Radiation Chemical Studies of Methionine in Aqueous Solution: Understanding the Role of Molecular Oxygen

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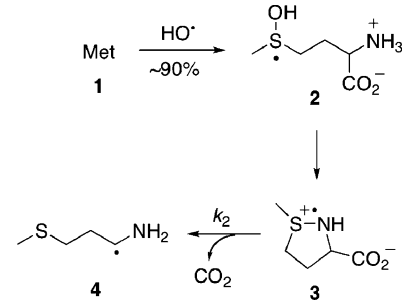
The oxidation of methionine is an important reaction in the biological milieu. Despite a few decades of intense studies, several fundamental aspects remain to be defined. We have investigated in detail the γ-radiolysis of free methionine in the absence and presence of molecular oxygen followed by product characterization and quantification. The primary site of attack by HO• radicals and H• atoms is the sulfur atom of methionine. We have disclosed that HO• radicals do not oxidize methionine to the corresponding sulfoxide in either the presence or the absence of oxygen; the oxidizing species is H_2O_2 derived either from the radiolysis of water or from the disproportionation of the byproduct $O_2^{\bullet-}$. 3-Methylthiopropionaldehyde is the major product of HO• radical attack in the presence of molecular oxygen. Together with the direct oxidation at sulfur as the major product, the potential of H• atoms is also proven to be highly specific for sulfur atom attack under anoxic and aerobic conditions. The major products derived from the H• atoms attack are found to be α-aminobutyric acid or homoserine, in the absence or presence of oxygen, respectively. All together, these results help clarify the fate of methionine related to a biological environment and offer a molecular basis for envisaging other possible pathways of *in vivo* degradation as well as other markers.

Introduction

One of the most important damages in proteins by reactive oxygen species (ROS) is the oxidation of methionine (Met, **1**) residues (*1, 2*). The major oxidation products are the two epimeric forms of sulfoxide (i.e., *R* and *S* epimers) that can be repaired enzymatically by methionine sulfoxide reductase (Mrs) (*3, 4*). For each epimeric residue, a specific enzyme exists (MrsA and MrsB for the *S* and *R* isomer, respectively). For this reason, Met residues in proteins are suggested to act as an antioxidant pool, and several studies support this vision (*5, 6*). The oxidation of Met occurs either by nonradical oxidants (like H_2O_2, HOONO, or HOCl) or by radical species (like HO•, $CO_3^{\bullet-}$, or $N_3^{\bullet}$) (*7–11*). Although the nonradical oxidation is site-specific with the formation of methionine sulfoxide, Met(O), the radical-based oxidation is believed to be more complex (*12*).

The reaction of HO• radicals with Met has been the subject of several investigations. Asmus and co-workers in a series of radiation chemical studies in the eighties disclosed the reaction mechanism (*13–15*). The initial step ($k_1 = 2.3 \times 10^{10}$ M^{-1} s^{-1}) is a competition process between addition of HO• radical to the sulfur atom (Scheme 1) and hydrogen abstraction. The hydroxyl adducts **2** immediately coordinate with nitrogen in a three-electron bond to yield species **3**. The optical spectra of this intermediate has been recorded and found to decay ($k_2 = 3.6 \times 10^6$ s^{-1}) directly into CO_2 and α-aminoalkyl radical (**4**). It is worth mentioning that the radiation chemical yield of CO_2 was found to be 0.49 ± 0.2 μmol J^{-1} in the pH range 6−8, which accounts for about 90% of the HO• radical attack to the sulfur atom (*13*). Although the direct detection of radical **2** by time-resolved spectroscopy is missing, the analogous sulfuranyl radical has been observed in aqueous solutions by replacing Met with $CH_3SCH_2C(O)OMe$ or CH_3SCH_3 and found to react with oxygen with a rate constant of 2×10^8 M^{-1} s^{-1} (*16–18*).

Scheme 1. Mechanism of Met (1) Attack by HO• Radicals in Neutral Solutions (*13–15*)



Although aliphatic sulfides react with HO• radicals in the presence of molecular oxygen affording the corresponding sulfoxides, it is not clear whether the analogous reaction of Met also affords the sulfoxide. Early works on radiolysis of Met showed the formation of sulfoxide among other products under deareated conditions, and more recently, the formation of sulfoxide in the radiolytic study of model peptides under aerobic conditions has been attributed to HO• radical attack (*19, 20*). Several reviews clearly indicate or imply that oxidation of Met occurs by HO• radicals (*7–11*). Is it possible that radical **2** in Scheme 1 be trapped by oxygen prior to the unimolecular rearrangement? On the basis of the above-mentioned rate constants, the answer is no. If this is true, how does the oxidation of Met by HO• radicals occur? It has become evident that the reaction of HO• radicals with Met in the absence or in the presence of molecular oxygen is not yet fully understood. In

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this paper, we will show that HO• radicals do not oxidize Met to Met(O) in either the absence or the presence of oxygen.

