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Type I Photosensitized Oxidation of Methionine†

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ABSTRACT

Methionine (Met) is an essential sulphur-containing amino acid, sensitive to oxidation. The oxidation of Met can occur by numerous pathways, including enzymatic modifications and oxidative stress, being able to cause relevant alterations in protein functionality. Under UV radiation, Met may be oxidized by direct absorption (below 250 nm) or by photosensitized reactions. Herein, kinetics of the reaction and identification of products during photosensitized oxidation were analyzed to elucidate the mechanism for the degradation of Met under UVA irradiation using pterines, pterin (Ptr) and 6-methylpterin (Mep), as sensitizers. The process begins with an electron transfer from Met to the triplet excited state of the photosensitizer (Ptr or Mep), to yield the corresponding pair of radicals, Met radical cation ($Met^{\bullet+}$) and the radical anion of the sensitizer ($Sens^{\bullet-}$). In air-equilibrated solutions, $Met^{\bullet+}$ incorporates one or two atoms of oxygen to yield methionine sulfoxide (MetO) and methionine sulfone ($MetO_2$), whereas $Sens^{\bullet-}$ reacts with O_2 to recover the photosensitizer and generate superoxide anion ($O_2^{\bullet-}$). In anaerobic conditions, further free-radical reactions lead to the formation of the corresponding dihydropterin derivatives (H_2Ptr or H_2Mep).

KEYWORDS. Photosensitization; Methionine; Electron-transfer; Type I/Type II photooxidation; Pterins

INTRODUCTION

Proteins have been shown to represent important targets for photodamage under UV and visible irradiation, and tryptophan (Trp), tyrosine (Tyr), methionine (Met) and histidine (His) are amino acids particularly susceptible to a variety of oxidizing agents (1). UV-A (320-400 nm) and visible radiation induce oxidation reactions through photosensitized processes. These processes involve excitation of the photosensitizers to yield singlet and triplet excited states (2). The latter, having longer lifetimes, may undergo both electron transfer with different substrates, mechanism known as type I photooxidation, or energy transfer to molecular oxygen (O_2) forming singlet oxygen ($^1O_2(^1\Delta_g)$, denoted as $^1O_2$) responsible for type II photooxidation reactions (3).

Met, a non-polar sulphur-containing α-amino acid (Scheme 1), is more easily oxidized than the other amino acids and highly susceptible to oxidation by reactive oxygen species (ROS) (4,5,6). Met is an essential amino acid and its deficiency leads to a failure in the synthesis of critical proteins. Depending on the redox environment, Met oxidation undergo by one- or two-electron transfer to yield Met radical cations. After one-electron oxidation of Met, an unstable radical cation is formed, which is later converted in several products, some of them irreversible. Met oxidation products are mainly methionine sulfoxide (MetO) or methionine sulfone ($MetO_2$) (Scheme 1). *In vivo*, MetO may be enzymatically reduced by methionine sulfoxide reductase, and then the oxidation of Met is repair (7). The accumulation of MetO occurs over the years or in some pathologies as Alzheimer's disease (8). On the contrary, $MetO_2$ is an irreversible product which cannot be repaired enzymatically (7).

<Scheme 1>

Typical type I photosensitized oxidation of Met has been reported in the presence of a suitable sensitizer and UV or visible radiation (9,10,11). Pterins are a family of heterocyclic compounds widespread in biological systems, which have been identified as efficient photosensitizers by type I and II mechanisms (12,13). Under UV-A excitation (320-400) pterins photoinduce photodegradation of various biomolecules such as DNA (14,15), nucleotides (16,17), proteins (18), amino acids (19,20,21) and lipids enriched in polyunsaturated fatty acids (PUFAs) (22). Pterins are present in human epidermis in a low concentration at normal physiological conditions, but during skin disorders, such as vitiligo, pterins concentration is much higher (23).

In the present study, we evaluated the capability of two pterin derivatives, pterin (Ptr) and 6-methylpterin (Mep), to photosensitize the degradation of Met in aqueous solutions exposed to UV-A irradiation under different experimental conditions. In this spectral region, pterins are the species that absorb radiation, whereas Met does not (Fig. 1). The study was performed in the pH range 5.5-6.0, so that more than 99% of the pteridine (pK_a ~ 8) (24) was in the acid form, the predominant form at physiological pH. In this work, UV/Visible spectrophotometry, HPLC with UV-visible and mass detection, and an enzymatic method for H_2O_2 determination were used to follow the photochemical reactions, and a mechanism for the photosensitized oxidation is proposed.

<Figure 1>

MATERIALS AND METHODS

General

Ptr and Mep (purity > 99%, Schircks Laboratories, Switzerland and Sigma-Aldrich) was used without further purification after checking for impurities by HPLC. Met and ammonium acetate (NH_4OAc) (Sigma Chemical Co) were of the highest purity available (> 98 %) and were used without further purification. Methanol (MeOH) and potasium iodide (KI) were purchased from J. T. Baker and Laboratorios Cicarelli, respectively. Other chemicals were from Sigma Chemical Co.

Solutions were prepared dissolving the pterine (Ptr or Mep) and Met 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. Concentration ranges used for the experiments were 50-150 μM and 600-800 μM for the pterine and Met, respectively.

Steady-state irradiation.

