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Histidine oxidation photosensitized by pterin: pH dependent mechanism

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ABSTRACT

Aromatic pterins accumulate in the skin of patients suffering from vitiligo, a chronic depigmentation disorder, due to the oxidation of tetrahydrobiopterin, the biologically active form of pterins. In this work, we have investigated the ability of pterin, the parent compound of aromatic pterins, to photosensitize the oxidation of histidine in aqueous solutions under UV-A irradiation. Histidine is an α-amino acid with an imidazole functional group, and is frequently present at the active sites of enzymes. The results highlight the role of the pH in controlling the competition between energy and electron transfer mechanisms. It has been previously demonstrated that pterins participate as sensitizers in photosensitized oxidations, both by type I (electron-transfer) and type II mechanisms (singlet oxygen ($^1O_2$)). By combining different analytical techniques, we could establish that a type I photooxidation was the prevailing mechanism at acidic pH, although a type II mechanism is also present, but it is more important in alkaline solutions.

1. Introduction

Proteins are targets for oxidative damage, the individual amino acid residues being oxidized at different rates depending on their individual physicochemical properties. Oxidation of amino acids mediated by radiation occurs through two main pathways: (i) absorption of UV-B radiation by the amino acid residues and subsequent reaction of their excited states and (ii) absorption of UV-A and/or visible radiation by endogenous sensitizers (such as flavins, porphyrins, pterins) or exogenous sensitizing drugs [1], that yield reactive long-lived triplet excited states. Subsequently, the latter may react with biological molecules by electron-transfer or hydrogen-abstraction processes (type I mechanism) or transfer their energy to O_2 yielding singlet oxygen ($^1O_2(^1\Delta_g)$, denoted as $^1O_2$) (type II mechanism) [2].

Pterins are a family of heterocyclic compounds present in all living systems, tetrahydro derivatives being, in general, the biologically active forms. These compounds have fundamental functions in the synthesis of melanin [3], the pigment responsible of the protection of the skin against UV radiation. The synthesis of melanin, the main pigment in the skin, starts with the hydroxylation of aromatic amino acids [4] and the enzymes that catalyzes these reactions use 5,6,7,8-tetrahydrobiopterin (H_4Bip) as cofactor [3]. Vitiligo is a skin disorder characterized by the acquired loss of pigmentation [5]. In this disease the protection against UV radiation fails due to the lack of melanin. In addition, the H_4Bip metabolism is altered [6] and unconjugated oxidized pterins accumulate in the affected tissues. These oxidized pterins do not play any biological function in the skin, but are well-known sensitizers under UV-A radiation [7], and they may act through both type I [8] and type II [9] photooxidation mechanisms.

It was recently demonstrated that free tryptophan (Trp) and tyrosine (Tyr) in aqueous solution are degraded under UV-A (350 nm) radiation in the presence of pterin (Ptr), the parent unsubstituted compound of oxidized pterins [10,11]. The mechanism involved in these degradation reactions is initiated by an electron transfer reaction from the amino acid to the Ptr triplet excited state (type I mechanism). Moreover, other studies have suggested that the same mechanism leads to the degradation of Trp and Tyr residues in proteins and peptides [12,13].

Histidine, a naturally occurring α-amino acid, is one of the building blocks of proteins and contains an aromatic heterocycle (imidazole, in the side chain). This amino acid, free in aqueous solution, has 3 relevant acid-base forms, a protonated form ($HisH^+$), a neutral form (His) and anionic form ($His(-H)^-$), with pK_a values of 6.0 and 9.2 (Fig. 1) [14]. In the neutral form of histidine (His), the imidazole ring is not protonated, the net charge is zero but, as for the most common α-amino acids, a zwitterionic form predominates where the carboxylic and the amino groups of the amino acid function are respectively deprotonated ($-COO^-$) and protonated ($-N^+H_3$) (corresponding pK_a values of 9.2 and 2.2) [14]. Degradation of histidine under direct excitation by UV or visible radiation is irrelevant, but photosensitized processes were reported to oxidize His residues [1]. As previously published, the degradation of histidine is strongly pH dependent, both for type I mechanisms [15] or $^1O_2$-mediated oxidation [16]. In the reaction between histidine