In the radiolysis of water, together with radical (HO•) and nonradical oxidants (H_2O_2), primary reducing species like hydrated electrons (e_{aq}^-) and H• atoms are produced. Reductive radical stress has been less widely investigated than oxidative radical stress (*21*). The reaction of these reducing species with sulfur-containing residues in peptides and proteins has been studied in connection with biomimetic chemistry on tandem protein/lipid damages under reductive radical stress (*22–24*). In this paper, the reaction of H• atoms with Met ($k = 1.1 \times 10^9$ M^{-1} s^{-1}) will take part of the investigation, whereas the reaction of e_{aq}^- with Met, which takes place with a much lower rate constant ($k = 4.5 \times 10^7$ M^{-1} s^{-1}), will not be considered (*13*). Therefore, the main subject of this article is concerned with the identification and quantification of final products upon the reaction of HO• radicals or H• atoms (generated by γ-radiolysis of water) on Met in the absence or presence of molecular oxygen.

Materials and Methods

Materials. The following commercially available starting materials were obtained from Sigma-Aldrich Co. and used as received: Met, Met(O), α-aminobutyric acid (Aba), 3-methylthiopropylamine, 3-methylthiopropanaldehyde, and homoserine. Solvents were purchased from Merck (HPLC grade) and used without further purification. Water was purified with a Millipore system. All aqueous amino acid solutions were prepared immediately before use.

Continuous Radiolyses. Irradiations were performed at room temperature (22 ± 2 °C) using a ^{60}Co-Gammacell at different dose rates. The exact absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.61$ μmol J^{-1} (*25*). Mixtures of gases were obtained by an appropriate mixing, controlled by flow meter. The gas stream was obtained by a line connected with a needle inserted in the vessel, and the flow was adjusted to get a continuous bubbling during irradiation. Two hundred fifty milliliters of an Ar-purged aqueous solution of Met (the standard concentration of experiments was about 10 mM, pH 5.80) was saturated with the desired gas or gas mixture prior to γ-irradiation. The pH was registered for the solution at the final irradiation dose. HPLC analyses were recorded on an Agilent 1100 Liquid Chromatograph, equipped with a quaternary pump delivery system, a column thermostat, and a variable-wavelength detector. RP_{18} 5 μm columns were used as specified in each detection method.

OPA Derivatization of Irradiated Samples and HPLC Analysis. Aliquots of the irradiated Met solution were withdrawn at the specified doses for immediate conversion to the OPA (orthophthaldialdehyde) derivatives by adapting some reported procedures (*26, 27*). One hundred microliters of the sample was diluted in 250 μL of borate solution (0.4 M H_3BO_3, pH 10.2 adjusted with 4 M NaOH), 50 μL of the freshly prepared OPA reagent (10 mg/mL OPA and 10 mg/mL 2-mercaptoethanol) was added, and the solution was vortexed for 2 min and finally diluted with 320 μL of 1.5% v/v concentrated H_3PO_4. After derivatization, 20 μL of the reaction mixture was injected for HPLC analysis. A GraceSmart RP 18 5 μm column (150 mm × 4.6 mm) was used at 40 °C, with detection at $\lambda = 338$ nm. Mobile phase A was 10 mM NaH_2PO_4, adjusted to pH 7.5 with 4 M NaOH, while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v). The separation was obtained at a flow rate of 1 mL/min with the following gradient program: 0.5 min at 5% B, a 16.0 min increase of eluent B to 47% followed by a 21.0 min step increase of eluent B to 100%, then washing at 100% B and equilibration at 5% B. The total time was 25 min.

Recognition and Quantification of 3-(Methylthio)-propionaldehyde. Ten millimolar aqueous solutions of Met were irradiated for 500 Gy while flushing a very controlled stream of the appropriate gas or gas mixture. The aldehyde was recognized by NMR spectroscopy and 2,4-dinitrophenylhydrazine (2,4-DNPH) derivatization followed by HPLC analysis. For NMR analysis, the irradiated solution was extracted with deuterated chloroform, and the proton and carbon NMR spectra were recorded immediately after (see the Supporting Information). For recognition and quantification of the 2,4-dinitrophenylhydrazone of the aldehyde, a published protocol was employed and adapted to our system (*28*). The comparison was obtained from the same derivatization procedure performed with the commercially available compound followed by HPLC analysis and spike experiments.