Irradiation set-up. Aqueous solutions containing a pterine (Ptr or Mep) and Met (pH 5.5-6.0) were irradiated in 1 cm path length quartz cells at room temperature with a Rayonet RPR3500 lamps, with emission centered at 350 nm (fwhm bandwidth of *ca* 20 nm, Southern N.E. Ultraviolet Co.). The experiments were performed in the presence and in the absence of O_2. O_2-free solutions were obtained by bubbling with Ar during 20 min. The measurements were carried out under conditions of reduced environmental light.

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 ($q_{P,0}$) at the excitation wavelength. The method for the determination of $q_{P,0}$ has been described in detail elsewhere (25,26). Values of the photon flux absorbed ($q_{P,a}$), were calculated from $q_{P,0}$ ($q_{P,0}$ = 1.0 x 10^{-5} einstein L^{-1} min^{-1} at 350 nm) according to the Lambert-Beer law ($q_{P,a} = q_{P,0}$ $(1\text{-}10^{-A})$, where A is the absorbance of the sensitizer at the excitation wavelength).

UV/Visible 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.

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, column oven CTO-10AS VP, photodiode array detector SPD-M20A), was used to monitor and quantify the reactants and the photoproducts. Separation of the photosensitizer, the substrate and the products was performed on a Sinergy Polar-RP coPtrn column (ether-linked phenyl phase with polar endcapping, 150 x 4.6 mm, 5 μm; Phenomenex), using as mobile phase solutions containing 10 mM NH_4OAc aqueous solution (pH 6.8). HPLC runs were monitored by UV/Vis spectroscopy at different wavelengths. Absorption spectra of the reactants and products were registered at each retention time with the photodiode array detector of the equipment.

Data analysis. Determination of concentrations related to a given experiment were performed on the same day. Kinetic analyses were carried out for each experiment. Error of individual data is related to the detection methodology.

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 is detected by its absorbance in the visible region (27,28). This assay has high sensitivity and specificity due to the intense absorbance of the product at 505 nm and the enzymatic catalysis, respectively. The reactants were purchased from Wiener Laboratorios SAIC (cholesterol kit), and it was used as previously described elsewhere (19).

Mass spectrometry analysis

The analysis was performed using an UPLC chromatograph (ACQUITY UPLC from Waters) coupled to a quadrupole time-of-flight mass spectrometer (Xevo G2-QTof-MS from Waters) (UPLC-QTof-MS), described elsewhere (22). UPLC analyses were performed using an Acquity UPLC® BEH C18 (1.7 μm; 2.1 x 50 mm) column (150 mm, Waters), and isocratic elution with 0.1 % formic acid at a flow rate of 0.6 mL min^{-1}.

RESULTS

Irradiation of solutions containing Ptr and Met

Aqueous solutions containing Met (~ 600 μM) and the photosensitizer Ptr (~ 100 μM), equilibrated at different O_2 concentrations, were exposed to UV-A radiation (350 nm) for different periods of time. Considerable changes in the absorption spectra of the solutions were registered during irradiation even in the absence of O_2. Concentration profiles of reactants were obtained by HPLC. In aerated solutions, a decrease of Met concentration was observed as a function of the irradiation time, whereas Ptr concentration remained constant (Fig. 2a). On the other hand, in the absence of O_2, both Met and Ptr consumption were observed (Fig. 2b). Taking into account previous studies carried out with Ptr and other amino acids (19,20,21), the

behavior observed in the presence of O_2 was expected, but the results obtained in anaerobic conditions were surprising. In fact, studies carried out under anaerobic conditions with Trp and Tyr showed that neither the substrate nor the photosensitizer are consumed upon irradiation due to the recombination of radicals, which leads to the recovering of the reactants (19,20).

<Figure 2>

In air-equilibrated solutions H_2O_2 was detected as a product. In addition, HPLC analysis revealed the formation of a main product (P1), at a retention time (t_R) 3.6 min. On the other hand, in argon equilibrated solution, a different photoproduct (P2, t_R 16.5 min) was detected, being its absorption spectra similar to those reports for 7.8-dihyropterin derivatives (29), thus suggesting the reduction of Ptr in the photochemical process.

7,8-Dihydropterin (H_2Ptr) is not commercially available, and therefore it is not possible to confirm its formation. It was previously reported that, in anaerobic conditions, pteridines are photo-reduced to the corresponding 7,8-dihydroderivative in the presence of a suitable electron donor (30,31).

Although Ptr is the most studied pterin derivative in terms of photosensitizing properties, we changed to 6-methylpterin (Mep) in order to investigate a possible photoreduction of the photosensitizer. Mep is a compound with photochemical properties similar to Ptr (12), but its corresponding dihydroderivative, 6-methyl-7,8-dihydropterin (H_2Mep), is commercially available, stable, and well characterized by

chromatography. Therefore, after having observed the general behavior with Ptr, we performed the rest of the studies using Mep as photosensitizer.

