Side Chain Fragmentation of N-Terminal Threonine or Serine Residue Induced through Intramolecular Proton Transfer to Hydroxy Sulfuranyl Radical Formed at Neighboring Methionine in Dipeptides

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Abstract: The reaction of hydroxyl radicals with Ser-Met and Thr-Met at slightly acidic to neutral pH results in the side chain fragmentation of the Ser and the Thr moiety into formaldehyde and acetaldehyde, respectively. The efficiency of this process depends on the concentration of the peptide and protons with maximum yields at low peptide concentrations at near neutral pH. Significantly less aldehyde formation is observed for the reaction of hydroxyl radicals with Ala-Met, Val-Met, Gly-Ser-Met, Met-Ser, Gly-Met-Ser, Ser-Leu, Gly-Thr-Met, and Gly-Met-Thr. These results indicate that the formation of aldehyde requires (i) an N-terminal Ser or Thr residue and (ii) the presence of Met in the sequence. The underlying mechanism involves an intramolecular proton transfer from the protonated N-terminal amino group to an initially formed hydroxy sulfuranyl radical at the Met residue. This process leads to the elimination of water and the simultaneous formation of a three-electron-bonded $[>S\therefore NH_2]^+$-peptide intermediate which absorbs at λ_{max} = 385 nm and has been identified by pulse radiolysis. This intermediate decays with $t_{1/2}$ = 310 ns into aldehyde and an α-amino radical of the structure $H_2N-C^{\bullet}H-C(=O)NH-$*peptide*, which has been identified by ESR spectroscopy. Mechanistically, the latter process involves the formation of an intermediate nitrogen-centered radical cation which undergoes subsequent heterolytic scission of the C_α–C_β bond of the Ser or Thr side chain, respectively. One-electron oxidation of Thr-Met by $SO_4^{\bullet-}$ at slightly acidic pH (≈5.5–6) results in significantly lower yields of acetaldehyde as compared to the hydroxyl radical initiated process, indicating the importance of the intermediary formed hydroxy sulfuranyl radical. It is proposed, however, that generally sulfur-centered radical cations, derived through one-electron oxidation of the methionine sulfur, might convert into hydroxy sulfuranyl radicals which subsequently undergo the proton-transfer process with adjacent N-terminal Ser or Thr residues.

Introduction

Neighboring groups actively participate in the oxidation of organic thioethers.[1] This has been documented for a variety of functionalities such as sulfide, amino, hydroxyl, and carboxylate groups during oxidation processes carried out by molecular iodine,[2] electrochemistry,[3] and various free radicals,[4] in particular the hydroxyl radical, HO•. These neighboring groups generally act via providing electron lone pairs for the stabilization of cationic intermediates. The hydroxyl radical efficiently generates sulfur radical cations from organic sulfides[5] which subsequently form various three-electron-bonded intermediates with amino,[6] hydroxyl,[7] carboxylate,[7] and sulfide[5,8] groups. However, the hydroxyl radical induced generation of these sulfur-centered radical cations involves one preceding step, namely the initial formation of hydroxyl radical adducts (hydroxy sulfuranyl radicals), >S•-OH (**1**) (reaction 1).[5,9-11] These important

$$HO^{\bullet} + >S \rightarrow \underset{\mathbf{1}}{>S^{\bullet}\text{-}OH} \qquad (1)$$

intermediates, and consequently any influence of neighboring groups on their reactions, have received considerably less attention than their successors, the sulfur-centered radical cations. Recently, it was reported that hydroxy sulfuranyl radicals **1**, formed via addition of HO• to 2-(methylthio)methylacetate, could be stabilized via hydrogen bonding to an adjacent ester carbonyl oxygen,[10] resulting in an enhanced lifetime of $t_{1/2}$ = 12 μs (normally such adducts convert into sulfur-centered radical cations with $t_{1/2} \approx 0.4$ μs[5,9,11]). Moreover, the pronounced stability of this adduct was utilized to obtain evidence that such hydroxy sulfuranyl radicals react with molecular oxygen.[10] Another

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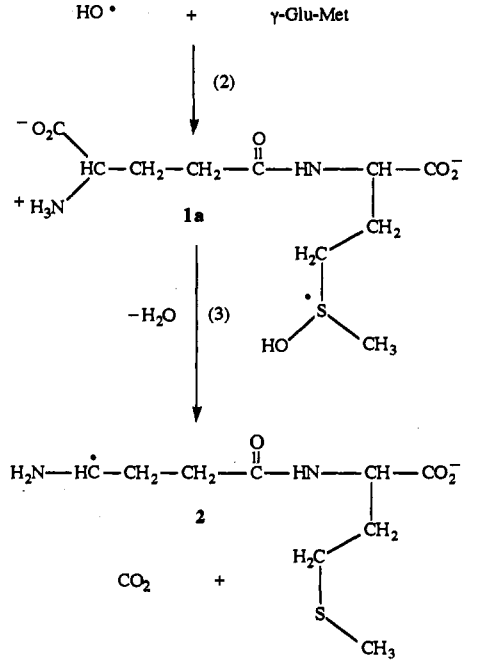
Scheme 1



example for neighboring group interactions was provided by the reaction of hydroxyl radicals with 2-(methylthio)ethanol leading to an extremely short-lived hydroxy sulfuranyl radical ($t_{1/2}$ < 20 ns)[11] which decomposes via proton/hydrogen transfer in a cyclic transition state.

