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Visible-light photochemistry of 6-formyl-7,8-dihydropterin in aqueous solution

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ABSTRACT

Pterins are a family of heterocyclic compounds present in a wide range of living systems and are involved in different photobiological processes. 6-Formyl-7,8-dihydropterin (H_2Fop), is a product of oxidation of several natural occurring pterin derivatives, and its absorption spectrum presents as special feature an intense band in the visible region. Studies of the photochemistry of H_2Fop in aqueous solutions under visible radiation and room temperature were performed. The photochemical reactions were followed by UV–vis spectrophotometry, electrochemical measurement of dissolved O_2, enzymatic methods for H_2O_2 determination and HPLC. When H_2Fop in air-equilibrated solution was exposed to light, O_2 was consumed, the reactant was oxidized to 6-formylpterin (Fop), and H_2O_2 released. When the photolysis took place in the absence of O_2, a red compound was generated. This product was rapidly oxidized to Fop on admission of O_2. The quantum yields of H_2Fop disappearance and of photoproducts formation are reported and the effect of pH is analyzed. The potential biological implications of the results obtained are also discussed.

1. Introduction

Pterins are a family of heterocyclic compounds that occur in a wide range of living systems and participate in relevant biological functions [1]. The most common pterin derivatives are 6-substituted compounds. The molecular weight and functional groups of these substituents vary considerably, *e.g.* pterins may have substituents with one carbon atom, with a short hydrocarbon chain or larger substituents containing a *p*-aminobenzoic acid moiety. Pterins can exist in different oxidation states and be divided into two classes according to this property: (a) oxidized or aromatic pterins and (b) reduced pterins. Within the latter group, 7,8-dihydropterins and 5,6,7,8-tetrahydropterins (denoted throughout as dihydropterins and tetrahydropterins, respectively) are the most important derivatives due to their biological activity, *e.g.* dihydroneopterin (H_2Nep) is secreted during the oxidative burst of stimulated macrophages [2] and dihydrobiopterin (H_2Bip) and tetrahydrobiopterin participate in the metabolism of aminoacids [3]. The chemistry of dihydropterins has been studied in detail [4].

Depending on their oxidation state, pterins have totally different reactivities towards oxidizing agents. In air-equilibrated aqueous solutions, tetrahydropterins are rapidly oxidized by dissolved molecular oxygen (O_2), whereas dihydropterins are more stable [5,6]. However, natural occurring dihydropterins, such as H_2Bip, H_2Nep and dihydrofolic acid (H_2PteGlu), undergoes slow oxidation by O_2 to yield dihydroxanthopterin (H_2Xap) and 6-formyl-7,8-dihydropterin (H_2Fop) [7]. These compounds are also formed in the reaction between H_2PteGlu and hydrogen peroxide (H_2O_2) [8]. Both H_2Fop and H_2Xap are stable in air-equilibrated aqueous solution [7].

H_2Bip, biopterin (Bip) and other pterin derivatives accumulate in the skin of patients suffering from vitiligo [9], a chronic depigmentation disorder. In the tissues affected by this disease, H_2O_2 is present in high concentrations and the cells undergo oxidative stress, deactivation of enzymes of the melanin biosynthesis takes place and the protection of the skin against UV and visible radiation fails because of the lack of melanin. Therefore, the photochemistry of pterins is of particular interest to this disease. Moreover, 6-carboxypterin (Cap), a product of photolysis of Bip that is not synthesized in the skin cells, has been isolated from the affected skin [10], thus proving that photooxidation of pterins occurs *in vivo* under pathological conditions. Although the photochemistry of aromatic pterins in air-equilibrated aqueous solutions has been thoroughly investigated [11,12], there is a lack of information on the photochemical behavior of dihydropterins.

In contrast to most aromatic and reduced pterin derivatives, H_2Fop can be excited by visible light due to its intense and broad absorption band in the spectral range 350–500 nm (Fig. 1). This particular spectral feature is caused by a charge transfer across the conjugated system of the pterin moiety [4]. Although H_2Fop may certainly be formed *in vivo* by oxidation of H_2Bip and absorbs visible

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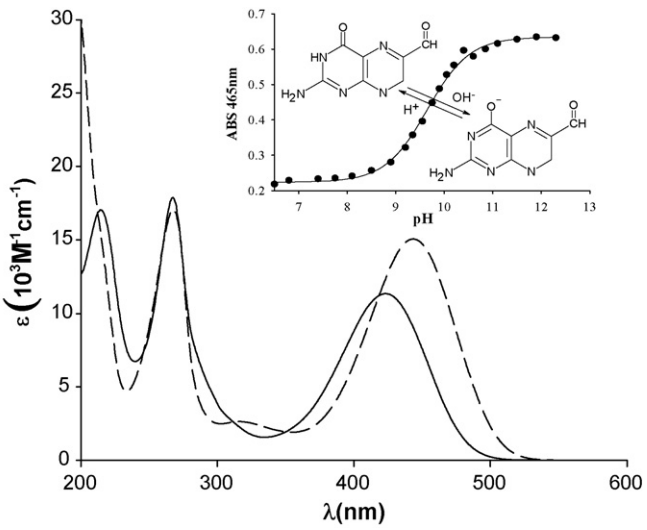


