized (or aromatic) pterins, dihydro and tetrahydro derivatives (Fig. 1). The most common natural pterin derivatives are 6-substituted compounds. According to the molecular mass and the functional groups of these substituents, pterins can be divided into two groups: (i) unconjugated pterins, containing substituents with one carbon atom or a short hydrocarbon chain, and (ii) conjugated pterins, with larger substituents containing a *p*-aminobenzoic acid (PABA) moiety. In Fig. 1, the molecular structures of the most common substituents are shown together with the names of the corresponding oxidized pterin derivatives. Analogous derivatives can be found for dihydro and tetrahydro pterins.

Pterins present several acid-base equilibria in aqueous solution, the main one from a biological point of view involves the equilibrium of the lactam group (pyrimidine ring) (Fig. 1). The pK_a of this equilibrium is *ca.* 8 for aromatic pterins and *ca.* 10 for dihydropterin derivatives [25–27]. Other functional groups of the pterin moiety (*e.g.* the 2-amino group or ring N-atoms) have pK_a values < 2 [28]. Pterin derivatives also exhibit tautomerism [29]. Theoretical calculations demonstrated that the 4-keto (lactam) tautomer is the most stable one, whereas the enol (lactim) structure is ~25 kJ/mol higher in energy, and three other neutral structures have energies within ~17 kJ/mol above that of the lactam. In addition, the studies revealed that the lactim anion is the most stable structure in the chemistry of deprotonated pterins [30]. Therefore, at pH > 5, the lactam form is in equilibrium with the corresponding lactim anion.

Pterins are present in human skin since 5,6,7,8-tetrahydrobiopterin (H_4Bip) is a cofactor for the aromatic amino acid hydroxylases [29,31] and participates in the regulation of melanin biosynthesis. Normal metabolism of H_4Bip is altered in the skin of patients suffering from vitiligo [32], a skin disorder characterized by the acquired loss of constitutional pigmentation [33], and dihydro and oxidized pterins accumulate in the affected tissues at concentrations that are significantly higher than those reported for healthy cells [34]. Due to the presence of oxidized pterins, patients affected by this pathology express a characteristic fluorescence in their white skin patches upon Wood's light examination [35]. Although intracellular concentrations were not determined, it can be assumed that pterins can reach any cellular compartment since pterins can freely cross phospholipid membranes [36]. Therefore, the photochemistry of pterins is important to understand the harmful effects of radiation on skin and is of particular interest in depigmentation disorders, such as vitiligo, where the protection against radiation fails due to the lack of melanin.

Dihydro and tetrahydropterins poorly absorb radiation in the UVA and visible range [25] and their excited singlet states deactivate fast, avoiding the formation of the long-lived triplet excited states [37,38]. That is why these pterin derivatives cannot act as efficient photosensitizers. Nevertheless, reduced pterins act as efficient scavenger of reactive oxygen species (ROS) and their antioxidant capability can be greater than known antioxidants like ascorbic acid and glutathione [39–41]. Oxidized conjugated pterins, such as folic acid (PteGlu), present intense absorption in the UVA, but the substituent acts as a strong "internal quencher" that efficiently deactivates the singlet excited states of the pterin moiety, which has been attributed to intramolecular photoinduced electron transfer [42,43]. For this reason, these compounds are not good photosensitizers either. On the other hand, unconjugated oxidized pterins also absorb in the UVA, but, in this case, they efficiently generate triplet excited states and, in consequence, they can act as photosensitizers. Reduced pterins in the presence of O_2 thermally and photochemically undergo oxidation to produce oxidized pterins [44–49]. Conjugated pterins, in turn, also undergo photooxidation to yield oxidized unconjugated pterins (*vide infra*) [50]. Therefore, pterin derivatives that are not photosensitizers, under oxidative conditions are converted into good sensitizers.

2. The photochemistry and photophysics of oxidized pterins

The pterin chromophore of oxidized unconjugated pterins [51] presents an absorption spectra with two main absorption bands in the range 230-500 nm. These absorption bands correspond to transitions from the singlet ground state of the pterin moiety (S_0) to singlet excited states (S_1, S_2). The wavelengths of the maxima of the high energy band ($S_0 \rightarrow S_2$) and of the low energy band ($S_0 \rightarrow S_1$) are in the ranges 250-285 nm and 340-370 nm, respectively [27] (Fig. 1). The exact wavelengths of the absorption maxima depend on the 6-substituent and the acid-base form.

S_2 rapidly decays to S_1 that is the only emissive excited state. The emission spectra in aqueous solution show a broad band centered at approximately 450 nm. For a given compound, fluorescence quantum yields (Φ_F) value does not depend on O_2 concentration, indicating that the quenching of S_1 by O_2 is negligible [27]. The fluorescence decays follow a first-order rate law with fluorescence lifetimes (τ_F) between 5 and 15 ns. Due to the fast non-radiative deactivation of S_1, Φ_F of conjugated pterins are very low (< 0.01) [27].

S_1 of unconjugated oxidized pterins undergoes intersystem crossing to generate two triplet excited states that derive from the lactim and lactam tautomers (*vide supra*) [52–55]. For Ptr in deaerated aqueous solutions, the lifetime (τ_T) of the former (0.4 (±0.1) μs) is significantly shorter than that of the latter (5 (±1) μs) [55,56]. Whereas the short-lived triplet excited state decays too fast for being significantly involved in dynamic processes, the long-lived triplet excited state is responsible for the photosensitizing properties of oxidized pterins (*vide infra*). Therefore all the photosensitized reactions that will be described in the next sections are triggered by this latter species. Consequently, from now, every time the triplet excited state of Ptr or a pterin derivative is mentioned in the text, we will be referring to the photosensitizer in the long-lived triplet excited state.

Although the τ_T and the energy of this excited state ($\Delta E_{0,0}$) depend on the pterin derivative and the experimental conditions, their values are high enough to participate in different reactions. As expected, taking into account the behavior of many other photosensitizers, triplet excited states of pterins are able to transfer energy to dissolved O_2 to yield $^1O_2$ [57–60]. The one-electron reduction potential of pterins in their triplet excited states (E(Pt*/Pt$^{\bullet-}$)), calculated from the values of the one-electron reduction potential of pterins in their ground states (E(Pt/Pt$^{\bullet-}$)~ -0.5 V *vs.* NHE) and $\Delta E_{0,0}$ (~ 300 kJ mol^{-1}) (Eq. 4), is higher than 2 V [61,62], which means, in terms of thermodynamic feasibility, that electronically excited pterins are able to oxidize many biomolecules.

$$E\left(Pt^*/Pt^{\cdot-}\right) = E(Pt/Pt^{\cdot-}) + \Delta E_{0,0}(eV) \tag{4}$$

6-Substituted oxidized pterins can undergo photoreduction in anaerobic conditions and in the presence of electron donors [63–65]. On the other hand, they undergo photooxidation in air-equilibrated aqueous solutions under UVA radiation. Many of these oxidations chemically modify the 6-substituent, but do not affect the pterin moiety. The products and quantum yields of photodegradation depend on the 6-substituent and the pH. In the absence of O_2 conjugated oxidized pterins, such as PteGlu (Fig. 1), are photostable [50]. On the other hand, excitation of PteGlu in the presence of O_2 leads to cleavage and oxidation of the molecule, yielding 6-formylpterin (Fop), PABA-glutamic acid and hydrogen peroxide (H_2O_2) as photoproducts (Fig. 1) [66–68]. Upon UVA irradiation, unconjugated pterins with oxygenated substituents, such as biopterin (Bip) and neopterin (Nep) (Fig. 1), undergoes oxidation yielding Fop and H_2O_2 [69,70]. The production of H_2O_2 in the photolysis of pterins is relevant for skin photobiology since this species is involved in the pathogenesis of vitiligo [34]. The photodegradation of the pterin moiety itself takes place upon irradiation of Ptr and pterin derivatives containing substituents that cannot be easily oxidized, such as 6-carboxypterin (Cap) and 6-methylpterin (Mep) (Fig. 1). These compounds are photostable under anaerobic conditions, whereas excitation in the presence of O_2 leads to oxidation, yielding non-pterinic photoproducts (cleavage of the pterin moiety) and H_2O_2 [71–73]. The quantum yields of Ptr, Mep, and Cap degradation in the presence of O_2 are much lower than the corresponding quantum yields determined for the derivatives bearing oxidizable substituents (PteGlu, Bip, Nep, etc) [27,70].

