Review

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Photosensitization of peptides and proteins by pterin derivatives

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Abstract: Proteins are one of the preferential targets of the photosensitized damaging effects of ultraviolet (UV) radiation on biological system. Pterins belong to a family of heterocyclic compounds, which are widespread in living systems and participate in relevant biological functions. In pathological conditions, such as vitiligo, oxidized pterins accumulate in the white skin patches of patients suffering this depigmentation disorder. It is known that pterins are able to photosensitize damage in nucleotides and DNA by type I (electron transfer) and type II (singlet oxygen) mechanisms. Recently, it has been demonstrated that proteins and its components may also be damaged when solutions containing both proteins and pterin are exposed to UV-A radiation. Therefore, given the biological and medical relevance of the photosensitizing properties of these molecules, we present in this article an overview of the capability of different pterin derivatives to photoinduce damage in proteins present in the skin, focusing our attention on the chemical modifications of tyrosine and tryptophan residues.

Keywords: electron transfer; photosensitization; proteins; pterins; UV-A radiation.

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Introduction

Solar radiation induces modifications in different biomolecules and is implicated in the generation of human skin cancer [1]. Most of the solar ultraviolet (UV) energy incidence on Earth's surface corresponds to UV-A radiation (320–400 nm), which can induce damage mostly through photosensitized reactions [2]. A photosensitized reaction is defined as a photochemical alteration occurring in one molecular entity as a result of the initial absorption of radiation by another molecular entity called photosensitizer [3]. Proteins and peptides are one of the preferential targets of the photosensitized damaging effects of UV radiation on biological systems [4]. These processes may be mediated by endogenous or exogenous photosensitizers and can take place through different mechanisms: the generation of radicals (type I mechanism), e.g. *via* electron transfer or hydrogen abstraction, and the production of singlet oxygen ($^1O_2$) (type II mechanism) [5, 6]. In general, it is accepted that the photosensitization of proteins occurs mainly through the reactions of $^1O_2$ with tryptophan (Trp), tyrosine (Tyr), histidine (His), methionine (Met) and cysteine (Cys) side-chains [7]. Nevertheless, in recent reports, it has been demonstrated that pterins photosensitize peptides [8, 9], proteins [10, 11] and their components [12–14] mainly through a type I mechanism.

Pterins, a family of heterocyclic compounds (Figure 1), are present in biological systems in multiple forms and play different roles ranging from pigments to enzymatic cofactors for numerous redox and one-carbon transfer reactions [15, 16]. The most common pterin derivatives are six-substituted compounds (Figure 1). According to the molecular weight and the functional groups of these substituents, pterins can be divided into two groups: (1) *unconjugated pterins*, containing substituents with one carbon atom or a short hydrocarbon chain, and (2) *conjugated pterins*, with larger substituents containing a *p*-aminobenzoic acid (PABA) moiety (Figure 1). Pterins can exist in living systems in different redox states and may be classified into three classes according to this property:

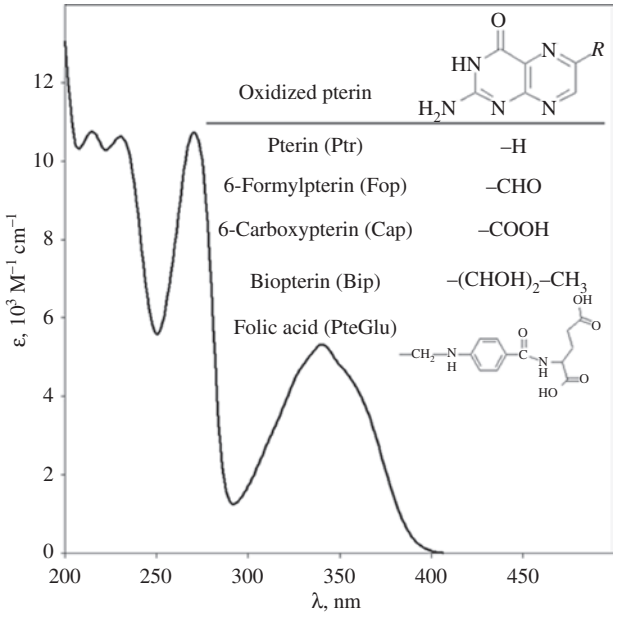


Figure 1: Molecular structures of common aromatic pterin derivatives and the absorption spectrum of pterin (Ptr) in air equilibrated aqueous solution at pH = 6.0.

fully oxidized (or aromatic) pterins, dihydro and tetrahydro derivatives. The latter are the most important pterins derivatives due to their biological activity.