Fig. 1. Absorption spectra of air-equilibrated aqueous solution of H_2Fop. Solid line: acid form; dashed line: basic form of H_2Fop. Inset: variation of the absorbance at 465 nm (circles) as a function of pH; the solid line represents the absorbance calculated by fitting the experimental data to Eq. (1).

light, to the best of our knowledge, no studies of the photochemistry of this compound have been reported in the literature.

In the context of our investigations on the photochemistry of pterin derivatives, we have performed a systematic study of the photolysis of H_2Fop under visible irradiation in aqueous solutions, in the presence and in the absence of O_2. The study was carried out for the neutral (or acid) form, which is the relevant form from a biological point of view, and for the monoanionic (or basic) form (Fig. 1). In particular, we have determined the quantum yields, investigated the production of reactive oxygen species (ROS), and analyzed the kinetics, under different experimental conditions. The results obtained are evaluated in connection with their biological implications and compared with those described for the photochemistry of other 6-substituted pterins.

2. Materials and methods

2.1. Chemicals

6-Formyl-7,8-dihydropterin (H_2Fop), 6-formylpterin (Fop) and other pterin derivatives were purchased from Schircks Laboratories (Switzerland) (purity >99.5%) and used without further purification. Other chemicals from Sigma–Aldrich were used as received. The pH of the aqueous solutions was adjusted, by adding drops of HCl and NaOH solutions from a micropipette. The concentration of the acid and the base used for this purpose ranged from 0.1 to 2 M. The ionic strength was approximately 10^{-3} M in all the experiments.

2.2. pK_a determination

pK_a values were determined from absorption changes. Measurements were performed at room temperature. The experimental absorption changes at a given wavelength can be fitted by the following equation:

$$A = \frac{(c \times l) \times (\varepsilon_a[H^+] + \varepsilon_b K_a)}{K_a + [H^+]} \quad (1)$$

where ε_a and ε_b are the molar absorption coefficients of the acid and basic forms of the species involved in the acid–base equilibrium, c is the total concentration of the substance (H_2Fop), l is the optical path length and K_a the acid dissociation constant. UV–vis spectra were registered on a Cary 5 (Varian) spectrophotometer using quartz cells (l = 1 cm). A more detailed description of pK_a determinations has been described elsewhere [13,14].

2.3. Steady-state irradiation

2.3.1. Irradiation set-up

The continuous photolysis of H_2Fop solutions (50–300 μM) was carried out in quartz cells (1 cm optical path length) at room temperature. Solutions were irradiated at 438 nm with a xenon/mercury arc (1 kW) through a water filter, focusing optics and a monochromator ISA Jobin-Yvon B204 (6 nm bandwidth). Photolysis experiments were performed in the presence and in the absence of air. Deaerated solutions were obtained by bubbling with argon for 20 min. This irradiation set-up was used for all the experiments except for those corresponding to the measurements of dissolved O_2. The latter experiments were performed on an optical bench equipped with a halogen lamp and a cutoff filter at 395 nm to minimize exposure to UV radiation.

2.3.2. Actinometry

Aberchrome 540 (Aberchromics Ltd.) was used as an actinometer for the measurements of the incident photon flux (P_0) at the excitation wavelength. Aberchrome 540 is the anhydride form of the (E)-α-(2,5-dimethyl-3-furylethylidene)(isopropylidene)succinic acid which, under irradiation in the spectral range 316–366 nm leads to a cyclized form. The reverse reaction to ring opening is induced by visible light (436–546 nm). A value of 3.2 (±0.1) × 10^{-6} Einstein L^{-1} min^{-1} was obtained for P_0 at 438 nm in the irradiation set-up described in the previous paragraph. The method for the determination of P_0 has been described in detail elsewhere [15,16]. Values of the photon flux absorbed (P_a) were calculated from P_0 according to the Lambert–Beer law:

$$P_a = P_0(1 - 10^{-A}) \quad (2)$$

where A is the absorbance of the reactant at the excitation wavelength.