3. Type I or type II photosensitizers?

According to the photophysical properties described in the previous section, upon UVA irradiation unconjugated oxidized pterins, named from here as pterins, form triplet excited states able to initiate both type I and type II photosensitized oxidations. Experiments using model biological targets in simple systems are useful to find out if a given compound can effectively act as a photosensitizer. Oxidizable small molecules soluble in H_2O, such as nucleotides and amino acids, are used for this purpose. If an air-equilibrated aqueous solution containing Ptr and Trp or 2'-deoxyguanosine 5'-monophosphate (dGMP) is exposed to UVA radiation, the substrate and O_2 are consumed, Ptr concentration does not change and H_2O_2 is produced [74,75]. On the other hand, if O_2 is removed from the solution before irradiation, the photodegradation of the substrate either does not occur or it does, but at a much lower rate. These simple experiments clearly demonstrate that pterins, in the presence of O_2, are able to photosensitize the oxidation of nucleobases and amino acids.

The elucidation of a mechanism is not simple. In first place the thermodynamic feasibility of an electron transfer from the substrate to the photosensitizer must be considered. This can be evaluated using Eq. 5 [76].

$$\Delta G(eV) = E\left(S^{\cdot+}/S\right) - E\left(Sens^*/Sens^{\cdot-}\right) - \left(e_0^2/4\Pi\varepsilon \mathrm{R} D^+A^-\right) \tag{5}$$

where *E(Sens*/Sens$^{\bullet-}$)* is given by Eq. 4 (Sens= Ptr), E(S$^{\bullet+}$/S) is the one-electron potential of the electron donor, the substrate (S), and the term $e_o{}^2/4\Pi\varepsilon RD^+A^-$ is the solvation energy of an ion pair D$^+$A$^-$ (S$^{\bullet+}$ and Ptr$^{\bullet-}$, in our case), which can be ignored in the case of strong polar solvents, such as H_2O. ΔG values, calculated with Eq. 5, for the reaction between excited pterins and Trp or dGMP are approximately -100 kJ mol^{-1} and -50 kJ mol^{-1}, respectively; indicating that a reaction initiated by an electron transfer cannot be discarded.

Whereas the four nucleobases of the DNA can undergo one-electron oxidation [77–79], it can be assumed that only guanine significantly reacts with $^1O_2$ [80,81] because the rate constant of the oxidation of guanine in different biomolecules by $^1O_2$ (k^{Δ}_{r-S} > 5×10^6 M^{-1}s^{-1}) is much higher than the corresponding rate constants for the other nucleobases (k^{Δ}_{r-S} < 10^5 M^{-1}s^{-1}) [75,82,83]. If the experiments considered before are carried out with 2'-deoxyadenosine 5'-monophosphate (dAMP), instead of dGMP, a similar behavior is observed, that is, consumption of the substrate and O_2, no change in the photosensitizer concentration and H_2O_2 production [83]. As adenine hardly reacts with $^1O_2$, this process cannot be assumed as a type II photosensitized oxidation. Moreover, Eq. 5 predicts that the oxidation of dAMP by excited Ptr is thermodynamically possible. In short, experiments performed with dAMP as a substrate suggest that pterins can also act as type I photosensitizers.

After the analysis presented in the previous paragraph, it is clear that the classification of the mechanism involved in the Ptr-photosensitized oxidation of Trp and dGMP deserves a deeper assessment. Given a substrate-photosensitizer system under certain conditions, how can one find out if both mechanisms are taking place and how to quantitatively evaluate the contribution of each one? In the next paragraphs some simple strategies to answer these questions will be explained.

A complete analysis of the pathways involving $^1O_2$ will be presented in the next section. Here we will only consider the processes that allow to assess the contribution of $^1O_2$–mediated oxidations in a given reaction system. The rate of the oxidation of a given substrate (S) by $^1O_2$ is given by Eq. 6:

$$\left(\frac{d[S]}{dt}\right)_{\Delta} = -k^{\Delta}_{r-S}\left[^1O_2\right][S] \tag{6}$$

where k^{Δ}_{r-S} is the rate constant of the chemical reaction between $^1O_2$ and S (Reaction 3). If a solution containing S and a photosensitizer is exposed to continuous UVA radiation, the steady-state concentration of $^1O_2$ can be estimated with Eq. 7:

$$\left[^1O_2\right] = \frac{q^{a,V}_{n,p}\ \Phi_{\Delta}}{k_d + k^{\Delta}_{t-Sens}[Sens] + k^{\Delta}_{t-S}[S]} \tag{7}$$

where $q^{a,V}_{n,p}$ and Φ_{Δ} are the photon flux absorbed by the photosensitizer and its quantum yield of $^1O_2$ production, respectively; k_d is the non-radiative deactivation rate constant [84]; k^{Δ}_{t-Sens} and k^{Δ}_{t-S} are the rate constants of $^1O_2$ total quenching by the photosensitizer and the target molecule, respectively (*vide infra*). For a given set of conditions (irradiation intensity, concentrations, etc), the experimental rate of substrate consumption ((d[S]/dt)$_{exp}$), determined by chromatography analysis (HPLC) or other techniques, is compared to (d[S]/dt)$_{\Delta}$ (Equations 6). If (d[S]/dt)$_{exp}$ is similar to (d[S]/dt)$_{\Delta}$, the predominance of a $^1O_2$–mediated mechanism can be inferred. In contrast, if (d[S]/dt)$_{\Delta}$ is much

Table 1
Values of rate constants of the chemical reaction with $^1O_2$ (k_r^Δ), $^1O_2$ total quenching (k_t^Δ) and the quenching of $^3Ptr^*$ (k_q^T) determined for nucleotides and amino acids in aqueous solutions.

	$^1O_2$		$^3Ptr^*$
	k_r^Δ ($M^{-1}s^{-1}$)	k_t^Δ ($M^{-1}s^{-1}$)	k_q^T ($M^{-1}s^{-1}$)
dGMP[a]	1.7×10^7 [75]	1.7×10^7 [75]	5.4×10^9 [56]
dAMP	8.0×10^3 [83]	4.1×10^5 [83]	1.4×10^9 [b]
dTMP	-	7×10^4 [85]	3.2×10^9 [b]
Trp	1.3×10^7 [86]	3.2×10^7 [86]	1.5×10^9 [87]
Tyr	$< 10^6$ [86]	2.7×10^7 [88]	3.1×10^9 [87]
His		$\sim 0.5 \times 10^7$ [89]	1.5×10^9 [87]
Met		1.3×10^7 [86]	0.6×10^9 [87]
Ptr	2.5×10^5 [73]	2.9×10^6 [90]	
O_2			1.6×10^9 [56]

[a] k_r^Δ and k_t^Δ values for dGMP strongly depend on the pH. The values correspond to slightly acidic media that is the pH condition of the experiments discussed in the text. [b] Data from Mariana P. Serrano PhD Thesis, Universidad Nacional de La Plata, Argentina, 2014.