Pterins are present in human epidermis given that 5,6,7,8-tetrahydrobiopterin (H_4Bip) is an essential cofactor for aromatic amino acid hydroxylases [17] and participates in the regulation of melanin biosynthesis [18]. In vitiligo, a skin disorder characterized by a defective protection against UV radiation due to the acquired loss of constitutional pigmentation [19], H_4Bip metabolism is altered [20]. Dihydro and oxidized pterin derivatives accumulate in the affected tissues at concentrations that are significantly higher than those reported for healthy cells [20, 21]. Although intracellular concentrations were not determined, it can be assumed that pterins can reach any cellular compartment since these compounds can freely cross phospholipid membranes [22].

The photochemistry of pterins is relevant to understand the harmful effects of radiation on skin and is of particular interest in depigmentation disorders, such as vitiligo. Pterins are photochemically reactive in aqueous solutions, and upon UV-A excitation, they can fluoresce, produce reactive oxygen species (ROS) [23–25] and, as mentioned above, photosensitize the oxidation of biomolecules [8–14].

In the context of our investigations on the photochemistry and photosensitizing properties of pterins, we present in this article an overview of the damage in peptides and proteins photoinduced by pterin derivatives. We have focused our attention on the chemical modifications of tyrosine (Tyr) and tryptophan (Trp) residues because these amino acids are particularly susceptible to a variety of oxidizing agents [26]. Moreover, the photosensitization of free Trp and Tyr by Ptr has previously been reported [12, 13]. The most relevant results, from a biological point of view, are summarized and discussed in this review.

General mechanism of photooxidation of biomolecules by pterins

In 1997, Ito and Kawanishi demonstrated for the first time that upon excitation with UV-A radiation, pterins are able to photoinduce DNA damage [27]. More recently, the mechanism involved in the photosensitization of biomolecules by pterins were investigated in a series of studies carried out with free nucleotides [28–30] and amino acids [12–14]. It was shown that pterins can act as photosensitizers through both type I and type II mechanisms and that the predominant one depends on a combination of many factors, such as quantum yields of $^1O_2$ production by the photosensitizer, ionization energy and reactivity of the substrate towards $^1O_2$ and presence of selective scavengers in the media.

Taking into account the studies mentioned in the previous paragraph, a set of competitive mechanisms can be summarized to explain the photooxidation of different biomolecules (S) by pterin derivatives (Pt) (reactions 1–12). After UV-A excitation of Pt and formation of its triplet excited state ($^3Pt^*$, reaction 1), three reaction pathways compete for the deactivation of the latter: intersystem crossing to singlet ground state (reaction 2), energy transfer to O_2 leading to the regeneration of Pt and the production of $^1O_2$ (reaction 11), and electron transfer from S to $^3Pt^*$ yielding the corresponding pair of radical ions (pterin radical anion ($Pt^{\bullet-}$) and biomolecule radical cation ($S^{\bullet+}$), reaction 3). $Pt^{\bullet-}$ is quenched by ground state O_2 to produce the superoxide anion ($O_2^{\bullet-}$) and regenerate Pt (reaction 4) [31, 32]. The spontaneous disproportionation of $O_2^{\bullet-}$ in aqueous solution leads to the formation of hydrogen peroxide (H_2O_2) (reaction 5) [33]. The radical $S^{\bullet+}$ (or its deprotonated form, $S(-H)^{\bullet}$) may react with $Pt^{\bullet-}$ (reaction 6) or/and $O_2^{\bullet-}$ (reaction 7) to regenerate the biomolecule (S). Alternatively, $S^{\bullet+}$ or $S(-H)^{\bullet}$ may react with O_2, H_2O or $O_2^{\bullet-}$ to yield oxidized products (reaction 8) or with other radicals to form dimeric products (reaction 9) or S-Pt adducts (reaction 10). On the other hand, the oxidation of S can occur by reaction with $^1O_2$ (reaction 12).

