Photophysics and Photochemistry of Pterins in Aqueous Solution

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ABSTRACT
Pterins belong to a family of heterocyclic compounds present in a wide range of living systems and participate in relevant biological functions. Interest in the photochemistry and photophysics of this group of compounds has increased since the participation of pterin derivatives in different photobiological processes has been suggested or demonstrated in recent decades. This account describes and connects basic studies on the fluorescence emission, the photooxidation, and the photosensitizing properties of oxidized six-substituted pterins in aqueous solution under UV-A irradiation. The biological implications of these studies are also discussed.

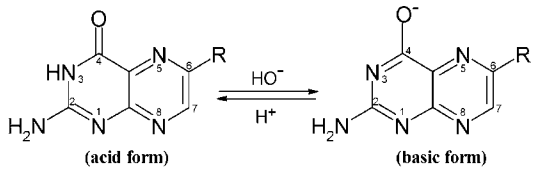
Introduction

Pterins (PTs), heterocyclic compounds widespread in biological systems, are derived from 2-aminopteridin-4(1*H*)-one or pterin (PT). The most common PT derivatives are six-substituted compounds (Scheme 1). The molecular weights and the functional groups of these substituents are quite different; for example, PTs may have substituents with one carbon atom, with a short hydrocarbon chain, or bigger substituents containing a *p*-aminobenzoic acid (PABA) moiety. Derivatives with the latter type of substituents are frequently called conjugated PTs.

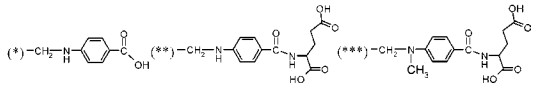
PTs behave as weak acids in aqueous solution. In general, the dominant equilibrium at pH > 5 involves an amide group (acid form) and a phenolate group (basic form).[1] As shown in Scheme 1, the pK_a of this equilibrium is ca. 8 for the different PT derivatives. Other functional groups of the PT moiety (e.g., the 2-amino group or ring N-atoms) have pK_a values <2.[1]

PTs participate in relevant biological functions. Some PT derivatives [e.g., xantopterin (6-hydroxypterin) and leucopterin (6,7-dihydroxypterin)] are present in butterflies[2] as natural pigments. Folic acid (FA) or pteroylglutamic acid, a conjugated PT, is a vitamin of the B group and acts as a coenzyme in reactions related to the synthesis of puric and pyrimidinic bases.[3] Tetrahydrobiopterin acts as a coenzyme in hydroxylation reactions of some amino acids metabolism[4] and is also relevant in nitric oxide metabolism.[5] Neopterin (NPT), a metabolite in the biosynthesis of tetrahydrobiopterin, is synthesized mainly in activated macrophages. The determination of the concentrations of PTs in body fluids has proven its clinical value; for example, high levels of NPT are related to activity in cell-mediated immune responses and are particularly apparent during infections caused by viruses and intracellular bacteria and parasites.[6]

Scheme 1. Molecular Structures of Common PT Derivatives and the Acid−Base Equilibrium in Aqueous Solution[a]



R	*Compound*	pK_a
-H	pterin (PT)	7.9
-CH_3	6-methylpterin (MPT)	8.3
-CH_3	6,7-dimethylpterin (DPT)	8.6
-CH_2OH	6-(hydroxymethyl)pterin (HPT)	8.1
-CHO	6-formylpterin (FPT)	7.3
-COOH	6-carboxypterin (CPT)	7.9
-$(CHOH)_2$-CH_3	biopterin (BPT)	8.1
-$(CHOH)_2$-CH_2OH	neopterin (NPT)	8.0
-$(CHOH)_3$-CH_3	rhamnopterin (RPT)	8.0
(*)	pteroic acid (PA)	8.5
(**)	folic acid (FA)	8.1
(***)	10-methylfolic acid (MFA)	8.4



[a] DPT has an additional methyl group at position 7 of the PT moiety.

The participation of PT derivatives in different photobiological processes has been suggested or demonstrated in past decades, and interest in the photochemistry and photophysics of this group of compounds has subsequently increased. The FA derivative 5,10-methenyltetrahydrofolate is present as the light-harvesting antenna in DNA photolyases,[7] involved in DNA repair after UV irradiation. Some reports suggested that PTs may act as blue antennas in superior plants[8] and play some role in photosynthesis.[9]

Interest in the photophysics of PTs has also increased since the fluorescence of these compounds has been used for analytical purposes. Some assays for analyzing the concentration of FA use the emission of 6-carboxypterin

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(CPT) generated after oxidation of FA with $KMnO_4$.[10] Many experimental assays for the determination of PT concentration by chromatographic methods use fluorescence detectors.[11] In addition, fluorescence is a useful tool to study nucleic acids and their interactions with proteins. DNA probes containing fluorophores are increasingly used to investigate different aspects of the physicochemical properties of DNA, such as the kinetics of interactions with other biomolecules and changes in structure. Recently, some pteridine-based fluorophores that are chemical analogues of the nucleosides of DNA have been developed.[12]