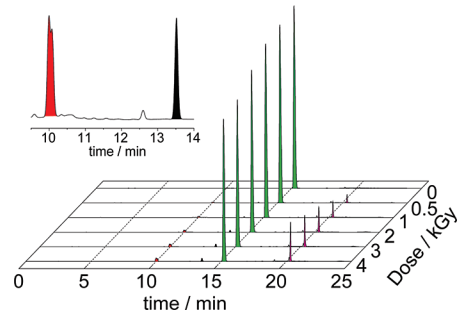


Figure 1. HPLC analyses of γ-irradiation of N_2O-saturated solutions of 10.16 mM Met (**1**) at natural pH (dose rate of ~6.5 Gy min^{-1}) after OPA derivatization of the amino compounds. The consumption of **1** (green peaks) led to the formation of three products, the major one being the amine **8** (violet peak at 20 min retention time). Inset: expansion of the chromatogram between 9.5 and 14 min of the 4 kGy irradiated sample; the red and black peaks correspond to Met(O) (**7**) and Aba (**6**), and the splitting of the red peak is due to the *R* and *S* epimers of sulfoxide.

Results and Discussion

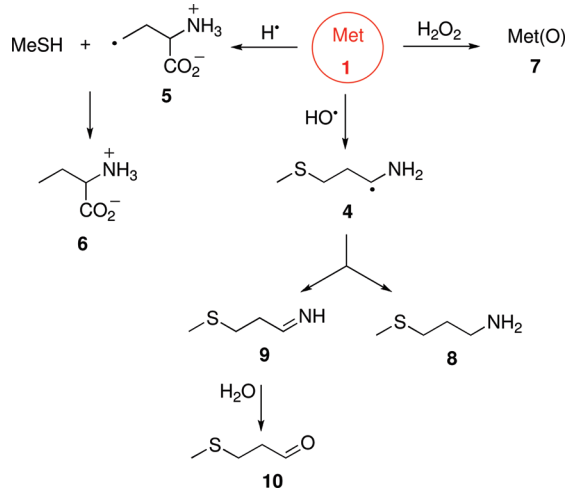
Reaction of HO• Radical and H• Atom with Met in Deaerated Aqueous Solutions. Radiolysis of neutral water leads to the reactive species e_{aq}^-, HO•, and H• together with H^+ and H_2O_2 as shown in eq 1. The values in parentheses represent the radiation chemical yields (G) in units of μmol J^{-1}. In N_2O-saturated solution (~0.02 M of N_2O), e_{aq}^- are efficiently transformed into HO• radicals via eq 2 ($k_2 = 9.1 \times 10^9$ M^{-1} s^{-1}), affording $G(HO^•) = 0.55$ μmol J^{-1}; that is, HO• radicals and H• atoms account for 90 and 10%, respectively, of the reactive species (*29, 30*).

$$H_2O \xrightarrow{\gamma} e_{aq}^- (0.27), HO^• (0.28), H^• (0.06), H^+ (0.27), H_2O_2 (0.07) \quad (1)$$

$$e_{aq}^- + N_2O + H_2O \rightarrow HO^• + N_2 + HO^- \quad (2)$$

Initially, γ-radiolysis experiments coupled with product studies were carried out under deaerated conditions. N_2O-saturated solutions containing Met (10.16 mM) at natural pH were irradiated under stationary state conditions with a dose rate of ca. 6.5 Gy min^{-1} followed by OPA derivatization (*26, 27*) of amino compounds and HPLC analysis. The consumption of **1** (green peaks, Figure 1) led to the formation of three new products, a major one and two minor ones. The two minor products that elute from the column before Met were identified as Aba (**6**) and Met(O) (**7**) by comparison with the corresponding OPA derivatives from the commercially available compounds (inset of Figure 1, black and red peaks, respectively). On the other hand, the major product was isolated from the crude reaction mixture and characterized by spectroscopic means to be the amine **8** (see Scheme 2). OPA derivatization of the commercially available amine **8** and HPLC analysis confirmed

Scheme 2. Reaction Mechanism for the γ-Irradiation of N_2O-Saturated Solution of Met (1)[a]



[a] For details of radical **4** formation, see Scheme 1.

the identity of the product at 20 min of retention time (Figure 1). As expected, the concentration of **1** decreased as the irradiation dose increased in the range of 0−4 kGy, whereas the concentrations of **6**, **7**, and **8** increased. After 4 kGy of irradiation, 27% of Met was consumed. The product yield (mol kg^{-1}) divided by the absorbed dose (1 Gy = 1 J kg^{-1}) gave the radiation chemical yield. Analysis of the data in terms of radiation chemical yield (G) gave $G(-\mathbf{1}) = 0.70$, $G(\mathbf{6}) = 0.03$, $G(\mathbf{7}) = 0.07$, and $G(\mathbf{8}) = 0.29$ μmol J^{-1} when the lines are extrapolated to zero dose (Figure 2).