Hydroxyl radicals are important intermediates in chemical and biological oxidations.[12] Among these the free radical induced oxidation of proteins currently receives attention due to its possible role in aging and the pathogenesis of several disease states.[13] Recently, emphasis has been placed on the chemical synthesis of transition metal complexes,[14,15] particularly such formed with peptide structures,[14c] which specifically bind to and subsequently cleave certain domains of proteins[14] or DNA.[15] The observed product patterns have led to the conclusion that hydroxyl radicals or hydroxyl radical like species are involved in these reactions.[14c] Effects of neighboring groups on the reactions of these radicals with particular peptide or protein domains may thus be of importance due to the presence of a manifold of possible participating functionalities such as carboxyl (from Asp or Glu), amine (from Lys), amide (from the peptide backbone), and hydroxyl groups (from Thr and Ser). As an example, it has been shown that the reaction of the hydroxyl radical with the methionine model peptide γ-Glu-Met[16] and S-alkyl glutathiones[16,17] does not only lead to the expected one-electron oxidized sulfur-centered radical cation but rather to the elimination of CO_2 from the N-terminal carboxylate group with parallel formation of α-amino-type radicals **2** (Scheme 1, reactions 2 and 3; representatively shown for the oxidation of γ-Glu-Met).[16,17]

In this paper we report the detailed mechanistic investigation of a novel side-chain fragmentation of N-terminal threonine (Thr) and serine (Ser) residues in Thr-Met or Ser-Met dipeptides, respectively. In both cases the fragmentation involves the initial formation of a hydroxy sulfuranyl radical at the methionine residue with subsequent intramolecular proton (or hydrogen) transfer from the N-terminal amino group, facilitating its intramolecular one-electron oxidation, followed by heterolytic cleavage of the side chain.

Experimental Section

Materials. The peptides Ser-Met, Ala-Met, Thr-Met, Val-Met, Met-Ser, Ser-Leu, and Gly-Met were obtained from Bachem Bioscience, Inc. (Philadelphia, PA) and were of highest commercially available purity. The peptides Gly-Thr-Met, Gly-Met-Thr, Gly-Ser-Met, and Gly-Met-Ser were synthesized by standard solid-phase methods using the Fmoc-protected amino acids. The peptides were characterized by FAB mass spectrometry, and their purity was checked by HPLC. The other chemicals were obtained as follows: *tert*-butyl alcohol (p.a.), acetonitrile (p.a.), ascorbic acid (p.a.), formaldehyde solution (37%), acetaldehyde, and (2,4-dinitrophenyl)hydrazine (2,4-DNPH, p.a.) from Merck (Germany); $K_2S_2O_8$ (potassium persulfate, p.a.) and DL-dithiothreitol (p.a.) from Sigma Chemical Company (St. Louis, MO); 1,1′-dimethyl-4,4′-bipyridinium dichloride (methyl viologen dichloride) from Aldrich (Milwaukee, WI).

Solutions. All solutions were made up freshly in Millipore water (18 MΩ), and their respective pH's were adjusted through addition of NaOH or $HClO_4$. They were subsequently purged for at least 20 min per 5-mL sample with the desired gas, N_2 or N_2O, before irradiation.

γ-Radiolysis. The γ-radiolysis experiments were carried out in the field of a 6000-Ci ^{60}Co-source at the Hahn-Meitner-Institut Berlin (Germany). The employed dose rates were determined through Fricke dosimetry.[18] The applied radiation doses were adjusted such as that the radiolytic conversion of starting material was <15% in order to avoid secondary reactions of radiolytically produced radicals with reaction products.

Pulse Radiolysis. All pulse radiolysis experiments were performed by applying 5-ns pulses of high-energy electrons from the Notre Dame 7-MeV ARCO LP-7 linear accelerator or 0.3–1.0-μs pulses of high-energy electrons (1.55 MeV) from the Van-de-Graaff accelerator of the Hahn-Meitner-Institut Berlin (for details see refs 19 and 20). Absorbed doses were on the order of 2–4 Gy (1 Gy = 1 J/kg), corresponding to an average concentration of radicals of (1.2–2.5) × 10^{-6} M for a radiation chemical yield of G = 6.0 (G denotes the number of species formed or converted per 100 eV of absorbed energy; G = 1.0 corresponds to 0.1036 μmol per joule of absorbed energy in aqueous solution; for practical purposes the G-unit rather than the SI-unit is used throughout this paper).[21]

Electron Spin Resonance. The in situ radiolysis ESR spectra[22,23] of steady-state radical populations were recorded during continuous electron irradiation of a flowing, cooled, N_2O-saturated aqueous solution, pH 6.2, containing 2.0 × 10^{-3} M Thr-Met. The 2-mA dc electron beam was provided by a Van-de-Graaff accelerator with further details of the method described elsewhere.[24] X-band (9.2-GHz) spectra were recorded in second-derivative presentation using 100-kHz and 200-Hz field modulation. NMR methods were used for field measurements. The solution flow rate was approximately 15 mL/min. The solution temperature was kept at 13 °C. The line positions were determined by a nonlinear least squares fit of the digitally recorded spectrum with a second-derivative Gaussian line shape.