PTs exist in different redox states in vivo. The molecular structures shown in Scheme 1 correspond to the oxidized state of PTs. Many active derivatives that participate as enzymatic cofactors are tetrahydropterins. This reduced state is labile in solution and can react with O_2 to yield dihydro and oxidized derivatives.[13] Physiological or pathological situations can lead to the accumulation of PTs in regions exposed to sunlight. Vitiligo, a depigmentation disorder, is an interesting example from a biomedical point of view. Because of the accumulation of oxidized PTs, patients affected by this pathology express a characteristic fluorescence in their white skin patches upon Wood's light examination.[14] Thus, the photochemistry and photophysics of oxidized PTs become of importance since these compounds accumulate in zones of the skin where the protection against UV radiation fails due to the lack of melanin.

Despite their evident importance, many aspects of the photophysical and photochemical properties of PTs and their biological implications remained mostly unknown for many decades. Thus, questions about the properties of the excited states, about the relationship between the photophysical and photochemical behavior and the chemical structure, about the photoinduced production of reactive oxygen species, and about the capacity to photosensitize biomolecules had no answers. However, systematic studies that deal with such matters have been developed in the past decade. Our account focuses on those recent basic studies of the photochemistry and the photophysics of six-substituted oxidized PTs in aqueous solution under UV-A radiation. In particular, we discuss their absorption and emission properties and the various pathways of their photooxidation. Finally, we describe briefly some photosensitizing properties such as photosensitized production of singlet molecular oxygen ($^1O_2$) and the photosensitization of biomolecules by PTs.

Absorption Spectra

The absorption spectra of PT and of most of the oxidized PT derivatives with substituents at position 6 containing a single carbon atom or short hydrocarbon chains typically show two main absorption bands in the range of 230−500 nm.[15,16] These absorption bands correspond to transitions from the singlet ground state of the PT moiety (S_0) to singlet excited states (S_1, S_2). The wavelengths of the maxima of absorption of a series of PT derivatives are listed in Table 1. The high energy band ($S_0 \rightarrow S_2$) of the acid form of these compounds ($\lambda_{max} \sim 270-285$ nm) is less intense and red-shifted in comparison with the corresponding band of the basic form ($\lambda_{max} \sim 250-260$ nm) (Figure 1a). On the other hand, the low energy band ($S_0 \rightarrow S_1$) of the acid form ($\lambda_{max} \sim 340-350$ nm) is less intense but blue-shifted in comparison with the corresponding band of the basic form ($\lambda_{max} \sim 355-370$ nm) (Figure 1a).

Table 1. Wavelengths of the Absorption Maxima (λ_{max}, nm) of PT Derivatives in Aqueous Solution[15,16]

		PT moiety		
compound	form	high energy band	low energy band	PABA moiety
PT	acid	270	340	
	basic	252	358	
MPT	acid	271	347	
	basic	252	363	
HPT	acid	275	345	
	basic	254	364	
FPT	acid	276	346	
	basic	280	370	
CPT	acid	286	346	
	basic	264	364	
DPT	acid	273	344	
	basic	250	358	
BPT	acid	274	346	
	basic	254	363	
NPT	acid	274	346	
	basic	254	363	
RPT	acid	273	344	
	basic	254	363	
FA	acid	285	354	285
	basic	255	365	285
PA	acid	279	347	279
	basic	257	366	277
MFA	acid	281	∼345	303
	basic	255	365	303

The spectra of conjugated PTs having a PABA moiety in position 6 (Scheme 1) show an additional absorption band not affected by the pH (Table 1). In the basic forms, the PABA band is clearly separated from the PT bands (Figure 1b). The corresponding acid forms show a PABA band completely or partially superimposed with the high energy band due the pH-induced shift of the PT bands (Figure 1b).

Emission Properties

The emission spectra of PTs, upon excitation into the low energy PT band (350 nm), correspond to the transition ($S_1 \rightarrow S_0$) (reaction 1, Scheme 2) and show a broad band centered at approximately 450 nm.[15−17] The emission spectra of the basic forms are red-shifted by approximately 10 nm in comparison with those of the acid forms obtained by excitation at the same wavelength (see Figure 2 for PT). The wavelengths of the fluorescence maxima (λ_F) are listed in Table 2.