$$Pt \xrightarrow{hv} {}^1Pt^* \xrightarrow{ISC} {}^3Pt^* \quad (1)$$

Type I mechanism

$${}^3Pt^* \rightarrow Pt \quad (2)$$

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

$$Pt^{\bullet -} + O_2 \rightarrow Pt + O_2^{\bullet -} \quad (4)$$

$$2H^+ + 2O_2^{\bullet -} \rightarrow H_2O_2 + O_2 \quad (5)$$

$$Pt^{\bullet -} + S^{\bullet +} \rightarrow Pt + S \quad (6)$$

$$S^{\bullet +} + O_2^{\bullet -} \rightarrow S + O_2 \quad (7)$$

$$S^{\bullet +} \xrightarrow{O_2/H_2O/O_2^{\bullet -}} S(ox) \quad (8)$$

$$2S^{\bullet +} \rightarrow S_2(-2H) + 2H^+ \quad (9)$$

$$S^{\bullet +} + Pt^{\bullet -} \rightarrow S - Pt \quad (10)$$

Type II mechanism

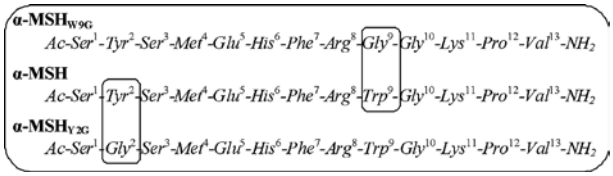
$${}^3Pt^* + {}^3O_2 \rightarrow Pt + {}^1O_2 \quad (11)$$

$$S + {}^1O_2 \rightarrow \rightarrow S(ox) \quad (12)$$

Photodamage to α-melanocyte-stimulating hormone (α-MSH) and related peptides

The photosensitization of amino acids in biological peptides using Ptr as photosensitizer was investigated [8, 9]. We performed studies with α-melanocyte-stimulating hormone (α-MSH) as a substrate. This hormone is a short peptide that stimulates the production and release of melanin by melanocytes in the skin and hair. The amino acid sequence of α-MSH is shown in Scheme 1. When aerated aqueous solutions containing α-MSH and Ptr (pH 5.5) were exposed to UV-A radiation (350 nm), the peptide consumption and H_2O_2 generation were observed, but without significant change in the photosensitizer concentration. It is important to mention that under these experimental conditions, only Ptr was excited (Figure 1).

Changes in Trp and Tyr residues were studied by fluorescence measurements [8]. The analysis of emission

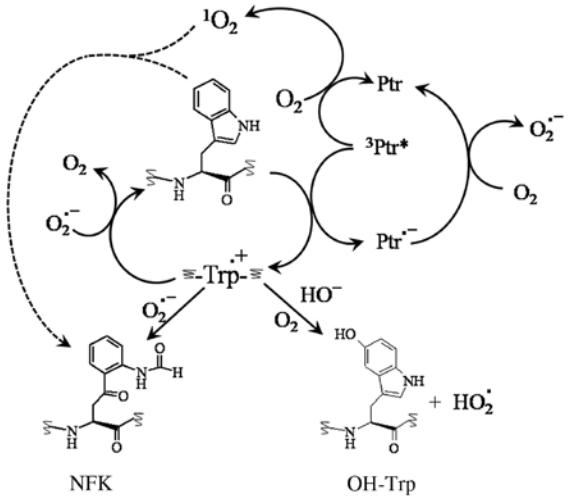


Scheme 1: Molecular structures of α-MSH and the modified peptides: α-MSH$_{W9G}$ and α-MSH$_{Y2G}$, in which the Trp and the Tyr residues of α-MSH where mutated to Gly residues, respectively.

spectra indicated that both residues were damaged and new fluorescent compounds were formed. One of these compounds exhibits an emission band with a peak maximum coinciding with that expected for tyrosine dimers (Tyr_2). The cross-linking between two Tyr residues was confirmed by ultra-high performance liquid chromatography coupled to a mass spectrometry detector (UPLC-MS). In addition, analysis of mass spectra of irradiated solutions showed new peaks with *m/z* values corresponding to monooxygenated and dioxygenated products. Surprisingly, the evolution of the concentration of each amino acid residue as a function of irradiation time indicated that in α-MSH, the Tyr residue reacted faster than the Trp residue.

To investigate specifically the reactivity and photoproducts of Trp and Tyr residues, we used two peptides in which the amino acid sequence of α-MSH was mutated (Scheme 1) [9]. In the peptide named α-MSH$_{W9G}$, the Trp residue in position 9 was mutated to a glycine (Gly), whereas in the peptide named α-MSH$_{Y2G}$, the Tyr residue in position 2 was mutated, also to a Gly residue. In these studies aqueous solutions containing Ptr and α-MSH$_{W9G}$ or α-MSH$_{Y2G}$ were exposed to UV-A radiation.