HPLC Analysis of Reaction Products. The HPLC analysis of reaction products was performed employing a Gynkotek HPLC, equipped with a diode array detector and an SGE 250 × 4.6 mm^2 Hypersil C18 column. Formaldehyde and acetaldehyde were analyzed as described previously.[11] The product Gly-Met was analyzed by the direct injection of an irradiated solution onto the HPLC column and detection at 214 nm (chromophore is the peptide bond). A satisfactory separation of small concentrations

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of Gly-Met (radiation chemical yield $<5.0 \times 10^{-6}$ M) from excess Ser-Met (remaining peptide concentration after irradiation $>9.0 \times 10^{-4}$ M) was achieved employing an isocratic elution with a mixture of acetonitrile/water (5:95, v/v), containing 0.02% trifluoroacetic acid. Under these conditions Gly-Met (t_R = 17.9 min) eluted as a distinct peak on a small residual tailing of the Ser-Met peak (t_R = 14.4 min). Quantification was achieved by chromatography of mixtures of defined added quantities of Gly-Met (0–2.5×10^{-5} M) to solutions containing excess Ser-Met (1.0×10^{-3} M). A satisfactory resolution of low concentrations of Gly-Met from excess Thr-Met was not possible.

Results

1. γ-Radiolysis. The radiolysis of water leads to the formation of the highly reactive species shown in reaction 4.[21] In N_2-

$$H_2O \rightarrow e_{aq}^-,\ HO^\bullet,\ H^\bullet \qquad (4)$$

saturated solutions, taking the employed peptide concentrations, the radiation chemical yields of these species are $G^\circ(e_{aq}^-) = 2.75$, $G^\circ(HO^\bullet) = 2.75$, and $G^\circ(H^\bullet) = 0.6$.[25] In N_2O-saturated solutions ($[N_2O]_{sat} \approx 2 \times 10^{-2}$ M[19]), the hydrated electrons are converted into hydroxyl radicals according to reaction 5 ($k_5 = 9.1 \times 10^9$

$$e_{aq}^- + N_2O \rightarrow HO^\bullet + HO^- + N_2 \qquad (5)$$

$M^{-1}\ s^{-1}$[19]), giving the total initial yield of hydroxyl free radicals of $G^{\circ\prime}(HO^\bullet) = 5.5$. At pH < 4 the diffusion-controlled reaction of e_{aq}^- with protons becomes important (reaction 6, $k_6 = 2.0 \times$

$$e_{aq}^- + H^+ \rightarrow H^\bullet \qquad (6)$$

$10^{10}\ M^{-1}\ s^{-1}$[26]), resulting in a pH-dependent reduced yield of hydroxyl free radicals.

The reaction of $H^\bullet$ atoms with organic thioethers generally proceeds via hydrogen abstraction at the α-(alkylthio) carbon–hydrogen bond, yielding α-(alkylthio)alkyl radicals (reaction 7;

$$H^\bullet + -CH_2-S-CH_2- \rightarrow H_2 + -^\bullet CH-S-CH_2- \qquad (7)$$

for methionine, $k_7 = 3.5 \times 10^8\ M^{-1}\ s^{-1}$[26]).

As will be discussed below, the formation of α-(alkylthio)alkyl radicals does not interfere with the mechanism under discussion so that the reactions of hydrogen atoms do not have to be further considered in our systems.

2. Formation of Aldehydes through the Reaction of Hydroxyl Radicals with Peptides. A. Identification of Aldehydes. γ-Irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 5×10^{-4} M Ser-Met or Ala-Met, respectively, results in the formation of formaldehyde. The formaldehyde yields depend linearly on the applied dose (Figure 1, curves a and b), and from the respective slopes of the plots of [produced formaldehyde] vs dose, the radiation chemical yields $G = 0.66 \pm 0.05$ (Ser-Met) and $G = 0.24 \pm 0.05$ (Ala-Met) are calculated. Taking $G^{\circ\prime}(HO^\bullet) = 5.5$, it follows that formaldehyde is produced with a 12% yield from Ser-Met and a 4.4% yield from Ala-Met. Comparably low formaldehyde yields ($G < 0.2$) are obtained upon γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 5×10^{-4} M Gly-Ser-Met or Gly-Met-Ser, respectively. No formaldehyde is obtained upon γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 5×10^{-4} M Ser-Leu ($G < 0.01$; Figure 1, curve c) or 5×10^{-4} M Met-Ser ($G < 0.02$), respectively. The γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 5×10^{-4} M Thr-Met or Val-Met, respectively, results in the formation of formaldehyde with $G = 0.1 \pm 0.05$ (Thr-Met) and $G = 0.3 \pm 0.05$ (Val-Met). Most important, however, the irradiation of the Thr-Met solution leads