For unconjugated PTs, the fluorescence spectrum (normalized relative to the maximum emission value for comparative purposes) remained unchanged, irrespective of the excitation wavelength in the range of 230−350 nm, suggesting that only S_1 of the PT is the emissive state. However, the fluorescence intensities decreased when

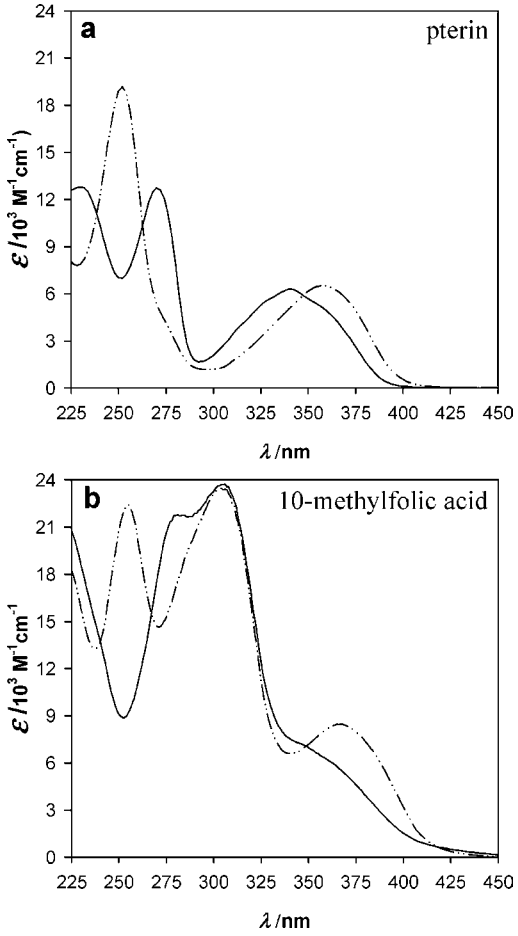
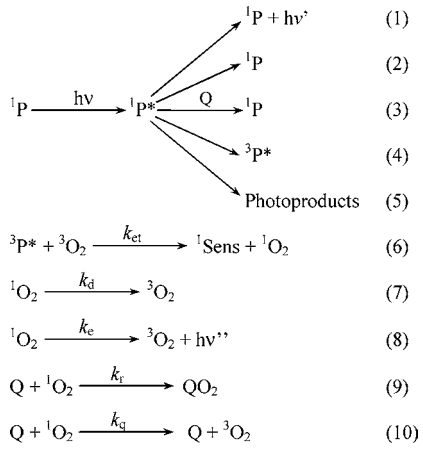


FIGURE 1. Absorption spectra of air-equilibrated aqueous solutions of PT[15] and MFA;[16] solid lines, acid forms (pH 5.5); dashed−dotted lines, basic forms (pH 10.5).

Scheme 2. Processes Initiated by the Excitation of the Low Energy PT Band (350 nm)[a]



[a] P, PT derivatives.

exciting into the high energy absorption bands (vide infra). In the case of conjugated PTs, corrected fluorescence spectra were registered by excitation between 250 and 320 nm, where both the PT high energy band and the PABA substituent are excited. Under these conditions, the fluorescence spectra of the acid and basic forms have two emission bands (Figure 3 for PA), i.e., a band centered at

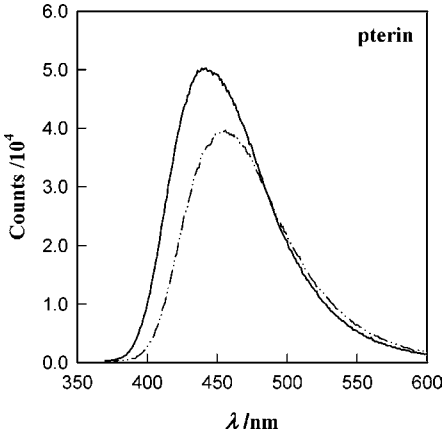


FIGURE 2. Corrected fluorescence spectra of PT obtained by excitation at 350 nm[15] [spectra were recorded using solutions of equal absorbance (<0.12) at the excitation wavelength]; solid lines, absorption spectra of acid form (pH 5.5); dashed−dotted lines, absorption spectra of basic form (pH 10.5).

Table 2. Wavelengths of Fluorescence Maxima (λ_F), Fluorescence Quantum Yields (Φ_F), Fluorescence Lifetimes (τ_F), and Bimolecular Quenching Rate Constants (k_q) for the Quenching of the Fluorescence of PT Derivatives by Phosphate and Acetate[15−17] [a]

compound	form	λ_F (nm) (±3)	Φ_F	τ_F (ns) (±0.4)	10^9 M^{-1} s^{-1} k_q[b]	10^9 M^{-1} s^{-1} k_q[c]
PT	acid	439	0.33 ± 0.01	7.6	1.6	2.1
	basic	456	0.27 ± 0.01	5.0	0.18	
MPT	acid	448	0.61 ± 0.01	13.3		
	basic	460	0.61 ± 0.04	11.2		
HPT	acid	449	0.53 ± 0.02	11.0		
	basic	457	0.46 ± 0.01	8.4		
FPT	acid	446	0.12 ± 0.01	7.9	1.2	1.3
	basic	454	0.07 ± 0.01	2.2		
CPT	acid	439	0.28 ± 0.01	5.8	0.93	1.6
	basic	451	0.18 ± 0.01	4.1		
DPT	acid	433	0.85 ± 0.01	13.5		
	basic	445	0.84 ± 0.02	11.6		
BPT	acid	441	0.36 ± 0.01	9.1	1.5	1.6
	basic	455	0.29 ± 0.01	7.6	0.11	
NPT	acid	440	0.38 ± 0.01	8.9	1.3	1.5
	basic	454	0.31 ± 0.01	7.4	0.09	
RPT	acid	441	0.47 ± 0.01	10.7		
	basic	455	0.40 ± 0.01	7.5		
FA	acid	445	<0.005			
	basic	455	<0.005			
PA	acid	450	(6.1 ± 0.5) × 10^{-3}			
	basic	460	(7.9 ± 0.3) × 10^{-3}			
MFA	acid	455	~0.001			
	basic	465	~0.001			

