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Production and quenching of reactive oxygen species by pterin derivatives, an intriguing class of biomolecules*

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Abstract: Pterins, a family of heterocyclic compounds derived from 2-aminopteridin-4(1*H*)-one, are widespread in living systems and participate in important biological functions, such as metabolic redox processes. Under UV-A excitation (320–400 nm), aromatic pterins (Pt) can generate reactive oxygen species (ROS), as a consequence of both energy- and electron-transfer processes from their triplet excited state. Quantum yields of singlet oxygen ($^1O_2$) production depend largely on the nature of the substituents on the pterin moiety and on the pH. Formation of the superoxide anion by electron transfer between the pterin radical anion and molecular oxygen leads to the production of significant amounts of hydrogen peroxide (H_2O_2) by disproportionation. Dihydropterins (H_2Pt) do not produce $^1O_2$ but are oxidized by this species with high rate constants yielding pterins as well as H_2O_2. In contrast to aromatic derivatives, H_2Pt are oxidized by H_2O_2, and rates and products strongly depend on the nature of the substituents on the H_2Pt moiety. Aromatic pterins have been found in vivo under pathological conditions, e.g., biopterin or 6-carboxypterin are present in the skin of patients affected by vitiligo, a depigmentation disorder. The biomedical implications of the production of ROS by pterin derivatives and their reactivity with these species are discussed.

Keywords: hydrogen peroxide; photochemical reactivity; pterin derivatives; quantum yields; rate constants; reactive oxygen species (ROS); singlet oxygen; superoxide anion.

INTRODUCTION

Pterins, a family of heterocyclic compounds (Table 1), are present in biological systems in multiple forms and play different roles ranging from pigments to enzymatic cofactors for numerous redox and one-carbon transfer reactions [1,2]. These compounds are derived from 2-aminopteridin-4(1*H*)-one or pterin (Ptr). The most common pterin derivatives are 6-substituted compounds (Table 1). According to the molecular weight and the functional groups of these substituents, pterins can be divided into two groups: (1) *unconjugated pterins*, containing substituents with one carbon atom or a short hydrocarbon

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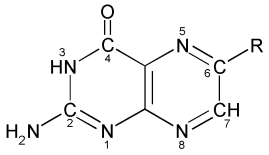
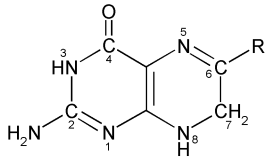
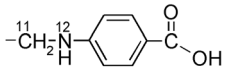
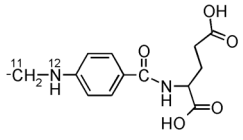
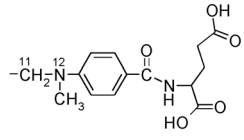
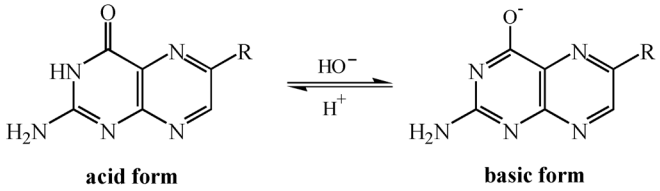
chain, and (2) *conjugated pterins*, with larger substituents containing a *p*-aminobenzoic acid (PABA) moiety.

Table 1 Molecular structures of pterin derivatives investigated and corresponding pK_a values (Dmp and H_2Dmp have an additional methyl group at position 7 of the pterin moiety).

R	Aromatic pterin	pK_a	7,8-Dihydropterin	pK_a
				
UNCONJUGATED PTERINS				
–H	pterin (Ptr)	7.9[a]	–	
$-CH_3$	6-methylpterin (Mep)	8.3[a]	6-methyl-7,8-dihydropterin (H_2Mep)	10.85[c]
$-CH_3$	6,7-dimethylpterin (Dmp)	8.6[a]	6,7-dimethyl-7,8-dihydropterin (H_2Dmp)	11.09[c]
$-CH_2OH$	6-hydroxymethylpterin (Hmp)	8.1[a]	–	
–CHO	6-formylpterin (Fop)	7.3[a]	6-formyl-7,8-dihydropterin (H_2Fop)	9.68[b]
–COOH	6-carboxypterin (Cap)	7.9[a]	–	
$-(CHOH)_2-CH_3$	biopterin (Bip)	8.1[a]	7,8-dihydrobiopterin (H_2Bip)	10.85[d]
$-(CHOH)_2-CH_2OH$	neopterin (Nep)	8.0[a]	7,8-dihydroneopterin (H_2Nep)	10.62[e]
$-(CHOH)_3-CH_3$	rhamnopterin (Rap)	8.0[a]	–	
=O	–		7,8-dihydroxanthopterin (H_2Xap)	9.91[e]
$-CO-CHOH-CH_3$	–		sepiapterin (Sep)	9.95[c]
CONJUGATED PTERINS				
$-CH_2$–PABA	pteroic acid (Pte)	8.5[a]	–	
$-CH_2$–PABAGlu	folic acid (PteGlu)	8.1[a]	7,8-dihydrofolic acid (H_2PteGlu)	10.41[d]
$-CH_2$–MePABAGlu	10-methylfolic acid (MePteGlu)	8.4[a]	–	
				
$-CH_2$–PABA	$-CH_2$–PABAGlu		$-CH_2$–MePABAGlu	

[a][12]
[b][38]
[c][31]
[d][3]
[e]Unpublished results.