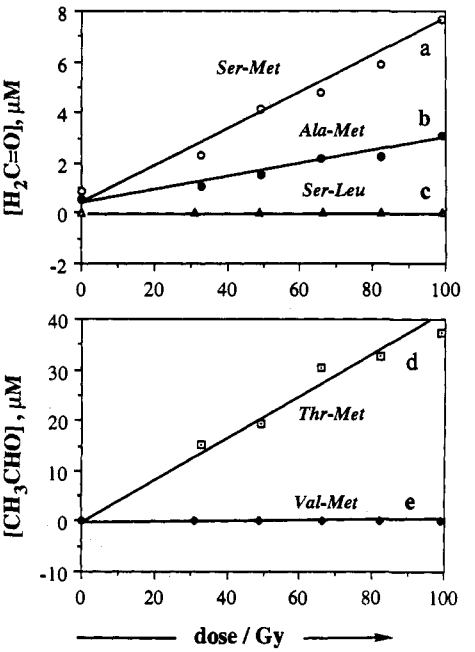

Figure 1. Absolute concentrations of (a–c) formaldehyde and (d and e) acetaldehyde measured after γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 5.0×10^{-4} M peptide at various doses.

additionally to the formation of acetaldehyde (CH_3CHO) with the high yield of $G = 3.3 \pm 0.2$ (60% related to $G^{\circ\prime}(HO^\bullet)$; Figure 1, curve d). This product is not detected in the Val-Met system (Figure 1, curve e) and in any other of the investigated peptide systems. The γ-irradiation of N_2O/O_2 (4:1, v/v) saturated aqueous solutions, pH 6.0, containing 5×10^{-4} M Thr-Met leads to the formation of acetaldehyde with $G = 1.60 \pm 0.3$, i.e. a yield being lower by 50% compared to the deoxygenated (N_2O-saturated) system. Considerably less acetaldehyde is quantified after γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 5×10^{-4} M Gly-Thr-Met ($G < 0.6$) or Gly-Met-Thr ($G < 0.4$), respectively.

B. Aldehyde Formation as a Function of Peptide Concentration. The final yields of formaldehyde, measured as a function of peptide concentration, after γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing Ser-Met or Ala-Met at concentrations between 1.5×10^{-4} and 2.0×10^{-3} M, respectively, are illustrated in Figure 2a. Whereas the yields derived from Ala-Met hardly show any variation with changing peptide concentration, the radiation chemical yields of formaldehyde from Ser-Met *increase* progressively upon decreasing the initial peptide concentration from 2.0×10^{-3} M ($G_{H_2CO} = 0.42 \pm 0.05$) to 1.5×10^{-4} M ($G_{H_2CO} = 1.1 \pm 0.1$).

A behavior similar to that for Ser-Met is observed for the yields of acetaldehyde derived from Thr-Met which increase progressively upon decreasing the initial concentration of peptide from 2.0×10^{-3} M ($G_{CH_3CHO} = 1.77 \pm 0.2$) to 1.5×10^{-4} M ($G_{CH_3CHO} = 3.5 \pm 0.3$) (Figure 2b).

C. Aldehyde Formation as a Function of pH. The final yields of formaldehyde and acetaldehyde, measured after γ-irradiation of N_2O-saturated aqueous solutions at various pH values between 2.0 and 8.0, containing 5.0×10^{-4} M Ser-Met or Thr-Met, are shown in Figure 3, curves a and b, respectively. Taking into account the competition between reactions 5 and 6, all final formaldehyde yields were normalized to the total initial radiation chemical yield of hydroxyl radicals, $G^{\circ\prime}(HO^\bullet)$, and are given as efficiencies f ($f = G_{aldehyde}/G^{\circ\prime}(HO^\bullet) = G_{aldehyde}/[G^\circ(HO^\bullet) + G^\circ(e_{aq}^-)\{k_5[N_2O]/k_5[N_2O] + k_6[H^+]\}]$). A progressive increase of the efficiency of aldehyde formation from Ser-Met and Thr-Met with increasing pH is observed on going from pH 3.0 to 8.0. Irradiations at pH > 8.0 have not been carried out in order to

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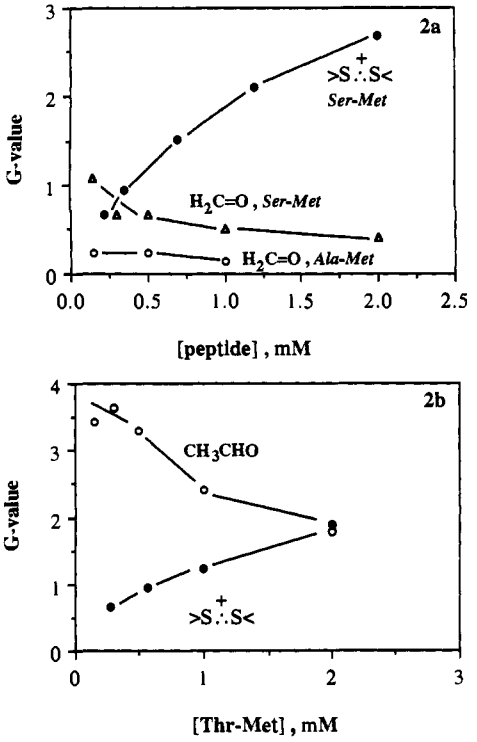