During the photosensitization of α-MSH$_{Y2G}$, the Trp residue was consumed, and at least three major products were detected by chromatographic methods. The spectroscopic characterization of these products suggested that the Trp residue was oxidized to N-formylkynurenine (NFK) and hydroxy-tryptophan (HO-Trp) (Scheme 2). The analysis of irradiated solutions by UPLC-MS revealed products with incorporation of one ([M + O]), two ([M + 2O]) or three ([M + 3O]) oxygen atoms. The oxidation of the Trp moiety to OH-Trp and NFK involves the incorporation of one and two oxygen atoms, respectively. The product with molecular weight [M + 3O] might correspond to a peptide with NFK and an additional oxygen atom in another residue of the peptide sequence. A potential target for Ptr-photosensitization, besides Trp, is the His residue because we have recently reported that Ptr is able to photo-induced the oxidation of free His [14].

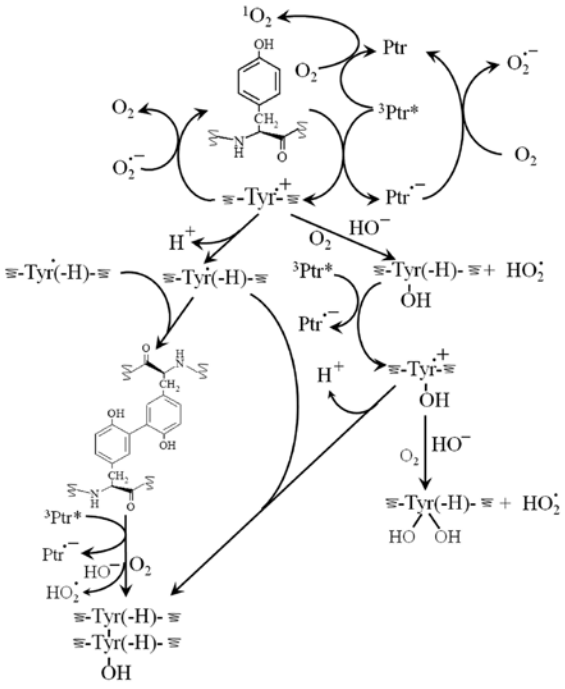


Scheme 2: Main reaction pathways proposed for the Ptr photosensitized degradation of the Trp residue in peptides.

The photosensitization of α-MSH_{W9G} revealed that the Tyr residue was consumed during irradiation. As in the case of α-MSH and α-MSH_{Y2G}, H_2O_2 was generated and its concentration increased as a function of irradiation time. The analysis of the consumption rates of each amino acid revealed that the consumption of Trp in α-MSH_{Y2G} is slightly faster than that of Tyr in α-MSH_{W9G}. As we mention above, the opposite behaviour was observed when both amino acid residues were in the same peptide. These facts indicate that small differences in the composition of the peptides may change to some extent the relative reactivity of both amino acid residues.

The analysis of the products of α-MSH_{W9G} revealed that two major products were formed; one of them has spectroscopic properties similar to that reported for Tyr_2 (Scheme 3) [34]. The mass spectrometry analysis confirmed the formation of the Tyr_2 moiety. The mass spectra of other peaks revealed that at least, two photoproducts incorporated one oxygen atom ([M + O]), but only one bore two oxygen atoms ([M + 2O]), suggesting that there are two main different positions for the incorporation of oxygen in this peptide. In addition, dimeric products with incorporation of one, two and three oxygen atoms were also detected (Scheme 3). These results indicated that upon irradiation in the presence of Ptr, α-MSH_{W9G} undergoes two simultaneous processes: dimerization and incorporation of oxygen.

For the Ptr photosensitization of Trp and Tyr residues in α-MSH, the predominant mechanism is type I (*vide supra*). The process is initiated by an electron transfer from the peptide to the triplet excited stated of Ptr ($^3Ptr^*$) (reaction 3). The resulting radicals, mainly located on



Scheme 3: Main reaction pathways proposed for the Ptr photosensitized degradation of the Tyr residue in peptides.

the Trp ($Trp^{\bullet+}$) and Tyr ($Tyr^{\bullet+}$) residues, undergo further reactions to yield the products depicted in Schemes 2 and 3. The role of $^1O_2$ (type II mechanism) was negligible or minor, depending on the amino acid residue involved. However, NFK is a typical product of oxidation of Trp by $^1O_2$ and in this case, type II mechanism might contribute as a minor pathway.