2.4. Analysis of irradiated solutions

2.4.1. UV–vis analysis

UV–vis absorption spectra were registered on a Varian Cary-5 spectrophotometer or on a Hewlett-Packard 8452A diode array spectrophotometer. Absorption spectra of the solutions were recorded at regular intervals of irradiation time, using quartz cells of 1 cm of optical path length. Experimental difference (ED) spectra were obtained by subtracting the spectrum at time t = 0 from the subsequent spectra recorded at different times. Each ED spectrum was normalized relative to the maximum absolute value of the absorbance difference yielding the normalized experimental difference (NED) spectrum. Reference-difference (RD) spectra and normalized-reference-difference (NRD) spectra were obtained from aqueous solutions of commercial standards. The comparison between NED and NRD spectra allows the characterization of the major photolysis products. The analysis based on these difference spectra is described elsewhere [17].

2.4.2. High-performance liquid chromatography (HPLC)

Two chromatographic systems were employed for monitoring the reactions: (I) a Series 1100 equipment from Hewlett-Packard (photodiode array detector HP 1100 DAD), with a RP 18 LiChro CART 125-4 column; (II) a Prominence equipment from Shimadzu (photodiode array detector SPD-M20A), with a Pinnacle-II C18 column (250 mm × 4.6 mm, 5 μm; Restek). Solutions containing 0–10% of acetonitrile and 90–100% of potassium phosphate aqueous solution

(20 mM, pH 5.5) were used as eluents. Aqueous solutions of commercial standards were employed for obtaining calibration curves of reactants and products.

It should be noted that in the case of H_2Fop the peak of the reactant could not be well separated from that of the corresponding oxidized product (Fop). Therefore, integrations of the peaks at different wavelengths were performed. Assuming that the peak considered is only due to the reactant and one known product, the concentration of both compounds can be calculated by resolving sets of equations as follows:

$$\text{Area}_{\lambda 1} = f^{R}_{\lambda 1} C^{R} + f^{P}_{\lambda 1} C^{P} \quad (3)$$

$$\text{Area}_{\lambda 2} = f^{R}_{\lambda 2} C^{R} + f^{P}_{\lambda 2} C^{P} \quad (4)$$

where $\text{Area}_{\lambda 1}$ and $\text{Area}_{\lambda 2}$ are the values resulting from integration of the chromatogram peaks at analysis wavelengths λ1 and λ2, C^R and C^P are the concentrations of the reactant and the product, $f^R_{\lambda 1}$, $f^P_{\lambda 1}$, $f^R_{\lambda 2}$ and $f^P_{\lambda 2}$ are the factors obtained from the calibration curves for the reactant and the product at λ1 and λ2. Although only two equations are required for calculating C^R and C^P, more equations were used in order to check the results obtained.

2.4.3. H_2O_2 determination

For determination of H_2O_2, a Cholesterol Kit (Wiener Laboratories) was used. H_2O_2 was quantified after reaction with 4-aminophenazone and phenol [18,19]. Briefly, 400 μL of irradiated solution of H_2Fop was added to 1.8 mL of reagent. The absorbance at 505 nm of the resulting mixture was measured after 30 min at room temperature, using the reagent as a blank. Aqueous solutions of H_2O_2, prepared from commercial standards, were employed for obtaining the corresponding calibration curves.

2.4.4. Determination of the concentration of O_2

The O_2 consumption during irradiation was measured with an O_2-selective electrode (Orion 37-08-99). The solutions and the electrode were placed in a closed glass-cell of 130 mL.

2.5. Quantum yield determinations

The quantum yields of reactant disappearance (Φ_{-R}) and product formation (Φ_P) were determined in experiments performed under different conditions. Values were obtained using the following equations:

$$\Phi_{-R} = -\frac{(d[R]/dt)_0}{P_a} \quad (5)$$

$$\Phi_P = \frac{(d[P]/dt)_0}{P_a} \quad (6)$$

where $(d[R]/dt)_0$ and $(d[P]/dt)_0$ are the initial rates of reactant consumption and product formation, respectively, and P_a is the photon flux absorbed by the reactant (Eq. (2)). For determining the initial rates, the experiments were carried out using solutions of relatively high concentration of reactant (H_2Fop). Under these conditions the time evolution of the concentrations of H_2Fop and products followed a pseudo-zero order rate law during the period of time within which the change of P_a was negligible. The initial rates were obtained from the slope of the corresponding plots of concentration *vs*. irradiation time within such time windows. The evolution of the concentrations during irradiation was followed by HPLC (*vide supra*).

2.6. Singlet oxygen ($^1O_2$) measurements

The photosensitized production of $^1O_2$ by H_2Fop in D_2O solutions was determined by direct analysis of the weak $^1O_2$ near-infrared (NIR) phosphorescence at 1270 nm [20–22]. D_2O was chosen as a solvent, since the $^1O_2$ lifetime (62 ± 3 μs) is much longer than in H_2O (3.8 μs), leading to stronger luminescence signals [23]. Measurements were carried out under continuous irradiation of the solutions using the irradiation set-up described before (xenon/mercury lamp). The main features of the method and equipment have already been described in detail [24,25]. Briefly, the $^1O_2$ luminescence was collected with a mirror, chopped, and after passing through a focusing lens, a cutoff filter (1000 nm), and an interference filter (1271 nm) was detected at 90° with respect to the incident beam using a cooled NIR photomultiplier (Hamamatsu R5509-42).