Figure 2. (a) Radiation chemical yields (*G*-values) of formaldehyde obtained after γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing various concentrations of Ser-Met (△) and Ala-Met (○) and radiation chemical yield of $[>S.\therefore S<]^+$-type adducts measured upon pulse irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing various concentrations of Ser-Met (●). (b) Radiation chemical yields of acetaldehyde obtained after γ-irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing various concentrations of Thr-Met (○), and radiation chemical yield of $[>S.\therefore S<]^+$-type adducts measured upon pulse irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing various concentrations of Thr-Met (●).

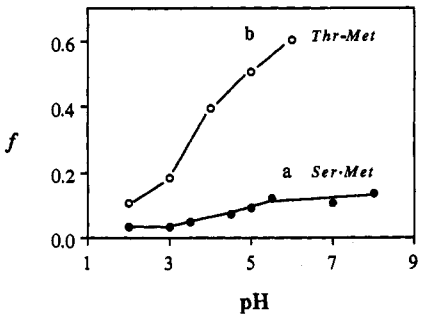

Figure 3. Efficiency *f* of aldehyde formation upon γ-irradiation of N_2O-saturated aqueous solutions at various pH's containing 5.0×10^{-4} M Ser-Met (formaldehyde; a) or Thr-Met (acetaldehyde; b), respectively. For calculation of *f* see text.

avoid complications arising from the additional reaction of hydroxyl radicals with the deprotonated N-terminal amino group of the peptides (N-terminal amino groups of dipeptides show pK_a-values on the order of 8.2[27]).

3. Formation of Acetaldehyde through One-Electron Oxidation of Thr-Met by $SO_4^{\bullet-}$. Generally, the initial step in the reaction of the hydroxyl radical with an organic sulfide constitutes an addition to the sulfur (reaction 1), followed by the decomposition of this adduct into hydroxide ion or water, and a sulfur-centered radical cation (see also Scheme 4; reactions 29 and 30).[5,9-11] Thus, in principle, both intermediates, the hydroxyl radical adduct or the subsequently formed sulfur-centered radical cation, might be responsible for the formation of the aldehydes from the peptides.

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The thioether radical cation induced formation of acetaldehyde from Thr-Met[28] can be investigated employing a typical one-electron oxidant such as $SO_4^{\bullet-}$.

The radiolysis of an N_2-saturated aqueous solution, pH 5.5, containing 5.0×10^{-3} M $S_2O_8^{2-}$ and 0.5 M *tert*-butyl alcohol leads to the formation of $SO_4^{\bullet-}$ according to reaction 8 ($k_8 = 1.2 \times 10^{10}$ M^{-1} s^{-1} [26]) while the hydroxyl radicals are scavenged by *tert*-butyl alcohol (reaction 9, $k_9 = 5.0 \times 10^8$ M^{-1} s^{-1} [26]). The $SO_4^{\bullet-}$ radical reacts via electron transfer[29] with organic thioethers (reaction 10a; for methionine at pH 7.0: $k_{10} = 1.1 \times 10^9$ M^{-1} s^{-1} [30]) and with Thr-Met (reaction 10b; see *pulse radiolysis*) whereas it abstracts hydrogen atoms from *tert*-butyl alcohol (reaction 11, $k_{11} \approx 6.0 \times 10^5$ M^{-1} s^{-1} [30]).

$$e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{2-} + SO_4^{\bullet-} \quad (8)$$

$$HO^\bullet + CH_3C(CH_3)_2OH \rightarrow H_2O + {}^\bullet CH_2C(CH_3)_2OH \quad (9)$$

$$SO_4^{\bullet-} + >S \rightarrow SO_4^{2-} + >S^{\bullet+} \quad (10a)$$

$$SO_4^{\bullet-} + \text{Thr-Met}(>S) \rightarrow SO_4^{2-} + \text{Thr-Met}(>S^{\bullet+}) \quad (10b)$$

$$SO_4^{\bullet-} + CH_3C(CH_3)_2OH \rightarrow H^+ + SO_4^{2-} + {}^\bullet CH_2C(CH_3)_2OH \quad (11)$$