Pterins are present in living systems mainly at three different redox states: fully oxidized (or aromatic) pterins, and dihydro and tetrahydro derivatives. They behave as weak acids in aqueous solution. The dominant equilibrium at pH > 5 involves the lactam group (pyrimidine ring) (Scheme 1). The pK_a of this equilibrium is ca. 8 for the aromatic pterins and ca. 10 for dihydropterin derivatives (Table 1). Other functional groups of the pterin moiety (e.g., the 2-amino group or ring N-atoms) have pK_a values <2 [4].



Scheme 1 Main acid–base equilibrium of pterin derivatives.

Pterins participate in relevant and diverse biological functions. Some pterin derivatives [e.g., xanthopterin (6-hydroxypterin), leucopterin (6,7-dihydroxypterin)] are present in butterflies as natural pigments [1]. Folic acid (PteGlu) or pteroyl-L-glutamic acid, a conjugated pterin, is the B9 vitamin and is essential to cellular functions as a coenzyme in reactions related to the synthesis of purine and pyrimidine bases [5]. Tetrahydrobiopterin, the main unconjugated pterin in vertebrates, acts as a coenzyme in hydroxylation reactions of some amino acids metabolism [6] and is also relevant in the nitric oxide metabolism [7]. H_2Nep and Nep are secreted by human macrophages upon stimulation with interferon-γ [8,9]. When the cellular immune system is activated, the concentration of Nep increases in body fluids and, consequently, measurements of its concentration allows sensitive monitoring of the degree of immune activation [10,11].

The interest in the photochemistry and photophysics of pterin derivatives has been increasing steadily during the past decade, due to the implication of these compounds in various photobiological processes. Under UV-A excitation (320–400 nm), these biomolecules can fluoresce, undergo photooxidation to produce different products, and generate reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) [12]. Physiological or pathological situations can lead to the accumulation of pterins in the skin exposed to sunlight. Vitiligo, a depigmentation disorder, is an interesting example from a biomedical point of view. Some pterin derivatives (e.g., biopterin, 6-carboxypterin) accumulate in the skin of patients affected by this pathology, where the protection against UV-radiation fails due to the lack of melanin [13]. Different studies performed on this disease indicate that excited states of pterins are photogenerated in vivo [6,14]. In addition, the photodegradation in vivo of folic acid, a conjugated pterin, has also been demonstrated in independent investigations [15]. The folic acid derivative 5,10-methenyltetrahydrofolate is present as the light-harvesting antenna in DNA photolyases [16], involved in DNA repair after UV irradiation. Some reports suggested that pterins may act as blue antennas in superior plants [17] and play some role in photosynthesis [18].

In this paper, we present an overview of the variety of pathways resulting in the production of ROS by pterin derivatives. Photochemical production of $^1O_2$, superoxide anion ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) is most relevant for understanding the photosensitizing properties of these heterocycles. The reactivity of pterin derivatives with the different ROS shows the complexity that may result from successive steps involving both oxidized and reduced pterins, as well as more than one ROS. The considerable structural and pH dependence of the mechanisms involved in both the production and quenching of ROS by pterin derivatives has important biological implications, and it is shown how the results obtained contribute to a better understanding of the role of these compounds from a biomedical point of view.

PRODUCTION OF SINGLET OXYGEN BY PTERIN DERIVATIVES

Singlet oxygen [$O_2(^1\Delta_g)$], the lowest electronic excited state of molecular oxygen, is an important oxidizing intermediate in chemical processes and one of the main ROS responsible for the damaging effects of light on biological systems (photodynamic effects) [19,20]. Photosensitization is primarily responsible for the production of $^1O_2$ in vivo [21]. In this process, $^1O_2$ is most often produced by energy transfer from the excited triplet state of a sensitizer (^{3}Sens*) to dissolved molecular oxygen (reactions 2 and 3). Subsequently, $^1O_2$ relaxes to its ground state ($^3O_2$) through solvent-induced radiationless and radiative pathways (reactions 4 and 5). It may also be deactivated by a physical quencher (reaction 6) and/or oxidize an acceptor molecule (reaction 7) [22].

