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Article

Early Events of Photosensitized Oxidation of Sulfur-Containing Amino Acids Studied by Laser Flash Photolysis and Mass Spectrometry

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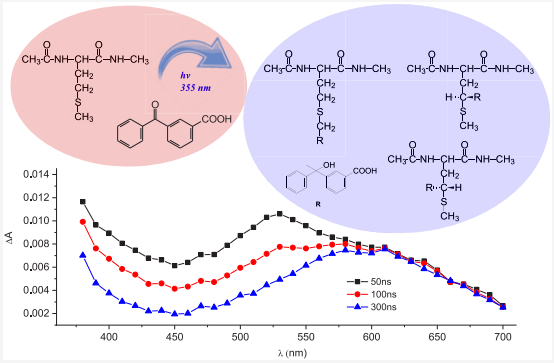
ABSTRACT: The mechanism of photooxidation of methionine (N-Ac-Met-NH-CH_3, **1**) and methyl-cysteine (N-Ac-MeCys-NH-CH_3, **2**) analogues by 3-carboxybenzophenone triplet (3CB*) in neutral aqueous solution was studied using techniques of nanosecond laser flash photolysis and steady-state photolysis. The short-lived transients derived from 3CB and sulfur-containing amino acids were identified, and their quantum yields and kinetics of formation and decay were determined. The stable photoproducts were analyzed using liquid chromatography coupled with high-resolution mass spectrometry. Substantial differences in the mechanisms were found for methionine and *S*-methyl-cysteine analogues for both primary and secondary photoreactions. A new secondary reaction channel (back hydrogen atom transfer from the ketyl radical to the carbon-centered α-thioalkyl radical yielding reactants in the ground states) was suggested. The detailed mechanisms of 3CB* sensitized photooxidation of **1** and **2** are proposed and discussed.



INTRODUCTION

The mechanisms of photosensitized and radiation-induced oxidation of amino acids and peptides have been investigated mainly due to the biological significance of such processes.[1−8] One of the sites primarily attacked by oxidative agents such as short-lived excited states, free radicals, or reactive oxygen species is the thioether moiety of methionine (Met) residues. Met oxidation can cause serious consequences during oxidative stress;[1] however, despite the numerous studies focused on the one-electron oxidation processes of the methionine residue, some aspects of the process still remain unclear or controversial (e.g., the fate of free radicals leading to stable modifications of the amino acids[9,10]). One-electron oxidation of Met-containing peptides and proteins in solution occurs easily, e.g., by using strongly oxidizing hydroxyl radicals ($^{\bullet}OH$) from water radiolysis or through photosensitization using carboxybenzophenone (CB) excited triplets as electron acceptors.[3,6,11] The transients formed in the oxidation of Met-containing peptides by various one-electron oxidants have been well-characterized.[3,6,11−15] The initially formed sulfur radical cation can interact with electron-rich atoms (O, N, or S), yielding two-centered three-electron bonds. It can also irreversibly deprotonate, yielding a carbon-centered, α-(alkylthio)alkylmet-containing radical (αS) as presented in Scheme 1.

Similar to the Met-containing peptides, the mechanisms of photoinduced and radiation-induced oxidation of peptides containing *S*-alkyl-cysteine residues (i.e., *S*-alkylglutathiones) were also studied due to their significant biological role in living organisms (e.g., see refs 14 and 26). Despite many reports devoted to the detailed mechanisms of radical reactions and their transformations in the sensitized photooxidation of Met- and S-Me-Cys peptides, some doubts remain as to their final fates (i.e., the stable oxidation products). Surprisingly, there are only a few reports that combine complementary time-resolved and steady-state techniques in the photoinduced and radiation-induced oxidation of Met-containing peptides.[9,10,16] It is, therefore, rational to use relatively simple model structures, such as the compounds investigated in this paper (see Figure 1), to carry out these complementary time-resolved laser flash photolysis and stationary photochemical irradiations experiments.

In this work, we investigated the mechanism of one-electron, photosensitized oxidation of two synthetic amino acids containing a thioether moiety N-Ac-Met-NH-CH_3, **1**, and N-Ac-MeCys-NH-CH_3, **2** (see Figure 1 for structures) in aqueous

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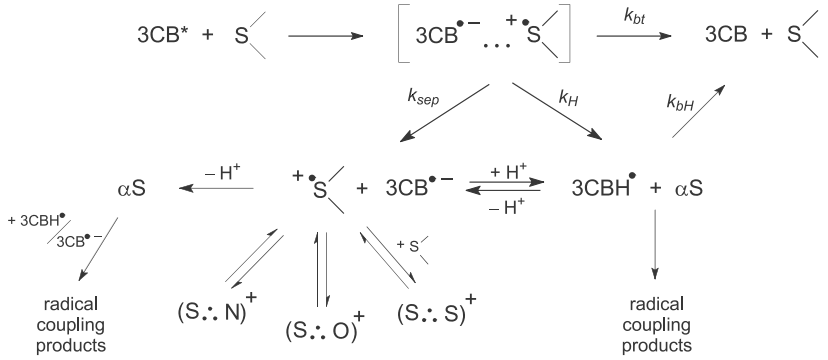
Scheme 1. General Scheme for 3CB Triplet Photosensitized Oxidation of S-Containing Compounds[a]



[a]3CB denotes 3-carboxybenzophenone; 3CBH•, ketyl radical; 3CB•−, radical anion; S<, sulfur-containing compound; αS, carbon-centered α-thioalkyl radical; (S.∴N)+, intramolecular two-centered three-electron bonded species; (S.∴S)+, intermolecular two-centered three-electron bonded species; (S.∴O)+, intramolecular two-centered three-electron bonded species.

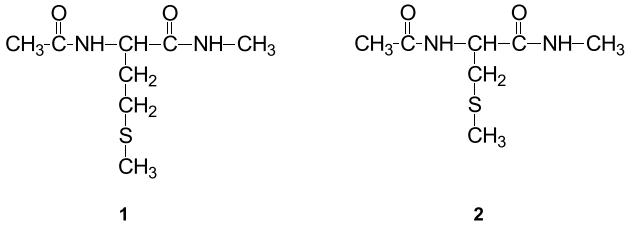


Figure 1. Structures of peptide model compounds used in this work: N-Ac-Met-NH-CH_3 (**1**) and N-Ac-MeCys-NH-CH_3 (**2**).

solutions. The compounds studied in this paper are better models for internal Met and S-MeCys residues in peptides and proteins than previously investigated 3-(methylthio)-propylamine (3-MTPA) and *N*-acetyl-3-(methylthio)-propylamine (3-AcMTPA)[17,18] since they possess the peptide-like bonds on both C and N terminals, ruling out the possibility for decarboxylation and the possibility of proton transfer from the protonated N-terminal group.[4,18,19] Both short-lived transients and the stable products from photosensitized irradiations of aqueous solutions of the studied compounds were identified in the current work showing significant differences between Met and S-MeCys analogues. Time-resolved and steady-state experimental approaches allowed us, based on the model compounds, to suggest the mechanism of one-electron oxidation which is important for unraveling the redox chemistry in sulfur-containing amino acids, peptides, and proteins.