After addition of 1.0×10^{-3} M Thr-Met to an N_2-saturated aqueous solution, pH 5.5, containing 5.0×10^{-3} M $S_2O_8^{2-}$ and 0.5 M *tert*-butyl alcohol, a radiation chemical yield of acetaldehyde, $G_{\text{acetaldehyde}} = 0.4 \pm 0.03$, is obtained. Taking an initial yield of hydrated electrons of $G^\circ(e_{aq}^-) = 2.75$ and $k_{10a} = 1.1 \times 10^9$ M^{-1} s^{-1}, standard competition kinetics predict that the one-electron oxidation of Thr-Met should theoretically occur with an initial radiation chemical yield of $G = 2.12$. This was separately confirmed by time-resolved pulse radiolysis experiments which gave a radiation chemical yield of one-electron-oxidized Thr-Met of $G = 2.10$ (vide infra: *pulse radiolysis*). Division of the measured acetaldehyde yield by the latter value yields an efficency of acetaldehyde formation of $f = 0.19$. In contrast, the hydroxyl radical induced formation of acetaldehyde from Thr-Met (1.0×10^{-3} M) at pH 5.5 occurs with $f = 0.45$.

These results show that some acetaldehyde is indeed formed through one-electron oxidation of Thr-Met by $SO_4^{\bullet-}$. However, significantly higher amounts are derived from the hydroxyl radical induced process. The only difference between the $SO_4^{\bullet-}$ and the hydroxyl radical induced oxidation is that the latter reaction involves the direct formation of the well-characterized hydroxy sulfuranyl radical.[5,9-11] The significant higher acetaldehyde yield from the hydroxyl radical initiated process is, therefore, ascribed to the subsequent reaction of a hydroxy sulfuranyl radical.

(28) It has been found (Bobrowski, K.; Schöneich, Ch. Unpublished results) that α-(alkylthio) methyl radicals (which are likely products from the deprotonation of peptide sulfur-centered radical cations) can reduce $S_2O_8^{2-}$. Such process would lead to the formation of α-(alkylthio)carbocations which subsequently add HO^- to form the corresponding thiohemiacetal. The latter decomposes into free thiol and formaldehyde and might lead to an erroneous interpretation of the total formaldehyde yields. Such processes can by no means generate acetaldehyde in the peptide systems under investigation.

(29) The reaction of $SO_4^{\bullet-}$ radicals with 2,2'-thiodiethanoic acid leads stoichiometrically to decarboxylation of this acid (Bobrowski, K.; Schöneich, Ch. Unpublished results). This process has been shown to involve initial one-electron oxidation of the sulfur followed by subsequent electron transfer from the carboxylate moiety to the sulfur-centered radical cation.[44] In contrast, hydrogen abstraction from the α-(alkylthio) C–H bond did not result in the formation of CO_2 which, in turn, identifies the $SO_4^{\bullet-}$ radical as a one-electron oxidant of organic thioethers.

(30) Neta, P.; Huie, R. E.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 1027.

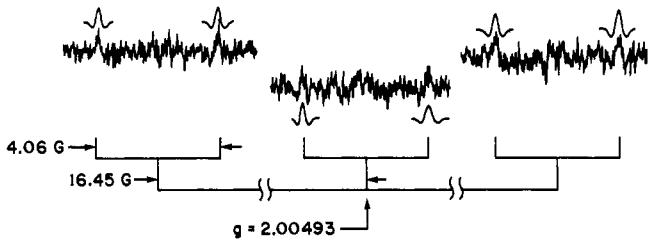
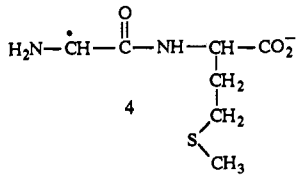


Figure 4. Second-derivative X-band (9.2-GHz) in situ radiolysis ESR spectrum of radical **4** derived from Thr-Met, showing absorption lines, best fit second-derivative Gaussian lines (above or below experimental spectrum), and stick diagram showing nitrogen hyperfine coupling (16.45 G) and proton hyperfine coupling (4.06 G). The *g*-factor is 2.004 93. The lines represent intensity for two transitions of the Thr-Met ESR spectrum; unit intensity lines are below the noise floor and are not directly observed.

4. ESR Spectroscopy. A possible heterolytic side chain cleavage of a nitrogen-centered radical cation[31] at the peptide N-terminus may yield the respective aldehydes and peptide-C_α-centered radicals of the general structure **4**[32] (see also Scheme 3, reactions 18–22; in the following, the $-CH(CO_2^-)CH_2CH_2-SCH_3$ moiety of the Met residue is denoted as *met*).

ESR spectroscopic measurements have been carried out in order to identify radical **4**. Since the expected yields of **4** are



higher in the Thr-Met system than in the Ser-Met system (compare the aldehyde yields), these studies were representatively carried out for the oxidation of Thr-Met. The ESR spectrum recorded during continuous irradiation of an N_2O-saturated aqueous solution, pH 6.2, containing 2.0×10^{-3} M Thr-Met is shown in Figure 4. The strong features of the spectrum are a 1:1:1 triplet of doublets, indicating the interaction of the unpaired electron with a nitrogen nucleus, $a(N,NH_2) = 16.45 \pm 0.07$ G, and a proton, $a(H,CH) = 4.06 \pm 0.07$ G. The spectral center corresponds to a *g*-factor of $2.004\,93 \pm 0.000\,03$. This very high *g*-factor indicates that a very high percentage of the unpaired spin population resides on an oxygen atom (in nonpolar solvents a larger spin population would be expected to reside on the methylenic carbon[33]). The hyperfine couplings and the *g*-factor lead to an assignment of the observed spectrum to $H_2N-C^{\bullet}H-C(=O)R$, i.e. the structure of radical **4**. There should also be finite hyperfine couplings to the two inequivalent protons on the terminal nitrogen, but the relatively poor spectral signal to noise ratio necessitated by the low sample concentration obscures the outer lines of the resulting quartet.