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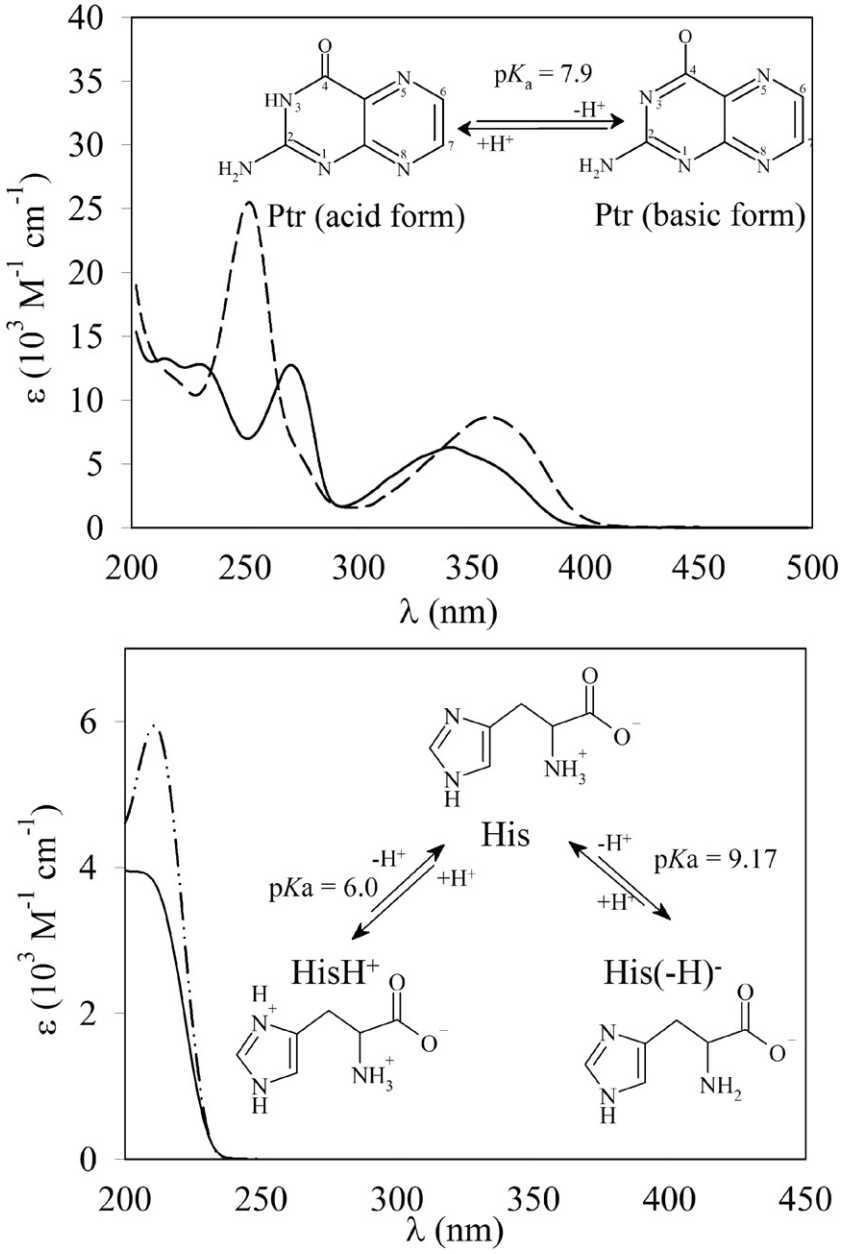

Fig. 1. Molecular structures of pterin and histidine, and corresponding absorption spectra in air-equilibrated aqueous solutions; solid line: pH = 5.5; dashed line: pH = 10.5.

and $^1O_2$ only the neutral and anionic forms (Fig. 1) are significantly reactive. The mechanism proposed for the type I photosensitized oxidation in the pH range 4–12 involves a proton-coupled electron transfer from histidine to different types of photosensitizers, in their triplet excited states [14,15,17].

In the present work the degradation of histidine in the presence of Ptr under UV-A irradiation (350 nm) in aqueous solution was investigated under two different pH conditions. The only UV-A absorbing species was Ptr since histidine does not absorb radiation in the UV-A region (Fig. 1). The experiments were performed at pH 5.5 and pH 10.5, so that more than 99% of the pterin (pKa = 7.9) [18] was in the acid or in the alkaline form, respectively. Histidine was present as a mixture of the neutral form His and the protonated form $HisH^+$ at pH 5.5, and as $His(-H)^-$ at pH 10.5 (Fig. 1). The photochemical reactions were followed by UV/visible spectrophotometry, chromatography (HPLC-UV) and an enzymatic method for H_2O_2 determination. The role of molecular oxygen, in its ground triplet state (O_2) and in its first singlet excited state ($^1O_2$), was investigated. Mechanistic aspects of the photodegradation of histidine are discussed, taking into consideration the acid-basic forms of both Ptr and histidine.

2. Experimental section

2.1. General

Pterin (Ptr) (purity > 99%, Schircks Laboratories, Switzerland and Sigma-Aldrich) was used without further purification after checking for impurities by HPLC. Histidine and ammonium acetate (NH_4OAc) (Sigma Chemical Co) were of the highest purity available (>98%) and were used without further purification. Methanol (MeOH) and KI were purchased from J. T. Baker and Sigma, respectively. Other chemicals were from Sigma Chemical Co. Solutions were prepared by dissolving Ptr and histidine in water. The final pH of the solutions was adjusted by adding drops of HCl or NaOH solutions (0.1–0.2 M) with a micropipette. The ionic strength was *ca.* 10^{-3} M in all experiments.

Concentrations used for the experiments were approximately ~100 μM for both Ptr and histidine.