[a] Measurements were carried out for acidic and basic forms in the pH ranges 5.0−5.5 and 10.0−10.5, respectively (in air-equilibrated aqueous solutions of PT derivatives; excitation wavelength, 350 nm; standard deviations are given in parentheses). [b] Fluorescence quenching by phosphate. [c] Fluorescence quenching by acetate.

ca. 450−470 nm, which corresponds to the emission of the PT S_1 state, and a band below 400 nm due to the PABA emission.

In general, the fluorescence quantum yields (Φ_F) upon excitation at 350 nm in Ar-saturated solutions (Table 2) of the acid forms are higher than the corresponding values for the basic forms and are considerably affected by the nature of the six-substituent.[15−17] Φ_F values of conjugated PTs (FA, PA, and MFA) in both acidic and alkaline media are very low (<0.01) in comparison with those of the rest

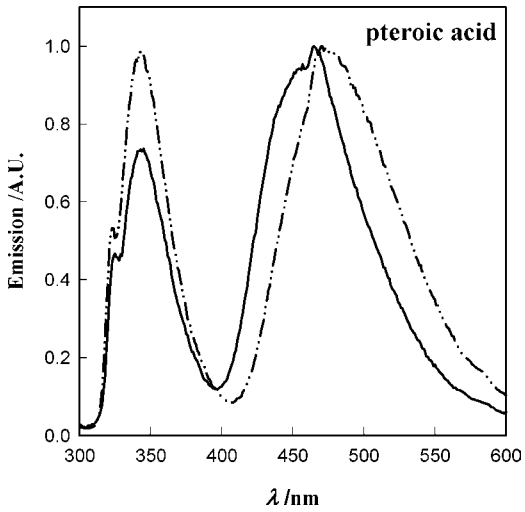


FIGURE 3. Corrected and normalizated fluorescence spectra of air-equilibrated aqueous solutions of PA obtained by excitation at 278 nm;[17] solid lines, absorption spectra of acid form (pH 5.5); dashed—dotted lines, absorption spectra of basic form (pH 10.5).

of the PT derivatives studied ($0.07 \leq \Phi_F \leq 0.85$). The relatively long chain substituent containing a PABA unit of conjugated PTs might act as an "internal quencher", enhancing the radiationless deactivation of the singlet excited state (S_1) (reaction 2, Scheme 2). Except for DPT, the differences between the Φ_F values determined in argon-saturated, air-equilibrated, and oxygen-saturated solutions are not significant, indicating that quenching of the S_1 state by O_2 is negligible.[15−17]

The excitation spectra recorded by monitoring the fluorescence at 450 nm of unconjugated and conjugated PTs show different features.[16] For the former group, the maxima of the excitation and of the absorption spectra are similar. However, the intensity ratio of the high energy to that of the low energy band is much lower than the corresponding ratio in the absorption spectra. The Φ_F values obtained by excitation into the high energy bands are much lower than those corresponding to the low energy bands.[15,16] These results suggest that only a fraction of the energy of the upper excited state(s) (S_2, S_n) is dissipated through internal conversion to the lowest singlet excited state (S_1). Therefore, a photophysical (e.g., intersystem crossing to the triplet manifold) or/and a photochemical process should occur from the upper excited singlet states. The excitation spectra of conjugated PTs also show two bands with wavelength ranges that match those of the absorption bands of the PT moiety.[16] In contrast, the band corresponding to the absorption of the substituent is missing in the excitation spectra. Therefore, we conclude that excitation of the substituent does not populate the S_1 state of the PT moiety.

The fluorescence decay upon excitation at 350 nm and analyzed at 450 nm ($S_1 \rightarrow S_0$) followed a first-order rate law for all of the compounds studied.[15−17] Reported fluorescence lifetimes (τ_F) are listed in Table 2.

Under specific experimental conditions (pH, anion concentration), some anions are able to significantly reduce the emission of PTs in aqueous solutions.[17] The acid forms of five PTs [PT, CPT, NPT, 6-formylpterin (FPT), and biopterin (BPT)] undergo dynamic quenching by phosphate and acetate (k_q in Table 2) (reaction 3, Scheme 2; Q, phosphate or acetate). These results are of importance from the technical and analytical points of view because measurements of the fluorescence of PT derivatives for a variety of purposes are often performed in the presence of salts, e.g., buffers, and significant quenching of the PT fluorescence by the buffer might lead to errors in interpretation and erroneous conclusions.