5. Formation of Gly-Met through the Hydroxyl Radical Induced Oxidation of Ser-Met in the Presence of Dithiothreitol. The ESR experiments positively identify the formation of radical **4** in the course of the hydroxyl radical induced oxidation of Thr-Met. Similarly, radical **4** can be expected from the oxidation of Ser-Met, but with much lower yield. Abstraction of hydrogen by **4** from any appropriate hydrogen donor would then lead to the formation of Gly-Met.

The γ-irradiation of an N_2O-saturated aqueous solution, pH 5.5, containing 1.0×10^{-3} M Ser-Met does not lead to the formation of Gly-Met. This seems yet not surprising, since the formation of Gly-Met from radical **4** would require a disproportionation (reaction 12), followed by the protonation of the product carbanion (reaction 13). However, in competition to this disproportionation (reaction 12), the radicals **4** might as well recombine to yield peptide dimers.

$$2\,\mathbf{4} + 2\,H^+ \rightarrow {}^+H_3N-C^{(-)}H-CONH-\textit{met} + {}^+H_3N-C^{(+)}H-CONH-\textit{met} \quad (12)$$

$${}^+H_3N-CH^{(-)}-CONH-\textit{met} + H^+ \rightarrow {}^+H_3N-CH_2-CONH-\textit{met} \quad (13)$$

The irradiation of the above system in the presence of (1.0–3.0) $\times 10^{-4}$ M dithiothreitol [DTT; $HS-CH_2CH(OH)CH(OH)-CH_2-SH$] results in the formation of Gly-Met, showing increasing Gly-Met yields with increasing concentrations of DTT. Employing 3.0×10^{-4} M DTT, a final yield of Gly-Met of $G = 0.1$ is obtained. Regarding that in these systems the hydroxyl radicals react with both solutes, Ser-Met ($k \approx 1.0 \times 10^{10}$ M^{-1} s^{-1} [34]) and DTT ($k = 1.5 \times 10^{10}$ M^{-1} s^{-1} [35]), the fraction of hydroxyl radicals reacting with Ser-Met amounts to $G^{\circ\prime}(HO^{\bullet}) = 0.69 \times 5.5 = 3.8$. Thus, the efficiency of Gly-Met formation from Ser-Met in the presence of 3.0×10^{-4} M DTT amounts to $f = 0.024$, i.e. a value being lower by 75% than the efficiency of formaldehyde formation from Ser-Met in the absence of DTT ($G = 0.52 \pm 0.05$; $f = 0.094$). This deviation is likely caused by the fact that radical **4** does not react quantitatively with DTT (reaction 14). This is already indicated by the yield of Gly-Met being dependent on the employed concentration of DTT and can further be rationalized by the analysis of the structure of radical **4**.

$$\mathbf{4} + DTT \rightarrow {}^+H_3N-CH_2-CONH-\textit{met} + DTT(-H^{\bullet}) \quad (14)$$

The radical **4** is of the type D–C•H–A, in which D denotes an electron-donating substituent (the α-amino group) and A denotes an electron-accepting substituent (the amide group). Such structures are highly stabilized through the combined effect of both substituents[36] and have often been discussed in terms of the so-called capto-dative effect.[37] Calculations performed for a structure closely related to **4**, the $c\text{-}C_5H_{10}N-C^{\bullet}H-C(=O)-Ph$ radical, have revealed that the latter exhibits a radical stabilization energy (RSE) of 33 kcal/mol as compared to that of the reference $^{\bullet}CH_3$ radical.[36] From the comparison of another series of derivatives, $PhS-C^{\bullet}H-C(=O)R$ with R = OEt (RSE = 19 kcal/mol), R = Me (RSE = 21 kcal/mol), and R = Ph (RSE = 23.5 kcal/mol),[36] it evolves that the variation of R does not drastically affect the total RSE of these molecules as long as the carbonyl function is preserved. Thus, at maximum, also the

(31) (a) Ci, X.; Whitten, D. G. In *Photoinduced Electron Transfer. Part C*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, The Netherlands, 1988; pp 553–577. (b) Ci, X.; Lee, L. Y. C.; Whitten, D. G. *J. Am. Chem. Soc.* **1987**, *109*, 2536–2538. (c) Ci, X.; Whitten, D. G. *J. Am. Chem. Soc.* **1987**, *109*, 7215–7217. (d) Ci, X.; Whitten, D. G. *J. Am. Chem. Soc.* **1989**, *111*, 3459–3461. (e) Ci, X.; Kellet, M. A.; Whitten, D. G. *J. Am. Chem. Soc.* **1991**, *113*, 3893–3904. (f) Haugen, C. M.; Bergmark, W. R.; Whitten, D. G. *J. Am. Chem. Soc.* **1992**, *114*, 10293–10297. (g) Lucia, L. A.; Burton, R. D.; Schanze, K. S. *J. Phys. Chem.* **1993**, *97*, 9078–9080.