2.2. Steady-state irradiation

Irradiation set-up. Aqueous solutions containing Ptr (~100 μM) and His (~100 μM) were irradiated in 1 cm path length quartz cells at room temperature with Rayonet RPR3500 lamps with emission centered at 350 nm (Southern N.E. Ultraviolet Co.) at a variable distance between 0 cm and 2 cm. The experiments were performed at pH 5.5 and 10.5, in the presence and in the absence of O_2. Oxygen-free solutions were obtained by bubbling with Ar during 20 min. The measurements were carried out under conditions of reduced environmental light.

2.3. Actinometry

Aberchrome 540 (Aberchromics Ltd.), the anhydride form of the (E)-*R*-(2,5-dimethyl-3-furylethylidene)(isopropylidene)-succinic acid, was used as an actinometer for the measurements of the incident photon flux (P_0) at the excitation wavelength. The method for the determination of P_0 has been described in detail elsewhere [19,20]. Values of the photon flux absorbed (P_a) were calculated from P_0 according to the Lambert–Beer law ($P_a = P_0\,(1-10^{-A})$, where A is the absorbance of the sensitizer at the excitation wavelength).

2.3.1. UV/visible spectrophotometric analysis

UV-visible absorption spectra were registered on a Shimadzu UV-1800 spectrophotometer. Measurements were made in quartz cells of 0.4 and 1 cm optical path length.

2.3.2. High Performance Liquid Chromatography (HPLC)

A Prominence equipment from Shimadzu (solvent delivery module LC-20AT, on-line degasser DGU-20A5, communications bus module CBM-20, auto sampler SIL-20A HT, oven CTO-10AS VP and photodiode array detector SPD-M20A) was used to monitor and quantify the reactants and the photoproducts. Separation was performed on a Sinergy Polar-RP column (150 × 4.6 mm, 5 μm; Phenomenex) using an aqueous solution containing 10 mM NH_4OAc (pH 7.0) as a mobile phase. HPLC runs were monitored by UV/vis spectroscopy at different wavelengths.

2.3.3. Detection and quantification of H_2O_2

H_2O_2 was determined by its reaction with 4-aminophenazone and phenol catalyzed by the enzyme peroxidase to yield 4-(*p*-benzoquinone monoimino)phenazone, which was detected by its absorbance in the visible region [21,22]. This assay has high sensitivity and specificity due to the intense absorbance of the product at 505 nm and to the enzymatic catalysis, respectively. The reactants were purchased from Wiener Laboratorios SAIC (cholesterol kit). Briefly, 500 μl of irradiated solution was added to 600 μl of reagent. The absorbance of the resulting mixture at 505 nm was measured after 30 min at room temperature, under conditions of reduced environmental light, using the reagent as a blank. Aqueous H_2O_2 solutions prepared from commercial standards were employed for obtaining the corresponding calibration curves. In all cases in which H_2O_2 was detected and quantified using the technique described above, controls with catalase, the enzyme that catalyzes specifically the decomposition of H_2O_2 to H_2O and O_2, were also carried out. Catalase was added after irradiation and before mixing the analyzed solution with the reactants. Thus, the absence of absorbance at 505 nm in these controls confirmed the formation of H_2O_2 in the studied reactions.

3. Results

3.1. Irradiation of solutions containing pterin and histidine

Solutions containing Ptr (~100 μM) and histidine (~100 μM) were exposed to UV-A (350 nm) radiation for different periods of time. The experiments were performed in air-equilibrated aqueous solutions at pH 5.5 and 10.5. The reactions were monitored by UV-Vis spectrophotometry and the concentrations of histidine, Ptr and H_2O_2 were determined by HPLC (Experimental Section).

In both acidic and alkaline media, significant changes in the absorption spectra of the aerated solutions were observed upon irradiation (Fig. 2), thus indicating that chemical changes took place as a consequence of the treatment. For both pH conditions, a decrease of the histidine concentration was observed during the irradiation time (Fig. 3), whereas the Ptr concentration did not change (data not shown) in the analyzed time-window. HPLC measurements also showed that several products were formed under both pH conditions. Additionally, production of H_2O_2 was detected and its concentration increased with the irradiation time. However, at pH 5.5 the initial rate of H_2O_2 production was equal within the experimental error to the initial rate of histidine consumption, whereas for pH 10.5 the former rate was much lower than the latter (Fig. 4).