Irradiation of solutions containing Mep and Met

Experiments similar to those described in the previous section were performed using Mep instead of Ptr as a photosensitizer. In air-equilibrated solutions, the behavior observed upon irradiation was equivalent to that observed with Ptr, that is, consumption of the amino acid, no consumption of the photosensitizer, formation of H_2O_2 (Fig. 3a) and formation of a product at 3.6 minutes of retention time (Fig. 3b). The addition of commercial MetO to irradiated solutions results in an increase in the peak at 3.6 minutes (Fig. 3b), thus indicating that P1 is this Met oxidation product. According to previous studies, MetO is a common product of thermal and photochemical oxidation of Met.(8,9,32,33,34)

<Figure 3>

In deaerated solutions, both Met and Mep, as in the case of Ptr, were also consumed, and a peak at retention time 16.1 min, and similar spectroscopic characteristics as P2 (t_R 16.5 min) was detected whose concentration increased as a function of irradiation time (Fig. 4). The addition of commercial H_2Mep as a standard, resulted in an increase of the area of the peak at 16.1 min (Fig. 4, dash red line). Moreover, the absorption spectra recorded at 16.1 min (Fig. 4, inset c) was coincident with the absorption spectra of commercial H_2Mep (Fig. 4, inset b). These results

strongly suggest the reduction of Mep upon UVA irradiation in the presence of Met. In this case, as expected, no MetO was detected.

<Figure 4>

In addition, control experiments showed that reactions between a pterine (Ptr or Mep) and Met in the dark could be discarded. Moreover, no chemical modification of the amino acid was detected when Met (~600 μM) solutions were irradiated at 350 nm in the absence of the photosensitizer, under aerobic or anaerobic conditions, thus excluding spurious effects of light absorption by Met.

Mechanistic analysis

Kinetic analyses on the Mep-photosensitized degradation of Met were performed at different experimental conditions in order to determine the mechanism. Iodide (I^-) is an efficient and selective quencher of triplet-excited states of pterins at micromolar concentrations (31,35). Thus, to evaluate the participation of triplet-excited state of Mep (^{3}Mep*), photosensitization experiments were carried out in aqueous solutions containing Met and Mep at pH 5.5 in the presence of 400 μM KI, both in air-equilibrated and argon–equilibrated solutions. At this KI concentration, the quenching of the singlet excited state of Mep (^{1}Mep*) is less than 3% (τ_{F0} = 8.9 10^{-9} s, k_F = 6.2 x 10^9 $M^{-1}s^{-1}$ (35)), and therefore any change in the rate of Met consumption is not due to deactivation of ^{1}Mep*. The rate of consumption of Met, both in the presence and in the absence of O_2, was lower in the

presence of I^- (Fig. 5), indicating that ^{3}Mep* is involved in the mechanism of photodegradation of Met.

<Figure 5>

Since Met is oxidized by $^1O_2$ (33) and pterins are relatively good $^1O_2$-photosensitizers (12), we investigated the role of this ROS in the oxidation of Met photosensitized by Mep in aerobic conditions. Therefore, experiments using D_2O as solvent were carried out and the results were compared to those obtained in H_2O. The $^1O_2$ lifetime (τ_Δ) is much longer in D_2O than in H_2O (*c.a.* 60 μs and 4 μs, respectively) (36). Therefore, if $^1O_2$ is involved in the oxidation of Met, the rate of consumption should be about 20-times faster in D_2O than in H_2O. The determined rate of Met consumption was higher in D_2O (Fig. 6), but lower than expected considering a pure type II mechanism. Previously, it has been reported the rate constant value of the total quenching (k_T) of $^1O_2$ by Met (1.3×10^{-7} M^{-1} s^{-1}) and that the quenching is almost completely a chemical process ($k_r \sim k_T$) (37). Accordingly, in a given experiment, the contribution of $^1O_2$ to the Mep-photosensitized oxidation of Met can be evaluated by comparing the experimental initial rate of Met consumption ($(d[Met]/dt)_{0,exp}$) to the calculated initial rate of the reaction between $^1O_2$ and Met ($(d[Met]/dt)_{0,calc}$, equation 1).

$$-(d[Met]/dt)_{0,calc} = k_r [Met] [^1O_2] \qquad (1)$$

The steady-state concentration of $^1O_2$ during irradiation of a solution containing Mep and Met is given by:

$$[^1O_2] = q_{P,a} \Phi_\Delta / (k_d + k_t^{Mep} [Mep] + k_t^{Met} [Met]) \qquad (2)$$

where $q_{P,a}$ (einstein L^{-1} s^{-1}) is the photon flux absorbed by Mep; Φ_Δ is the quantum yield of $^1O_2$ production by Mep (Φ_Δ = 0.10 ± 0.02) (12); k_d is the rate constant of $^1O_2$ deactivation by the solvent (2.6 x 10^5 s^{-1}); k_t^{Mep} and k_t^{Met} are the overall rate constants of $^1O_2$ quenching by Mep (8.0 x 10^6 M^{-1} s^{-1} (12)) and Met (1.3 x 10^7 M^{-1} s^{-1} (37)), respectively. Considering the experimental conditions of Fig. 6 (120 μM Mep, 700 μM Met), $(d[Met]/dt)_{0,calc}$, was 1.5 μM min^{-1}, considerably lower than $(d[Met]/dt)_{0,exp}$ (5.7 μM min^{-1}), calculated by HPLC analysis. The difference observed between $(d[Met]/dt)_{0,calc}$ and $(d[Met]/dt)_{0,exp}$ indicates that the chemical reaction between Met and $^1O_2$ does not contribute significantly to the photosensitized oxidation of Met. This behavior was already observed for the oxidation of Trp and Tyr sensitized by Ptr (19,20) which is consequence of the significant difference between the rate constants of the reaction of Met with $^1O_2$ (1.3 x 10^7 M^{-1} s^{-1} (37)), and with ^{3}Ptr* (3 x 10^9 $M^{-1}s^{-1}$ (35)), that is, Met reacts faster with ^{3}Ptr*, to yield $Met^{\bullet+}$, than with $^1O_2$.