3. Results and discussion

3.1. Determination of the pK_a

Pterins exhibit several dissociation equilibria (Fig. 1), but above pH 4 the lactam (amide) group is the only relevant ionizable group (Fig. 1) [26]. The pK_a values of the other functional groups of the pterin moiety, *e.g.* 2-amino group, are lower than 2. For several dihydropterin derivatives, the pK_a value of the lactam group was reported to be higher than 10 [4]. However, to the best of our knowledge, the corresponding pK_a value for 6-formyl-7,8-dihydropterin (H_2Fop) has not been reported in the literature. Therefore we determined this pK_a and a value of 9.68 ± 0.04 was found (Fig. 1), which is a little lower than those reported for other dihydropterins [4,27]. This fact is very likely due to the presence of the formyl group. Moreover, a similar behavior was observed for aromatic pterins, *i.e.* 6-formylpterin (Fop) is more acidic (pK_a = 7.3) [17] than other aromatic pterin derivatives ($pK_a \sim 8$) [14].

Taking into account these results and to avoid interferences between acid and basic forms, we performed our experiments at pH < 7.5, where H_2Fop is present at more than 99% in the acid (neutral) form, and in the range 10.8–11.8, where H_2Fop is present at more than 90% in the basic form (monoanion). No experiment was carried out at pH values above this range because, in strongly alkaline solutions, H_2Fop undergoes a slow thermal reaction, thus indicating that the reactant is not stable under such pH conditions.

3.2. Photolysis of H_2Fop in the presence of O_2

Neutral or slightly acidic air-equilibrated aqueous solutions of H_2Fop (pH = 5–7) were irradiated at 438 nm during different periods of time. Fast changes in the absorption spectra of the solutions were observed during irradiation (Fig. 2a), with isosbestic points at 278 and 379 nm. The resulting spectrum at long irradiation times, *e.g.* more than 90 min in the experiment shown in Fig. 2a, was similar to that corresponding to Fop. Moreover, the normalized experimental difference (NED) spectra obtained at different irradiation times (Section 2.4, *UV–vis analysis*) were almost identical to the NRD spectrum (obtained by subtracting the spectrum of a standard solution of H_2Fop from that of a standard solution containing Fop) (Fig. 2a). Therefore spectral analysis strongly suggests that Fop is the main photoproduct of photolysis of H_2Fop. No further changes were detected when irradiated solutions were stored in the dark for several hours, thus indicating that no thermal reactions took place after interrupting the irradiation.

HPLC analysis revealed that Fop was present in H_2Fop solutions exposed to light and that its concentration steadily increased as a function of irradiation time. In several experiments carried out with different initial concentrations of the reactant, the rate of H_2Fop consumption was equal, within experimental error, to the corresponding rate of Fop formation and, accordingly, the sum of the concentrations of the reactant and the product remained constant during the experiments (Fig. 3a). In agreement with these