On the basis of the known rate constants for the reactions of H• atom and HO• radical with Met (see above), the following observations are underlined (cf. Scheme 2): First, taking into account that $G(HO^•) + G(H^•) + G(H_2O_2) = 0.68$ μmol J^{-1} is similar to $G(-\mathbf{1}) = 0.70$, this suggests that all HO• radicals, H• atoms, and H_2O_2 react with **1**; second, the equality $G(H_2O_2) = G(\mathbf{7}) = 0.07$ μmol J^{-1} indicates that H_2O_2 reacts quantitatively with Met to give the corresponding sulfoxide (see the next section); third, we note that $G(\mathbf{6}) = 0.03$ is the half of $G(H^•) = 0.06$ and $G(\mathbf{8}) = 0.29$ is nearly half of $G(HO^•) = 0.55$, attributing the lower value of $G(\mathbf{8})$ to the radical−radical termination of radical **4** to give **8** and **9**, the latter promptly hydrolyzed to aldehyde **10**. The presence of aldehyde **10** was confirmed in the reaction mixture by chloroform-D extraction and analyses by ^{1}H and ^{13}C NMR and GC/MS in comparison with the authentic sample. Moreover, by a sequence of extraction from the crude mixture, 2,4-DNPH derivatization (*28*), and HPLC analysis, the formation of aldehyde **10** was quantified, and the radiation chemical yield, $G(\mathbf{10}) = 0.26$, was obtained. Therefore, the sum of various identified products accounts for ∼93% of the consumed Met.

Further support of the reaction path of HO• radical attack depicted in Schemes 1 and 2 was obtained from an independent experiment, where the reaction is repeated in the presence of 0.1 mM thiol to trap the intermediate radical **4** [$k \geq 2 \times 10^9$ M^{-1} s^{-1} has been estimated for the reaction of radical **4** with cysteine (*13*)]. In particular, 0.1 mM β-mercaptoethanol was added at 0 and 0.5 kGy of irradiation, and the crude mixture was analyzed after 0.5 and 1 kGy by the protocol reported above. Analysis of the data in terms of radiation chemical yield (G) gave $G(-\mathbf{1}) = 0.68$, $G(\mathbf{6}) = 0.03$, $G(\mathbf{7}) = 0.05$, and $G(\mathbf{8}) = 0.50$ μmol J^{-1}. It is gratifying to see that the formation of amine **8** corresponds to about 90% percentage of the HO• radical attack at the sulfur atom, which is the same resulting from the radiation chemical yield of CO_2 (*13*).

In summary, in the absence of molecular oxygen, (i) the addition of HO• radicals to the sulfur atom of Met (the major path) does not afford Met(O) but exclusively the radical **4**, whose fate depends on the reaction conditions, and (ii) the main reaction of H• atoms with Met is the homolytic substitution at sulfur with the formation of Aba (**6**).

Reaction of H_2O_2 and Superoxide with Met in Oxygenated Aqueous Solutions. From the previous section, it is noticeable that the amount of H_2O_2 produced from the radiolysis of water corresponds exactly to the formation of Met(O). It is well-known that H_2O_2 oxidizes Met to Met(O), and a rate constant of $\sim 1 \times 10^{-2}$ M^{-1} s^{-1} has been reported in phosphate buffer at pH 6−8 (*31*), which fits very well with our findings. Indeed, the half-life for the oxidation of 10 mM Met is estimated to be $t_{1/2} \approx 2$ h, and our irradiation time is ∼ 10 h (dose rate ∼ 6.5 Gy min^{-1} for a total dose of 4 kGy, cf. Figure 1). To simulate the steady-state formation of H_2O_2, 500 μL of 1 M aqueous H_2O_2 was introduced by syringe pump (flow rate of 100 μL/h) to an aqueous solution of Met (250 mL, 10.41 mM, pH 5.8) under stirring for 5 h (H_2O_2 final concentration = 2 mM). Samples were withdrawn each hour, followed by OPA derivatization of amino compounds and HPLC analysis. The oxidation of Met to Met(O) occurs readily and quantitatively (see the Supporting Information).