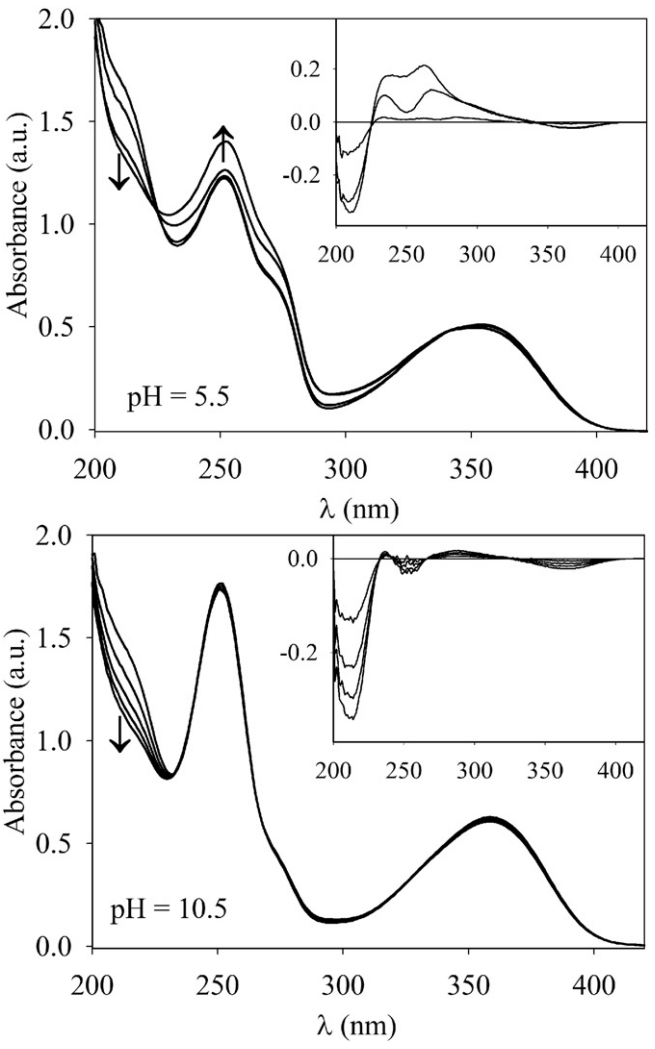

Fig. 2. Evolution of the absorption spectra of air-equilibrated solutions of histidine (100 μM) in the presence of Ptr (100 μM), as a function of the irradiation time at pH 5.5 (0, 5, 20 and 35 min) and pH 10.5 (0, 5, 10, 15 and 20 min). Arrows indicate the changes observed at different wavelengths. Insets: experimental-difference spectra.

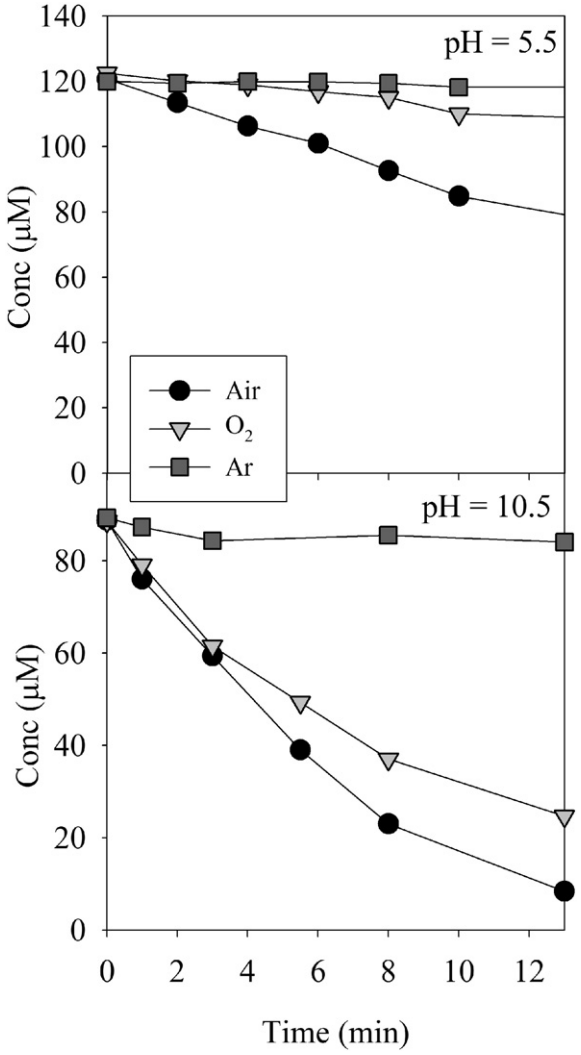

Fig. 3. Evolution of the histidine concentration in aerated, Ar- and O_2-saturated aqueous solutions under UV-A irradiation (350 nm) as a function of the irradiation time at pH 5.5 and 10.5 (concentrations were determined by HPLC analysis). $\lambda_{exc} = 350$ nm, pH = 5.5; $[Ptr]_0$ ~ 100 μM, $[His]_0$ ~ 100 μM.