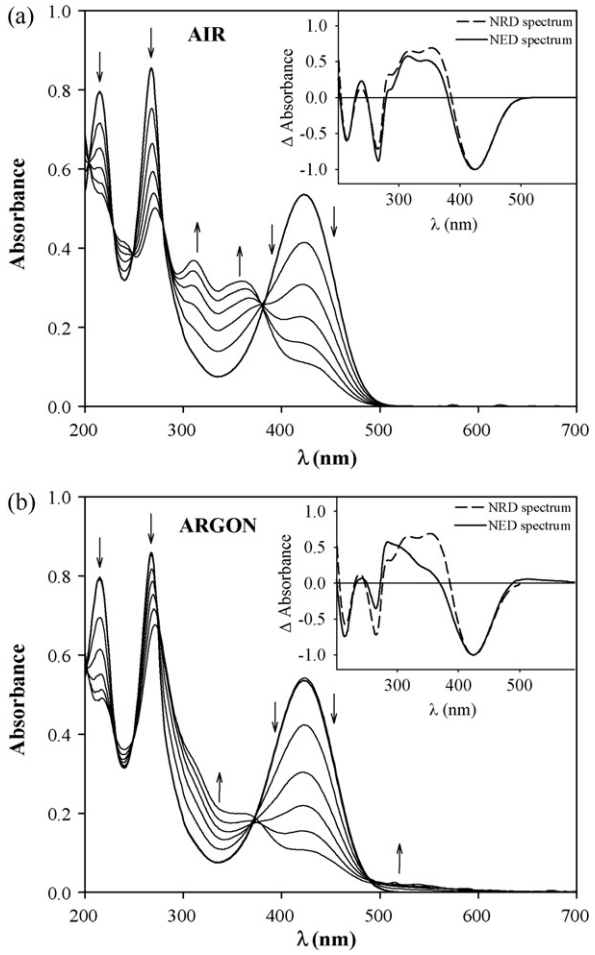


Fig. 2. Time evolution of the absorption spectra of solutions of the acid form of H_2Fop (47.3 μM) exposed to visible light (438 nm). Optical path length = 10 mm; spectra recorded every 10 min; arrows indicate the changes observed at different wavelengths. (a) Air-equilibrated solution, and (b) argon-equilibrated solution. Inset: NED spectrum obtained by subtracting the initial H_2Fop spectrum from the corresponding spectrum of an irradiated solution, and NRD spectrum obtained by subtracting the spectrum of a standard solution of H_2Fop from the spectrum of a standard solution of Fop, at the same concentration and pH.

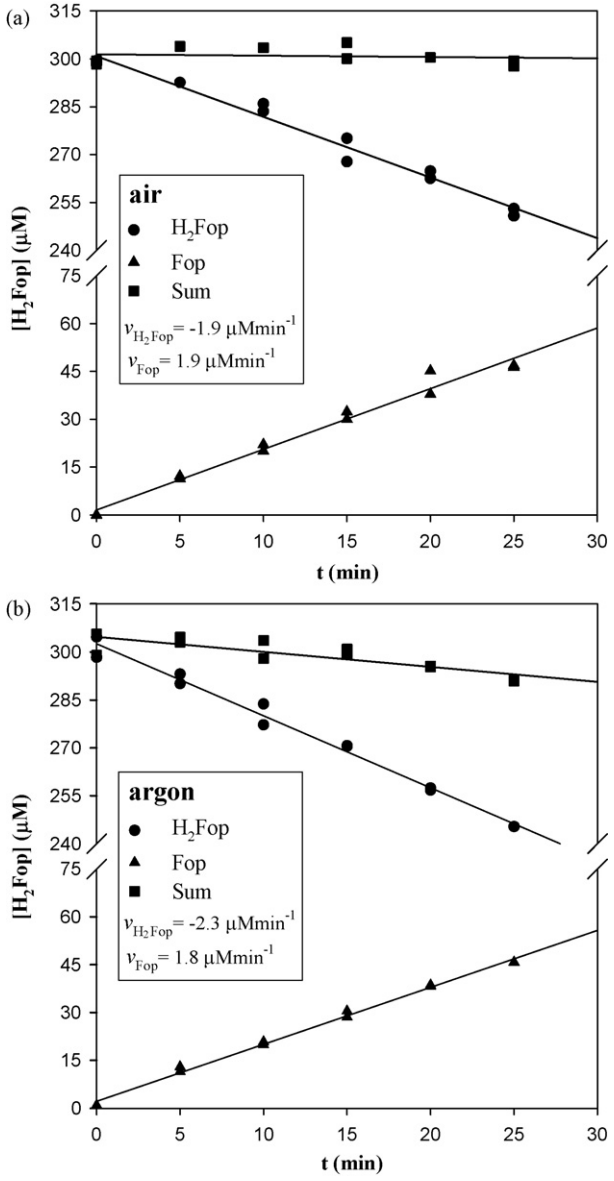


Fig. 3. Time evolution of H_2Fop and Fop concentrations in irradiated solutions of H_2Fop (300 μM, pH 7, λ_{irr} = 438 nm). (a) Air-equilibrated solutions and (b) argon-equilibrated solutions; (■) sum of the H_2Fop and Fop concentrations in μM, at each time (concentrations were determined by HPLC analysis).

results, no additional product was detected by HPLC analysis. Under these experimental conditions, values of 9.9 (±0.8) × 10^{-3} and 9.6 (±0.8) × 10^{-3} were obtained for the quantum yields of H_2Fop consumption (Φ_{-H_2Fop}) and Fop formation (Φ_{Fop}), respectively.

The evolution of the O_2 concentration during irradiation was monitored with an O_2 electrode in a closed cell. In these experiments, the concentration of H_2Fop was measured by HPLC before and after irradiation. A significant decrease in O_2 concentration with irradiation time was observed (Fig. 4), thus indicating that O_2 was consumed during the photooxidation of H_2Fop. In addition, the relationship between O_2 and H_2Fop consumptions ($\Delta[O_2]/\Delta[H_2Fop]$) was calculated for different irradiation times and values close to 1 were obtained.