Superoxide radical anions ($O_2^{-•}$) are generated as the exclusive reactant upon O_2-saturated solutions containing >0.1 M HCOONa at pH ∼ 7 (*32*). Indeed, in O_2-saturated solution (1.34 mM of O_2), e_{aq}^- and H• are efficiently converted into $O_2^{-•}$ via eqs 3 ($k_3 = 1.9 \times 10^{10}$ M^{-1} s^{-1}) and 4 ($k_4 = 2 \times 10^{10}$ M^{-1} s^{-1}), the pK_a(HOO•) being 4.8 (eq 5). On the other hand, HO• and H• are transformed first to $CO_2^{-•}$ via eqs 6 ($k_6 = 3.5 \times 10^9$ M^{-1} s^{-1}) and 7 ($k_7 = 1.3 \times 10^8$ M^{-1} s^{-1}) and then to $O_2^{-•}$ via eq 8 ($k_3 = 2.4 \times 10^9$ M^{-1} s^{-1}).

$$e_{aq}^- + O_2 \rightarrow O_2^{-•} \quad (3)$$

$$H^• + O_2 \rightarrow HOO^• \quad (4)$$

$$HOO^• \rightleftharpoons O_2^{-•} + H^+ \quad (5)$$

$$HO^• + HCO_2^- \rightarrow CO_2^{-•} + H_2O \quad (6)$$

$$H^• + HCO_2^- \rightarrow CO_2^{-•} + H_2 \quad (7)$$

$$CO_2^{-•} + O_2 \rightarrow CO_2 + O_2^{-•} \quad (8)$$

An O_2-saturated aqueous solution of 0.5 M HCOONa and 10.55 mM Met at pH 7.3 is γ-irradiated, while oxygen is bubbling throughout the reaction time. Aliquots (4 mL) are withdrawn at each specified dose for immediate OPA derivatization and HPLC analysis (Figure 3). The pH registered for the solution at the final dose (4 kGy) is 7.3. The concentrations of Met (**1**) decreased, whereas that of Met(O) (**7**) increased, as the dose increases in the range of 0−4 kGy. Analysis of the data in terms of radiation chemical yields (G) affords $G(-\mathbf{1}) = 0.34$ and $G(\mathbf{4}) = 0.35$ μmol J^{-1} when the lines are extrapolated to zero dose (inset of Figure 3), indicating a quantitative transformation. The analysis of these values is straightforward, taking into account that $G(H_2O_2) = 0.07$ and $G(O_2^{-•}) = 0.61$ μmol J^{-1} and considering that two molecules of $O_2^{-•}$ are needed for each Met oxidation. Reported findings clearly argue against

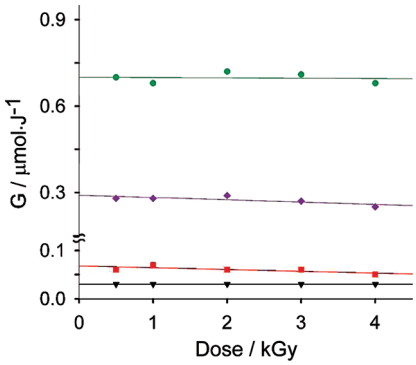


Figure 2. Radiation chemical yields (G) of **1**, **8**, **7**, and **6** vs dose; $G(-\mathbf{1}) = 0.70$, $G(\mathbf{8}) = 0.29$, $G(\mathbf{7}) = 0.07$, and $G(\mathbf{6}) = 0.03$ μmol J^{-1} are obtained when the lines are extrapolated to zero dose.

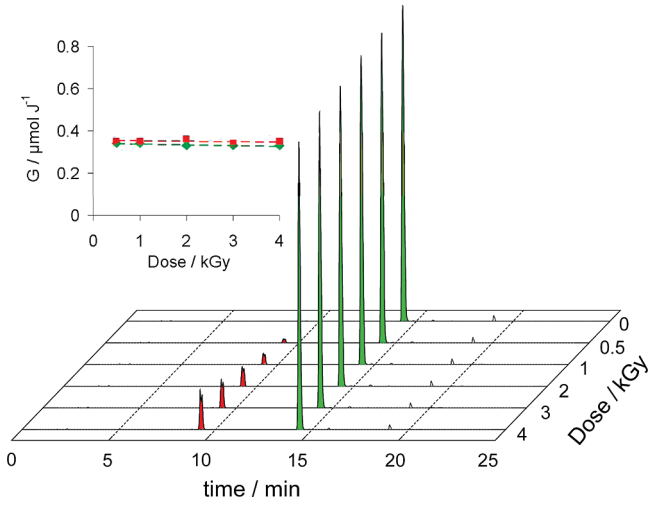


Figure 3. HPLC analyses of irradiated (dose rate of ~6.5 Gy min^{-1}) O_2-saturated solutions of 0.5 M HCOONa and 10.55 mM Met (**1**) at pH 7.3 after OPA derivatization of amino compounds. Inset: Radiation chemical yields (G) of **1** and **7** vs dose; $G(-\mathbf{1}) = 0.34$ and $G(\mathbf{7}) = 0.35$ μmol J^{-1} obtained when the lines are extrapolated to zero dose.