EXPERIMENTAL SECTION

The synthetic procedure, as well as spectral characterization, of S-MeCys and Met-analogues is described in the Supporting Information.

3-Carboxybenzophenone (3CB) was obtained commercially from Sigma-Aldrich as the best available grade and was used as received. The deionized water for the experiments was purified using a commercial system from Millipore, model Simplicity (Billerica, MA, USA).

The laser flash photolysis (LFP) setup used in this work has been described in detail elsewhere.[13] Samples for LFP experiments were excited using 355 nm, the third harmonic of a Nd:YAG laser (Spectra Physics Mountain View, CA, USA, model INDI 40-10) with pulses of 6−8 ns duration. The monitoring system consisted of a 150 W pulsed Xe lamp with a lamp pulser (Applied Photophysics, Surrey, U.K.), a monochromator (Princeton Instruments, model Spectra Pro SP-2357, Acton, MA, USA), and a R955 model photomultiplier (Hamamatsu, Japan), powered by a PS-310 power supply (Stanford Research System, Sunnyvale, CA, USA). The data processing system consisted of real time acquisition using a digital oscilloscope (WaveRunner 6100A, LeCroy, Chestnut Ridge, NT, USA) which was triggered by a fast photodiode (Thorlabs, DET10M, ∼1 ns rise time). The data from the oscilloscope were transferred to a computer equipped with software based on LabView 8.0 (National Instruments, Austin, TX, USA) which controls the timing and acquisition functions of the system. Data acquired on the nanosecond laser setup were analyzed using Origin 8.0 fitting functions. For the determination of the quantum yields of the transients, relative actinometry was used according to the procedure described in ref 20, taking 3CB in aqueous solution as the actinometer and ε_{520} = 5400 M^{-1} cm^{-1} for the T−T absorption of 3CB.[11]

Steady-state photochemical irradiation experiments were performed in a 1 cm × 1 cm rectangular cell on an optical bench irradiation system using a Genesis CX355 STM OPSL laser (Coherent), with a 355 nm emission wavelength (the output power used was set at 20 mW). Absorption spectra were measured using a Cary 5000 UV/vis spectrophotometer. A benzophenone-benzhydrol actinometer was used for the determination of the quantum yields of amino acid disappearance.[21] The MS experiments were carried out using a hybrid QTOF instrument (Impact HD, Bruker). Ions were generated by electrospray ionization (ESI). MS/MS fragmentation mass spectra were produced by collisions (CID, collision-induced dissociation) with nitrogen gas in the Q2 section of the spectrometer. The MS instrument was coupled with an HPLC chromatographic system (Ultimate 3000, Thermo/Dionex) equipped with an autosampler, a vacuum degasser, and a diode-array detector. Separation was achieved using a C18 reversed-phase analytical column (2.6 μm, 2.1 mm × 100 mm, Thermo-Scientific) eluted with a gradient from 3% to 60% of acetonitrile and water (with 0.1% formic acid) at a flow rate of 0.3 mL/min for 30 min.

All LFP and stationary irradiation experiments were performed in oxygen-free aqueous solutions at neutral pH.

RESULTS AND DISCUSSION

The general mechanism of primary reactions in the one-electron oxidation of methionine and *S*-methylcysteine

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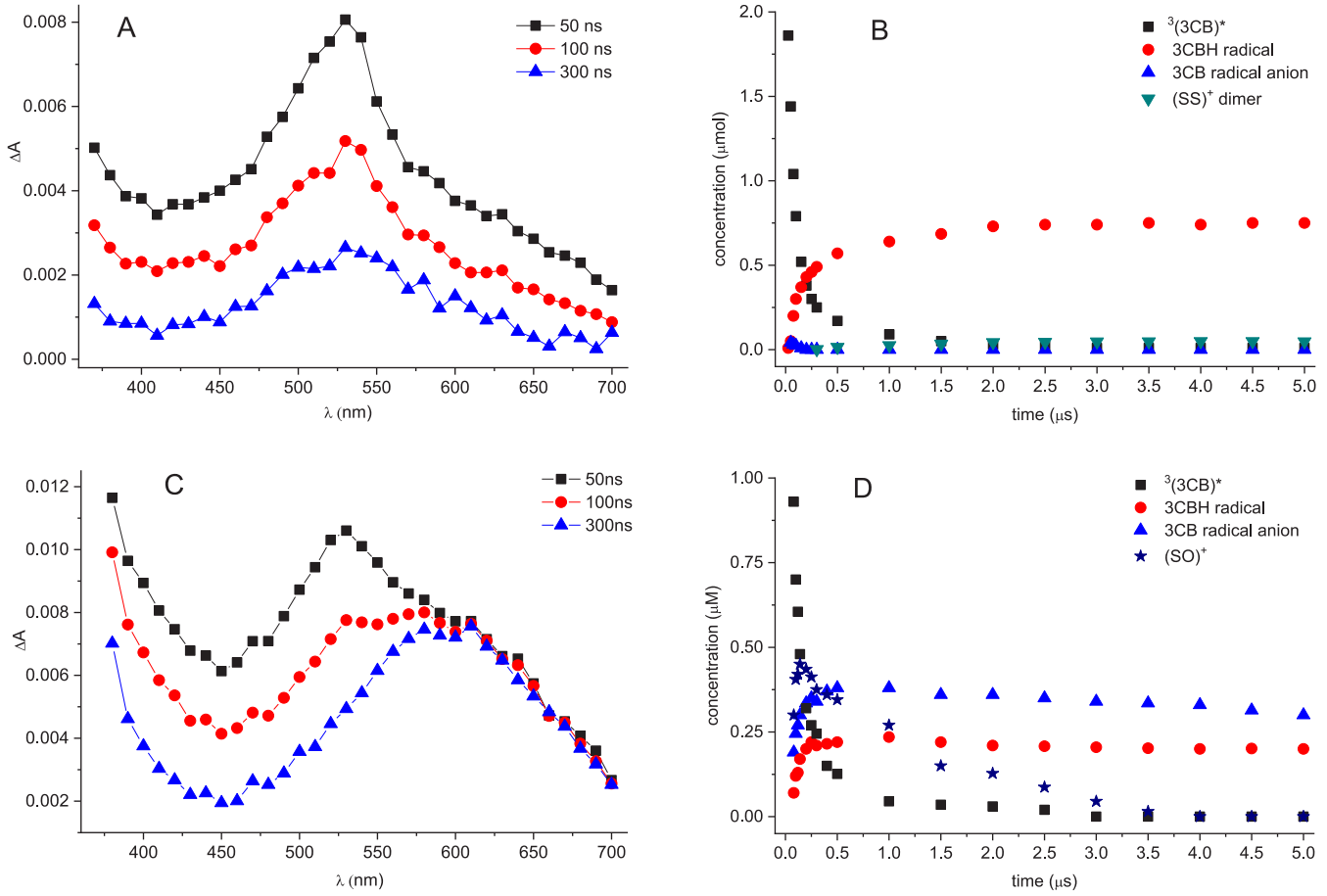