In addition, the photooxidation of H_2Fop produced H_2O_2. Its concentration was determined at different times during the irradiation of a H_2Fop solution (120 μM, pH 7.0). The rate of production of H_2O_2 (1.3 (±0.1) μM/min) was very close to the rate of H_2Fop consumption (1.4 (±0.1) μM/min), showing that for each molecule of H_2Fop consumed, one molecule of H_2O_2 was generated.

Results presented so far demonstrate that the photolysis of the acid form of H_2Fop in air-equilibrated solutions consists in a process in which the reactant is oxidized by dissolved O_2 to yield the corresponding aromatic analogue and H_2O_2 (Reaction (7)):

$$H_2Fop \xrightarrow{h\nu, O_2} Fop + H_2O_2 \quad (7)$$

Since H_2Fop can be present in the skin (Section 1), it is important to evaluate the biological implications of this reaction. The photochemical generation of H_2O_2 may be deleterious because this reactive oxygen species (ROS) is in part responsible for the inhibition of enzymes of the melanin biosynthesis in vitiligo [28,29]. In addition, in contrast to H_2Fop [30], Fop is a good singlet oxygen ($^1O_2$) photosensitizer upon irradiation in the UV-A spectral region (320–400 nm) [31].The lowest electronic excited state of O_2 ($^1\Delta_g$) is an important oxidizing intermediate in chemical processes and one of the main chemical species responsible for the damaging effects of light on biological systems (photodynamic effects) (see *e.g.* [32]). The main source of $^1O_2$ production *in vivo* is photosensitization (see *e.g.* [33]).

Therefore, if H_2Fop were irradiated in the UV-A, its photooxidation product (Fop) would also absorb part of the incident radiation,

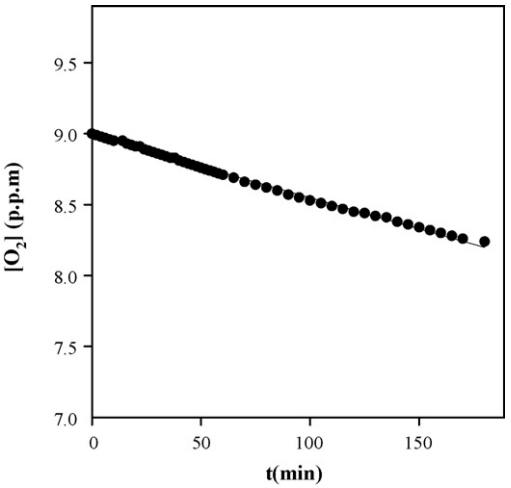


Fig. 4. Time evolution of O_2 concentration during irradiation of a H_2Fop aqueous solution (162 μM, pH 7, halogen lamp and a cutoff filter at 395 nm).

resulting in its excitation and the subsequent production of $^1O_2$. In order to check this assumption, the $^1O_2$ emission in the near-infrared (NIR) was followed during the irradiation of H_2Fop (Section 2.6). Experiments were performed irradiating H_2Fop solutions in D_2O at the same concentration and pH (90 μM, pD 7.0) at two different wavelengths: (i) 438 nm, where Fop does not absorb, and (ii) 372 nm, where both reactant and product absorb. Upon irradiation at the former wavelength, the signal was very low indicating a negligible production of $^1O_2$, as expected because H_2Fop is not a $^1O_2$ sensitizer and Fop hardly absorbed at 438 nm. On the other hand, upon irradiation at 372 nm a fast increase in the NIR emission was observed (Fig. 5), thus revealing that, under these conditions, $^1O_2$ was produced and its steady-state concentration increased with irradiation time.

Although the basic form of H_2Fop is not relevant from a biological point of view, some experiments at pH values higher than 10.8 (Section 3.1) were performed in order to characterize its photochemical behavior. The results in air-equilibrated solutions were comparable to those found for the acid form: H_2Fop and O_2 were consumed, whereas Fop and H_2O_2 were produced. However, the

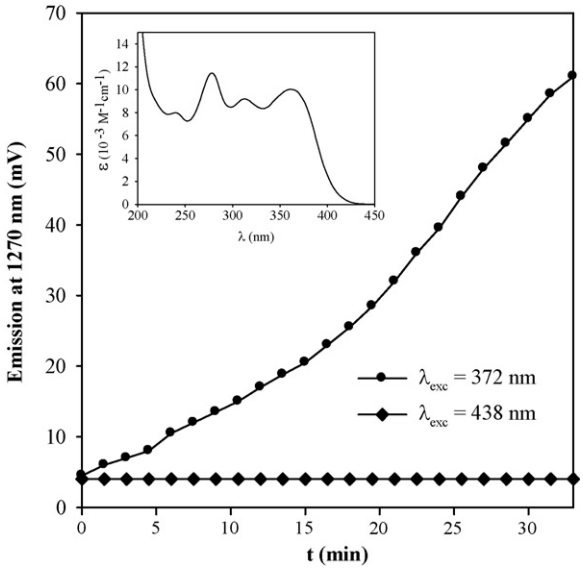


Fig. 5. Emission at 1270 nm observed from D_2O solutions of H_2Fop under continuous irradiation at different wavelengths. [H_2Fop] = 90 μM, pD 7.0. Inset: absorption spectra of an air-equilibrated aqueous solution of Fop (pH 7.2).