a direct oxidation of Met by superoxide (*33*). On the other hand, the reactivity of $HO_2^{\bullet}/O_2^{-\bullet}$ in aqueous solution is fully understood, and disproportionation is expected to produce H_2O_2 (eq 9) (*32*). Therefore, a total 0.37 μmol J^{-1} radiation chemical yield of H_2O_2 is expected during the experiments, which fits very well with the corresponding values of Met disappearance and Met(O) formation (Figure 3).

$$O_2^{-\bullet} + HO_2^{\bullet} + H_2O \rightarrow H_2O_2 + O_2 + HO^- \qquad (9)$$

Reaction of HO$^{\bullet}$ Radicals with Met in Oxygenated Aqueous Solutions. To investigate the reaction of HO$^{\bullet}$ radicals with Met in the presence of molecular oxygen, three different experimental conditions were investigated. In particular, about 10 mM Met solutions were saturated by N_2O/O_2 (90:10, v/v) or by air (N_2/O_2 = 80:20, v/v) or by pure O_2 during the irradiation.

An air-saturated aqueous solution of 10.43 mM **1** at natural pH (5.8) was irradiated under stationary state conditions with a dose rate of ca. 6.5 Gy min^{-1}, while air is continuously bubbled throughout the reaction time. At different doses, 4 mL aliquots were withdrawn followed immediately by OPA derivatization of amino compounds and HPLC analysis. A pH of 7.8 was registered for the solution at the final dose of 3.5 kGy. The consumption of **1** (green peaks, Figure 4) led to the formation of two new products, which elute at shorter retention times.

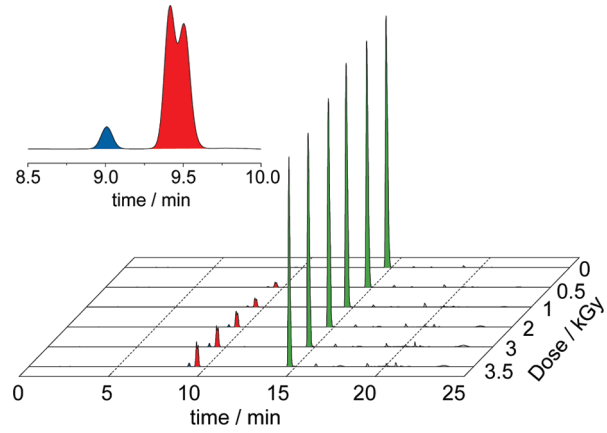


Figure 4. HPLC analyses of γ-irradiated air-saturated solutions of 10.43 mM Met at natural pH (dose rate of ~6.5 Gy min^{-1}) after OPA derivatization of amino compounds. The consumption of Met (green peaks) led to the formation of Met(O) (red peaks) and homoserine (blue peaks). Inset: Expansion of the chromatogram between 8.5−10 min of the 3.5 kGy irradiated sample; the splitting of the red peak is due to the R and S epimers of sulfoxide.

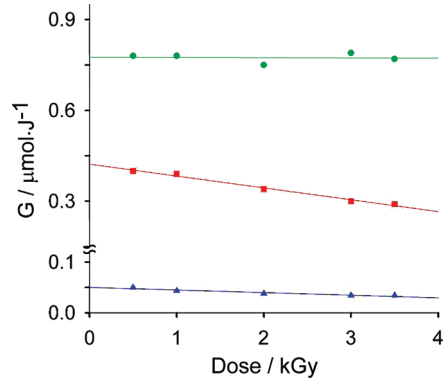


Figure 5. Radiation chemical yields (G) of Met (**1**), Met(O) (**7**), and homoserine (**12**) vs dose obtained from the experiment reported in Figure 4; $G(-\mathbf{1}) = 0.78$, $G(\mathbf{7}) = 0.42$, and $G(\mathbf{12}) = 0.05$ μmol J^{-1} are obtained when the lines are extrapolated to zero dose.