<Figure 6>

Further experiments were carried out in the presence of superoxide dismutase (SOD), an enzyme that catalyzes the conversion of $O_2^{\bullet-}$ into H_2O_2 and O_2 (38). The experiments revealed an increase in the rate of Met consumption when SOD was present in the solution (Fig. 7) indicating that $O_2^{\bullet-}$ is involved in the mechanism.

<Figure 7>

Considering all these kinetic results, and previous articles on photosensitization of biomolecules with pterins, we propose a mechanism (Scheme 2) for Met degradation during UV-A irradiation in the presence of Mep (or Ptr). Upon UV-A excitation Mep yields a singlet excited state (^{1}Mep*, Reaction 1) which, in turn, by intersystem crossing (ISC), leads to the formation of the reactive triplet excited state (^{3}Mep*, Reaction 2). ^{3}Mep* undergoes ISC to the ground state (Reaction 3) or reacts with Met in an electron transfer (ET) reaction yielding the corresponding radical ions, $Mep^{\bullet-}$ and $Met^{\bullet+}$ (Reaction 4, type I mechanism).

<Scheme 2>

Based on the behavior of $Met^{\bullet+}$, the investigation of photoreduction of pterins (30) and the results observed in the absence of O_2, we proposed that a second electron transfer reaction takes place in which H_2Ptr is formed (Reaction 6). Considering previous reports on photosensitized degradation of methionine by flavins, a molecular mechanism could be proposed (Scheme 3) (9).

<Scheme 3>

In air-equilibrated solutions, energy transfer to O_2 leads to the regeneration of Ptr and the formation $^1O_2$ (Reaction 9) (39,40), which competes with reactions 3 and 4. It was previously published (19), that during irradiation of solutions containing Ptr, $O_2^{\bullet-}$ is generated due to the reaction between $Ptr^{\bullet-}$ and O_2 (Reaction 8), and Ptr is recovered. Later, $O_2^{\bullet-}$ may disproportionate with its conjugated acid $HO_2^{\bullet}$ to form H_2O_2 (Reactions 9). Also,

$O_2^{\bullet-}$ may react with $Met^{\bullet+}$ to regenerate Met (Reaction 10), preventing its further oxidation, which is evidence by the increase of Met oxidation while $O_2^{\bullet-}$ is eliminated from the solution. Finally, Met or $Met^{\bullet+}$ is oxidized to different products by both type I and type II Mechanism (Reactions 11 and 12, respectively), but in our reaction system type I mechanism predominates.

Analysis of products by mass spectrometry

In UPLC-QTof-MS analysis of non-irradiated solutions, performed in positive ion mode (ESI^+), the intact molecular ions of Met and Mep as $[M + H]^+$ species at t_R 1.95 and 6.31 min, with *m/z* 150.0589 Da and 178.0732 Da were detected, in agreement with their molar weights, 149 and 177, respectively.

In aerated irradiated solutions, as well as the reactants, two products were detected. One product was detected at t_R = 1.42 min, with a mass of 166.0538 Da coincident the molecular ion $[Met + O + H]^+$, which corresponds to the incorporation of an atom of oxygen to Met to yield MetO. It was also observed the molecular ion $[Met + O + Na]^+$ at *m/z* = 188.0361 Da. In addition, at longer irradiation times, a less intense peak was detected at t_R = 1,61 min, with *m/z* 182.0309 Da, which corresponds to the molecular ion $[Met + 2O + H]^+$, that is the incorporation of two atoms of oxygen to Met, coincident with methionine sulfone ($MetO_2$).

In O_2-free irradiated solutions, the corresponding dihydro-reduced pterin was detected (H_2Mep) at t_R = 4,56, with a mass at 180.1011, identical to the commercial H_2Mep. No methionine oxidation products could be detected, although considering previous reports on both photosensitized degradation and enzymatic fermentation of

Met, $Met^{\bullet+}$ undergoes decarboxylation followed by a deamination to yield 3-(methylthio)propanal or methional (Scheme 3).(9,41)

CONCLUSIONS

The photosensitized oxidation of methionine (Met) by pterin (Ptr) and 6-methylpterin (Mep**)** was studied, both in aerated and deaerated aqueous solutions. Despite Met reacts with singlet oxygen ($^1O_2$, photosensitized type II mechanism), the mechanism of photosensitization takes place mainly by electron transfer mechanism (type I) even in the presence of O_2.

Summarizing, the process begins with the transfer of an electron from Met to the triplet excited state of the photosensitizer (Ptr or Mep), to yield the corresponding pair of radicals, Met radical cation ($Met^{\bullet+}$) and pteridine radical anion ($Ptr^{\bullet-}$ or $Mep^{\bullet-}$). The products of the amino acid are different depending on the availability of O_2. In the presence of O_2, $Met^{\bullet+}$ incorporates one or two atoms of oxygen to yield methionine sulfoxide (MetO) and methionine sulfone ($MetO_2$), and the pteridine radical anion ($Ptr^{\bullet-}$ or $Mep^{\bullet-}$) reacts with O_2 to recover the photosensitizer and generates superoxide anion ($O_2^{\bullet-}$). In the absence of O_2, pteridine radical anion is reduced to the corresponding dihydroderivative (H_2Ptr or H_2Mep) by a radical formed after decarboxilation of $Met^{\bullet+}$**.**

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Figure and Scheme Captions

Figure 1. Molecular structure of pterin (Ptr), 6-methylpterin (Mep) and methionine (Met), and their corresponding absorption spectra in air-equilibrated aqueous solutions (pH = 5.5). Solid line: acid form of Ptr; dotted line: acid form of Mep; dashed-dotted lines: Met.