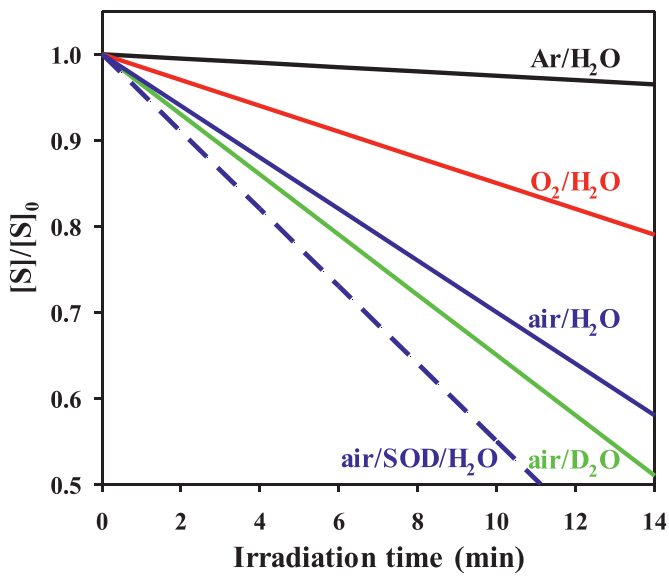

Fig. 2. Typical behavior of the time evolution of the concentration of an oxidizable substrate (S), such as Trp or dGMP, irradiated in the presence of Ptr under different experimental conditions.

lower than $(d[S]/dt)_{exp}$, it can be assumed that oxidation via type II mechanism has a minor contribution. This kinetic analysis allows a reliable method to assess the contribution of type II mechanism to the overall photosensitized oxidation of a substrate.

Returning to the photosensitization of Trp and dGMP by Ptr, for these compounds and others, k_r^Δ and k_t^Δ values have been determined (Table 1) and, in consequence, $(d[S]/dt)_\Delta$ (Eq. 6) can be estimated and compared to $d([S]/dt)_{exp}$. For both cases $d([S]/dt)_{exp}$ is much higher than $(d[S]/dt)_\Delta$, thus indicating that photooxidation mediated by $^1O_2$ does not contribute significantly to the consumption of neither Trp nor dGMP.

In addition, Fig. 2 schematically shows the comparison of the concentration profiles of the substrate (Trp or dGMP) obtained under different experimental conditions. It is clear that O_2 is needed for the consumption of the substrate, but high concentration (O_2-sturated solutions) causes a decrease in the rate of the process. Moreover, using D_2O as a solvent ($^1O_2$ lifetime ($\tau_\Delta = k_d^{-1}$) in D_2O is much longer than in H_2O) does not increase significantly the efficiency of the photooxidation. It is important to emphasize that even when $(d[S]/dt)_\Delta$ cannot be calculated, the analysis of simple experiments as those presented in Fig. 2 allows a rough assessment of the type II mechanism contribution. We have analyzed other substrates, and other pterin derivatives acting as photosensitizers and in most cases, although the substrate is oxidized by $^1O_2$ and the sensitizer produces this ROS, the predominant mechanism is type I [27,91,92].

Indeed, this analysis is not limited to pterins. Interestingly, we used this approach to investigate the photosensitized oxidation of Trp by phenalenone (1*H*-benzonaphthen-1-one, PN), a unique photosensitizer due to its Φ_Δ close to unity [93,94]. In addition, PN is photostable in most solvents and has a very low ability to deactivate $^1O_2$ (very low k_{t-Sens}^Δ). These properties have made PN one of the well-known $^1O_2$ photosensitizers [95]. In fact, it is widely used as a reference for the determination of Φ_Δ values and for investigating oxidations of biological compounds by $^1O_2$. Considering this and that Trp is oxidized by $^1O_2$ [86], one could easily accept that photosensitization of Trp by PN takes place through a purely type II mechanism. However, it was established that $^1O_2$ played a minor role and that the predominant mechanism of the photosensitization of Trp by PN involves an electron transfer process [96]. These studies call into question many reported photooxidation mechanisms and even the role of $^1O_2$ in processes involved in the photodynamic effects of a variety of photosensitizers in living systems.

4. Kinetic analysis

To understand the predominance of the type I mechanism from a kinetic point of view, the competitive pathways must be considered. The rate constant of Reaction 1 can be estimated from the bimolecular rate constant for the quenching of the triplet excited states of the sensitizer by a given substrate (k_{q-S}^T), assuming that the quenching is only due to an electron transfer process. These rate constants have been measured for the quenching of the triplet excited state of Ptr ($^3Ptr^*$) by Trp, dGMP and other substrates and are in the range of diffusion-controlled limit (Table 1). Reaction 1 competes with Reaction 2, that is, the energy transfer to O_2, whose rate constant ($k_{q-O_2}^T$) has also been determined and is of the same order of magnitude as k_{q-S}^T for dGMP and Trp (Table 1). Reaction 1 also competes with the decay of the triplet excited state to the ground state (Reaction 8).

$$^3Sens^* \rightarrow Sens \tag{8}$$

In a given reaction system, the fraction of the triplet excited state of the sensitizer quenched by a species X can be calculated as the rate of the reaction of the excited state with X divided into the overall rate of the excited state consumption. In the case of our experiments, the fractions of $^3Ptr^*$ quenched by the substrate (f_S^T) and O_2 ($f_{O_2}^T$) are given by Eqs. 9 and 10, respectively:

$$f_S^T = \frac{k_{q-S}^T\,[S]}{(\tau_T^0)^{-1} + k_{q-O_2}^T\,[O_2] + k_{q-S}^T\,[S] + k_{q-Ptr}^T\,[\mathrm{Ptr}]} \tag{9}$$

$$f_{O_2}^T = \frac{k_{q-O_2}^T\,[O_2]}{(\tau_T^0)^{-1} + k_{q-O_2}^T\,[O_2] + k_{q-S}^T\,[S] + k_{q-Ptr}^T\,[Ptr]} \tag{10}$$

where τ_T^0 is the lifetime of $^3Ptr^*$ in the absence of quenchers; in the conditions of our experiments the term k_{q-Ptr}^T [Ptr] can be neglected. In a steady-state experiment, if the concentration of the substrate is similar to that of O_2, f_S^T and $f_{O_2}^T$ are comparable and, in consequence, the rates of radicals and $^1O_2$ formation are also of the same order of magnitude. In Table 2 f_S^T and $f_{O_2}^T$ values obtained in experiments of photosensitization of dGMP and Trp with Ptr are listed, together with the fractions of $^3Ptr^*$ that decays to ground state (Reaction 8) ($f^{T\rightarrow S_0}$), calculated using the corresponding equation analogous to Eqs. 9 and 10.

As will be discussed later, there are reactions that can recover the substrate once the radical is form, but, if they are not significant, the

Table 2
Fractions of ^{3}Ptr* that decays to ground state ($f^{T\to So}$), is quenched by the substrate (f_S^T) or O_2 ($f_{O_2}^T$) in experiments performed at different O_2 concentrations; fractions of $^1O_2$ that decays to ground state ($f^{\Delta\to O_2}$) or is physically or chemically quenched by the substrate (f_{r-S}^{Δ} and f_{p-S}^{Δ}, respectively) in air-equilibrated solutions. The experiments were carried out under steady UVA irradiation using Trp or dGMP as a substrate (200 μM). [Ptr]= 100 μM, pH= 5.5-6.0.