Table 1. Radiation Chemical Yields (G) Extrapolated to Zero Dose from the Irradiation of Met 1 in Oxygenated Aqueous Solutions Under Various Conditions

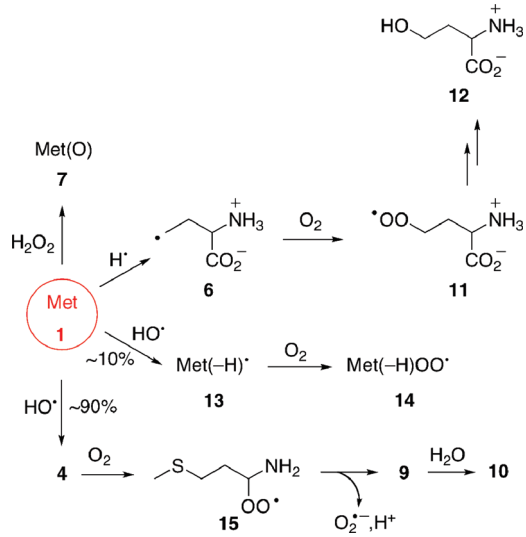
G[a]	N_2O:O_2 = 90:10	N_2:O_2 = 80:20[b]	O_2
$G(-\mathbf{1})$	1.10	0.78	0.79
$G(\mathbf{7})$	0.43	0.42	0.43
$G(\mathbf{12})$	0.034	0.05	0.027
$G(\mathbf{10})$[c]	0.63	0.31	0.33

[a] Units of G in μmol J^{-1}. [b] Air. [c] Radiation chemical yields determined after 0.5 kGy of irradiation by 2,4-DNPH derivatization method (an average of two measurements).

The major (red peak) and minor (blue) products were identified as Met(O) (**4**) and homoserine (**12**) respectively in comparison with the corresponding OPA derivatization of commercially available compounds.

As usual, the data derived from the experiments in Figure 4 are treated in terms of radiation chemical yields (G). Figure 5 shows that linear correlations do exist between G values and dose. By extrapolation of the lines to zero dose, the following values were obtained $G(-\mathbf{1}) = 0.78$, $G(\mathbf{7}) = 0.42$, and $G(\mathbf{12}) = 0.05$ μmol J^{-1}, which are reported in the third column of Table 1. By replacing air with pure oxygen or N_2O/O_2 (90:10, v/v) mixture, analogous experiments were performed, and the details of these experiments are reported in the Supporting Information. Also, in these cases, the consumption of Met led

Scheme 3. Reaction Mechanism for the γ-Irradiation of Oxygenated Solution of Met (1)



to the formation of Met(O) and homoserine, and the summary of the corresponding radiation chemical yields is also reported in Table 1.

The presence of aldehyde **10** in the reaction mixtures was considered next. In all cases, after the reaction is over, the presence of aldehyde **10** was identified by chloroform-D extraction followed by ^{1}H and ^{13}C NMR and GC/MS analyses and compared with the authentic sample. However, the quantification is not straightforward because during the continuous bubbling of gas throughout the reaction time, aldehyde may also be partially removed by the gas stream. For example, after 4 kGy irradiation (time ∼ 10 h) of N_2O/O_2 (90:10)-saturated 10 mM Met sample, followed by the above-described 2,4-DNPH derivatization and HPLC analysis, we were able to obtain a low limit value, $G(\mathbf{10}) \geq 0.32$. To get more favorable conditions for evaluating the formation of aldehyde **10**, new samples were irradiated only for 0.5 kGy followed by the 2,4-DNPH derivatization and HPLC analysis. The radiation chemical yields reported in Table 1 (last line) are the average of two runs. It is gratifying to see that the disappearance of Met equals the sum of the identified products including the aldehyde, viz., for N_2O/O_2 (90:10), $G(-\mathbf{1}) = 1.10$ vs $G(\mathbf{7}) + G(\mathbf{12}) + G(\mathbf{10}) = 1.09$ μmol J^{-1}, and for air, $G(-\mathbf{1}) = 0.78$ vs $G(\mathbf{7}) + G(\mathbf{12}) + G(\mathbf{10}) = 0.78$.

Table 1 shows that large amounts, and nearly the same concentration of Met(O) is formed in all cases. Interestingly, the results of air- or O_2-saturated solutions are similar, whereas much larger amounts of Met are consumed in the N_2O/O_2 (90:10) experiment with the formation of a larger amount of aldehyde **10**. In other words, in the experiments with air or O_2, the $G(-\mathbf{1})$ is about 15% larger than the sum of primary species [viz. $G(e_{aq}^-) + G(HO^\bullet) + G(H^\bullet) + G(H_2O_2) = 0.68$ μmol J^{-1}], whereas with N_2O/O_2 (90:10), it is about 62% higher. The main difference between N_2O/O_2 and air or O_2 is that in the former the e_{aq}^- are efficiently transformed to HO$^\bullet$ radical (eq 2), whereas in the latter two conditions, the e_{aq}^- are trapped by oxygen to give $O_2^{\bullet-}$ (eq 3).