Figure 2. Concentration evolution of (a) Ptr and (b) Met during irradiation of aqueous solution, equilibrated in air or argon, containing Met and Ptr ($[Met]_0$ = 600 µM, $[Ptr]_0$ = 100 µM, λ_{IR} = 350 nm, pH = 5.5). Errors on individual experimental points are ~ ± 4 µM.

Figure 3. UV-A irradiation of air-equilibrated solutions ($[Met]_0$ = 800 µM, $[Mep]_0$ = 120 µM, λ_{IR} = 350 nm, pH = 5.5) (a) Concentration of Met, Mep and H_2O_2 (inset) as function of irradiation time (errors on individual experimental points are ~ ± 4 µM). (b) Chromatograms obtained in HPLC, analysis at 220 nm, from irradiated solutions containing Met and Mep, without (solid black line) and with (solid red line) the additions of commercial MetO. Upper inset: evolution of MetO concentration; lower inset: absorption spectra at t_R =3.6 min (black line) and absorption spectra of commercial MetO (red line).

Figure 4. UV-A irradiation of Ar-equilibrated solutions ($[Met]_0$ = 610 µM, $[Mep]_0$ = 120 µM, λ_{IR} = 350 nm, pH = 5.5). Chromatograms obtained in HPLC, analysis at 330

nm from irradiated solutions containing Met and Mep, without (solid black line) and with (dash red line) the addition of commercial H_2Mep. Insets: (a): concentration evolution of Met, Mep and H_2Mep during irradiation time (errors on individual experimental points are ~ ± 4 μM); (b): absorption spectra of commercial H_2Mep; (c): absorption spectra of the peak at t_R =16,1 min.

Figure 5. Concentration evolution of Met, Mep and photoproducts during irradiation of air- and Ar-equilibrated aqueous solutions, containing Met and Mep in the absence and in the presence of KI ($[Met]_0$ = 600 μM, $[Mep]_0$ = 100 μM, λ_{IR} = 350 nm, pH = 5.5). Errors on individual experimental points are ~ ± 4 μM.

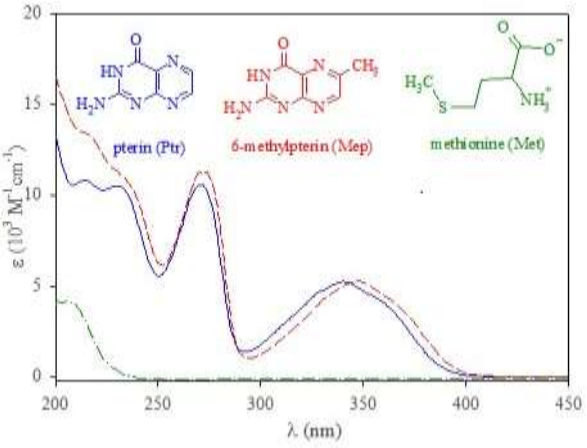
Figure 6. Concentration evolution of Met, Mep and photoproduct MetO during irradiation of D_2O and H_2O aerated solutions containing Met and Mep (λ_{IR} = 350 nm, pH = 5.5. ($[Met]_0$ ~ 700 μM; $[Mep]_0$ = 120 μM. Errors on individual experimental points are ~ ± 4 μM.

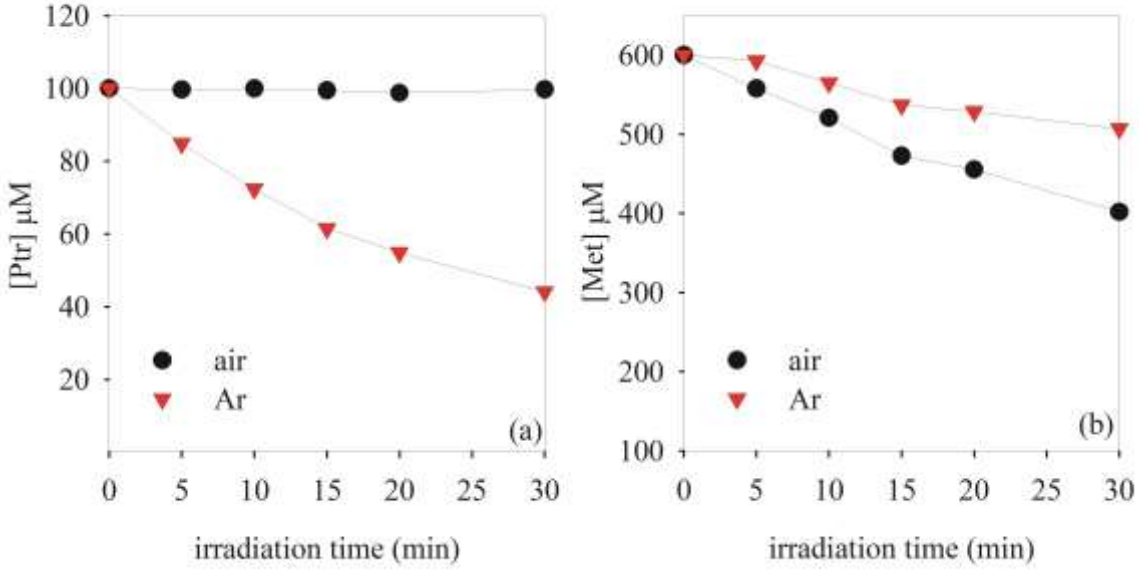
Figure 7. Concentration evolution of Met, Mep and photoproduct MetO during irradiation of aerated aqueous solutions containing Met and Mep (λ_{IR} = 350 nm; pH = 5.5; $[Met]_0$ ~ 670 μM; $[Mep]_0$ = 110 μM, in the absence and in the presence of SOD (50 U/ml). Errors on individual experimental points are ~ ± 4 μM.