Figure 2. (A) Optical transient absorption spectra of N-Ac-Met-NH-CH_3 (5 mM, pH = 6.8) recorded after the 355 nm laser pulse at 50, 100, and 300 ns delays; (B) concentration profiles calculated at different delay times with respect to the laser pulse for the reaction of 3CB excited triplet quenched by N-Ac-Met-NH-CH_3 (5 mM) in aqueous solution at pH = 6.8; (C) optical transient absorption spectra of N-Ac-Me-Cys-NH-CH_3 (5 mM, pH = 6.8) recorded after the 355 nm laser pulse at 50, 100, and 300 ns delays; (D) Concentration profiles calculated at different delay times with respect to the laser pulse for the reaction of 3CB excited triplet quenched by Ac-MeCys-NH-CH_3 (5 mM) in aqueous solution at pH = 6.8 (see text for details).

Table 1. Quantum Yields of Radical Species Generation from LFP Experiments and Quantum Yields of Amino Acids Disappearance from Stationary Irradiations[a]

	Φ (3CB$^{\bullet-}$)	Φ (3CBH$^{\bullet}$)	Φ (total)	Φ (S.∴O)$^+$	Φ_{dis}
N-Ac-Met-NH-CH_3	≤0.02	0.32	0.34	0	0.13
Ac-MeCys-NH-CH_3	0.34	0.24	0.58	~0.40	0.12

[a] ±15% experimental error.

analogues seems to be well-known[3,4] and is presented in Scheme 1. As can be seen in Scheme 1, the free radicals are generated in three primary processes: (i) electron transfer followed by charge separation (k_{sep}) yielding 3CB$^{\bullet-}$ radical anions (which are then involved in a water-assisted protonation reaction) and an >S$^{\bullet+}$ radical cation; (ii) electron transfer followed by proton transfer within the encounter complex (k_H) yielding a 3CBH$^{\bullet}$ radical and an αS radical; (iii) back electron transfer (k_{bt}) to regenerate the reactants in their ground states. It should be pointed out that the k_{sep} reaction path gives charged species while the k_H reaction path eventually yields neutral free radicals. The k_{bH} reaction of 3CBH$^{\bullet}$ with αS radicals leads to the formation of the reactants in their ground states (the exothermicity of this reaction (ΔH) is estimated to be approximately −60 kcal/mol). This value is in a good agreement with the exothermicity of cross-disproportionation of the alkyl radicals leading to double bond formation calculated by Benson[22] showing that this process (k_{bH}) remains in competition with radical coupling reaction.

The photosensitized oxidation of sulfur-containing compounds leads to a sulfur-centered radical cation (>S$^{+\bullet}$) as a primary intermediate. This radical cation can be reversibly stabilized by the formation of three-electron bonds with electron-rich nucleophilic centers (S, N, or O atom) or irreversibly deprotonate yielding a relatively stable carbon-centered radical (αS); see Scheme 1.

The spectra obtained from photosensitized oxidation (see Figure 2) of both amino acids were deconvoluted into individual components by using a linear regression technique:

$$\Delta A(\lambda_j) = \sum_{n}^{i=1} \epsilon_i(\lambda_j) a_i$$

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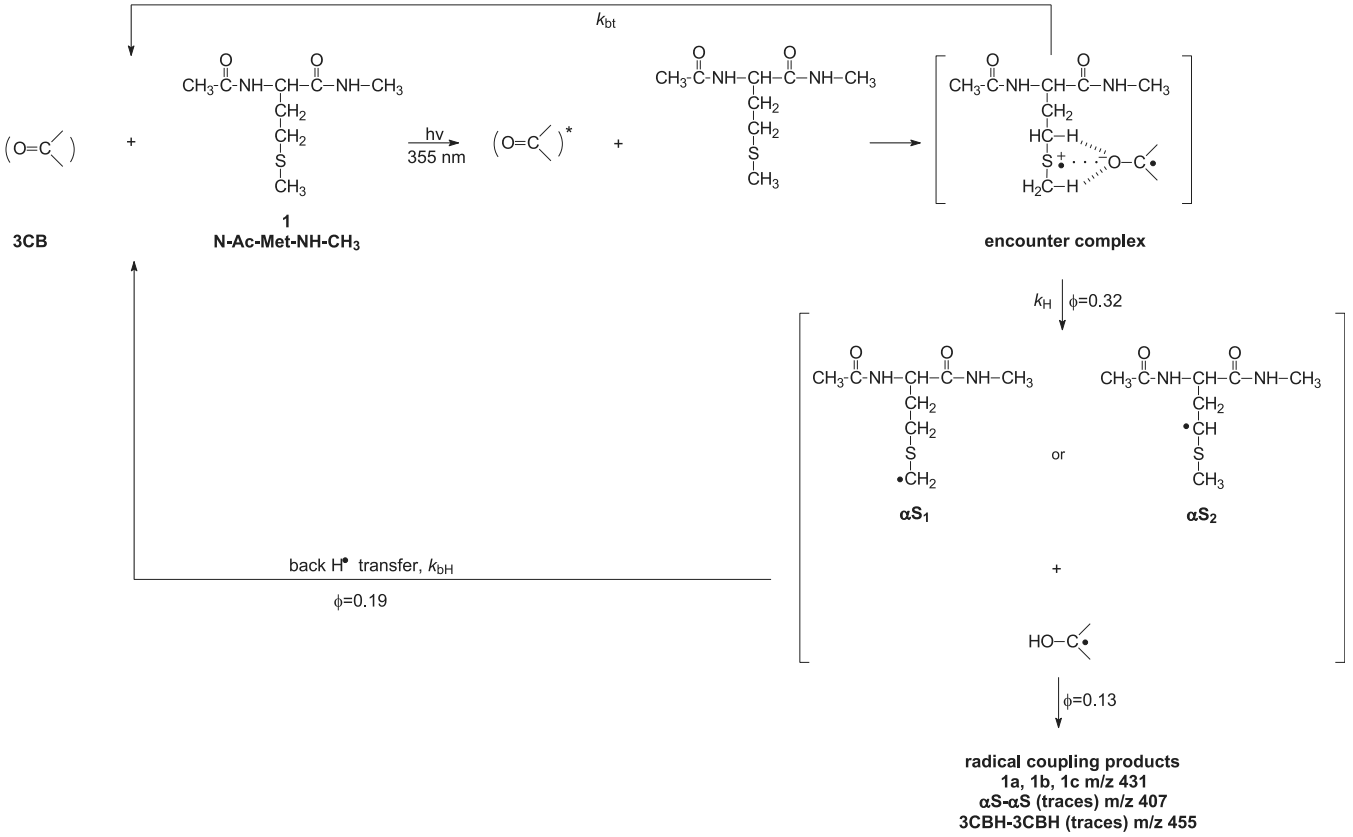
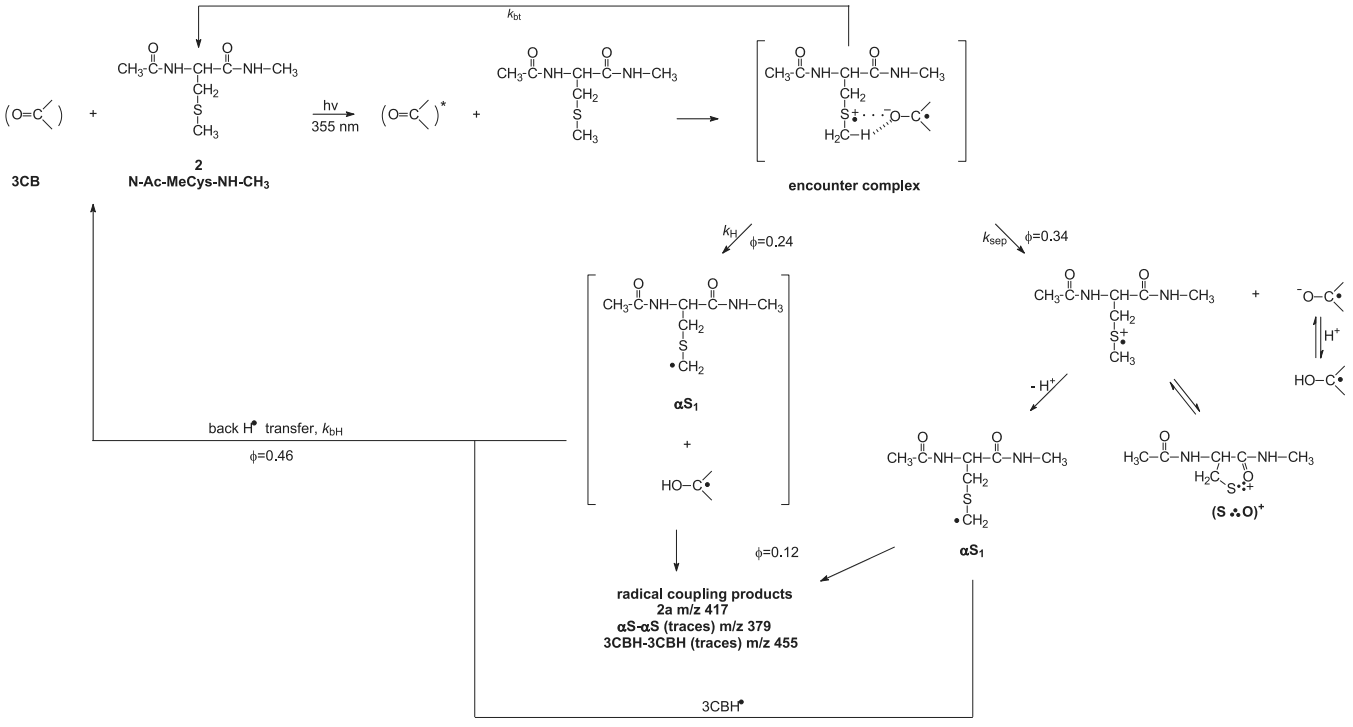
Scheme 2. Mechanism of 3CB* Photosensitized Oxidation of N-Ac-Met-NH-CH_3, Ultimately Leading to Radical-Coupling Reactions between αS and 3CB Ketyl Radicals Yielding Isomeric Photoproducts 1a, 1b, and 1c[a]