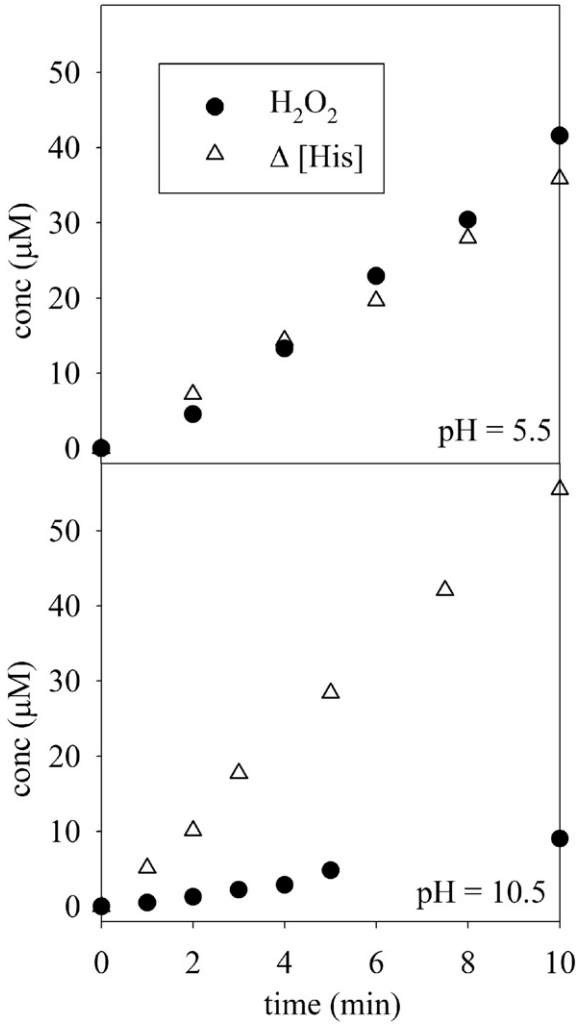

Fig. 4. Evolution of the H_2O_2 concentration and of the consumed histidine ($\Delta[His] = [His]_0 - [His]_t$) in aqueous solutions under UV-A irradiation as a function of the irradiation time. Experiments were performed in air equilibrated solutions, $\lambda_{exc} = 350$ nm, $[Ptr]_0$ ~ 100 μM, $[His]_0$ ~ 100 μM.

When the solutions containing Ptr and histidine (~100 μM) were previously bubbled with Ar, no significant changes were observed in the absorption spectra of the solutions after more than 30 min of irradiation, whatever the pH (acidic or basic). In these experiments lacking oxygen, the histidine concentration measured by HPLC did not decrease. Consequently, no photo-products could be detected.

Thermal reactions between Ptr and histidine were discarded after control experiments performed by keeping solutions containing both compounds in the dark. Moreover, no chemical modification of the amino acid could be detected when histidine solutions were irradiated at 350 nm in the absence of Ptr, thus excluding spurious effects of light absorption by histidine. These control experiments were carried out at both pH conditions. Therefore results presented so far clearly demonstrate that, under our experimental conditions, Ptr was able to photosensitize chemical changes in histidine under UV-A irradiation. To the best of our knowledge, this is the first time that a pH dependent photodegradation of histidine by a pterin is reported.

3.2. Mechanistic analysis

3.2.1. Participation of Ptr excited states

We have shown previously that the Ptr triplet excited state is selectively deactivated by iodide (I^-) at micromolar concentrations [23]. For that reason, experiments were carried out irradiating air-equilibrated aqueous solutions containing histidine and Ptr, at pH 5.5 and 10.5, in the presence of 400 μM KI. HPLC measurements demonstrated that the consumption of histidine in the presence of I^- was much slower than in its absence under both pH conditions (Fig. 5), revealing that the Ptr triplet excited state is involved in the consumption process.

3.2.2. Effect of the concentration of molecular oxygen

Equivalent experiments were performed in O_2-saturated solutions. The observed rate of consumption of histidine in O_2-saturated solutions was almost negligible in acidic media, compared to its consumption in air-equilibrated solutions at the same pH (Fig. 3). Since $^1O_2$ is formed by energy transfer from the triplet excited state of the sensitizer ($^3Ptr^*$) to dissolved O_2 [7], these results are not compatible with a predominant $^1O_2$-mediated mechanism at pH 5.5. In alkaline media however, similar rates of histidine consumption in air-equilibrated and in O_2-saturated solutions (Fig. 3) suggest that $^1O_2$ could be the main oxidizing species, provided that the quantum yield of $^1O_2$ production (Φ_Δ) is not affected by the variation in the O_2 concentration.

3.2.3. Contribution of singlet oxygen

To elucidate if $^1O_2$ is involved in the mechanism of histidine oxidation, equivalent experiments were performed in D_2O where the $^1O_2$ lifetime (τ_Δ) is higher than in H_2O (c.a. 60 μs and 4 μs, respectively) [24,25].