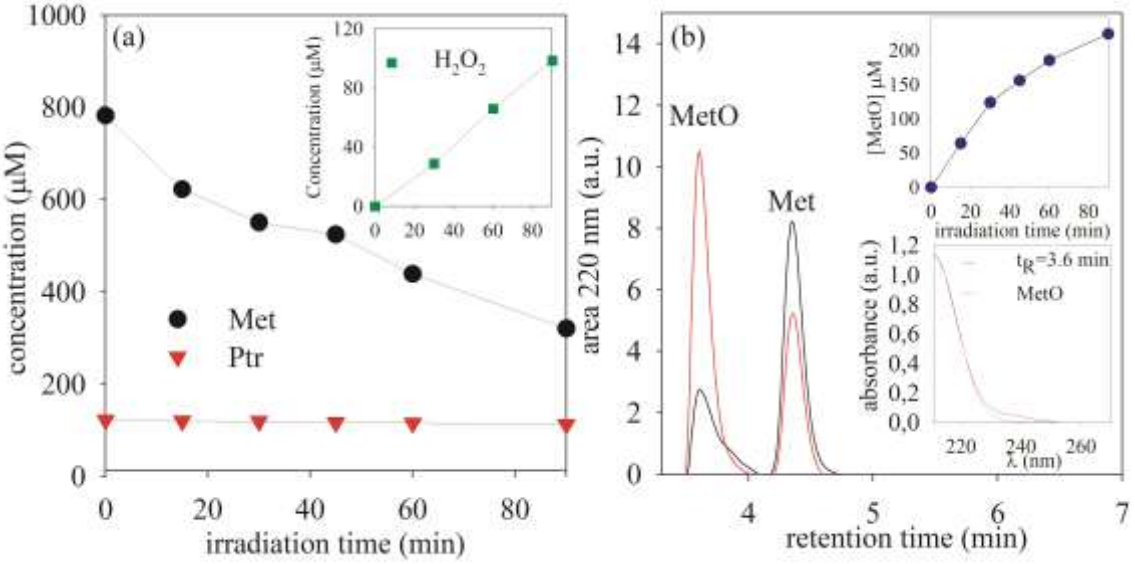
Scheme 1: Mechanism of Met oxidation

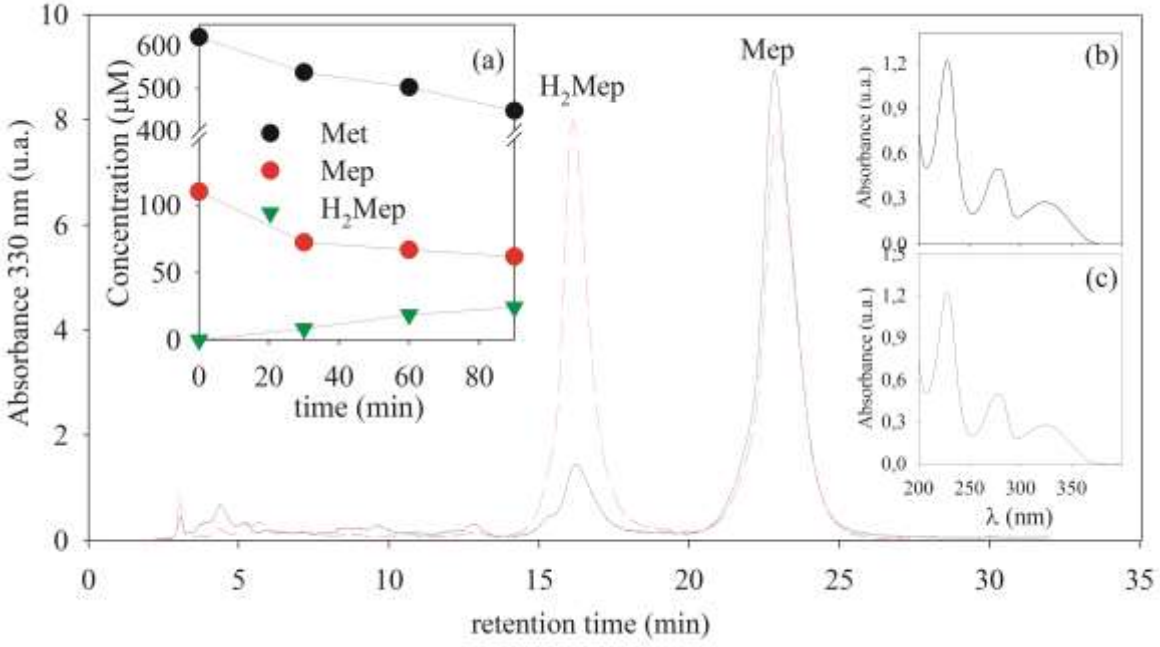
Scheme 2: Proposed mechanism for Ptr-photosensitized Met oxidation