Photoinduced chemical changes in serum albumin

Serum albumins are the most abundant plasma proteins, and their main biological function is the transport of a wide variety of molecules such as fatty acids, metabolites, and drugs [35]. In addition, albumin is present in human skin [36], where there is an autocrine synthesis and regulation [37]. It has been reported that in patients affected by vitiligo, epidermal albumin oxidation takes place, but the mechanism of this process has not been elucidated [38]. We have studied the capability of Ptr to photoinduce chemical and structural changes in bovine serum albumin (BSA) and human serum albumin (HSA). The results obtained for both proteins were very similar, as is expected due to the high structural homology between HSA and BSA [39, 40].

Air-equilibrated aqueous solutions of albumin (BSA or HSA) and Ptr (pH 6.0, KH_2PO_4 1 mM) were exposed to UV-A radiation for different periods of time. The treated samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and a decrease in the albumin concentration as a function of irradiation time was observed. The decrease of the intensity of the albumin peak was also observed by size-exclusion chromatography coupled with a light scattering detector (SEC-LS) (Figure 2). In the case of BSA, it was demonstrated that dimerization of the protein occurred, but larger products were not investigated [39]. For HSA, the molecular weights of the photoproducts were determined by SEC-LS, and it was found that oligomers with more than 10 HSA molecules were formed (Figure 2) [40].

The specific chemical modifications undergone by the albumin amino acids were evaluated by means of fluorescence measurements. To avoid the interference of the emission of the photosensitizer, the protein was isolated from Ptr using the high-performance liquid chromatography (HPLC) semipreparative technique [40]. In particular, the monomer and oligomer fractions were collected. The emission spectra of the oligomer fraction revealed the presence of Tyr_2, suggesting that this product is, at least in part, responsible for the bonds between albumin molecules. In some proteins, in particular globular ones, cross-linking of Tyr residues could lead to alteration of the conformation and of the biological activity [41]. However, the far- and near-UV CD spectra revealed that Tyr linkages did not produce modifications of the tertiary and secondary structures of the protein.

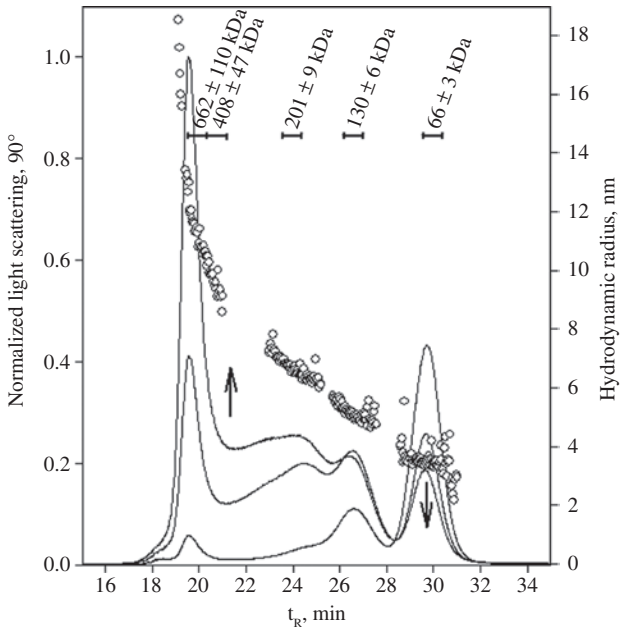


Figure 2: Chromatograms obtained using SEC-LS analysis of a solution of HSA irradiated in the presence of Ptr during different irradiation times (0 h, 1 h and 2 h). Circles represent the hydrodynamic radii of the molecules present in the sample after 2 h of irradiation. The corresponding molecular masses at different retention times are indicated in the upper part of the figure. Reprinted from Reference [40] (Reprinted with permission from Biochemistry 2016; 55(34): 4777–4786 Copyright 2016 American Chemical Society).