On the other hand, the fluorescence of the basic forms is neither quenched by these same anions or such a quenching is negligible in comparison with that for the acid forms. Charge effects might explain these results. Another explanation for the different behavior of acid and basic forms toward fluorescence quenching by anions has been suggested as follows: Quenching of the fluorescence of acid forms may occur by a proton-transfer mechanism.[17] This hypothesis is supported by the fact that anions of strong acids such as chloride, sulfate, and nitrate do not quench the fluorescence of PT derivatives. Moreover, k_q is readily correlated with the pK_b values of the anions.[17]

Table 3. Quantum Yields of Disappearance of PTs[a]

compound	Φ (acid forms)	Φ (basic forms)	type of reaction
FA	2.5×10^{-2}	5.1×10^{-3}	1
FPT	4.0×10^{-2}	9.0×10^{-3}	1
HPT	2.3×10^{-3}	1.8×10^{-2}	1
PT	8.2×10^{-4}	1.2×10^{-3}	2
CPT	5.1×10^{-3}	1.3×10^{-3}	2
MPT	2.4×10^{-4}	8.1×10^{-4}	2

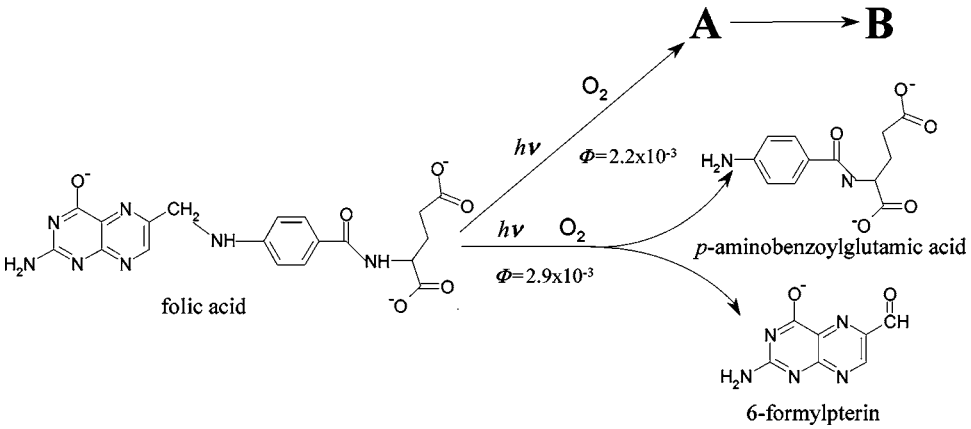
[a] Reaction types: 1, oxidation of the substituent; 2, oxidation of the PT moiety.

Photochemistry

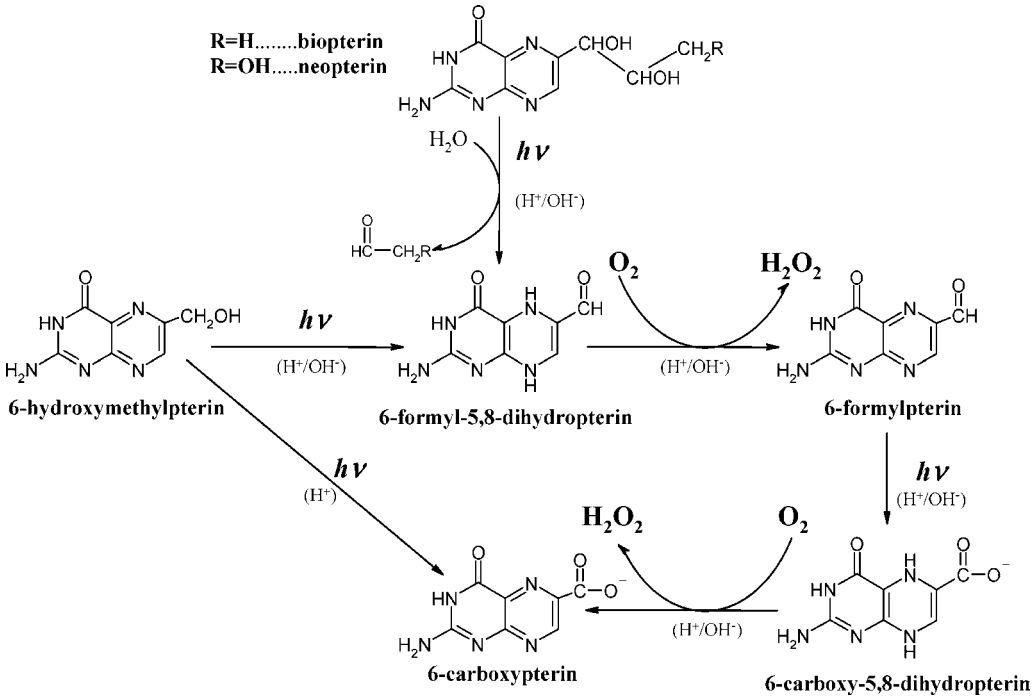
Six-substituted PTs undergo photooxidation in air-equilibrated aqueous solutions under UV-A radiation. Many of these oxidations chemically modify the six-subtituent but do not affect the PT moiety or at least do so to a smaller extent. The mechanism involved in the photooxidation of these compounds strongly depends on the nature of the substituent at position 6 as well as on the pH (i.e., the photochemistry of the acid and basic forms of the PT derivatives is different). The quantum yields of disappearance of both acid and basic forms of several PTs are listed in Table 3.