$$^1\text{Sens}_0 + h\nu \rightarrow {}^1\text{Sens}^* \quad (1)$$

$$^1\text{Sens}^* \rightarrow {}^3\text{Sens}^* \quad k_{ISC} \quad (2)$$

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

$$^1O_2 \rightarrow {}^3O_2 \quad k_d \quad (4)$$

$$^1O_2 \rightarrow {}^3O_2 + h\nu'' \quad k_e \quad (5)$$

$$Q + {}^1O_2 \rightarrow Q + {}^3O_2 \quad k_q \quad (6)$$

$$Q + {}^1O_2 \rightarrow QO_2 \quad k_r \quad (7)$$

Recent systematic studies of the production of $^1O_2$ by oxidized and reduced pterins in aqueous solution revealed that the oxidation state of the pterin derivative, the nature of the 6-substituent on the pterin moiety, as well as the pH, considerably affect the quantum yields of $^1O_2$ production (Φ_Δ) [23–26]. In these studies, Φ_Δ values (Table 2) were determined by analysis of the weak $^1O_2$ NIR phosphorescence at 1270 nm, produced upon excitation of the pterins with UV-A radiation, as described in, e.g., [23,26,27].

In general, aromatic unconjugated pterins produce significant amounts of $^1O_2$, both in their acid and basic forms (pH ranges 5.0–6.0 and 10.0–11.0, respectively). Although values of Φ_Δ for the basic forms are higher than those for the corresponding acid forms, Φ_Δ of these compounds are mainly affected by the 6-substituent on the pterin moiety. The poorest $^1O_2$ sensitizer of the series is Dmp with $\Phi_\Delta \leq 0.10$, whereas several pterins of the series (such as Fop, Bip, Nep) are efficient sensitizers with $\Phi_\Delta \geq 0.30$. Therefore, aromatic unconjugated pterins could contribute to photodynamic processes in vivo.

Interestingly, biologically active pterin derivatives (aromatic conjugated pterins and dihydropterins) do not produce $^1O_2$ (Table 2). The very low Φ_Δ values (≤0.02) for the conjugated derivatives, such as folic acid (the most important aromatic pterin in mammalians), was explained by the efficient radiationless deactivation of the S_1 state of the pterin moiety by the large 6-substituent (PABA), acting as an internal fluorescence quencher [30]. Subsequently, intersystem crossing (reaction 2) becomes inefficient and these compounds behave as poor $^1O_2$ sensitizers.

Dihydropterins at physiological pH, conditions where these compounds are in their neutral form ($pK_a \geq 9.5$) [31], are not $^1O_2$ sensitizers ($\Phi_\Delta \leq 10^{-3}$ [26]). This result shows the drastic effect of the oxidation state of the pterin derivative: the loss of aromaticity in the pyrazine ring is responsible for a considerable decrease of the quantum yield of intersystem crossing (Φ_{ISC}) and thus of Φ_Δ.

Table 2 Quantum yields of $^1O_2$ production (Φ_Δ) by pterin derivatives, rate constants of $^1O_2$ total quenching ($k_t = k_r + k_q$), physical quenching (k_q) and rate constants of the chemical reaction with $^1O_2$ (k_r) in air-equilibrated aqueous solutions (data from refs. [23–26,28]).

Compound	Φ_Δ[a]	Φ_Δ[a]	k_t[c]	k_r[d]	k_q[f]
	Acid form pD = 5.5	Basic form pD = 10.5	10^6 M^{-1} s^{-1}	10^6 M^{-1} s^{-1}	10^6 M^{-1} s^{-1}
AROMATIC UNCONJUGATED PTERINS (all k_i for basic form, pD = 10.5)					
Ptr	0.18 ± 0.02	0.30 ± 0.02	2.9 ± 0.3	0.25 ± 0.03	2.6 ± 0.3
Cap	0.27 ± 0.03	0.37 ± 0.02	1.4 ± 0.2	–	–
Fop	0.45 ± 0.05	0.47 ± 0.02	1.4 ± 0.2	–	–
Bip	0.34 ± 0.01	0.40 ± 0.03	2.4 ± 0.3	–	–
Nep	0.23 ± 0.01	0.34 ± 0.04	2.3 ± 0.3	–	–
Mep	0.10 ± 0.02	0.14 ± 0.02	8.0 ± 0.6	4.9 ± 0.7	3 ± 1
Rap[b]	0.13 ± 0.02	0.16 ± 0.02	3.6 ± 0.4	2.4 ± 0.2	1.2 ± 0.6
Hmp	0.15 ± 0.02	0.21 ± 0.01	3.1 ± 0.4	1.2 ± 0.1	1.9 ± 0.5
Dmp	0.04 ± 0.02	0.10 ± 0.02	31 ± 3	10 ± 2	21 ± 5
AROMATIC CONJUGATED PTERINS (all k_i for basic form, pD = 10.5)					
PteGlu	≤0.02	≤0.02	30 ± 3	2.8 ± 0.3	27 ± 3
MePteGlu	≤0.02	≤0.02	44 ± 4	1.9 ± 0.2	42 ± 4
Pte	≤0.02	≤0.02	67 ± 7	12 ± 2	55 ± 9
7,8-DIHYDROPTERINS (all data for acid form, pH or pD = 7.0-7.2)	Φ_Δ[a]		k_t[c] 10^8 M^{-1} s^{-1}	k_r[d,e] 10^8 M^{-1} s^{-1}	k_q[f] 10^8 M^{-1} s^{-1}
H_2Fop	≤0.001		2.1 ± 0.2	3.2 ± 0.4[g]	0.7 ± 0.1
H_2Bip	≤0.001		3.7 ± 0.3	3.1 ± 0.4	–
H_2Nep	≤0.001		4.6 ± 0.4	4.2 ± 0.5	–
H_2Xap	≤0.001		6.8 ± 0.4	7.6 ± 0.8	–
Sep	≤0.001		1.9 ± 0.2	3.5 ± 0.4[g]	0.3 ± 0.1
H_2PteGlu	≤0.001		5.5 ± 0.9	5.3 ± 0.6	–