To study the effect of the photochemical process on the Trp residue, emission spectra of the protein fraction (monomer and oligomers together) were recorded. Upon irradiation, the intensity of the Trp emission of the albumin decreased as a function of irradiation time, indicating the modification of this amino acid residue. It is known that Trp fluorescence properties are affected by the polarity of its surrounding environment [42]. When a Trp residue in an apolar medium (as in the case of Trp-214 of albumin) becomes hydrogen bonded or exposed to water, the emission shifts to longer wavelengths, and its fluorescence quantum yield decreases. In our system, although the emission of Trp residues decreases as a function of irradiation time, the corresponding maximum did not change, suggesting that the decrease in the emission is mainly due to a net consumption of Trp residues, and changes in the environment are negligible. In addition, NFK was identified by fluorescence measurements as one of the photooxidation products of albumin. This result showed for the first time that NFK is produced in albumin by the photosensitized oxidation of the Trp residue and could explain the presence of oxidized albumin in the white skin patches in vitiligo patients.

Association with a protein can modify the properties of a photosensitizer and, as a result, the efficiency and the mechanism of the photodamage to the macromolecule. This fact can be particularly important for albumins because of their high physiological concentrations and binding capacity [43]. We have demonstrated that the affinity of albumin for Ptr is relatively low [10, 40], suggesting that in the photosensitization process a contribution of a static mechanism initiated by Ptr associated with the protein is unlikely. To investigate further this point, irradiation experiments were performed at various temperatures and the Trp emission was measured. The rate of Trp consumption increased with temperature, thus confirming that the photodamage to the Trp residues of albumin by Ptr takes place *via* a purely dynamic mechanism [10, 40].

The analysis of the mechanism suggested, as it was observed for the peptides, that the ^{3}Ptr* initiated the photosensitization process *via* an electron transfer reaction. Moreover, the interaction of ^{3}Ptr* with HSA was confirmed by measuring the rate constant of quenching of ^{3}Ptr* by HSA ($k^{HSA} = (4.5 \pm 0.7) \times 10^9$ $M^{-1}s^{-1}$) [40].

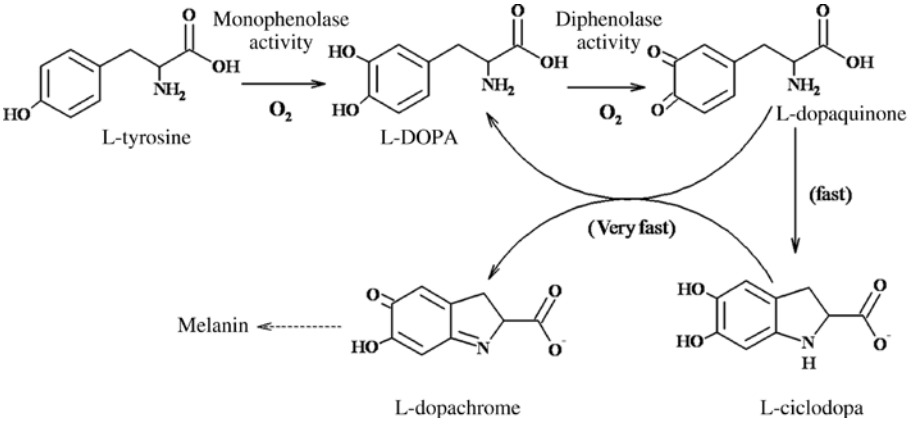
It is accepted that the photosensitization of proteins occurs mainly through oxidation by $^1O_2$[7] and NFK has been reported as a typical product of the oxidation of Trp by $^1O_2$ [44]. However, pterins are able to photosensitize biomolecules principally by a type I mechanism. In order to confirm this point, the contribution of oxidation by $^1O_2$ was evaluated by measuring the NFK formation and albumin degradation in comparative experiments using H_2O and deuterium oxide (D_2O) as solvents (the $^1O_2$ lifetime is much longer in the latter than in the former solvent). The results indicate that the reaction with $^1O_2$ is present in our reaction system, but it is not the only contribution for the formation of NFK, thus suggesting that this compound can be also formed by a type I mechanism. Protein damage evaluated by SDS-PAGE showed that the intensity of the protein band decreased only slightly faster in D_2O than in H_2O, suggesting that the contribution of type II mechanism is minor in the photosensitization of albumin by Ptr.