	S: Trp			S: dGMP		
	Ar	air	O_2	Ar	air	O_2
f_S^T	0.65	0.35	0.13	0.88	0.68	0.36
$f_{O_2}^T$	0.00	0.46	0.80	0.00	0.23	0.59
$f^{T\to So}$	0.35	0.19	0.07	0.12	0.09	0.05
f_{r-S}^{Δ}		0.010			0.013	
f_{p-S}^{Δ}		0.015			~ 0	
$f^{\Delta\to O_2}$		0.97			0.98	

rate of Reaction 1 is the rate of oxidation via type I photosensitization. On the other hand, the rate of Reaction 2 is the rate of oxidation *via* type II photooxidation only in the case that every molecule of $^1O_2$ reacts with the substrate. To assess this, the reactions of $^1O_2$ have to be considered. $^1O_2$ relaxes to its ground state through solvent induced radiationless and radiative pathways (Reactions 11 and 12). It may also be deactivated by a physical quencher (Reaction 13) and/or oxidize an acceptor molecule (Reaction 14) [97]. Q in Reactions 13 and 14 is the substrate (then Reaction 14 is Reaction 3), the photosensitizer or another compound present in the solution.

$$^1O_2 \rightarrow {}^3O_2 \tag{11}$$

$$^1O_2 \rightarrow {}^3O_2 + h\nu \tag{12}$$

$$Q + {}^1O_2 \rightarrow Q + {}^3O_2 \tag{13}$$

$$Q + {}^1O_2 \rightarrow QO_2 \tag{14}$$

The rate constants of the physical (k_p^{Δ}) and chemical quenching (k_r^{Δ}) of $^1O_2$ by Trp, dGMP and Ptr have been determined (Table 1) (73,75,86). As a matter of fact, k_r^{Δ} and k_t^{Δ} are experimentally determined and k_p^{Δ} are calculated with Eq. 15. The fractions corresponding to the different fates of $^1O_2$ can be calculated in a given photolysis experiment. The fraction of $^1O_2$ that reacts with the substrate (f_{r-S}^{Δ}) to yield oxidized products is given by Eq. 16. Values listed in Table 2 show that only a very low proportion of the $^1O_2$ formed reacts with the substrate to give oxidized products.

$$k_t^{\Delta} = k_r^{\Delta} + k_p^{\Delta} \tag{15}$$

$$f_{r-S}^{\Delta} = \frac{k_{r-S}^{\Delta}\,[S]}{(\tau_{\Delta})^{-1} + k_{t-S}^{\Delta}\,[S] + k_{t-Ptr}^{\Delta}\,[\mathrm{Ptr}]} \tag{16}$$

In conclusion, in the analyzed substrate/sensitizer systems although the rate of radicals and $^1O_2$ generated are comparable, only a low percentage of this ROS leads to oxidized products and, therefore, the rate of products formation *via* oxidation by $^1O_2$ is much lower than the rate of radical formation. Whereas the fraction of the triplet excited state that leads to radicals is f_S^T, the fraction of the triplet excited state that yields oxidized products *via* $^1O_2$ oxidation is $f_{O_2}^T \times f_{r-S}^{\Delta}$, which is 4.6×10^{-3} and 3.0×10^{-3} for Trp and dGMP, respectively, in the experiments presented in Table 2. The main kinetic aspect for this result is that whereas type I mechanism involves a single step to chemically modify the substrate, which takes place with a diffusion-limited rate constant (k_{q-S}^T), type II involves two steps, the first one at diffusion-limited rate ($k_{q-O_2}^T$) and the second one with a much lower rate constant (k_{r-S}^{Δ}).

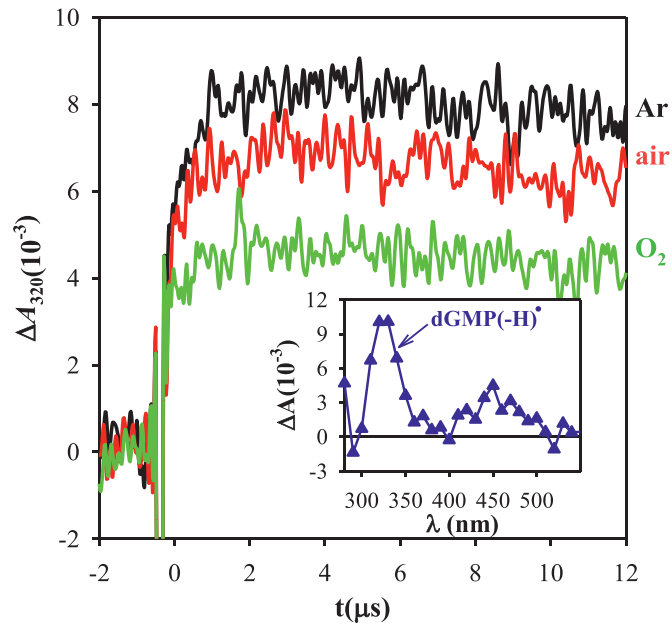

Fig. 3. LFP experiments. Formation of dGMP(-H)• monitored by the time-evolution of the ΔA at 320 nm after the laser pulse at various O_2 concentrations. Experiments performed in Ar-, air- and O_2-saturated aqueous solutions. Inset: Differential transient absorption spectra (ΔA), recorded after 10 μs of the laser pulse. Excitation wavelength 355 nm, [Ptr] = 100 μM, [dGMP] = 1 mM. Adapted from Ref. 56.

5. The oxygen paradox

Whereas the presence of O_2 is mandatory for a $^1O_2$-mediated oxidation (Reactions 2 and 3), the formation of radicals does not involve O_2 (Reaction 1). Moreover, under anaerobic conditions, O_2 does not compete with the substrate for the excited state of the sensitizer, *e.g.* f_S^T value increases when O_2 concentration decrease (Table 2). The question that naturally arises is: why when O_2 is removed from the solution, the consumption of the substrate is negligible compared to that observed in the presence of O_2 (Fig. 2)? To explain this apparent contradiction, it is necessary to investigate the formation and fate of radicals. In this regard, we have studied the radicals generated in Ptr-dGMP system.

The radicals of 2´-(deoxy)guanosine and (d)GMP have characteristic spectral features and long lifetimes (>100 μs), allowing their detection in different systems [98–102]. In our reaction system (aqueous solutions containing Ptr and dGMP exposed to UVA irradiation), we investigated the dGMP neutral radical (dGMP(-H)•) formation by laser flash photolysis (LFP) [56,75]. Under our experimental conditions, the differential transient absorption spectra showed the characteristic narrow absorption band centered at 320 nm of 2´-deoxyguanosine radicals (Inset Fig. 3). The traces recorded at 320 nm followed first-order kinetics and the lifetime ($\tau_{G\bullet}$) was equal, within the experimental error, to that obtained for the decay of ^{3}Ptr* (τ_T), thus indicating, as expected, that the formation of dGMP(-H)• is due to the reaction of dGMP with ^{3}Ptr*. Fig. 3 shows several traces registered in LFP experiments, performed under the same conditions, except O_2 concentration. Once the *plateau* is reached, the dGMP(-H)• concentration is proportional to the ΔA_{320} intensity. The higher the O_2 concentration, the lower the dGMP(-H)• concentration reached after excitation of Ptr. These results are in agreement with the data presented in Table 2 and confirms that O_2 partially prevents the formation of dGMP(-H)• due to the quenching of ^{3}Ptr*. This explains why the dGMP consumption is lower in O_2-saturated than in air-equilibrated solutions, but it emphasizes the controversial fact that under anaerobic conditions the dGMP consumption is negligible.