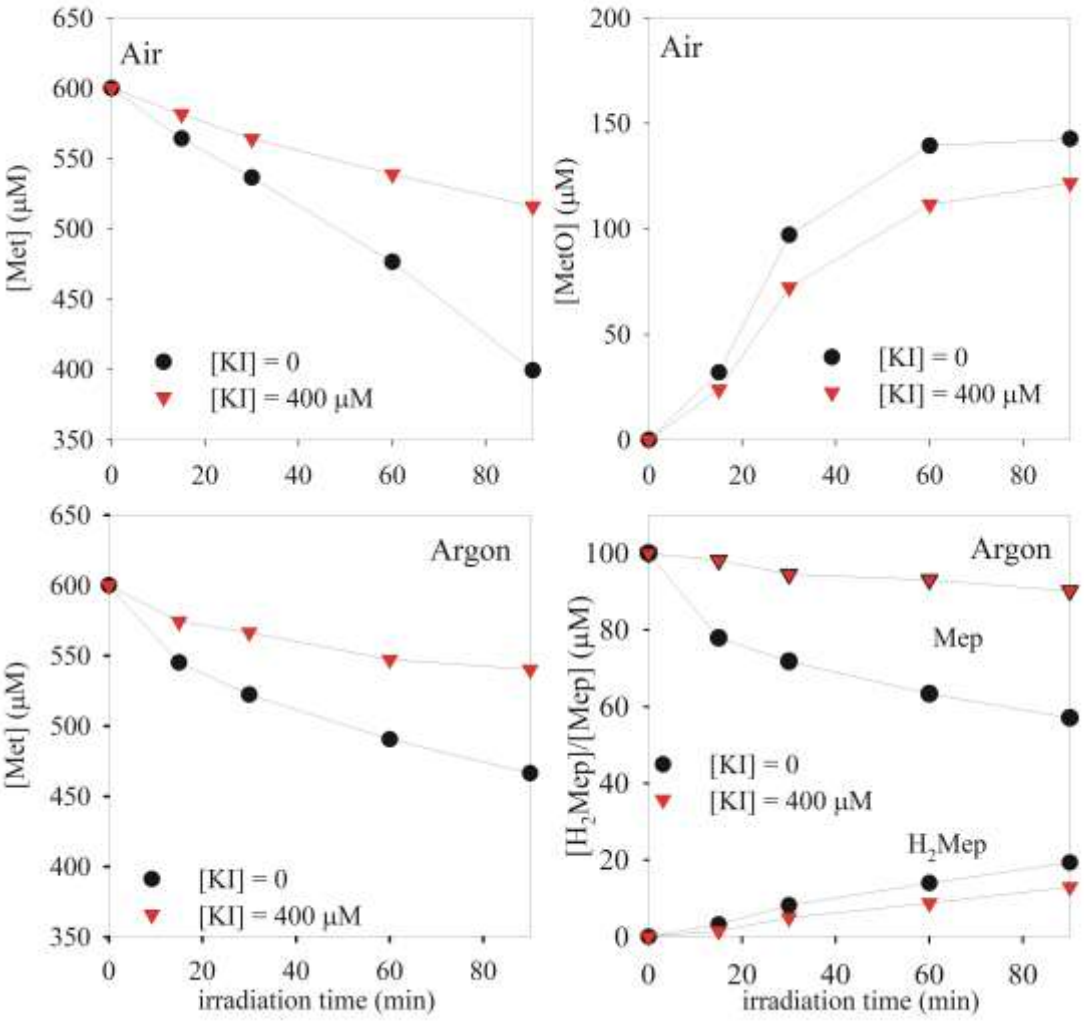
Scheme 3: Proposed mechanism of Met degradation in O_2-free aqueous solutions (according to the mechanism proposed in Ref (9))