In the absence of oxygen, in both acidic and alkaline solutions, FA is photostable.[18,19] On the other hand, excitation of the acid form of FA in the presence of oxygen leads to cleavage and oxidation of the molecule, yielding FPT and *p*-aminobenzoylglutamic acid as photoproducts; this reaction is the only pathway of photooxidation.[18] Evidence suggesting the participation of $^1O_2$ in the mechanism of this process has been reported. In the photooxidation of the basic form (Scheme 3), an additional reaction pathway exists, leading to an unknown compound having a molecular weight (455) higher than that of FA (441).[19] This increase in the molecular weight may be due to the incorporation of an oxygen atom into the

Scheme 3. Photooxidation of FA in Alkaline Aqueous Solution[19]



Scheme 4. Photochemistry of BPT, NPT, HPT, and FPT in Aqueous Solution



folate anion and the loss of two hydrogen atoms. This reaction does not involve a cleavage of the folate anion.

Some PT derivatives are not photostable even in the absence of O_2. Red compounds are generated when Ar-purged solutions of BPT, NPT, FPT, and 6-hydroxymethylpterin (HPT) are exposed to UV-A radiation.[18,20–22] It has been proposed that these compounds with long wavelength ($\lambda_{max} \sim 480$ nm) absorption bands of low intensity are 6-acyl-5,8-dihydropterins (Scheme 4).[20] Therefore, the reactions observed imply the reduction of the PT moiety and the oxidation of the substituent through an intramolecular redox reaction. The 6-acyl-5,8-dihydropterins are thermally oxidized quickly on admission of O_2 to yield the corresponding oxidized PTs and hydrogen peroxide (H_2O_2) (Scheme 4). The production of H_2O_2 in the photolysis of PTs is important from a biomedical point of view and is particularly relevant for some skin diseases such as vitiligo. Indeed, H_2O_2 participates in the pathogenesis of vitiligo.[23] The accumulation of PTs derivatives in the skin of patients who suffer this disease has been confirmed as mentioned above.[14,24] Therefore, PTs might be involved in the pathogenesis of vitiligo in contributing to the production of significant levels of H_2O_2.[24] It is noteworthy that the photolysis of the acid form of FPT yields CPT as the main photoproduct, instead of FPT (Scheme 4).[25]

The photochemistry of the PT moiety itself has been investigated using PT[26] and PT derivatives containing substituents that cannot be easily oxidized, e.g., CPT[27] and 6-methylpterin (MPT).[28] Acid and basic forms of the PT moiety are photostable in these cases in the absence of O_2, whereas excitation in the presence of O_2 leads to oxidation, yielding nonpterinic photoproducts (cleavage of PT moiety) and H_2O_2. Although PTs are good $^1O_2$ sensitizers and are able to quench $^1O_2$ (vide infra), the chemical reaction between both species is not the only pathway of PT moiety photooxidation.

The quantum yields of PT, MPT, and CPT disappearance in both acidic and alkaline media in the presence of oxygen are much lower than the corresponding quantum yields determined for the other derivatives (Table 3).

Table 4. Quantum Yields of $^1O_2$ Production (Φ_Δ) and Rate Constants of $^1O_2$ Total Quenching (k_t) in Air-Equilibrated Solutions[22,28,35]

	Φ_Δ^{app} pD = 5.5	Φ_Δ pD = 10.5	Φ_Δ pD = 12.6	$M^{-1}\ s^{-1}$ k_t	$M^{-1}\ s^{-1}$ k_r
PT	0.18 ± 0.02	0.30 ± 0.02	0.08 ± 0.02	$(2.9 \pm 0.3) \times 10^6$	$(2.5 \pm 0.3) \times 10^5$
CPT	0.27 ± 0.03	0.37 ± 0.02	0.09 ± 0.02	$(1.4 \pm 0.1) \times 10^6$	
FPT	0.45 ± 0.05	0.47 ± 0.02	0.26 ± 0.02	$(1.4 \pm 0.1) \times 10^6$	
FA	≤ 0.02	≤ 0.02	<0.02	$(3.0 \pm 0.3) \times 10^7$	
BPT	0.34 ± 0.01	0.40 ± 0.03	0.08 ± 0.02	$(2.4 \pm 0.2) \times 10^6$	
NPT	0.23 ± 0.01	0.34 ± 0.04	0.08 ± 0.02	$(2.3 \pm 0.2) \times 10^6$	
HPT	0.15 ± 0.02	0.21 ± 0.01	0.10 ± 0.02	$(3.1 \pm 0.4) \times 10^6$	
MPT	0.10 ± 0.02	0.14 ± 0.02	0.07 ± 0.01	$(8.0 \pm 0.6) \times 10^6$	$(4.9 \pm 0.7) \times 10^6$
DPT				$(4 \pm 1) \times 10^7$	$(1.0 \pm 0.2) \times 10^7$