Table 1
Quantum yields of H_2Fop consumption (Φ_{-H_2Fop}) and Fop formation (Φ_{Fop}) determined under different experimental conditions.

	Acid form		Basic form	
	Air	Argon	Air	Argon
Φ_{-H_2Fop} (10^{-3})	9.9 (±0.8)	11.3 (±0.9)	1.1 (±0.1)	1.2 (±0.1)
Φ_{Fop} (10^{-3})	9.6 (±0.8)	9.8 (±0.6)	0.57 (±0.05)	0.59 (±0.05)

comparison between the rates of disappearance and formation suggests that the photochemical process according to Reaction (7) can explain only part of the H_2Fop consumption. For comparative purposes, the values of the corresponding quantum yields (Φ_{-H_2Fop} and Φ_{Fop}) are presented in Table 1, together with those corresponding to the acid form of H_2Fop. It should be noted that Φ_{-H_2Fop} is about one order of magnitude lower for the basic form, revealing that this species is much less photosensitive than the corresponding acid form. In addition, values obtained for Φ_{-H_2Fop} and Φ_{Fop} indicate that the formation of Fop corresponds to approximately 50% of the reactant consumption for the basic form.

3.3. Photolysis of H_2Fop in the absence of O_2

The spectral changes observed in H_2Fop solutions after irradiation under anaerobic conditions were different from those observed in air-equilibrated solutions (Fig. 2b). In the absence of oxidant, the formation of Fop as a photoproduct could in any case be discarded. In the anaerobic experiments, the solutions initially yellow took up an orange hue. Time evolution of the spectra indicates that this fact is due to the formation of a compound absorbing above 450 nm, with a broad band partially superimposed to the low energy band of H_2Fop. In this case, the NED spectra obtained at different irradiation times were quite different to the NRD spectrum, obtained from standard solutions of H_2Fop and Fop (Fig. 2b).

As soon as air was introduced into the cell, a fast reaction occurred in the dark, which was observed by the disappearance of the absorption above 450 nm (Fig. 6). The NED spectra, obtained by subtracting the spectra of non-irradiated solutions of the reactant from those of irradiated and then immediately aerated solutions, are comparable to the NRD spectrum obtained from standard solutions of H_2Fop and Fop (Fig. 6). Therefore, the latter compound appears to be the main final product after introducing O_2 in H_2Fop solutions irradiated under argon.

These results indicate that irradiation under anaerobic conditions leads to the formation of a "red product" (RP) with a broad absorption band in the visible region that reacts very rapidly with O_2 to yield Fop. This behavior is similar to that previously described for the photolysis of a group of aromatic pterins bearing a CHOH group (Bip, Nep and 6-hydroxymethylpterin (Hmp)) or a CO group on the first C-atom of the substituent [17,34,35]. Taking into account these studies, the photoproduct formed in the absence of O_2 (RP) could be 6-formyl-5,8-dihydropterin. Further investigation is needed to unambiguously identify this unstable compound.

HPLC analysis confirmed that Fop is the main product obtained by photolysis of H_2Fop in the absence of O_2 and followed by immediate aeration. An additional product was also detected, with a spectrum compatible with an aromatic pterin derivative. However, its corresponding retention time did not match with any of the available standards. In particular, the formation of Hmp and 6-carboxypterin (Cap) was discarded. In agreement with the presence of an additional product, the rates of Fop production were a little lower than the corresponding rates of H_2Fop consumption (Fig. 3b). Under these experimental conditions, values of $1.13\ (\pm 0.09) \times 10^{-2}$ and $9.8\ (\pm 0.6) \times 10^{-3}$ were obtained for Φ_{-H_2Fop} and Φ_{Fop}, respectively. These values are similar to those obtained under aerobic conditions (Table 1).