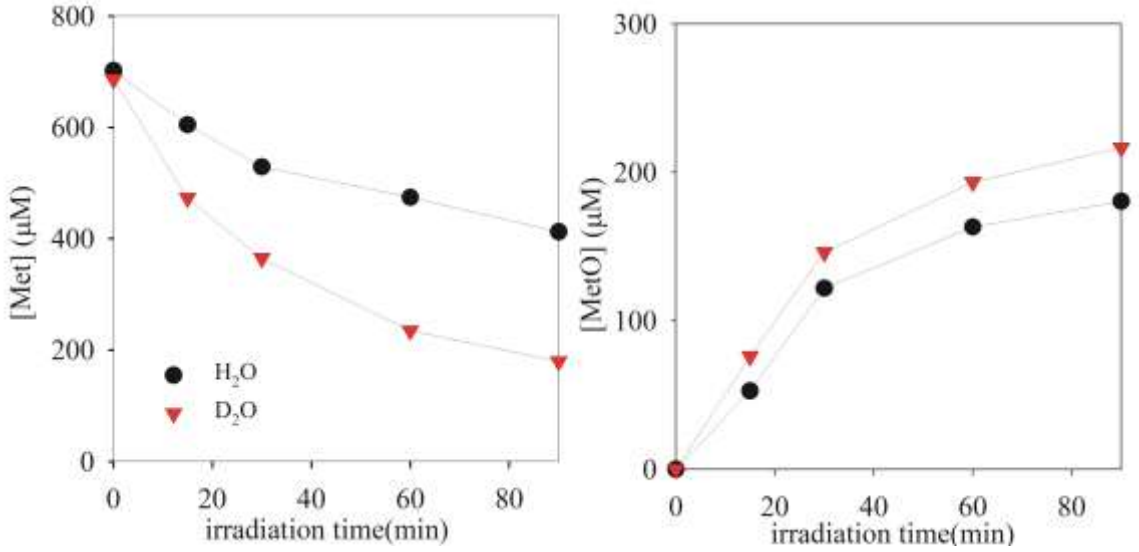


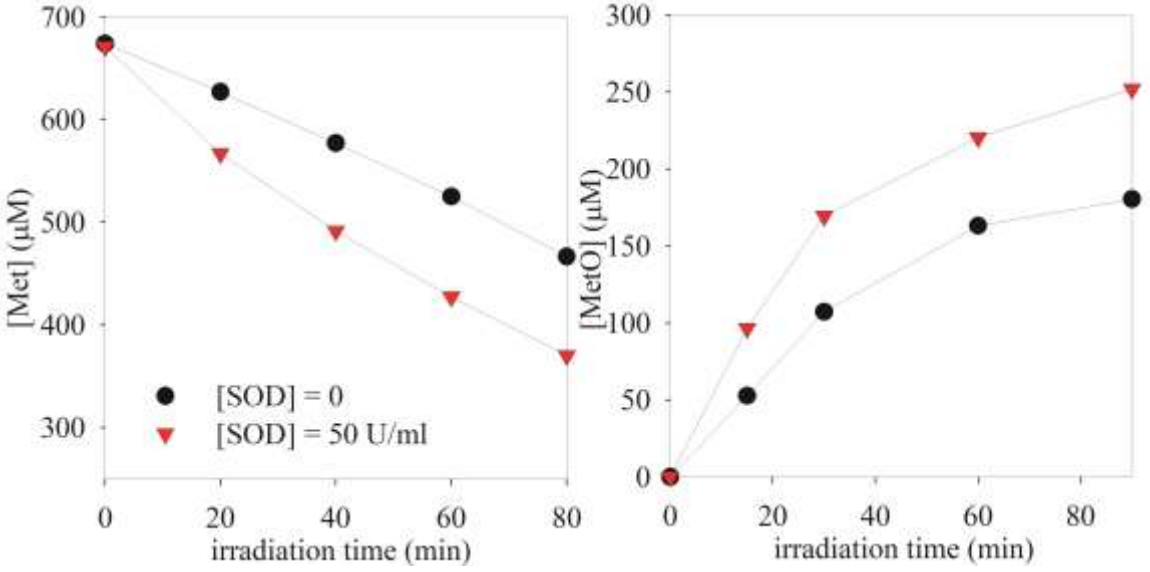


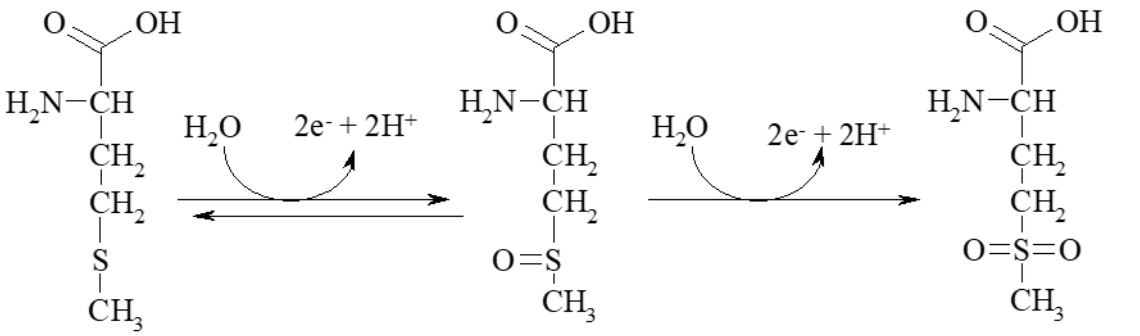












methionine (Met) | methionine sulfoxide (MetO) | methionine sulfone ($MetO_2$)

$$Ptr \xrightarrow{h\nu} {}^1Ptr^* \quad (1)$$

$$^1Ptr^* \xrightarrow{ISC} {}^3Ptr^* \quad (2)$$

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

$$^3Ptr^* + Met \longrightarrow Ptr^{\bullet-} + Met^{\bullet+} \quad (4)$$

$$Ptr^{\bullet-} + H^+ \longrightarrow PtrH^{\bullet} \quad (5)$$

Anaerobic condition:

$$PtrH^{\bullet} + Met^{\bullet+} \longrightarrow PtrH_2 + Met(ox) \quad (6)$$

Aerobic condition:

$$^3Ptr^* + O_2 \longrightarrow Ptr + {}^1O_2 \quad (7)$$

$$Ptr^{\bullet-} + {}^3O_2 \longrightarrow Ptr + O_2^{\bullet-} \quad (8)$$

$$O_2^{\bullet-} + HO_2^{\bullet} \longrightarrow H_2O_2 + O_2 \quad (9)$$

$$Met^{\bullet+} + O_2^{\bullet-} \longrightarrow Met + O_2 \quad (10)$$

$$Met + {}^1O_2 \longrightarrow Met(ox) \quad (11)$$

$$Met^{\bullet+} \xrightarrow{O_2^{\bullet-}/\,H_2O} Met(ox) \quad (12)$$