[a]In D_2O where the $^1O_2$ lifetime ($\tau_\Delta = 1/k_d$) is much longer (62 μs) than in H_2O (3.8 μs) [29]; $^1O_2$ sensitizers of known Φ_Δ employed as standards.
[b]Unpublished Φ_Δ values.
[c]Determined in D_2O by Stern–Volmer analysis of the $^1O_2$ NIR phosphorescence.
[d]Determined by following the disappearance of the substrate by HPLC.
[e]In H_2O.
[f]Obtained by subtracting the k_r values from the corresponding k_t values in D_2O.
[g]$k_r(D_2O) = (1.4 \pm 0.3) \times 10^8$ M^{-1} s^{-1} and $(1.6 \pm 0.3) \times 10^8$ M^{-1} s^{-1} for H_2Fop and Sep, respectively.

REACTIVITY OF PTERIN DERIVATIVES WITH SINGLET OXYGEN

The study of the reactivity of $^1O_2$ with biomolecules is an important tool to analyze their antioxidant capability. If a biological compound is able to deactivate $^1O_2$ efficiently by means of physical quenching, such a compound may have a protective role against $^1O_2$ in vivo, whereas an efficient chemical reaction with $^1O_2$ may be beneficial or harmful to biological systems, depending on the nature of the oxidized products. The efficiencies of these processes may be evaluated by determining the rate constants of $^1O_2$ physical quenching and of the chemical reaction with $^1O_2$ (k_q and k_r, reactions 6 and 7, respectively). Values of these rate constants have been published for aromatic conjugated and unconjugated pterins, as well as for 7,8-dihydropterins, in aqueous solutions [23–25,28,32] (Table 2). Oxidation prod-

ucts have also been investigated to evaluate potential consequences in vivo. The results show striking differences in efficiencies and mechanisms depending on the structural features of the pterin derivatives.

Reactivity of aromatic pterins with singlet oxygen

Unconjugated pterins

The rate constants of the chemical reaction between $^1O_2$ and unconjugated pterin derivatives (k_r) is strongly affected by the nature of the 6-substituent (k_r from 2.5×10^5 to 10^7 M^{-1} s^{-1}, Table 2). The values increase with the electronic activation of the C6=C7 bond of the pyrazine ring, suggesting that the electrophilic attack of $^1O_2$ takes place preferentially on this bond, in agreement with the known reactivity of $^1O_2$ with C=C [33]. For example, as expected according to the electron donor effects of the substituents, values of k_r increase in the order k_r(Ptr) << k_r(Mep) < k_r(Dmp). The oxidation of these compounds by $^1O_2$ leads to the cleavage of the pyrazine ring and the formation of several non-pterinic products. In contrast to k_r, the rate constants of $^1O_2$ physical quenching (k_q in the range $1.2–3 \times 10^6$ M^{-1} s^{-1} for Ptr, Hmp, Rap, and Mep) do not show a direct dependence on the electronic activation of the C6=C7 bond. Charge-transfer deactivation by the amino group at position 2 may contribute significantly to $^1O_2$ physical quenching [34].

Conjugated pterins

These compounds are the most efficient $^1O_2$ quenchers among aromatic pterins, with k_t values an order of magnitude larger than those of 6-substituted unconjugated pterins (Table 2). This result is mainly due to the efficient $^1O_2$ physical quenching through charge-transfer-induced quenching by the aromatic amino group of the PABA unit, in agreement with published data for different types of amines [34,35]. In contrast to unconjugated pterins, k_q values for conjugated derivatives are larger than corresponding k_r values (Table 2).

Two different processes may be distinguished in the chemical reaction of $^1O_2$ with conjugated pterins: (a) the attack of $^1O_2$ on the pterin moiety responsible for the formation of non-pterinic products as in the case of unconjugated derivatives; (b) the attack of $^1O_2$ on the secondary amino group [N(12)-atom] of the PABA unit (Table 1), leading to the oxidation of the amine to an imine with concomitant H_2O_2 elimination (reactions 8 and 9 [36]); hydrolysis of the imine group in aqueous solution induces cleavage of the 6-substituent, yielding Fop and PABA or PABAGlu as products for Pte or PteGlu, respectively. In the case of MePteGlu, the substitution of N(12) by a methyl group prevents this reaction. It should be noted that, although Pte or PteGlu do not produce significant amounts of $^1O_2$, their reaction with $^1O_2$ leads not only to H_2O_2 production, but also to the formation of Fop, an unconjugated pterin with a strong absorption in the UV-A and an efficient $^1O_2$ sensitizer (Table 2).