[a]See Figure 4 for final products structures. Square brackets denote the geminate radical species.

Scheme 3. Mechanism of 3CB* Photosensitized Oxidation of Ac-MeCys-NH-CH_3, Ultimately Leading to Radical-Coupling Reactions between αS and 3CB Ketyl Radicals Yielding Isomeric Photoproduct 2a[a]



[a]See Figure 4 for final products structures. Square brackets denote the geminate radical species.

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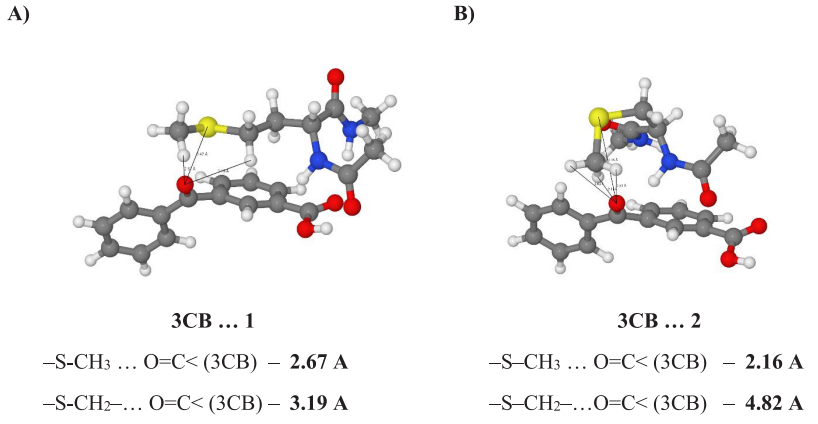

Figure 3. Calculated (DFT) O–H distances for the most stable conformations for encounter complexes, [3CB···$^{+\bullet}$S<]: (A) N-Ac-Met-NH-CH_3 (**1**); (B) Ac-MeCys-NH-CH_3 (**2**).

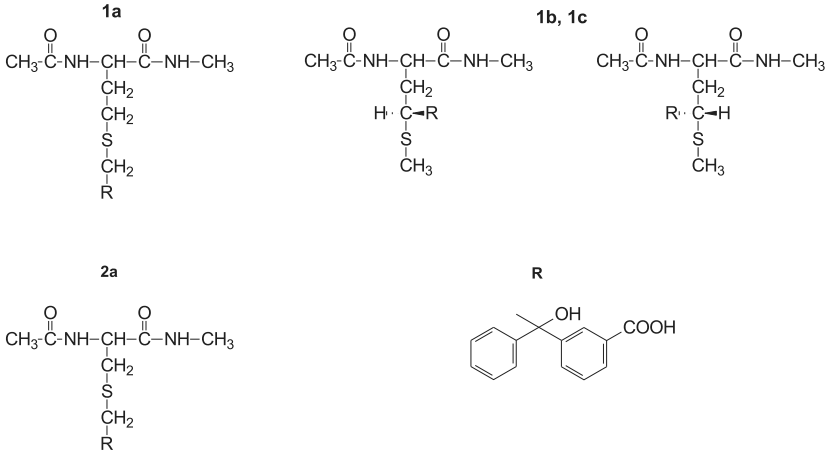

Figure 4. Structures of main stable products of 3CB* photosensitized reaction with N-Ac-Met-NH-CH_3 (products **1a**, **1b**, **1c**) and Ac-MeCys-NH-CH_3 (**2a**).