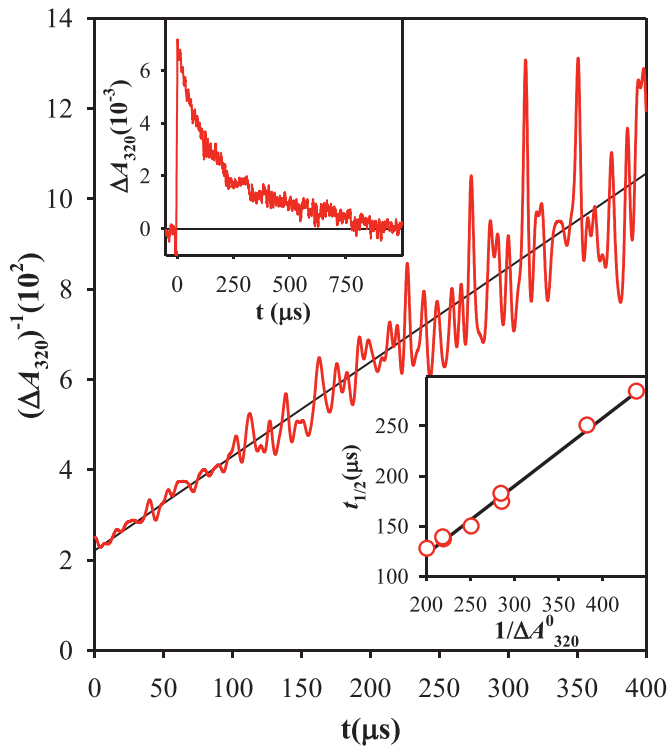

Fig. 4. LFP experiments. Decay of dGMP(-H)• monitored by the time-evolution of the ΔA at 320 nm (ΔA_{320}) after the laser pulse in Ar-saturated aqueous solutions. Plot of $\Delta A_{320}{}^{-1}$ *vs.* time. Upper inset: $\Delta A_{320}{}^{-1}$ *vs.* time. Lower inset: Dependence of $t_{1/2}$ with the initial amount of radical formed, evaluated as $\Delta A^0{}_{320}$. Excitation wavelength 355 nm, [Ptr] = 100 μM, [dGMP] = 200 μM. Adapted from Ref. 56.

After dGMP(-H)• is formed and reached a maximum concentration, its evolution can also be followed by monitoring ΔA_{320}, but in a longer time window. Whereas in the absence of O_2, ΔA_{320} decays to a negligible value at infinite time, in the presence of O_2 ΔA_{320} decays to a residual value. This behavior is in agreement with the fact that O_2 is needed for the consumption of dGMP (Fig. 2) and the consequent formation of products. Reaction 17 is the recombination of the radicals that leads to the recovery of the reactants. Therefore, if dGMP(-H)• does not participate in another reaction, the total recombination of the radicals to completely recuperate the reactants should be observed. Under anaerobic conditions, the decay of dGMP(-H)• follows a second order rate law: the traces plotted as $1/\Delta A_{320}$ *vs.* time are linear and the half life ($t_{1/2}$) increases with the decrease of the initial amount of dGMP(-H)• formed after the flash (Fig. 4). This kinetic behavior is expected if dGMP(-H)• is consumed only by Reaction 17, in which the stoichiometric ratio between dGMP(-H)• and Ptr•− is 1:1.

$$dGMP(-H)^{\cdot} + Ptr^{\cdot -} + H^+ \rightarrow dGMP + Ptr \tag{17}$$

$$Ptr^{\cdot -} + O_2 \rightarrow Ptr + O_2^{\cdot -} \tag{18}$$

$$dGMP(-H)^{\cdot} \xrightarrow{H_2O/O_2} dGMP(ox) \tag{19}$$

In the presence of O_2, the Ptr radical anion (Ptr•−) may react with O_2 to regenerate Ptr and produce superoxide anion ($O_2^{\cdot -}$) (Reaction 18). This is a common reaction for radical anions [103], the sensitized production $O_2^{\cdot -}$ by pterins and related compounds has been proved in different systems [85,65,104]. If one assumes rate constants of the same order, in air-equilibrated solution, Reaction 18 will be faster than Reaction 17 due to the higher concentration of O_2 compared to dGMP(-H)• concentration. Therefore, in the presence of O_2 there is a minor recovery of dGMP (Reaction 17), if any at all, and, in consequence, dGMP(-H)• evolves to products. Many chemical transformations of the guanine radicals (dGMP•+ and dGMP(-H)•), involving initial hydration, reaction with O_2 and other oxidants, lead to a complicated net of competitive pathways that eventually yield oxidized products (Reaction 19) [105,106]. In the case of photosensitization with Ptr a set of oxidized products has been identified [107].

Experiments performed in O_2-saturated solutions of dGMP show a slower consumption of dGMP compared to that registered in air-equilibrated solutions (Fig. 2) [75]. This behavior has also been observed with other substrates photosensitized by Ptr and related compounds [74,83,108–110]. The higher the O_2 concentration, the faster the Reaction 2; the higher $f^T_{O_2}$, the smaller f^T_S (Table 2), which implies that less radicals are formed and, in consequence, the oxidation through the dominant type I mechanisms becomes slower.

In conclusion, O_2 concentration plays a key role in the efficiency of type I mechanism; even though it does not participate in the first steps of the process. Considering the Ptr-photosensitization of Trp, dGMP and other substrates under anaerobic conditions, the quantum yield of radical formation is maximum, but, contradictorily, the overall consumption of substrate is negligible. In air-equilibrated solutions, O_2 by reacting with Ptr•− (Reaction 18) avoids the recombination of radicals (Reaction 17) and allows the further reaction of substrate radicals (Reaction 19). At high O_2 concentration the quenching drastically decreases the amount of ^{3}Ptr* available and both the formation of radical and the overall consumption of substrate drop. The complex set of competitive pathways that explains the paradoxical role of O_2 in the oxidation of dGMP photosensitized by Ptr is depicted in Fig. 5.

6. The role of superoxide anion

$O_2^{\cdot -}$ disproportionates with its conjugated acid $HO_2^{\cdot}$ to form H_2O_2 and O_2 (summarized by Reaction 20). The reactivity of $O_2^{\cdot -}$, $HO_2^{\cdot}$ and H_2O_2 is low toward most biomolecules [111]. However, in some cases these species can contribute to extend the oxidative damage. For example, the photoinactivation of tyrosinase, enzyme present in human skin and involved in the biosynthesis of the melanin, by Ptr and other pterin derivatives takes place through a type I mechanism and H_2O_2, photochemically generated, reacts with the active site of the enzyme and contributes to its inactivation [112,113].

Although $O_2^{\cdot -}$ poorly reacts with non-radical species, it can react with other radicals. In particular, it reacts with guanine radicals to yield 2,5-diamine imidazolone or simply imidazolone (Iz) as the main product [106,114]. In the dGMP-Ptr system a product bearing the Iz moiety linked to the 2'-deoxyribose monophosphate unit was detected (dIzMP) (Reaction 21) (Fig. 5) [107]. The role of $O_2^{\cdot -}$ in the mechanism can be assessed in photolysis experiments performed in the presence of superoxide dismutase (SOD), an enzyme that catalyzes Reaction 20 [115]. In the photosensitization of dGMP with Ptr, SOD causes a decrease in the rate of dIzMP formation and an increase in the rate of dGMP consumption (Fig. 2). This behavior reveals that Reactions 20 and 21 compete with another pathway that leads to the restoration of the guanine moiety (Reaction 22). This reaction has been also described for other reaction systems [116–118]. Reactions 20-22 were added to Fig. 5 to complete the simplified map of competitive pathways that occur in the oxidation of dGMP photosensitized by Ptr.