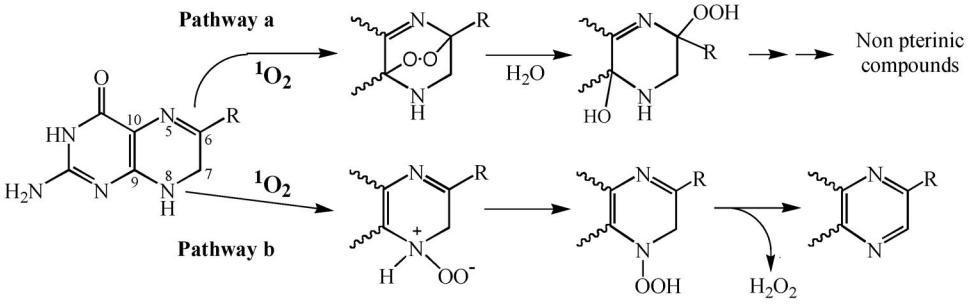
$$RCH_2–N(H)R' + {}^1O_2 \rightarrow RCH_2–N^+(H)R'–OO^- \rightarrow H_2O_2 + RCH=NR' \quad (8)$$

$$RCH=NR' + H_2O \rightarrow RCHO + H_2NR' \quad (9)$$

Reactivity of 7,8-dihydropterins with singlet oxygen

The values of k_t for the dihydropterin derivatives investigated lie in the range from 1.9×10^8 to 6.8×10^8 M^{-1} s^{-1} (Table 2) [26]). These values are one to two orders of magnitude larger than those obtained for aromatic pterin derivatives, indicating that the dihydropyrazine ring is much more efficient in quenching $^1O_2$ than the pyrazine one. Moreover, in contrast to aromatic pterins, which are predominantly $^1O_2$ physical quenchers, 7,8-dihydropterins are very efficient $^1O_2$ acceptors (k_r values larger than 10^8 M^{-1} s^{-1} and very similar to those of k_t, Table 2).

At least two chemical pathways have to be considered for the reaction of $^1O_2$ with dihydropterins (Scheme 2): (a) the oxidation of the dihydropterin moiety to yield an endoperoxide (that could result from the attack of $^1O_2$ to the azadienic system), followed by hydrolytic cleavage into non-pterinic sub-



Scheme 2 Mechanisms proposed for the reaction of $^1O_2$ with the dihydropterin moiety; Pathway (a): reaction of $^1O_2$ with the azadienic system [–C(9)=C(10)–N(5)=C(6)–]; Pathway (b): aromatization via charge-transfer reaction between the N(8)-atom and $^1O_2$ (adapted from [26]).

stances; (b) the oxidation of the dihydropyrazine ring through the charge-transfer reaction between the N(8)-atom and $^1O_2$ followed by subsequent aromatization with concomitant H_2O_2 elimination (reaction 8 in the dihydropyrazine ring). Similarly to the remark made above for Pte and PteGlu, although dihydropterins are not $^1O_2$ sensitizers, their reaction with this species may lead to H_2O_2 production and to the formation of corresponding aromatic pterins, which may be more or less efficient $^1O_2$ sensitizers (Table 2).

PRODUCTION OF SUPEROXIDE ANION AND HYDROGEN PEROXIDE

UV-A irradiation of aqueous solutions containing aromatic pterins results in the production, not only of $^1O_2$, but also of $O_2^{\bullet-}$ and H_2O_2. Since these latter species participate in the physiopathology of many diseases [37], the photochemical production of these species by pterins is biologically relevant. Aromatic pterins are able to generate $O_2^{\bullet-}$ and H_2O_2 through two different photochemical mechanisms:

(a) In neutral or slightly acidic aqueous solutions, *photoinduced electron transfer* from an electron donor (D), which can be the pterin itself or a different compound such as EDTA, to the triplet state of pterin (^{3}Pt*) may initiate a series of reactions leading to the formation of $O_2^{\bullet-}$ and H_2O_2. The main steps of the mechanism are summarized by reactions 10–13 [38], reactions 14–17 being competing pathways. The main results supporting this mechanism are: electron paramagnetic resonance (EPR) analysis in the presence of the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) shows the characteristic signal of the adduct between DMPO and $O_2^{\bullet-}$; the formation of H_2O_2 has been proven by a colorimetric method and its amount increased in the presence of superoxide dismutase (SOD) which catalyzes the $O_2^{\bullet-}$ disproportionation (reaction 13); it also increased with the pterin concentration and decreased when the O_2 concentration increased (D = Pt).