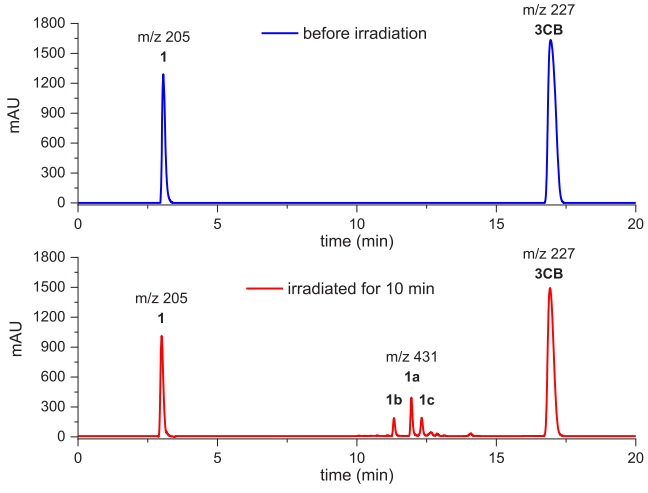

Figure 5. LC–MS chromatogram of 355 nm irradiated (lower panel) and nonirradiated (upper panel) aqueous solution containing 3CB (2 mM) and N-Ac-Met-NH-CH_3 (5 mM) at pH = 6.8. The numbers above the peaks indicate the m/z values of the parent MH^+ ion (for exact masses, refer to the text).

The details of this deconvolution procedure has been described earlier in refs 20 and 23, together with the reference spectra of all of the expected transients. The optical spectra of both $(S.\therefore N)^+$ and $(S.\therefore O)^+$ are very similar and exhibit their absorption maxima around λ_{max} = 390 nm.[14,24−26] Hence, the analysis of the obtained transient absorption spectra for compound **2** was based on the approximation that the transients at λ_{max} = 390 nm were attributed to the $(S.\therefore O)^+$ species (vide infra).

An important observation from the nanosecond laser flash photolysis experiments (see the concentration profiles in Figure 2) is that there was an efficient stabilization of $>S^{\bullet+}$ through the formation of two-centered three-electron bonded cyclic intermediates observed for N-Ac-MeCys-NH-CH_3 (compound **2**). The most rational explanation for the formation of the 390 nm absorbing transient is that the sulfur-centered radical cation was intramolecularly stabilized by the formation of a three-electron bond with a nitrogen atom (five-membered ring intermediate, $(S.\therefore N)^+$) or with an oxygen (six- or five-membered ring, $(S.\therefore O)^+$). This stabilization remains in competition with the deprotonation of the $>S^{\bullet+}$, the primarily formed intermediate, eventually leading to the formation of a much more stable, carbon-centered α-thioalkyl radical (αS). Interestingly, no intermolecular stabilization by the formation of $(S.\therefore S)^+$ was observed in these studies. This is due to the negligibly small yield of $>S^{\bullet+}$ formation for **1** and competing processes of $>S^{\bullet+}$ reactions for **2** (see Scheme 3), resulting in relatively low transient concentrations.

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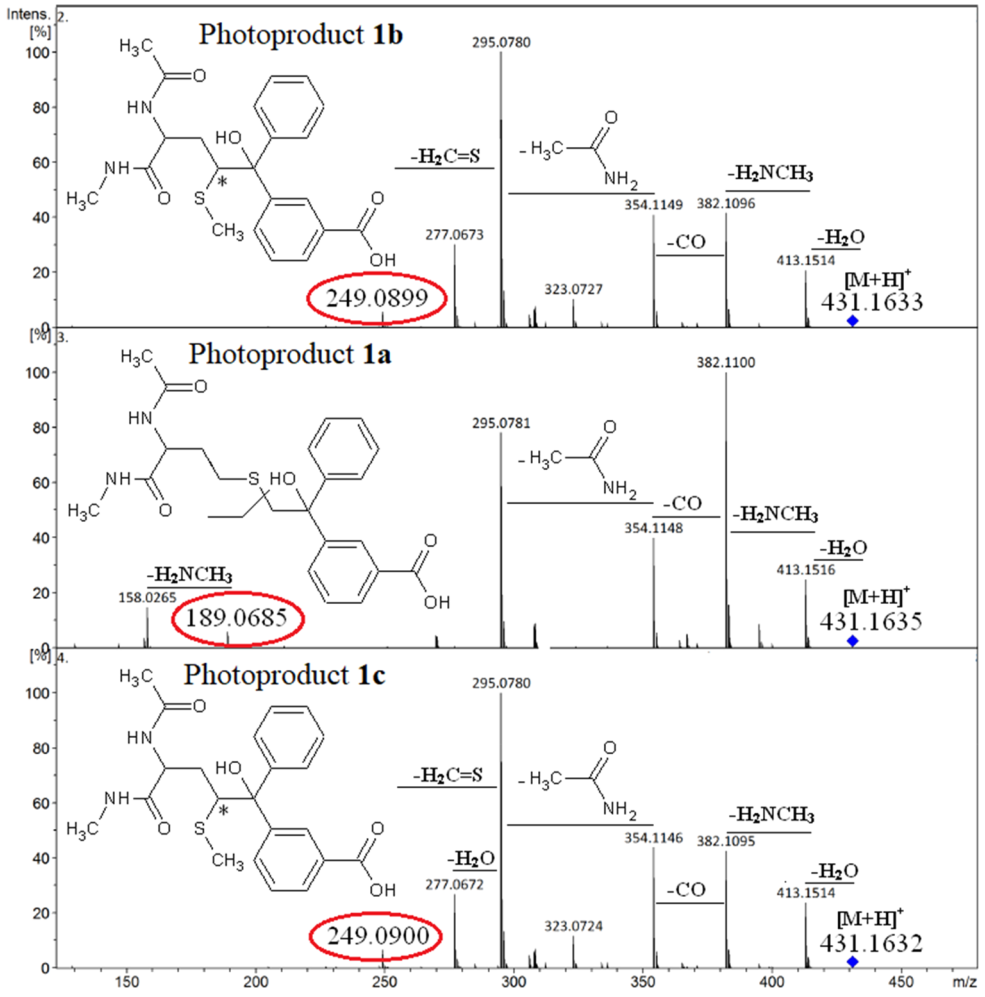

Figure 6. Product ion spectra of $[M + H]^+$ (m/z = 431) ions of the photoproduct **1a** (middle) and the diastereomeric photoproducts **1b** and **1c** (top and bottom) of N-Ac-Met-NH-CH_3 oxidation at pH = 6.8. Red circles indicate the diagnostic product ions for each isomer (see Table 2 for details).