$$2\ H^+ + 2O_2^{\cdot -} \rightarrow H_2O_2 + O_2 \tag{20}$$

$$dGMP(-H)^{\cdot} + O_2^{\cdot -} \quad \rightarrow \quad dIzMP \tag{21}$$

$$dGMP(-H)^{\cdot} + H^+ + O_2^{\cdot -} \quad \rightarrow \quad dGMP + O_2 \tag{22}$$

The increase in the rate of consumption of substrate in the presence of SOD has been observed in the oxidation of amino acids sensitized by Ptr, such as Trp and tyrosine (Tyr), free in solution [74,108] and in peptides [119]. These studies suggest that the reactions of the substrate

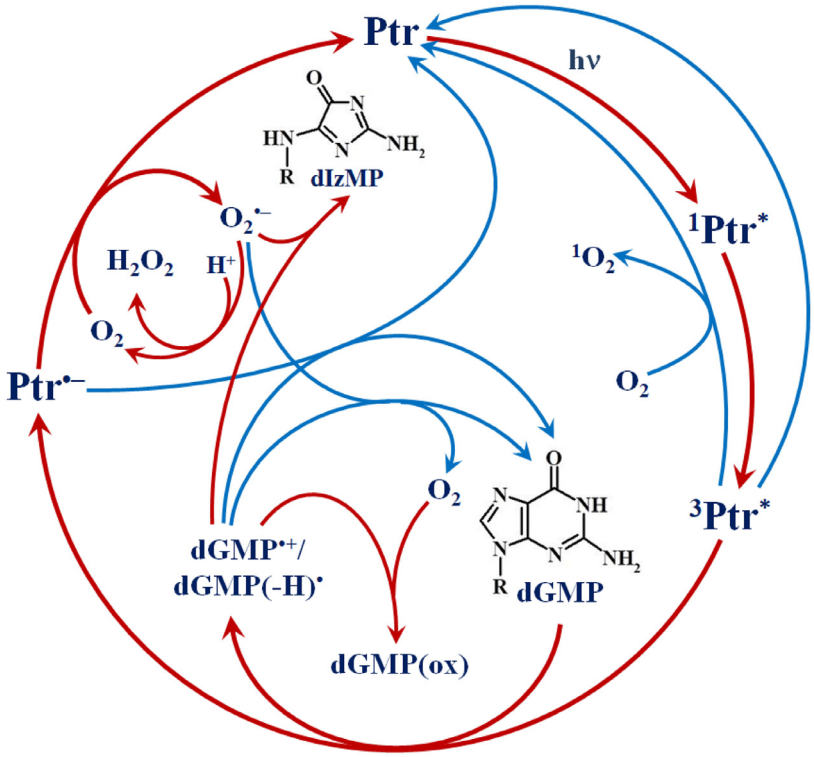

Fig. 5. Mechanism of the type I photooxidation of dGMP using Ptr as a photosensitizer. Red arrows: generation of radicals and reactions leading to products; blue arrows: recovery of reactants.

radicals with $O_2^{\bullet-}$ should be always considered and that such reactions can lead to oxidized products or to recover the substrate. In short, $O_2^{\bullet-}$ can play a protective role against photooxidation via type I mechanism or contribute to the evolution of radicals to oxidized products. Both roles can coexist, as in the case of dGMP (Fig. 5), and the overall effect of $O_2^{\bullet-}$ on a given mechanism will depend on the rate constants of the competitive reactions and on the experimental conditions.

In this sense, it is intriguing the effect of SOD. This enzyme is widely accepted as an antioxidant because it removes a ROS avoiding its reaction with oxidizable substrates or its evolution to other ROS, such as H_2O_2 or hydroxyl radical. However, in type I mechanisms as shown in Fig. 5, SOD plays a pro-oxidant role, favoring the overall oxidation of the substrate (Fig. 2) by reducing the contribution of a restoration pathway. It would be interesting to investigate the effect of SOD in this type of processes at a cellular level.

7. Tyrosine dimer: a photosensitized product that acts as a photosensitizer

Among the type I photosensitized oxidations investigated using pterins as photosensitizers, the case of Tyr deserves a special comment. As mentioned above, Ptr under UVA irradiation in aqueous solution is able to generate Tyr radicals (Reactions 23 and 24) [120] and the set of competitive pathways undergone by the radicals formed is similar to that investigated for dGMP (Fig. 5), with a minor, but significant difference: besides oxygenated products (Reaction 25), a dimer is formed. This dimer (Tyr_2) is the product of the coupling of two long-lived tyrosyl radicals (Tyr(-H)$^{\bullet}$) [121] (Reaction 26).

$$^3Ptr^* + Tyr \rightarrow Ptr^{\cdot-} + Tyr^{\cdot+} \quad (23)$$

$$Tyr^{\cdot+} \rightleftarrows Tyr(-H)^{\cdot} + H^+ \quad (24)$$

$$Tyr(-H)^{\cdot} \xrightarrow{H_2O/O_2/O_2^{\cdot-}} Tyr(ox) \quad (25)$$

$$2\,Tyr(-H)^{\cdot} \rightarrow Tyr_2 \quad (26)$$

Actually, six possible products may be produced by pairing the different radicals generated in the one-electron oxidation of Tyr. However, due to the instability of the intermediates formed or the steric hindrance, the most stable products are the carbon-oxygen-carbon and the carbon-carbon dimers, being the last one the most abundant [122]. During the photosensitization of Tyr using Ptr as photosensitizer, the *o,o′*-dityrosine was the only dimer product detected [120]. While self-reactions of radicals derived from the one-electron oxidation of nucleobases are not relevant, dimerization is an important pathway in the case of amino acids. In fact, the photosensitized formation of Trp dimers [123–125] and Trp-Tyr cross links have also been investigated [126].

The Tyr cross-link has been extensively studied [127,128] and affects the solubility and elastic properties of proteins [129,130]. Ptr-photosensitized generation of Tyr_2 has been investigated in free Tyr [108] and in Tyr residues in peptides [119] and proteins [131,132]. In studies using human serum albumin (HSA) as a model protein, it was demonstrated that HSA undergoes cross-linking photoinduced by Ptr, a process that is not just a dimerization, but an oligomerization that yields large protein structures with more than 10 HSA molecules [131]. Based on Ptr-mediated photosensitization, a novel method to prepare Tyr_2 was developed [122]. Several studies have indicated that the photosensitized oxidation of Tyr gives rise to other products such as DOPA, being the yield of this compound much higher than that of Tyr_2 [133,134]. However, in the case of Ptr-photosensitized generation of Tyr_2, the yield is about 30-40 %, depending on the experimental conditions [118], which indicates that the generation of this photoproduct, under certain condition, might be significant.