$$^3Pt^* + D \rightarrow Pt^{\bullet-} + D^{\bullet+} \quad (10)$$

$$Pt^{\bullet-} + O_2 \rightarrow Pt + O_2^{\bullet-} \quad (11)$$

$$O_2^{\bullet-} + H_2O \rightarrow HO_2^{\bullet} + HO^- \quad (12)$$

$$HO_2^{\bullet} + O_2^{\bullet-} \rightarrow HO_2^- + O_2 \quad (13)$$

$$Pt^{\bullet-} + D^{\bullet+} \rightarrow Pt + D \quad (14)$$

$$D^{\bullet+} + O_2^{\bullet-} \rightarrow D + O_2 \text{ (and/or } D_{ox}) \quad (15)$$

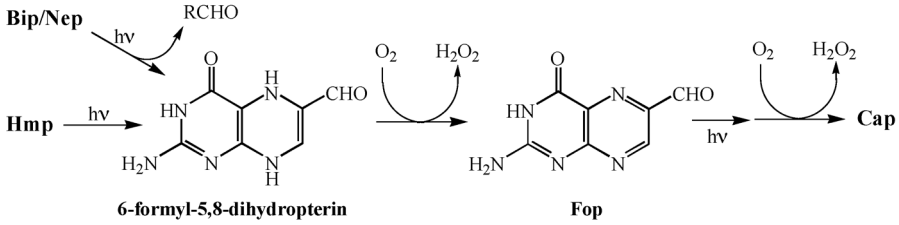
$$^3Pt^* + O_2 \rightarrow Pt + O_2 \ (^1O_2) \quad (16)$$

$$^3Pt^* \rightarrow Pt \quad (17)$$

(b) *Oxidation of 5,8-dihydroderivatives* has been shown to be also a source of H_2O_2. Hence, it was demonstrated that Bip, Nep, or Hmp exposed to UV-A radiation formed a red intermediate, very likely 6-formyl-5,8-dihydropterin, generated in an O_2-independent process (Scheme 3) [39,40].

The red intermediate was rapidly oxidized on admission of O_2 to yield Fop and $O_2^{\bullet-}$, the latter being disproportionated to form H_2O_2. Finally, Fop is photolyzed to Cap, producing more H_2O_2 in the process. The mechanism proposed in Scheme 3 is in agreement with the suggestion that Bip may be a source for H_2O_2 generation in vitiligo [41].

It should be noted that H_2O_2 is also generated by reaction of aromatic conjugated pterins and some dihydropterins with $^1O_2$ (vide supra).



Scheme 3 Photochemistry of Bip, Nep, Hmp, and Fop in aqueous solution (adapted from [12], R = CH_3 and CH_2OH for Bip and Nep, respectively).

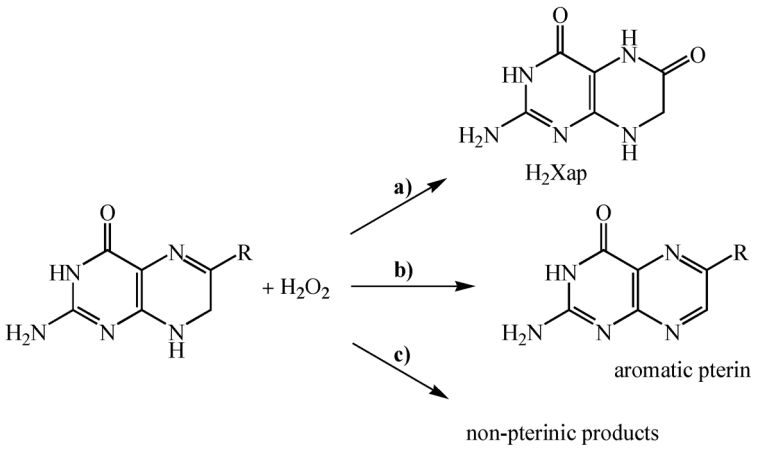
REACTIVITY OF PTERIN DERIVATIVES WITH HYDROGEN PEROXIDE

Since pterins and H_2O_2 accumulate in the skin of patients affected by vitiligo (Introduction), the reactivity of pterins with this oxidant was evaluated. Aromatic pterins are stable in solutions containing H_2O_2, whereas dihydropterins (H_2Pt) undergo oxidation [42]. In air-equilibrated aqueous solution (pH = 7.0–7.2), the rates of oxidation by H_2O_2 were much faster than those by O_2 in its ground state [43]. The rate constants of the reaction with H_2O_2, as well as the products formed, strongly depend on the nature of the substituents on the H_2Pt moiety (Table 3).

Table 3 Rate constants of the chemical reaction between 7,8-dihydropterins and H_2O_2 ($k_{H_2O_2}$) in air-equilibrated aqueous solutions (pH = 7.0 ± 0.1; 37 °C) [42].