Therefore, the PTs are less photolabile in the absence of oxidizable substituents. Finally, the acid form of CPT undergoes, in addition to the photooxidation of the PT moiety, a decarboxylation to yield PT, through an O_2-independent photochemical process.[27]

PTs and Singlet Molecular Oxygen ($^1\Delta_g$)

Photophysical time-resolved studies performed more than 10 years ago suggested that PTs may generate singlet molecular oxygen[29,30] [$O_2(^1\Delta_g)$, denoted throughout as $^1O_2$]. $^1O_2$, the lowest electronic excited state of molecular oxygen, is an important oxidizing species in chemical processes and one of the main activated species responsible for the damaging effects of light on biological systems (photodynamic effects).[31] This activated metastable state is much more reactive than the ground triplet state [$O_2(^3\Sigma_g^-)$, denoted throughout as $^3O_2$] and has been attracting interest in both practical and fundamental aspects.[31,32]

Photosensitization is primarily responsible for the production of $^1O_2$ in vivo.[33] This process can be summarized as follows: A sensitizer is promoted by the light absorption to an electronically excited singlet state and subsequently undergoes intersystem crossing to generate a long-lived triplet state (reaction 4, Scheme 2). $^1O_2$ may then be produced by energy transfer to dissolved molecular oxygen (reaction 6, Scheme 2). $^1O_2$ relaxes to ground state $^3O_2$ by both radiationless and radiative pathways (reactions 7 and 8, Scheme 2, respectively). If a substance in solution is able to quench or trap $^1O_2$, chemical reaction and physical quenching must be considered (reactions 9 and 10, Scheme 2, respectively).

In 1996, Neverov et al.[34] reported the quantum yields of $^1O_2$ production (Φ_Δ) sensitized by 6,7-dimethylpterin (DPT) (Φ_Δ = 0.10) and 6-tetrahydroxybutylpterin (Φ_Δ = 0.17). More recently, systematic studies of the photochemical production of $^1O_2$ by oxidized PTs in aqueous solution afforded Φ_Δ values from the direct analysis of the weak $^1O_2$ near-infrared luminescence at 1270 nm, produced upon excitation of the PTs with continuous UV-A radiation.[22,28,35,36] Because of the short lifetime of $^1O_2$ ($\tau_\Delta = 1/k_d$) in H_2O (3.8 μs), D_2O was used as solvent, where τ_Δ is much longer (62 μs).[37] Experiments were performed in the pD ranges 5.0−6.0 and 10.0−11.0 for determining Φ_Δ values of acidic and basic forms, respectively. Singlet oxygen sensitizers of known Φ_Δ values were used as references. The main features of the method have been described in detail.[38]

Significant $^1O_2$ emission was detected for unconjugated PT derivatives, in both acidic and alkaline media (Table 4).[22,28,35] This general result is important from a biological point of view and reveals that the participation of unconjugated PTs in biological photodynamic processes should be taken into account. There are significant differences between the values (Table 4), revealing that the nature of the substituent at position 6 and the pH considerably affect the capacity of PTs to produce $^1O_2$. In general, values of Φ_Δ for the basic forms (pD = 10.5) are higher than those for the corresponding acidic forms (pD = 5.5). Φ_Δ did not increase when the solutions were saturated with oxygen. Therefore, all excited triplet states are quenched by oxygen already under air-equilibrated conditions.

Values of Φ_Δ for FA and other conjugated PTs are much lower than those for unconjugated PT derivatives. If the substituents of these compounds enhance the radiationless deactivation of the singlet excited state [reaction 2, Scheme 2 (vide supra)], intersystem crossing (reaction 4, Scheme 2) becomes very inefficient and conjugated PTs behave as poor $^1O_2$ sensitizers.

The variation of Φ_Δ with pH (pD) in the range 4−13 was analyzed in detail for PT and CPT.[35] For both compounds, Φ_Δ showed little variation within experimental error for pD values in the ranges 4−6.5 and 9−11, i.e., under conditions where only the acidic or the basic form is present. However, a strong decrease of Φ_Δ was observed at pD higher than 11. This was also observed for other PT derivatives, and values found at pD 12.6 are listed in Table 4. Nevertheless, under these pH conditions, there is no new acid−base equilibrium that could explain those changes. Once again, the reason for this behavior can be found considering the results obtained in the fluorescence studies. The fluorescence of PTs upon excitation at 350 nm is efficiently quenched by hydroxide ions (HO^-) above pH 11 (reaction 3, Scheme 2; Q, HO^-).[15] Therefore, deactivation of singlet excited states by HO^- reduces the intersystem crossing efficiency (Φ_{ISC}) and, hence, the quantum yield of $^1O_2$ production ($\Phi_\Delta = \Phi_{ISC}\,\eta_{et}$, where η_{et} is the efficiency of energy transfer from the triplet excited state to molecular oxygen).