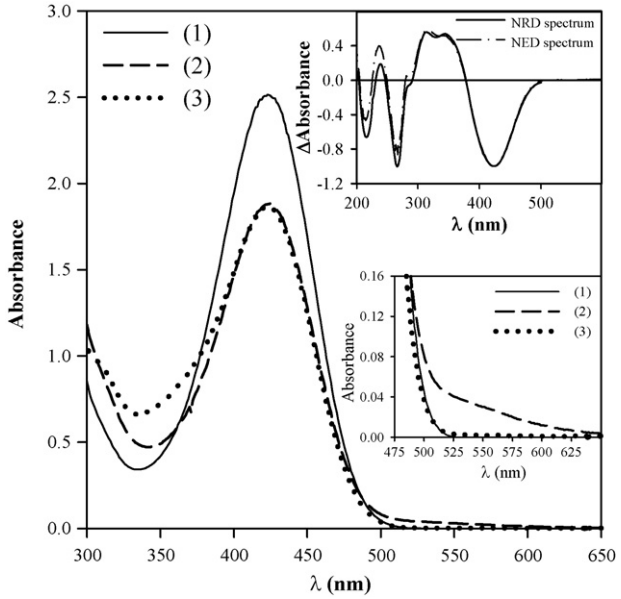
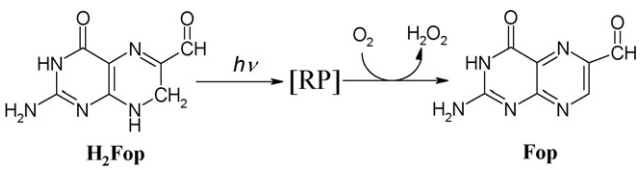


Fig. 6. (1) Spectrum of a non-irradiated solution of H_2Fop; (2) spectrum of a H_2Fop solution irradiated for 25 min under anaerobic conditions; (3) spectrum of a H_2Fop solution irradiated for 25 min under anaerobic conditions, and then aerated ($[H_2Fop]_0$ = 220 μM, pH 5.5, optical path length = 10 mm). Lower inset: detail of the spectral changes in the region 475–650 nm. Upper inset: NRD spectrum obtained by substracting the spectrum of H_2Fop from that of Fop at the same concentration and pH, and NED spectrum obtained by subtracting the initial spectrum (1) from the corresponding spectrum (3).

The determination of H_2O_2 in solutions of H_2Fop, immediately aerated after different times of irradiation under anaerobic conditions, revealed that this ROS is also generated. H_2O_2 cannot be produced during anaerobic photolysis, therefore, these results demonstrate that this ROS is formed in the thermal reaction between O_2 and RP. The concentrations of H_2O_2 generated and of the reactant consumed were of the same order of magnitude.

Taking into account the results presented in this section, Scheme 1 shows the main reaction pathway of the photolysis of H_2Fop under anaerobic conditions followed by aeration. In addition, considering that Fop is also the main product of the aerobic photolysis, with similar quantum yields, it can be inferred that Scheme 1 can also represent the mechanism under aerobic conditions. In this case, RP formed in air-equilibrated solutions would be immediately oxidized by dissolved O_2, explaining why RP can be detected only under anaerobic conditions.

A set of similar experiments was performed for the basic form of H_2Fop (pH 11.4). In this case, after irradiation in the absence of O_2, no increase in the absorbance of the solution above 450 nm was observed. The H_2Fop solutions exposed to visible light under anaerobic conditions and aerated immediately after interrupting the irradiation were analyzed by spectrophotometry and HPLC. Results were similar to those found for air-equilibrated solutions: Fop was formed, but only approximately 50% of the reactant consumed was oxidized to this aromatic pterin. The values calculated for Φ_{-H_2Fop} and Φ_{Fop} were equal, within experimental error, to those obtained in the presence of O_2 (Table 1).



Scheme 1.

4. Conclusion

6-Formyl-7,8-dihydropterin (H_2Fop) presents in aqueous solution an acid–base equilibrium that involves the lactam group (Fig. 1), with a pK_a value of 9.68 ± 0.04. We have investigated the photochemistry of 6-formyl-7,8-dihydropterin (H_2Fop) in aqueous solutions exposed to visible radiation at room temperature. Photolysis of H_2Fop in air-equilibrated solutions in the pH range 5–7, where the neutral (acid) form is predominant, yielded 6-formylpterin (Fop) and hydrogen peroxide (H_2O_2) as final products. Values of 9.9×10^{-3} and 9.6×10^{-3} were obtained for the quantum yields of H_2Fop consumption (Φ_{-H_2Fop}) and Fop formation (Φ_{Fop}), respectively. When the photolysis took place in the absence of O_2, a red compound was generated. This product was rapidly oxidized upon admission of O_2 to yield Fop and H_2O_2. Under these experimental conditions, the values of Φ_{-H_2Fop} and Φ_{Fop} (for the combined photolysis and subsequent O_2 oxidation) were similar to those obtained for photolysis in the presence of O_2. In both aerobic and anaerobic conditions, Φ_{-H_2Fop} values were about one order of magnitude lower for the basic form (pH > 10.8). In addition, the formation of Fop was only about the 50% of the reactant consumption in this latter case.

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