Compound	$k_{H_2O_2}/M^{-1}s^{-1}$	Products
H_2Fop	$(1.5 \pm 0.1) \times 10^{-2}$	H_2Xap (>90 %)
H_2Bip	$(2.7 \pm 0.2) \times 10^{-2}$	H_2Xap (>90 %)
H_2Nep	$(3.7 \pm 0.4) \times 10^{-2}$	H_2Xap (>90 %)
H_2Xap	$(2.5 \pm 0.2) \times 10^{-4}$	Xap (<5 %); non-pterinic compounds
H_2Mep	0.66 ± 0.03	Mep (~10 %); non-pterinic compounds
H_2Dmp	0.32 ± 0.02	Dmp (~5 %); non-pterinic compounds
H_2PteGlu	$(6.1 \pm 0.7) \times 10^{-2}$	H_2Xap (~60 %); PteGlu (~40 %)

At least three different pathways were observed (Scheme 4): (a) the cleavage of the substituent and oxidation of the C6 atom to yield H_2Xap (>90 % for H_2Fop, H_2Bip, and H_2Nep); (b) the oxidation of the pyrazine ring to yield the corresponding oxidized pterin derivative; and (c) oxidation and cleavage of the dihydropterin to yield non-pterinic substances.



Scheme 4 Products of the reaction between dihydropterins and H_2O_2 (the percentage of each product depends on the substituent in position 6, see Table 3).

CONCLUSION

Pterin derivatives are a family of interesting heterocyclic biomolecules commonly found in living systems in small amounts. In this overview, we have summarized the variety of pathways likely to lead to the production of ROS (in particular, singlet molecular oxygen, H_2O_2, and superoxide anion), when these compounds are excited by UV-A radiation in aqueous solutions in the presence of molecular oxygen. It has been demonstrated that their capability to produce these ROS depends on the redox state of the pterin, on the chemical nature of the substituents, as well as on the pH. These findings contribute to the understanding of the mechanisms of the photosensitizing properties of pterins, both in model systems (nucleotides) and in biological media [44–46]. Much remains to be learnt about these intriguing biomolecules, and further work will indeed bring new insights into their photophysical and photochemical properties, and their possible consequences in living systems.

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REFERENCES

1. W. Pfleiderer. In *Chemistry and Biology of Pteridines and Folates*, J. E. Ayling, M. G. Nair, C. M. Baugh (Eds.), pp. 1–16, Plenum Press, New York (1993).
2. T. J. Kappock, J. P. Caradonna. *Chem. Rev.* **96**, 2659 (1996).
3. G. Maharaj, B. S. Selinsky, J. R. Appleman, M. Perlman, R. E. London, R. L. Bakley. *Biochemistry* **29**, 4554 (1990).
4. A. Albert. *Biochem. J.* **54**, 646 (1953).