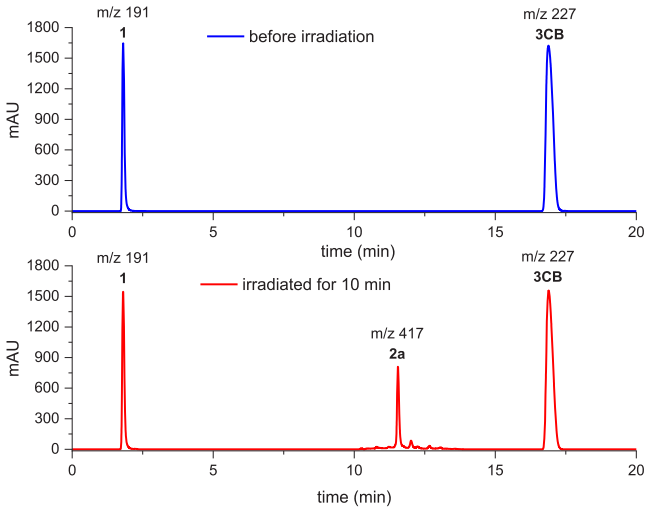

Figure 7. LC−MS chromatogram of 355 nm irradiated (lower panel) and nonirradiated (upper panel) aqueous solution containing 3CB (2 mM) and Ac-MeCys-NH-CH_3 (5 mM) at pH = 6.8. The numbers above the peaks indicate the m/z values of the parent MH^+ ion (for exact masses please refer to the text).

The sulfur-centered radical cation $>S^{\bullet+}$ can be, in fact, a precursor of the $(S.\therefore N)^+$ or $(S.\therefore O)^+$ intermediate. The formation of six-membered $(S.\therefore O)^+$ intermediates was postulated earlier by Schoeneich et al.[27,28] for radiation-induced oxidation of the methionine amide N-Ac-Met-NH_2, a compound similar in structure to N-Ac-Met-NH-CH_3 studied here. However, as it was shown in this work for compound **1** and in the sensitized photooxidation of N-Ac-Met-OCH_3 by Pedzinski et al.,[13] the $(S.\therefore N)^+$ and $(S.\therefore O)^+$ intermediates were not detected in neutral aqueous solutions. However, in the case of Ac-MeCys-NH-CH_3 (compound **2**) the formation of five-membered ring, $(S.\therefore O)^+$, intermediates was postulated by the analogy to the transient observed for the oxidation of S-Me-glutathione, analogue of compound **2**.[26] Moreover, it is difficult to directly compare the experimental results from LFP (present work) and pulse radiolysis[27,28] since the initial steps in the mechanism of the photolysis and radiolysis are different (electron transfer quenching vs OH radical addition), and this difference affects the secondary reactions.

The significant difference in the initial reaction paths of compounds **1** and **2** can also be explained by the structure of the encounter complex formed as a result of collisional quenching of 3CB* by **1** or **2**. The transient absorption spectra and the concentration profiles of intermediates obtained from LFP experiments are presented in Figure 2. Although the pH

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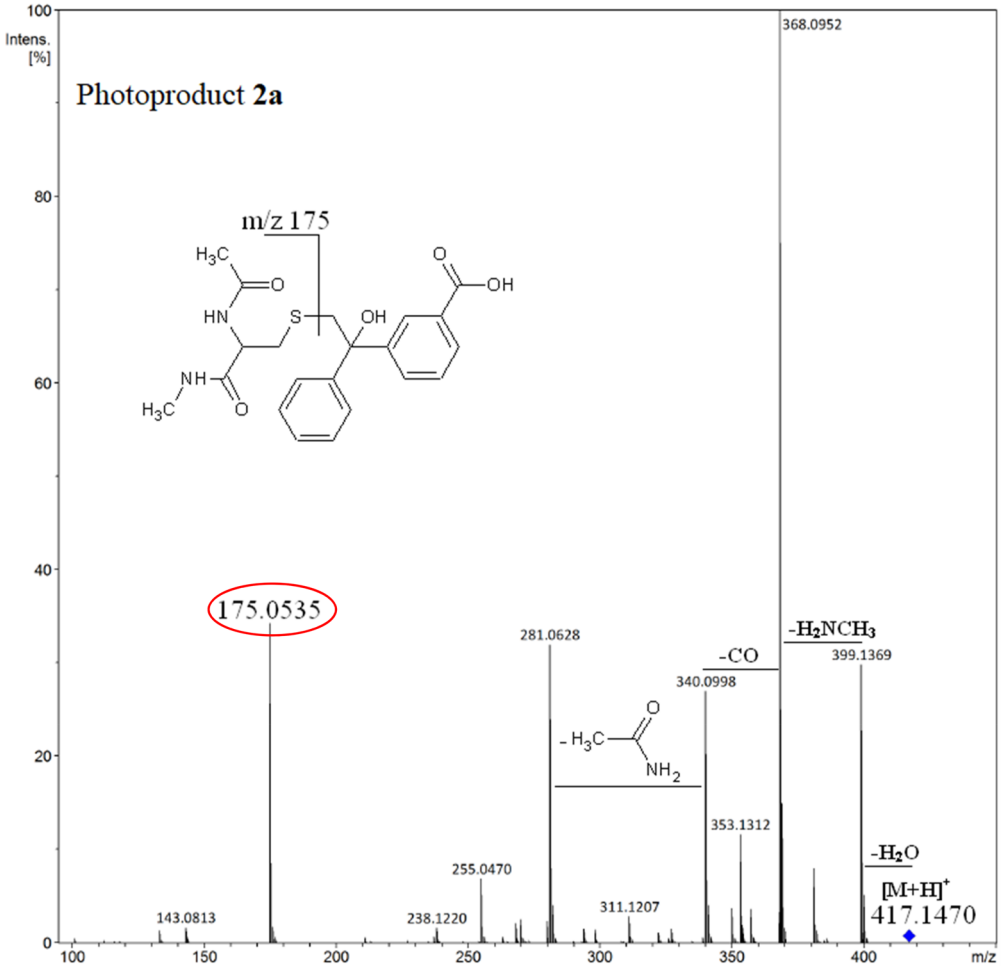

Figure 8. Product ion spectrum of $[M + H]^+$ (m/z 417) ion of photoproduct **2a** of Ac-MeCys-NH-CH_3 oxidation at pH = 6.8.