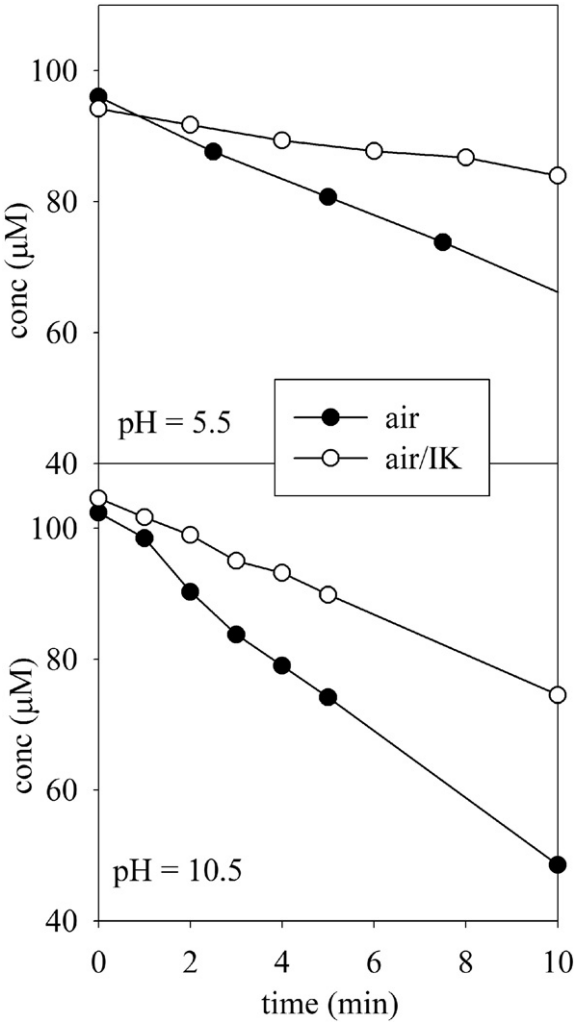

Fig. 5. Evolution of the histidine concentration in aerated aqueous solutions in the absence and in the presence of IK (400 μM), under UV-A irradiation (350 nm) as a function of the irradiation time at pH 5.5 and 10.5 (concentrations were determined by HPLC analysis). λ_{exc} = 350 nm, $[Ptr]_0$ ~ 100 μM, $[His]_0$ ~ 100 μM.

If a $^1O_2$ mediated oxidation were dominant, the degradation of histidine should be much faster in D_2O than in H_2O. The experiments revealed that the corresponding rate of histidine consumption in both media was higher in D_2O than in H_2O, being in alkaline media much faster (Table 1).

In view of previously reported values for the rate constants of the total quenching (k_t) of $^1O_2$ by histidine, and assuming that the quenching is due to chemical reaction between $^1O_2$ and histidine (k_t assumed to be equal to k_r) [16], (k_t = ~0.5 × 10^7 $M^{-1}s^{-1}$ and ~1 × 10^8 $M^{-1}s^{-1}$, for pH 5.5 and 10.5 respectively), the contribution of $^1O_2$ to the photosensitized oxidation of histidine by Ptr can be evaluated by comparing the experimental initial rate of histidine consumption to the calculated initial rate of the reaction between $^1O_2$ and histidine, using Eq. (1) [24]:

$$(d[His]/dt) = k_r^{His}[His][^1O_2] \quad (1)$$

The steady-state concentration of $^1O_2$ during irradiation of a solution containing Ptr and histidine is given by Eq. (2),

$$[^1O_2] = P_a\Phi_\Delta / \left(k_d + k_t^{Ptr}[Ptr] + k_t^{His}[His]\right) \quad (2)$$

where P_a (Einstein L^{-1} s^{-1}) is the photon flux absorbed by Ptr and Φ_Δ the quantum yield of $^1O_2$ production by Ptr (0.18 ± 0.02 and 0.30 ± 0.02 at pD 5.5 and pD 10.5, respectively) [7]; k_d (s^{-1}) is the rate constant of $^1O_2$ deactivation by the solvent (= $1/\tau_\Delta$); the overall (physical and reactive) rate constants of $^1O_2$ quenching ($k_t = k_q + k_r$) by Ptr and histidine are: k_t^{Ptr} = 2.9 × 10^6 $M^{-1}s^{-1}$ and k_t^{His} = 0.5 × 10^7 $M^{-1}s^{-1}$ and 1 × 10^8 $M^{-1}s^{-1}$ for acidic and alkaline solutions, respectively [7,16]. The initial rate of the reaction between $^1O_2$ and histidine, $(d[His]/dt)_{calc}$, for an individual experiment was calculated using Eqs. (1) and (2), and it was compared to the corresponding initial rate of histidine consumption determined experimentally by HPLC analysis, $(d[His]/dt)_{exp}$ (Table 1).

Considering the results presented in this section, at acidic pH (5.5) the main mechanism of the degradation of histidine photosensitized by Ptr is not a $^1O_2$-mediated mechanism. However, in alkaline solutions $^1O_2$ plays a significant role in the oxidation of histidine.

3.2.4. Role of the superoxide anion

It has been demonstrated for Trp [10] and Tyr [11] that the degradation of these amino acids at acidic pH is predominantly initiated by an electron transfer from the amino acid to the triplet excited stated of Ptr ($^3Ptr^*$). It is well established that, in a typical type I process, ground state O_2 readily traps the resulting organic radical anion to produce the superoxide anion ($O_2^{\bullet-}$) [26–28]. The detected H_2O_2 (vide supra) can then be the product of the spontaneous disproportionation of $O_2^{\bullet-}$ in aqueous solution [29]. Therefore, we have investigated the participation of $O_2^{\bullet-}$ in the process, by performing experiments at pH 5.5 and 10.5 in the presence of superoxide dismutase (SOD), an enzyme that catalyzes the conversion of $O_2^{\bullet-}$ into H_2O_2 and O_2 [30]. The rate of degradation of histidine in the absence or in the presence of SOD, under both pH conditions, was the same, within the experimental error (Fig. 6), indicating that $O_2^{\bullet-}$ does not contribute to the degradation of histidine.