5. R. L. Blakley. *The Biochemistry of Folic Acid and Related Pteridines*, North-Holland, Amsterdam (1969).
6. C. A. Nichol, G. K. Smith, D. Duch. *Annu. Rev. Biochem.* **54**, 729 (1985).
7. J. M. Hevel, M. A. Marletta. *Biochemistry* **31**, 7160 (1992).
8. C. Huber, J. R. Batchelor, D. Fuchs, A. Hausen, A. Lang, D. Niederwieser, G. Reibnegger, P. Swetly, J. Troppmair, H. Wachter. *J. Exp. Med.* **160**, 310 (1984).
9. C. Huber, D. Fuchs, A. Hausen, R. Margreiter, G. Reibnegger, M. Spielberger, H. Wachter. *J. Immunol.* **130**, 1047 (1983).
10. D. Fuchs, G. Weiss, H. Wachter. *Crit. Rev. Clin. Lab. Sci.* **29**, 307 (1992).
11. D. Fuchs, G. Weiss, H. Wachter. *Int. Arch. Allergy Immunol.* **101**, 1 (1993).
12. C. Lorente, A. H. Thomas. *Acc. Chem. Res.* **39**, 395 (2006).
13. K. U. Schallreuter, J. Moore, J. M. Wood, W. D. Beazley, E. M. Peters, L. K. Marles, S. C. Behrens-Williams, R. Dummer, N. Blau, B. Thöny. *J. Invest. Dermatol.* **116**, 167 (2001).
14. K. U. Schallreuter, J. M. Wood, M. R. Pittelkow, M. Gütlich, K. R. Lemke, W. Rödl, N. N. Swanson, K. Hitzemann, I. Ziegler. *Science* **263**, 1444 (1994).
15. R. F. Branda, J. W. Eaton. *Science* **201**, 625 (1978).
16. J. E. Hearst *Science* **268**, 1858 (1995).
17. P. Galland, H. Senger. *Photochem. Photobiol.* **48**, 811 (1988).
18. R. C. Fuller, G. W. Kidder, N. A. Nugent, V. C. Dewey, N. Rigopoulos. *Photochem. Photobiol.* **14**, 359 (1971).
19. M. C. DeRosa, R. J. Crutchley. *Coord. Chem. Rev.* **233–234**, 351 (2002).
20. J. Cadet, J. L. Ravanat, G. R. Martinez, M. H. G. Medeiros, P. Di Mascio. *Photochem. Photobiol.* **82**, 1219 (2006).
21. M. Tarr, D. P. Valenzeno. *Photochem. Photobiol. Sci.* **2**, 355 (2003).
22. C. Schweitzer, R. Schmidt. *Chem. Rev.* **103**, 1685 (2003).
23. A. H. Thomas, C. Lorente, A. L. Capparelli, C. G. Martínez, A. M. Braun, E. Oliveros. *Photochem. Photobiol. Sci.* **2**, 245 (2003) and refs. therein.
24. F. M. Cabrerizo, A. H. Thomas, C. Lorente, M. L. Dántola, G. Petroselli, R. Erra-Balsells, A. L. Capparelli. *Helv. Chim. Acta* **87**, 349 (2004).
25. F. M. Cabrerizo, C. Lorente, M. Vignoni, R. Cabrerizo, A. H. Thomas, A. L. Capparelli. *Photochem. Photobiol.* **81**, 793 (2005).
26. M. L. Dántola, A. H. Thomas, A. M. Braun, E. Oliveros, C. Lorente. *J. Phys. Chem. A* **111**, 4280 (2007).
27. A. M. Braun, E. Oliveros. *Pure Appl. Chem*. **62**, 1467 (1990).
28. F. M. Cabrerizo, M. L. Dántola, G. Petroselli, A. L. Capparelli, A. H. Thomas, A. M. Braun, C. Lorente, E. Oliveros. *Photochem. Photobiol.* **83**, 526 (2007) and refs. therein.
29. F. Wilkinson, H. P. Helman, A. B. Ross. *J. Phys. Chem. Ref. Data* **24**, 663 (1995).
30. F. M. Cabrerizo, G. Petroselli, A. H. Thomas, C. Lorente, A. L. Capparelli, A. M. Braun, E. Oliveros. *Photochem. Photobiol.* **81**, 1234 (2005).
31. W. Pfleiderer. In *Biochemical and Clinical Aspects of Pteridines*, Vol. 5, W. Pfleiderer, H. Wachter, J. A. Blair (Eds.), pp. 3–21, Walter de Gruyter, Berlin (1987).
32. F. M. Cabrerizo, M. L. Dántola, A. H. Thomas, C. Lorente, A. M. Braun, E. Oliveros, A. L. Capparelli. *Chem. Biodiv.* **1**, 1800 (2004).
33. E. L. Clennan, A. Pace. *Tetrahedron* **61**, 6665 (2005).
34. B. M. Monroe. *J. Phys. Chem.* **14**, 1861 (1977).
35. (a) A. P. Darmanyan, W. S. Jenks, P. Jardon. *J. Phys. Chem. A* **102**, 7420 (1998); (b) Erratum: A. P. Darmanyan, W. S. Jenks, P. Jardon. *J. Phys. Chem. A* **102**, 9308 (1998).
36. K. Gollnick, J. H. E. Lindner. *Tetrahedron Lett.* **21**, 1903 (1973).
37. M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, J. Telser. *Int. J. Biochem. Cell Biol.* **39**, 44 (2007).

38. M. L. Dántola, M. Vignoni, C. González, C. Lorente, P. Vicendo, E. Oliveros, A. H. Thomas. *Free Rad. Biol. Med.* **49** 1014 (2010).
39. M. Vignoni, F. M. Cabrerizo, C. Lorente, A. H. Thomas. *Photochem. Photobiol.* **85**, 365 (2009).
40. M. Vignoni, M. L. Salum, R. Erra-Balsells, A. H. Thomas, F. M. Cabrerizo. *Chem. Phys. Lett.* **484**, 330 (2010).
41. H. Rokos, W. D. Beazley, K. U. Schallreuter. *Biochem. Biophys. Res. Commun.* **292**, 805 (2002).
42. M. L. Dántola, T. M. Schuler, M. P. Denofrio, M. Vignoni, A. L. Capparelli, C. Lorente, A. H. Thomas. *Tetrahedron* **64**, 8692 (2008).
43. M. L. Dántola, M. Vignoni, A. L. Capparelli, C. Lorente, A. H. Thomas. *Helv. Chim. Acta* **91**, 411 (2008).
44. K. Hirakawa, H. Suzuki, S. Oikawa, S. Kawanishi. *Arch. Biochem. Biophys.* **410**, 261 (2003).
45. G. Petroselli, R. Erra-Balsells, F. M. Cabrerizo, C. Lorente, A. L. Capparelli, A. M. Braun, E. Oliveros, A. H. Thomas. *Org. Biomol. Chem.* **5**, 2792 (2007).
46. G. Petroselli, M. L. Dántola, F. M. Cabrerizo, A. L. Capparelli, C. Lorente, E. Oliveros, A. H. Thomas. *J. Am. Chem. Soc.* **130**, 3001 (2008).