The rate constants of $^1O_2$ total quenching (physical and chemical) ($k_t = k_r + k_q$, see reactions 9 and 10, Scheme 2) by various PT derivatives, obtained by Stern−Volmer analysis of the $^1O_2$ luminescence quenching using rose

bengal as a sensitizer, are listed in Table 4 for the basic PT forms.[22,28,35,39] FA is a good $^1O_2$ scavenger since it has a relatively high value of k_t and is a poor $^1O_2$ sensitizer.

Several mechanisms of quenching of $^1O_2$ have been described in the literature.[40] On the basis of the high triplet energy[35] and the oxidation potentials of PT derivatives,[41] of the values of k_t[22,28,35] and k_r (vide infra), and of the systematic studies on others families of heterocyclic compounds,[40] a charge-transfer-induced process should be considered as the main mechanism involved in the quenching of $^1O_2$ by PTs.[41]

The PT moiety is able to react with $^1O_2$ to yield oxidized nonpterinic products.[26,28,41] The reactivity of the PT moiety strongly depends on the nature of the six-substituent. The rate constant of the chemical reaction between the $^1O_2$ and the basic form of PT, MPT, and DPT (k_r) was determined from the analysis of compound disappearance (HPLC analysis) during the photosensitized oxidation using RB as sensitizer (Table 4). Because $^1O_2$ is an electrophilic species, the higher electronic density in the pterinic ring given by the methyl group(s) explains the difference between the k_r values determined for the different compounds. Detailed mechanistic investigations are currently under progress.[41]

Photoinduced Damage of DNA

It is well-known that solar UV radiation is mutagenic and carcinogenic. However, the mechanism involved in DNA damage depends on the wavelength.[42] UV-B radiation (280−320 nm), which represents a low proportion of the solar radiation at the surface of the earth, can be directly absorbed by the DNA and other biomolecules. Excited DNA molecules subsequently undergo chemical modifications. On the contrary, UV-A radiation (320−400 nm) represents a larger proportion of the solar UV radiation and cannot be directly absorbed by DNA. Nevertheless, UV-A radiation is also able to damage the DNA molecule.[43] However, in this case, the mechanism involves photosensitized reactions. It is important to evaluate the capacity of endogenous substances to photoinduce DNA damage.

PTs are present in human cells and, in some diseases such as vitiligo, they are accumulated in zones of the skin with loss of melanin and, therefore, with deficiency in protection against UV radiation.[14] In addition, the excited states of PTs can be generated by means of absorption of UV-A radiation and visible light and those excited states are able to generate reactive oxygen species, such as $^1O_2$, that can oxidize DNA and other biomolecules.[31] Indeed, the interaction of many planar heterocyclic compounds with DNA has been demonstrated.[44]

K. Ito and S. Kawanishi[45] demonstrated in 1997 that fragments of double-stranded DNA exposed to UV-A radiation in the presence of different PT derivatives (PT, CPT, BPT, NPT, and FA) in air-equilibrated aqueous solutions are damaged. The main chemical PT-induced modification of DNA involves the hydroxylation of guanine yielding 7,8-dihidro-8-oxo-2′-deoxyguanosine as a major product. This damage is sequence-specific: The oxidation takes place at the 5′ site of 5′-GG-3′ sequences. In a later study, photoinduced cleavage of plasmid pUC18 (plasmids are small circular double-stranded DNA molecules) by UV-A light in the presence of PT was demonstrated.[46] In this work, the conversion of the supercoiled plasmid to its relaxed form at short irradiation times and to linear DNA at long irradiation times was described. The accumulation of single strand breaks within the DNA helix may explain the observed behavior. Finally, a very recent investigation, performed using CPT as photosensitizers, has proposed that the mechanism involved in these processes is a photoinduced electron transfer that produces a PT anion radical and a guanine cation radical.[47]

Conclusions

PTs are a family of heterocyclic biomolecules that have a profuse and amazing photochemistry. We have summarized the photochemical behavior of oxidized PTs in aqueous solutions and pointed out their biological implications. PTs excited by UV-A radiation can fluoresce, undergo photooxidation to produce different photoproducts, generate reactive oxygen species (in particular singlet molecular oxygen), and photosensitize the oxidation of biomolecules such as DNA. These properties depend both on the pH and on the chemical nature of the substituents. Thus, the quantum yields of fluorescence (Φ_F), of consumption in photolysis reactions, and of reactive oxygen species production (Φ_Δ) found for these compounds cover wide ranges. A deeper knowledge of the photochemistry of PTs allows a better understanding of the role of these compounds in different photobiological processes important from a biomedical point of view.

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