Table 2. Obtained Mass Accuracies (Errors) for Diagnostic Product Ions for Fragmentation of 1 (m/z 431) and 2 (m/z 417)

diagnostic product	measured mass	exact mass	mass accuracy	composition
1a (-CH_2R)	189.0685	189.0698	−6.9 ppm	$C_7H_{13}N_2O_2S$
1b	249.0899	249.0916	−6.8 ppm	$C_{17}H_{13}O_2$
1c	249.0900	249.0916	−6.4 ppm	$C_{17}H_{13}O_2$
2a (-CH_2R)	175.0535	175.0541	−3.4 ppm	$C_6H_{11}N_2O_2S$

of the experiments is the same (neutral) and is well below the pK_a of 3CBH$^\bullet$/3CB$^{\bullet-}$ (pK_a = 9.5 [11]), there were noticeably more radical anions generated for Ac-MeCys-NH-CH_3 (compound **2**) as compared to the methionine analogue (compound **1**). The quantum yields for generating the ketyl radicals 3CBH$^\bullet$ and ketyl radical anions 3CB$^{\bullet-}$ reflecting the efficiencies of the primary photochemical reactions k_{sep} and k_H are summarized in Table 1. The quantitative results in Table 1 remain in excellent agreement with earlier studies on the compounds of similar structure.[4,7,13]

It can be assumed that at pH = 6.8 the protonation of initially formed 3CB radical anions (primary electron transfer product) will be relatively slow. In the time window of the LFP experiment (up to 5 μs), the equilibrium between the two forms of the ketyl radical (3CB$^{\bullet-}$ and 3CBH$^\bullet$) was still not established. This equilibration occurred on a longer time scale due to the low concentration of protons at neutral pH and a low value for the water-assisted protonation rate constant (the protonation rate constants by H^+, k_{H+} = 6.4 × 10^{10} M^{-1} s^{-1}, and water, k_{H2O} = 5.1 × 10^2 M^{-1} s^{-1}, are known and can be found in ref 11). Moreover, both radicals were involved in reactions with αS (radical-coupling) as shown in the reaction, Scheme 1. In other words, the ketyl radicals decayed due to the coupling reactions as well as due to slow equilibration between 3CB$^{\bullet-}$ and 3CBH$^\bullet$.

The concentration profiles of transients from LFP experiments (see Figure 2) clearly suggest that since very little 3CB$^{\bullet-}$ was observed for N-Ac-Met-NH-CH_3, the k_H reaction path dominated over k_{sep} for compound **1** (only negligible amounts of charge-separation products were observed). This means that the k_H pathway was the main primary photochemical reaction leading to the disappearance of the Met analogue ($k_H \gg k_{sep}$, see Scheme 2). On the other hand, for Ac-MeCys-NH-CH_3, higher yields of 3CB$^{\bullet-}$ generation in comparison with 3CBH$^\bullet$ were observed even at neutral pH showing that $k_{sep} > k_H$. A possible explanation of this behavior may be the difference in the primary photochemical reaction reactivity of both compounds (N-Ac-Met-NH-CH_3 (**1**) and Ac-MeCys-NH-CH_3 (**2**)) in the initial stages of the process, right after electron transfer in the solvent cage, within the encounter complex (vide infra). This argument is further supported by the observation of an intramolecular three-electron-bonded $(S\therefore O)^+$ species for Ac-MeCys-NH-CH_3 (from which charged radical species were produced: 3CB$^{\bullet-}$

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and >S$^{\bullet+}$ as presented in Scheme 3). No such stabilization was observed for N-Ac-Met-NH-CH_3 where the radicals observed to be formed from the separating encounter complex were the neutral radicals 3CBH$^{\bullet}$ and αS. This striking difference in reactivity between these two compounds, of such similar structure, can be reasonably explained by structural (steric) factors. One should notice that after electron transfer in the encounter complex, the hydrogen atoms attached to the carbon adjacent to the sulfur atom become acidic and therefore can possibly form hydrogen bonds, e.g., with the neighboring carbonyl oxygen of the sensitizer (as shown in Scheme 3). Due to the steric effect, such bonding would make the proton from the methylene group inaccessible for the k_H path of the reaction for the compound **2**, and consequently, no deprotonation at this carbon atom can occur. This steric effect of the sensitizer approaching the reaction center was additionally supported by the DFT calculation (Prof. Jacek Koput, private communication; see Figure 3) describing the structures of encounter complexes [3CB···$^{+\bullet}$S<] in the most stable conformations for Ac-MeCys-NH-CH_3 (**2**) and N-Ac-Met-NH-CH_3 (**1**). The distances between the oxygen atom of 3CB and the hydrogen atoms of the methyl and methylene groups neighboring the sulfur atom of **1** were both small and equal to 2.67 and 3.19 Å, whereas for compound **2** they were 2.16 and 4.82 Å for methyl and methylene groups, respectively.

The results discussed above show that the k_H reaction path in the oxidation of **1** involves proton transfer from both carbon atoms neighboring the sulfur atom, resulting in two αS-type radicals (αS_1 and αS_2 as shown in Scheme 2). The access to this "internal" proton in the encounter complex with Ac-MeCys-NH-CH_3 (see Scheme 3) is sterically more difficult, making the k_{sep} path more efficient in the case of **2** (Ac-MeCys-NH-CH_3). Consequently, only one type of αS radical (localized on the terminal carbon atom and depicted as αS_1) is being formed in this case (see stable product analysis).

The steady-state irradiations of the 3CB-amino acid systems (with **1** or **2**) led to the decomposition of the reactants and the generation of various stable products. The quantum yields of disappearance of the amino acids (Φ_{dis}) were found to be 0.13 and 0.12 (±0.02) for **1** and **2**, respectively. These values are significantly smaller than the quantum yields of the radicals obtained from LFP (see Table 1), indicating that only a fraction of the radical-coupling reactions in the radical pairs (as presented in Schemes 2 and 3) is involved in the formation of stable products. The remaining quantities of free radicals undergo back H atom transfer process (k_{bH}). In other words, only the fraction of the free radicals generated (and observed in LFP as shown in Table 1) generates stable products and the remaining radicals undergo the radical disproportionation reaction (k_{bH}) regenerating the initial reactants.

It is noteworthy that small amounts of the sulfoxide (>S═O) were detected after steady-state irradiation and LC−MS stable product analysis of **1**, while no sulfoxide was formed in the oxidation of **2**. Since the solutions were purged with high purity argon prior reaction, >S═O could not be produced in a reaction with molecular oxygen. The mechanism of sulfoxide formation from bimolecular αS radical disproportionation has been proposed earlier,[9,18] and it involves the γ-carbon-centered radical. This type of radical (αS_2) is present exclusively in the oxidation of **1**, while the photosensitized oxidation of compound **2** yields only one type of αS radical (αS_1, localized on the terminal C atom) and cannot be a precursor for any sulfoxide formation.

As explained above, the photosensitized oxidation reaction led to two major types of relatively stable, carbon-centered radicals: ketyl radicals from 3CB reduction and two types of αS radicals derived from amino-acid oxidation and/or subsequent > S$^{\bullet+}$ deprotonation (as presented in Scheme 1). These two radicals (αS and 3CBH$^{\bullet}$) are known to undergo radical-coupling reactions yielding the adduct-type photoproducts[10] of different structures depending on the exact structure of their precursors. Moreover, once the αS radicals are produced (either from the deprotonation of > S$^{\bullet+}$ or from the k_H reaction pathway as described above), the question arises on which of the two carbon atoms is the radical localized? To answer this question, the samples containing 3CB and the quencher were irradiated and subsequently analyzed using HPLC with a standard spectrophotometric detection and coupled with high-resolution MS and MS/MS detection. This stable product analysis is therefore very helpful in collecting information on the nature and structure of the free-radical species taking part in such photooxidation processes. High-resolution MS is a very powerful technique for analysis of the stable photoproducts since, from the measured exact masses (or more precisely m/z values) of the products, one can prove the molecular composition of the products. However, there is no structural information from such experiments. MSMS fragmentation experiment, on the other hand, may provide the structural information on the photoproducts and, after detailed analysis, their precursor radicals.