The photoinduced generation of Tyr_2 is an interesting case from a mechanistic point of view because, in contrast to the products detected and characterized for the Ptr-type I photosensitization of dGMP and dAMP, O_2 does not participate in any of the reactions involved in its formation. However, the rate of Tyr photosensitization and Tyr_2 formation is negligible or, at least, significantly lower under anaerobic conditions, compared to that registered in air-equilibrated solutions (Fig. 2). This behavior supports the hypothesis that O_2 by reacting with $Ptr^{\bullet-}$ avoids the recombination of radicals and pushes the process forward, towards

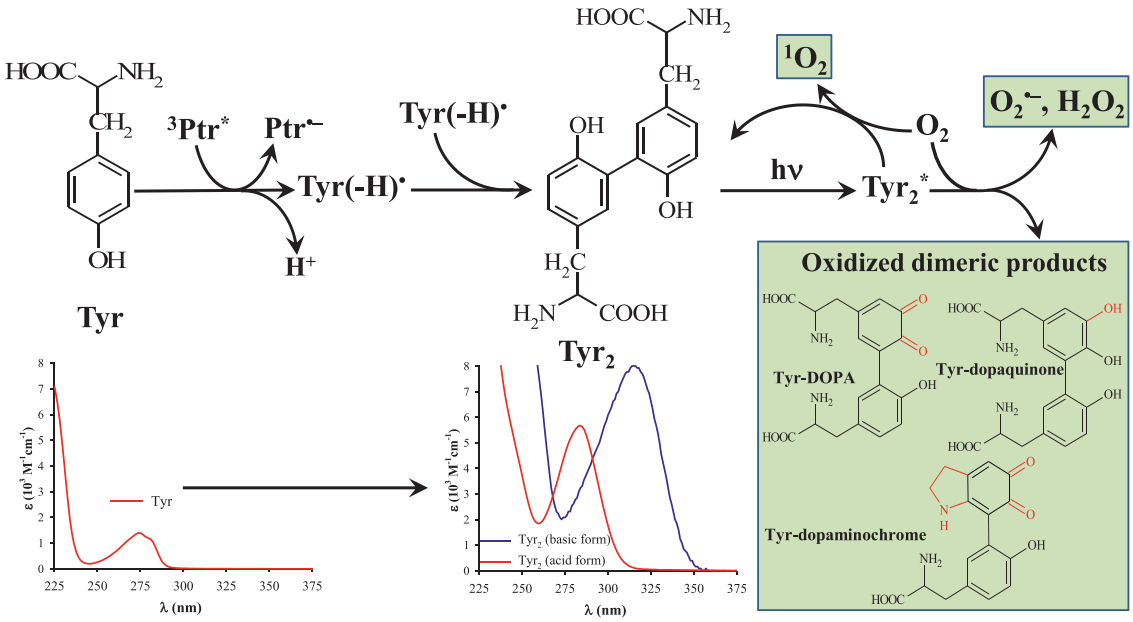

Fig. 6. Ptr-photosensitized generation of Tyr_2 and subsequent photochemical processes undergone by Tyr_2. Tyr and Tyr_2 absorption spectra.

products. Actually, the reaction of O_2 with $Ptr^{\bullet-}$ changes a radical ($Ptr^{\bullet-}$) by another radical ($O_2^{\bullet-}$) (Reaction 18). As discussed before, the latter reduces the substrate radical and recovers the reactant (Reaction 22 for the case of dGMP). However, the competition of this reaction, with the dismutation into H_2O_2 and O_2 (Reaction 20) and the reaction to yield oxygenated products (Reaction 21 for the case of dGMP), makes less efficient the restoration of the substrate via Reaction 22 and, in consequence, the overall substrate consumption becomes faster when $Ptr^{\bullet-}$ is eliminated by reaction with O_2.

Another interesting aspect is that the phenol groups of Tyr_2 are much more acidic than that of Tyr; i.e. pK_a values are 7.2 and 10.0, respectively [120,135]. In consequence, in contrast to Tyr, in aqueous solution at physiological pH, more than 50 % of Tyr_2 exists in its basic form, which absorbs in the UVB (280-320 nm) and UVA spectral regions (Fig. 6) [136]. As a result, when Tyr_2 is formed in a protein, a new chromophore appears, which is able to absorb, unlike natural amino acids, at wavelengths significantly present in solar radiation and artificial sources of light.

In the absence of O_2, Tyr_2 is photostable, whereas excitation in the presence of O_2 leads to its photodegradation, giving rise to oxidized products that conserve the dimeric structure (Fig. 6), that is, the chemical bond between the two Tyr moieties endures the UV exposure. What is even more interesting is that, upon excitation of the alkaline form of Tyr_2, H_2O_2, $O_2^{\bullet-}$ and $^1O_2$ are produced (Fig. 6) [136]. Therefore, although Tyr is a good $^1O_2$ photosensitizer in both monomeric [137] and dimeric form, with about the same quantum efficiency, the shift in the absorption spectrum of the Tyr chromophore in Tyr_2 generates an intrinsic photosensitizer, extending the active fraction of light towards the UVA range.

This is an interesting example of a product formed in a photosensitized process that can act as a photosensitizer itself leading to further photosensitized damage, thus amplifying the harmful effects of UV radiation on biological systems. This type of processes has been much more extensively studied for Trp, whose photoproducts (*N*-formylkynurenine, kynurenine, kynurenic acid) are well-known photosensitizers with demonstrated damaging effect on biological systems [18,133,138]. Speculate on the role of Tyr_2 as a photosensitizer *in vivo* and compare its photosensitizing activity with that of Trp photoproducts is not trivial. However, taking into account that Tyr residues are more abundant that Trp residues and that Tyr, due to its low ionization potential, sometimes acts as the final electron donor [139], even when the initial one-electron oxidation takes place in another amino acid, Tyr_2 photochemistry might be relevant and deserves further studies in complex systems.

8. Lipophilic pterins

In the mechanisms described up to now in this review, an encounter between the $^3Ptr^*$ and the target molecule takes place, that is, the process is dynamic and its rate is controlled by diffusion. All unsaturated lipids in cell membranes, including phospholipids, glycolipids and cholesterol, are well-known targets of oxidative damage [140], which can occur by type I and type II photosensitized oxidation [141,142]. In the case of vesicles dispersions, a hydrophilic photosensitizer will remain in the aqueous phase and the photosensitized oxidation of a target molecule in the membrane will be a dynamic process. On the other hand, if the photosensitizer is lipophilic, an association with a biomembrane is expected and, as the photosensitization is not limited by diffusion, the oxidation might be much faster [143–145].

Ptr can photoinduce the oxidation of polyunsaturated fatty acids (PUFAs) of phospholipids present in large unilamellar vesicles (LUVs), again predominantly through type I mechanism. Ptr does not bind to phospholipid membranes and, therefore, it is able to freely cross biomembranes [36]. Moreover, upon UVA irradiation in the presence of Ptr, the viability of *HeLa* cells decreases [146]. The photodynamic activity of Ptr affects, among other targets, the structural integrity of the cell membrane. In short, although Ptr remains in bulk water, it is able to photoinduce membrane damage in simple model systems (LUVs), as well as in eukaryotic cells.

In the search of better compounds that retain the photosensitizing properties of pterins and, at the same time, are able to bind to biomembranes, a series of decyl-pterin derivatives were synthesized [147]. Conjugation of a decyl chain to the pterin moiety drastically increases its solubility in common organic solvents and also enables its facile intercalation in LUVs. In addition, decyl-pterins present a more efficient intersystem crossing to the triplet excited state as compared to Ptr [147]. Among the decyl-pterins synthetized, due to its photochemical properties, 4-(decyloxy)pteridin-2-amine (*O*-decyl-Ptr) (Fig. 7) was chosen for further studies using phospholipid membranes with various compositions.