The mechanism conceived from the results of Table 1 is depicted in Scheme 3 for the following reasons:

(i) In 1 atm of N_2O/O_2 (90:10, v/v), e_{aq}^- are efficiently converted into HO$^\bullet$ (eq 2), whereas 20% of H$^\bullet$ are transformed into $O_2^{-\bullet}$ (eqs 4 and 5) and 80% react with Met. As we mentioned above, the reaction Met + HO$^\bullet$ is a competition process between addition of HO$^\bullet$ radical to the sulfur atom (∼90%) and hydrogen abstraction (∼10%). Therefore, $G(HO^\bullet) \approx 0.50$ and $G(HO^\bullet) \approx 0.05$ are associated with addition and hydrogen abstraction. We notice that the radiation chemical yield from disappearance of Met, $G(-\mathbf{1}) = 1.10$, matches very well with that from the sum of primary reactions of HO$^\bullet$ radicals, H$^\bullet$ atoms, and H_2O_2 with **1** [viz. $G(HO^\bullet) + G(H^\bullet) + G(H_2O_2) = 0.68$ μmol J^{-1}], plus secondary reactions derived from peroxyl radicals with **1** (viz. $G(\mathbf{11}) + G(\mathbf{14}) \approx 0.10$ μmol J^{-1}), plus the amount of H_2O_2 presumably derived from the disproportionation of superoxide (half of aldehyde **10**, viz. $G(H_2O_2) = 0.32$ μmol J^{-1}). Our proposal is in excellent agreement with the available rate constants from pulse radiolysis studies (*13, 16*), which suggest that the formation of radical **4** will be more than 2 orders of magnitude faster than the addition of sulfuranyl radical **2** to molecular oxygen (see the Introduction). We also notice that the amount of aldehyde **10** is higher than the amount of HO$^\bullet$ radicals produced, which suggests that the reaction of peroxyl radicals **11** and **14** with Met contributes to the formation of more aldehyde **10** and presumably of extra $O_2^{-\bullet}$ (*34*).

(ii) In 1 atm of air, e_{aq}^- are efficiently converted into $O_2^{\bullet-}$ (eq 3), whereas ∼30% of H$^\bullet$ are transformed into $O_2^{-\bullet}$ (eqs 4 and 5) and ∼70% react with Met to give homoserine (**12**). $G(HO^\bullet) \approx 0.25$ and $G(HO^\bullet) \approx 0.03$ μmol J^{-1} are associated with addition and hydrogen abstraction. The addition of HO$^\bullet$ radicals to sulfur, $G(HO^\bullet) \approx 0.25$, produces $G(\mathbf{10}) \approx 0.25$ and $G(O_2^{\bullet-}) \approx 0.25$. Peroxyl radical (viz. $G(\mathbf{11}) + G(\mathbf{14}) \approx 0.08$ μmol J^{-1}) also accounts for the extra formation of aldehyde **10** and $O_2^{-\bullet}$ (see above). Therefore, the amount of H_2O_2 derived from the disproportionation of superoxide oxidizes Met to Met(O). Analogous consideration can be made for O_2-saturated experiments, where the only difference is the varied percentage of H$^\bullet$ atom splitting, that is, ∼70% of H$^\bullet$ are transformed into $O_2^{-\bullet}$and ∼30% react with Met to afford homoserine (**12**). It is satisfying to see that going from the air- to oxygen-saturated experiments homoserine concentration is reduced by half.

In summary, in the presence of molecular oxygen, (i) the addition of HO$^\bullet$ radicals to the sulfur atom of Met (the major path) does not afford Met(O) but exclusively aldehyde **10**; (ii) H_2O_2, derived either from radiolysis of water or the disproportionation of superoxide, affords Met(O); and (iii) the reaction of H$^\bullet$ atoms with Met via homolytic substitution at sulfur is still effective, ensuring the formation of homoserine (**12**).

Conclusions

In this paper, we disclosed that HO$^\bullet$ radicals do not oxidize Met to the corresponding sulfoxide in either the absence or the presence of oxygen. H_2O_2 derived either from the γ-radiolysis of water (in the absence or presence of O_2) or from the disproportionation of the byproduct $O_2^{\bullet-}$ (in the presence of O_2) is the oxidizing species. The reaction of H$^\bullet$ atom (a primary species from radiolysis of water) with Met via homolytic substitution at sulfur is effective, ensuring the formation of Aba or homoserine in either the absence or the presence of O_2, respectively. These results help clarify the fate of Met under strictly biologically related conditions and offer molecular basis for envisaging other markers of Met degradation to be evaluated *in vivo*.

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Supporting Information Available: Detailed experimental part on product studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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