3.2.5. Thermodynamic feasibility of electron transfer

We have previously demonstrated that that $^3Ptr^*$ oxidizes Trp [10], Tyr [11], dGMP (2′-deoxyguanosine 5′-monophosphate) [31] and dAMP (2′-deoxyadenosine 5′-monophosphate) [32] via an electron transfer process conducting to the formation of the corresponding radicals. Considering the standard redox potential of histidine ($E(His^{\bullet+}$/

Table 1
Experimental and calculated initial rates of histidine consumption during irradiation of Ptr (100 μM) in aerated aqueous solutions (calculated initial rates were determined taking into account the reaction between $^1O_2$ and histidine according to Eqs. (1) and (2)).

Solvent	P_0	Experimental $(d[His]/dt)_0$	Calculated $(d[His]/dt)_0$ (for reaction with $^1O_2$)
H_2O, pH 5.5	1.40 10^{-5} einstein L^{-1} s^{-1}	−0.10 μM s^{-1}	−0.0031 μM s^{-1}
D_2O, pD 5.5		−0.21 μM s^{-1}	−0.051 μM s^{-1}
H_2O, pH 10.5		−0.20 μM s^{-1}	−0.11 μ M s^{-1}
D_2O, pD 10.5		−1.57 μM s^{-1}	−1.13 μM s^{-1}
H_2O, pH 10.5	9.8 10^{-6} Einstein L^{-1} s^{-1}	−0.038 μM s^{-1}	−0.082 μM s^{-1}
D_2O, pD 10.5		−0.67 μM s^{-1}	−0.57 μM s^{-1}

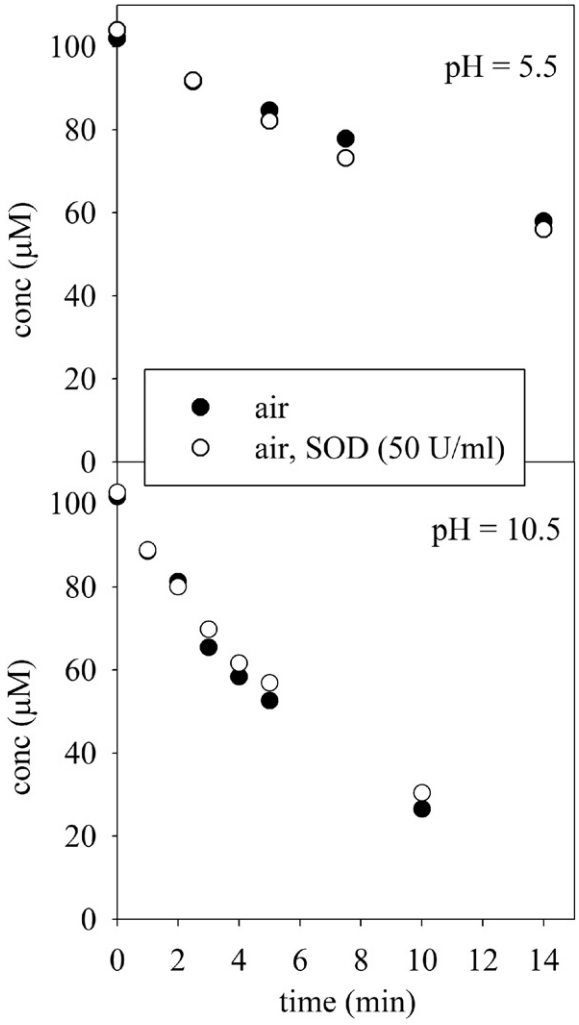

Fig. 6. Evolution of the histidine concentration in aerated aqueous solutions in the absence and in the presence of SOD (50 U/ml), under UV-A irradiation (350 nm) as a function of the irradiation time at pH 5.5 and 10.5 (concentrations were determined by HPLC analysis). $\lambda_{exc} = 350$ nm, $[Ptr]_0 \sim 100$ μM, $[His]_0 \sim 100$ μM.

His) of 1.17 V) [33], and the corresponding standard redox potentials for Trp, Tyr, dGMP and dAMP ($E(Trp^{\bullet+}/Trp) = 1.015$ V [34], $E(Tyr^{\bullet+}/Tyr) = 0.93$ V [35], $E(dGMP^{\bullet+}/dGMP) = 1.33$ V [35], and $E(dAMP^{\bullet+}/dAMP) = 1.44$ V) [36], the electron transfer from histidine to the $^3Ptr^*$ should be thermodynamically favored.