Photoinactivation of tyrosinase

To find out if the photoinduced chemical modifications that take place in albumins, used as models proteins, can affect the activity of enzymes, experiments using tyrosinase [TYR, L-tyrosine, L-dopa: oxygen oxidoreductase, EC 1.14.18.1)] as a substrate were performed [11]. This enzyme was chosen because it is an essential enzyme in the pigmentation of skin. TYR is a copper-containing glycoprotein that, in mammals, catalyzes the first and rate-limiting step in melanin biosynthesis, the hydroxylation of Tyr to 3,4-dihydroxy-L-phenylalanine (DOPA) (monophenolase activity) and the subsequent oxidation of DOPA to L-dopaquinone (diphenolase activity) (Scheme 4) [45]. The latter compound undergoes fast oxidation and rearrangement to yield L-dopachrome, which, in turn, spontaneously oligomerizes to form melanin, the natural pigment of skin (Scheme 4). Both types of activity are catalyzed by the same active site.

Aqueous solutions (1 mM KH_2PO_4, pH 6.0) containing TYR and Ptr were exposed to UV-A radiation during different periods of time. After the irradiation, the overall enzyme activity (oxidation of Tyr to L-dopachrome) was assayed according to the method of Pomerantz [46], which is based on the determination of L-dopachrome measured spectrophotometrically. The conversion of an inactive form of the catalytic site of the enzyme into an active form gives rise to a lag period before the reaction reaches maximal rate (Figure 3) [47]. Therefore, the enzyme activity (rate of formation of L-dopachrome, nM s^{-1}) was determined in the linear phase by measuring the slope of the absorbance curve at 475 nm *vs.* time (Figure 3). As shown in Figure 3, a fast inactivation of the enzyme was recorded. In another set of experiments, air-equilibrated solutions with the same concentration of TYR and different concentrations of Ptr were prepared. The samples were irradiated for the same period of time (12 min), and the activity of the enzyme was determined. Results showed a correlation between the Ptr concentration and the photoinactivation (Figure 3). These results provide evidence that processes photosensitized by pterins might affect the synthesis of melanin and, in consequence, play a key role in pigmentation disorders.

The two activities of the enzyme were measured separately. The monophenolase activity was determined by measuring the concentration of L-Tyr, DOPA and L-dopachrome at different irradiation times using the HPLC technique. To determine the diphenolase activity,



Scheme 4: First steps in melanin biosynthesis and structures of principal intermediates.

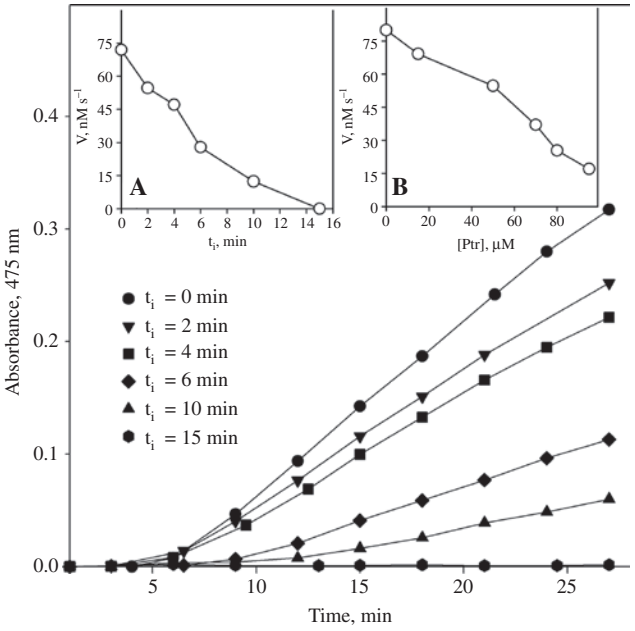
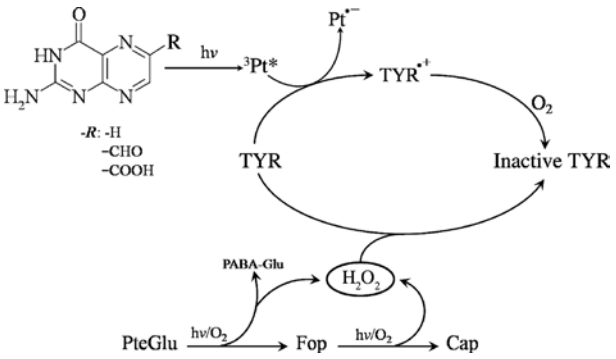


Figure 3: Determination of the tyrosinase activity in solutions (1 mM KH_2PO_4, pH 6.5) irradiated at room temperature in the presence of pterin (95 μM). For each sample, the formation of L-dopachrome was followed by measuring the absorbance at 475 nm as a function of the time elapsed after adding L-tyrosine as the substrate (37 °C). Inset: reaction rates (V) determined in the linear phase as a function of (A) irradiation time (t_i) ([Ptr] = 95 μM), (B) Ptr concentration after 12 min of irradiation.