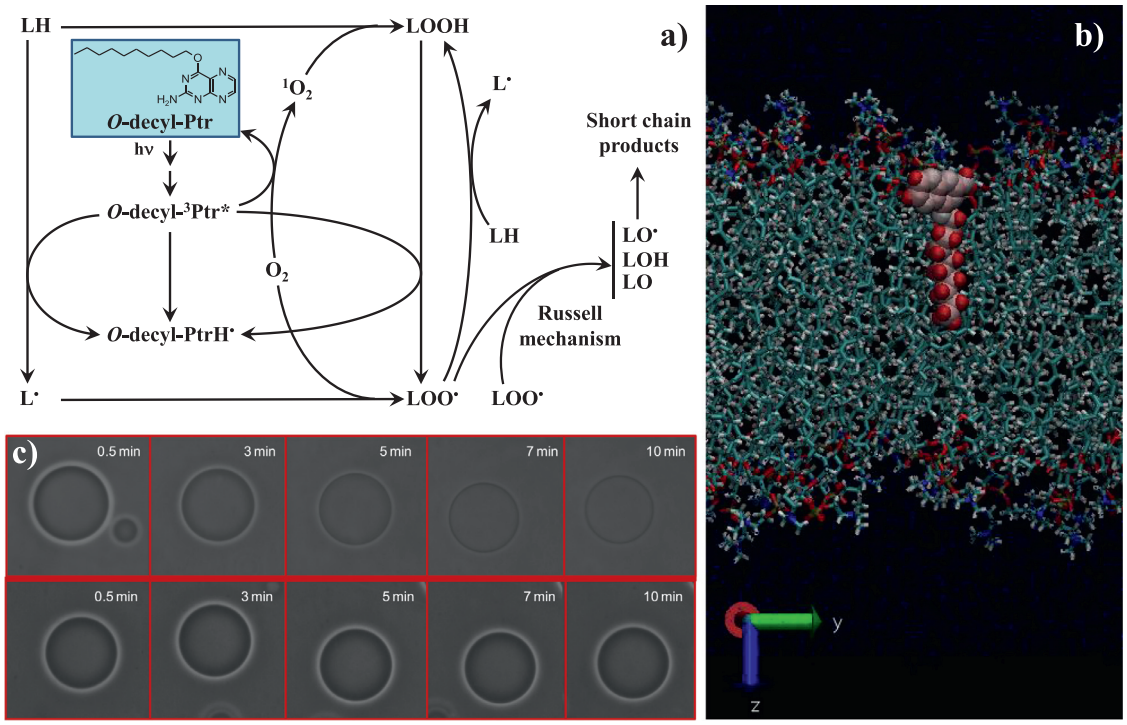

Fig. 7. a) Proposed mechanism of lipid peroxidation by photosensitization with *O*-decyl-Ptr. LH, phospholipid; LOOH, hydroperoxide; L•, alkyl lipid radical; LOO•, peroxyl lipid radical; LO•, alkoxyl lipid radical; LOH, hydroxyl derivatives; LO, carbonyl derivative. b) Molecular dynamic simulations representative snapshot of *O*-decyl-Ptr in its equilibrium position (after 200 ns) inserted into the membrane core. Lipid molecules (DOPC) are shown as sticks, with carbon, hydrogen, oxygen, nitrogen and phosphorous atoms in light blue, white, red, blue, and golden, respectively. Water molecules were omitted for simplicity. c) Representative phase contrast images of DLPC giant unilamellar vesicles (GUVs) recorded during photosensitization with *O*-decyl-Ptr (5 μM) (upper line) and Ptr (5 μM) (lower line). DOPC: 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DLPC: 1,2-Dilinoleoyl-*sn*-glycero-3-phosphocholine.

Upon UVA irradiation lipid peroxidation photosensitized by *O*-decyl-Ptr leads to the formation of hydroxyl derivatives, hydroperoxides and hydroxyhydroperoxides [148]. These photoproducts undergo a fast conversion into short-chain secondary products by cleavage of the fatty acid chains most likely due to further photosensitized processes (Fig. 7). These short-chain oxidized lipids are responsible for destabilizing the phospholipid bilayer and promoting membrane leakage.

The efficiency of photodamage, assessed in terms of oxidized products formation rate and membrane permeabilization, is much higher for *O*-decyl-Ptr than for free Ptr [148], which indicates that the intercalation of the alkyl-pterin to the membrane enhances the photosensitized reactions (Fig. 7). This is in agreement with several other reports in the literature pointing to the fact that membrane insertion is key to cause definitive membrane damage [149]. *O*-decyl-Ptr is also much more efficient than Ptr in the photodynamic activity on *HeLa* cells [150]. The photosensitizer location into the lipid bilayer of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was investigated by means of molecular dynamic simulations and the results revealed that *O*-decyl-Ptr is positioned right between the polar head and the beginning of the fatty acid chains of the phospholipids, overlapping with the C9–C10 double bond of the fatty acids (Fig. 7) [150].

9. Conclusions

Photosensitized oxidations, in part responsible for the harmful effect of UV and visible radiation on biological systems, can involve endogenous or exogenous photosensitizers and take place through type I (generation of radicals) or type II (singlet oxygen ($^1O_2$)) mechanisms. Within pterins, heterocyclic natural occurring compounds, oxidized unconjugated pterins are photochemically active and, in certain pathological conditions, such as vitiligo, accumulate in the skin. Since the photosensitizing properties of these compounds have been investigated in detail using different biomolecules as substrates, the oxidations photosensitized by pterins, under controlled conditions, are useful to understand the complex set of competitive pathways involved in the mechanisms of photoinduced processes.

Oxidized unconjugated pterins absorb in the UVA region and, in consequence, can be excited by solar radiation and artificial sources of light, which leads to the generation of long-lived triplet excited states. These excited states are able to oxidize many biomolecules either by one-electron transfer reactions or through energy transfer to O_2 to generate $^1O_2$, that is, pterins are able to act as photosensitizer through both types of mechanisms. A photosensitized reaction is frequently accepted to be a $^1O_2$-mediated oxidation if: i) O_2 is needed, ii) the photosensitizer generates $^1O_2$ upon irradiation and iii) the target molecule is sensitive to $^1O_2$. In this survey we present several cases using pterin (Ptr), the parent and unsubstituted derivative, as model photosensitizer, in which these three conditions are fulfilled, but the predominant mechanism is type I. Although the explanation for this fact is not simple, a key factor is that whereas type I mechanism involves a single step to chemically modify the substrate, which takes place with a diffusion-limited rate constant, type II involves two steps, the first one at diffusion-limited rate and the second one with a much lower rate constant. Interestingly, O_2 concentration plays a key role in the efficiency of type I mechanism. Although it does not participate in the first steps of the process, O_2 avoids the recombination of radicals and allows the further reaction of substrate radicals. Through this mechanism O_2 favors even the formation of products in which this species does not participate in any step, such is the case of the photosensitized formation of dityrosine (Tyr_2).

Ptr is also able to photosensitize the oxidation of phospholipids. A simple chemical modification of the Ptr structure to make it more lipophilic leads to the binding of the photosensitizer to lipid membranes,

which dramatically increases the efficiency of the photodamage. Likewise, lipophilic pterins are much more phototoxic on eukaryotic cell cultures than their hydrophilic precursors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors sincerely thank the valuable contribution of Gabriela Petroselli, M. Paula Denofrio, Carolina Castaño, Sandra Estébanez, Lara O. Reid, María N. Urrutia who have participated as graduated students in the studies presented in this work. The authors also thank the following researchers for their crucial collaboration: Esther Oliveros and Patricia Vicendo (Université Paul Sabatier/CNRS, France); André M. Braun (Karlsruhe Institute of Technology, Germany); Rosa Erra-Balsells and Sergio M. Bonesi (Universidad de Buenos Aires/CONICET, Argentina); Claudio D. Borsarelli (Universidad de Santiago del Estero/CONICET, Argentina); Niluksha Walalawela and Alexander Greer (City University of New York, USA); Rosangela Itri and Mauricio S. Baptista (Universidade de São Paulo, Brasil); Alejandro Vignoni, Virginie Lhiaubet-Vallet, Miguel A. Miranda and María Luisa Marin (Universitat Politecnica de Valencia/CSIC, Spain); Carla Layana (Universidad Nacional de La Plata (UNLP)/CONICET). The authors acknowledge the institutions that support their work: Consejo Nacional de Investigaciones Científicas y Tecnicas (CONICET), Agencia de Promoción Científica y Tecnológica (ANPCyT) and UNLP.

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