4. Discussion

In view of the results obtained in this work, we propose that the degradation of histidine photosensitized by Ptr, involves the following reactions steps (in the reaction scheme, histidine is the generic term for its three acid-base forms: $HisH^+$ / His / $His(-H)^-$, Fig. 1): excitation of Ptr results in the formation of the reactive triplet excited state ($^3Ptr^*$) by intersystem crossing from the singlet excited state (Reaction 3). Three reaction pathways compete for the deactivation of $^3Ptr^*$: intersystem crossing to the ground state (Reaction 4), energy transfer to molecular oxygen leading to the regeneration of Ptr and the formation of $^1O_2$ (Reaction 5), and electron transfer between histidine and $^3Ptr^*$ yielding the corresponding pair of radical ions (Reaction 6, Type I mechanism). Then $^1O_2$ may react with histidine, to yield oxidized products (Reaction 7, Type II mechanism). Depending on the pH, different acidic-basic forms of histidine must be considered. At pH 5.5, while Ptr is in its neutral form, histidine is present as a mixture of protonated ($HisH^+$) and neutral (His) forms. At pH 10.5, Ptr is in its basic (anionic) form and for histidine, the unprotonated ($His(-H)^-$) form is the only relevant species. Only His and $His(-H)^-$, are known to be reactive to $^1O_2$.

The relative rates of the type I and type II photosensitized reactions control the dominant mechanism (Type I or Type II) of the histidine oxidation process. Assuming that histidine consumption is mainly from a Type I process, an increase of 5 in O_2 concentration (O_2-saturated *vs* air-equilibrated solutions) would decrease considerably the proportion of $^3Ptr^*$ reacting with histidine by electron transfer. Therefore, the rate of histidine consumption should also be much slower, as observed in Fig. 3 at acidic pH. In acidic solutions (pH 5.5), the rate of histidine consumption in the presence of Ptr, is almost negligible in O_2 saturated solutions, evidencing that a type II oxidation of histidine has no relevance. Moreover, the rate of histidine consumption in D_2O acidic solutions is the same (within the experimental error), to the rate observed in H_2O acidic solutions. On the contrary, in alkaline solutions, the rate of degradation of histidine was much higher in D_2O alkaline solutions, but the rate decreased when O_2 was bubbled in the solutions, indicating that the degradation of histidine in the presence of Ptr it is not a purely type II mechanism.

$$Ptr \longrightarrow {}^1Ptr^* \longrightarrow {}^3Ptr^* \quad (3)$$

$$^3Ptr^* \longrightarrow Ptr \quad (4)$$

$$^3Ptr^* + {}^3O_2 \longrightarrow Ptr + {}^1O_2\,\Phi_\Delta \quad (5)$$

$$^3Ptr^* + \text{histidine} \longrightarrow Ptr^{\bullet-} + \text{histidine}^{\bullet+} \quad (6)$$

$$\text{histidine} + {}^1O_2 \longrightarrow \text{histidine(ox)/histidine} + {}^3O_2 \quad (7)$$

Depending on the histidine acid-base form, the electron-transfer mechanism may be coupled to a proton transfer or not. At pH <6.0, the imidazole group is protonated (Fig. 1), so a proton coupled electron transfer (PCET) is probably the main mechanism (Reaction 8). At neutral pH, the imidazole group is in its neutral form, and a direct electron transfer (ET) is probably much more efficient (Reaction 8′). At pH >10, with both molecules negatively charged, a proton coupled electron transfer may be more efficient (Reaction 8″)

$$^3Ptr^* + HisH^+ \xrightarrow[pH<6]{PCET} PtrH^{\bullet} + His^{\bullet+} \quad (8)$$

$$^3Ptr^* + His \xrightarrow[pH\sim7]{ET} Ptr^{\bullet-} + His^{\bullet+} \quad (8')$$

$$^3Ptr^* + His(-H)^- \xrightarrow[pH>10]{PCET} Ptr^{\bullet-} + His(-H)^{\bullet}. \quad (8'')$$

In the absence of O_2, the radical ions $PtrH^{\bullet}/Ptr^{\bullet-}$ and $His^{\bullet+}/His(-H)^{\bullet}$ formed in the electron transfer steps (Reaction 8, 8′ and 8″) recombine efficiently (Reaction 9). This recombination explains the nonexistence of histidine consumption in Ar-saturated solutions. In the presence of O_2, the electron transfer from $PtrH^{\bullet}/Ptr^{\bullet-}$ to O_2 regenerates Ptr and forms $O_2^{\bullet-}$ (Reaction 10), which latter disproportionate with its conjugated acid $HO_2^{\bullet}$ to form H_2O_2 (summarized by Reactions 11 and 12).

$$PtrH^{\bullet}/Ptr^{\bullet-} + His^{\bullet+}/His(-H)^{\bullet} \longrightarrow Ptr + \text{histidine} \quad (9)$$

$$PtrH^{\bullet}/Ptr^{\bullet-} + O_2 \longrightarrow Ptr + O_2^{\bullet-}(+H^+) \quad (10)$$

$$H_3O^+ + O_2^{\bullet -} \rightleftharpoons H_2O + HO_2^{\bullet} \quad (11)$$

$$HO_2^{\bullet} + O_2^{\bullet -} \longrightarrow HO_2^{\bullet -} + O_2 \quad (12)$$

Based on the analysis discussed in this section, we can conclude that an electron-transfer mediated oxidation of histidine (type I mechanism) takes place under UV-A radiation in the presence of Ptr. In acidic solutions (pH 5.5), the mechanism involves a type I oxidation, and the implication of $^1O_2$ is negligible. In alkaline solutions (pH 10.5), both type I and type II mechanisms are competing for the degradation of histidine.

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