Scheme 5: Reaction pathways proposed for the inactivation of Tyrosinase (TYR) photosensitized by different pterin derivatives.

the experimental procedure was exactly the same as that employed for the determination of the overall activity, but, in this case, DOPA was used as the substrate. The results obtained show that the photochemical process affects both activities of TYR (monophenolase and diphenolase). The mechanistic analysis suggested that the photoinactivation of TYR is initiated by an electron transfer reaction and takes place *via* a type I mechanism (Scheme 5) [11].

We extended our studies on the photoinactivation of TYR to folic acid (PteGlu) and its oxidation products because the photodegradation of PteGlu, an important vitamin, has been proposed as one of the reasons for the development of skin tanning in evolution [48–52]. Photooxidation of PteGlu under UV-A radiation may be divided into three stages: (i) in the first phase, *p*-aminobenzoylglutamic acid (PABA–Glu) and 6-formylpterin (Fop) are photogenerated, with a pseudo-zero-order kinetics; (ii) in the second phase, Fop photoinduces the photooxidation of PteGlu and its degradation process is accelerated; (iii) in the third phase, the degradation of Fop to 6-carboxypterin (Cap) is the dominating process (Scheme 5) [53–56]. Reactive oxygen species, such as H_2O_2 and $O_2^{\bullet-}$, are formed during the photooxidation of PteGlu into Fop, and in the reaction where Fop is converted into Cap (Scheme 5) [56].

Therefore, under irradiation of skin affected by vitiligo, Fop and Cap are formed, very likely, due to the photolysis of both PteGlu and H_4Bip [57–59]. In fact, Cap has been isolated from the skin of patients suffering from vitiligo [21].

The activity of TYR decreased significantly when the enzyme was exposed to radiation in the presence of PteGlu [60]. Nevertheless, the comparison of the kinetic profiles of PteGlu photodegradation and TYR photoinactivation indicated that in the first minutes of irradiation, the concentration of PteGlu did not change significantly, whereas no loss of the enzyme activity was registered. It can be assumed that in this time range, no photoproduct of PteGlu was generated, indicating that PteGlu itself presented a negligible capability to photoinactivate the enzyme. After the first minutes of irradiation, important increases in the rate of photodegradation of PteGlu and rates of formation of Fop and Cap were observed. Therefore, a significant proportion of light was absorbed by Fop and Cap. Simultaneously, a considerable decrease of the enzyme activity was observed. These results suggested that, in contrast to PteGlu, Fop and Cap photoinduce the inactivation of the enzyme. The comparison of the rates of photoinactivation using Fop, Cap and Ptr as photosensitizers revealed that, in fact, Fop is the most efficient sensitizer. This result explains why the rate of TYR inactivation increases when Fop is accumulated during the photooxidation of PteGlu.

Taking into account that under UV-A radiation, PteGlu and Fop generate H_2O_2 (Scheme 5), we explored the stability of TYR in the presence of H_2O_2 in the dark. In this experiment, we observed that the enzyme was inactivated by H_2O_2. In another set of experiments, air equilibrated solutions of TYR and Fop were exposed to UV-A radiation in the absence and in the presence of catalase, an enzyme that catalyzes specifically the decomposition of H_2O_2 into O_2 and H_2O, to eliminate H_2O_2 produced during irradiation. The data indicated that the photoinactivation

of TYR was much more efficient in the absence of catalase. These results clearly indicated that the inactivation of TYR in our reaction system takes place through two different pathways: photosensitized reaction and oxidation of the enzyme by H_2O_2 (Scheme 5).

Conclusions

Pterin derivatives are a family of interesting heterocyclic molecules commonly found in living systems in small amounts. Under pathological conditions, such as vitiligo, these compounds accumulate in the white skin patches, were the protection against the UV radiation fails. In this overview, we have summarized results that unequivocally prove the capability of pterins to photoinduce damage in proteins present in the skin, under UV-A radiation. The processes leading to the chemical changes in proteins are initiated by the triplet excited state of Ptr through production of singlet oxygen (type II mechanism) and electron transfer oxidation (type I mechanism), being the type I mechanism the main contribution in the photosensitization process. The photochemical reactions lead to the degradation of at least two amino acid residues: tryptophan (Trp) and tyrosine (Tyr).

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