The αS-type radicals produced in the oxidation of both compounds (αS_1 and αS_2 for **1** and only αS_1 for **2**) ultimately led to radical-coupling products with the 3CB ketyl radical (3CBH$^{\bullet}$) and traces of αS−αS dimeric products (as shown in the Supporting Information). As expected, only one main stable product was detected for **2** with m/z 417, while three isomers (one structural **1a** and two optical **1b** and **1c**) with m/z 431 were observed for **1** (see Figures 4−6).

Stable Product Analysis: N-Ac-Met-NH-CH_3 Oxidation. As can be seen on the chromatograms in Figure 5, three main photoproducts eluted with retention times of approximately 11−12.5 min were detected after 10 min, 355 nm irradiation. All products showed the same molecular composition at m/z 431.1633, suggesting their isomeric nature (photoproducts **1a**, **1b**, and **1c**; see Figure 4 for suggested structures).

The MSMS fragmentation was performed for the $[M + H]^+$ product ion at m/z 431, and as can be seen in Figure 6, the fragmentation revealed significant structural differences between the photoproducts. The main fragmentation pathways of **1a**, **1b**, and **1c** correspond to the decomposition of the methionine moiety. However, the diagnostic product ion at m/z 189 detected for photoproduct **1a** clearly indicates that the benzophenone moiety (depicted as R) was attached to the *S*-methyl group. Furthermore, the diagnostic product ions at m/z 249 detected for diastereomeric photoproducts **1b** and **1c** indicate that these two compounds possess the -S-CH_3 moiety; thus -R must be attached to the *S*-methylene group. It is therefore clear, that the photoproducts **1a**, **1b**, and **1c** were generated in a radical-coupling reaction between two types of radical species as described above (αS and the 3CBH$^{\bullet}$ ketyl radical). It is noteworthy that, as expected, traces of αS−αS radical-coupling products (m/z 405) were also detected (see small peaks at retention times 12−14 min in Figure 5), but these photoproducts were not analyzed in this work due to their small yields. Following the same line of reasoning, also

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the traces of benzpinacol-like products (3CBH−3CBH) were detected in the LC−MS analysis. The mechanism of 3CB* photosensitized oxidation of N-Ac-Met-NH-CH_3 is summarized in Scheme 2.

Stable Product Analysis: N-Ac-MeCys-NH-CH_3 Oxidation. As presented in Figure 7, only one main photoproduct eluting with a retention time of approximately 11.5 min was detected after irradiation. This photoproduct (**2a**) shows its MS spectrum with one clear peak at m/z 417, and the difference of 14 Da (CH_2 group) suggests that the structure of this product is very similar to the Met analogue discussed above. The MSMS fragmentation (Figure 8) of $[M + H]^+$ at m/z 417, analogically as for $[M + H]^+$ ion of photoproduct **1a**, corresponds to the decomposition of the cysteine moiety (loss of neutral molecules of water, methylamine, carbon monoxide, and acetamide). The diagnostic product ion at m/z 175, however, clearly indicates that R is attached to the *S*-methyl group. Therefore, the photoproduct **2a** is similar in structure to photoproduct **1a** (see Figure 4). As in the case of N-Ac-Met-NH-CH_3 oxidation, traces of αS-αS radical-coupling products (m/z 379) and benzpinacol-type products (m/z 455) were also detected for Ac-MeCys-NH-CH_3 (Scheme 3).

The exact masses of diagnostic ions from MSMS experiments that allowed us to suggest the structures of photoproducts are collected in Table 2.

On the basis of the data obtained in the flash photolysis and stationary irradiation followed by the LC−MS analysis of the stable photoproducts, the mechanism of 3CB* photosensitized oxidation of Ac-MeCys-NH-CH_3 is presented in Scheme 3.

CONCLUSIONS

The mechanisms for photosensitized oxidation of methionine (N-Ac-Met-NH-CH_3, **1**) and methyl-cysteine (*Ac-MeCys-NH-CH_3*, **2**) analogues by 3-carboxybenzophenone excited triplet (3CB*) in neutral aqueous solutions were shown to differ significantly. The differences observed for primary and secondary photoreactions are summarized below.

For primary photochemical reactions (Scheme 1), the following were observed.

- For compound **1** only one primary photoreaction, namely, electron transfer followed by proton transfer within the encounter complex, k_H leading to the formation of ketyl radical 3CBH$^\bullet$ and α-thioalkyl radical (αS) was observed with the quantum yield Φ(3CBH$^\bullet$) = 0.32.
- For compound **2** both k_H and k_{sep} (electron transfer followed by charge separation) primary photoreactions were observed with the quantum yields Φ (3CBH$^\bullet$) = 0.24 and Φ (3CB$^{\bullet-}$) = 0.34, respectively.

For secondary reactions leading to stable products (Schemes 2 and 3), the following were observed.

- For compound **1** both αS_1 and αS_2-type radicals were formed as proven by the detection of radical-coupling products with 3CB ketyl radicals (αS_1-3CBH and two diastereoisomers of αS_2-3CBH). This indicates that hydrogen atoms from the methyl and methylene groups attached to the sulfur atom in the amino acid side chain CH_3-S-CH_2- participate in the reaction mechanism,
- For compound **2** only one type of radical-coupling product (αS_1-3CBH) was found and only a hydrogen atom from the methyl group (CH_3-S-) was involved,
- The back H-atom transfer reaction of 3CBH$^\bullet$ with αS radicals (k_{bH}) leading to regeneration of reactants in the ground states was shown to compete with the radical-coupling reactions.

The differences in the photoreaction mechanisms were rationalized by the differences in geometry of the encounter complexes of 3CB with both amino acids (a steric effect for compound **2**).

In summary, it was demonstrated that a small change in the structure of the sulfur-containing amino acid (one methylene group less for *S*-methyl-cysteine analogue in comparison to methionine) led to significant changes in the mechanisms of the photosensitized oxidation of N-Ac-Met-NH-CH_3 (**1**) and *Ac-MeCys-NH-CH_3* (**2**) by the 3-carboxybenzophenone excited triplet in neutral aqueous solutions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.0c06008.

Comment S1 on the synthesis procedure; Figure S1 showing extracted ion chromatogram and MS spectrum of the >S═O photoproduct; Figure S2 showing αS−αS traces (MS spectra) detected for **1** and **2** (PDF)

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Conceptualization was by B.M., T.P., and P.F. Formal analysis was by B.M., T.P., P.F, and R.F. Funding acquisition was by B.M. Synthesis was by F.K. Investigation was by K.G., T.P., and P.F. Methodology was by T.P. and B.M. Validation was by T.P., R.F., and B.M.. For writing of the manuscript, the original draft preparation was by K.G. and T.P. and the review and editing were by T.P and B.M.

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Notes

The authors declare no competing financial interest.

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