(32) It has been demonstrated that the pK_a of the amino group in α-amino-substituted carbon-centered radicals is located around 3.85.[42] On the other hand, the pK_a of the N-terminal amino groups of dipeptides which do not contain α-amino-substituted C_α-radical centers is generally located at pH > 8.[27] Therefore, the amino group of all α-amino-substituted carbon-centered radicals formed under the experimental conditions of pH > 5 is generally displayed in the deprotonated state whereas the amino group of dipeptides which do not contain α-amino-substituted C_α-radical centers is shown in the protonated state.

(33) MacInnes, I.; Walton, J. C.; Nonhebel, D. C. *J. Chem. Soc., Chem. Commun.* **1985**, 712–713.

(34) Hiller, K.-O.; Masloch, B.; Göbl, M.; Asmus, K.-D. *J. Am. Chem. Soc.* **1981**, *103*, 2734–2743.

(35) Zhang, N.; Schuchmann, H.-P.; von Sonntag, C. *J. Phys. Chem.* **1991**, *95*, 4718–4722.

(36) Bordwell, F. G.; Zhang X.-M.; Alnajjar, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 7623–7629.

(37) Sustmann, R.; Korth, H.-G. *Adv. Phys. Org. Chem.* **1990**, *26*, 131–178.

hypothetical substitution of R = Ph by R = OMe in the c-$C_5H_{10}N$—C•H—C(=O)—Ph radical might reduce its RSE by only ca. 5 kcal/mol, and an effect of similar size might be expected for the substitution of R = Ph by R = NH-*met*. These considerations suggest that the carbon-centered radical **4** might as well have a relatively large RSE on the order of 25 ± 5 kcal/mol. An average RSE of similar magnitude, RSE ≈ 25 kcal/mol,[38] was determined for the pentadienyl radical, H_2C=CH—C•H—CH=CH_2. The latter does not react with thiols,[39,40] and by analogy, it cannot be expected that the radical **4** does efficiently abstract H-atoms from DTT.

No formation of Gly-Met was detected at pH 5.5 when 3.0×10^{-4} M ascorbate, an electron donor, instead of DTT was added to the Ser-Met system. This may reflect that, although, by analogy to •CH_2—CH(=O),[41] the •C_αH—C(=O)—NH-*met* moiety in **4** might be an oxidant per se, this propensity is compensated by the additional presence of the reducing[42] H_2N—•C_αH moiety.

Thus, at present, we shall not attempt a more detailed quantitative discussion of the obtained yields of Gly-Met in the presence of DTT. As an important result, Gly-Met has been qualitatively identified in the hydroxyl radical induced oxidation of Ser-Met in the presence of an H-atom donor.

6. Pulse Radiolysis. A. Optical Absorption Spectra of Long-Lived Radical Species. Identification of Transients. Parts a and b of Figure 5 display the optical absorption spectra obtained 60 μs after pulse irradiation of N_2O-saturated aqueous solutions, pH 5.5, containing 2.0×10^{-4} M Ala-Met or Ser-Met, respectively. They show distinct absorption maxima with λ_{max} = 283 ± 2 nm which are characteristic for α-(alkylthio)alkyl radicals, R–•CH–S–CH_2–R′.[11,43,44] Their radiation chemical yields, expressed as $G\epsilon_{283}$, are higher in the Ala-Met system ($G\epsilon_{283} \approx 13\,000$ M^{-1} cm^{-1}) than in the Ser-Met system ($G\epsilon_{283} \approx 11\,500$ M^{-1} cm^{-1}). Taking $\epsilon_{285} = 2500 \pm 500$ M^{-1} cm^{-1},[11,43,44] the radiation chemical yields for α-(alkylthio)alkyl radicals amount to G = 5.2 for Ala-Met and G = 4.6 for Ser-Met.

The pulse irradiation of an N_2O-saturated aqueous solution, pH 5.9, containing 2.0×10^{-4} M Thr-Met does not lead to the formation of the 283-nm maximum but rather to a broad uncharacteristic absorption band which shows no distinct λ_{max} at wavelenghts > 255 nm (Figure 5c). Its radiation chemical yield at 260 nm amounts to $G\epsilon_{260} = 12\,000$ M^{-1} cm^{-1}. Such spectra have been identified for a variety of α-amino-substituted radicals of the general structure RHN–•CH–R′.[42,45] Under similar experimental conditions the formation of radical H_2N–C•H–CONH-*met* (**4**) was positively identified by ESR (vide supra), and therefore, we tentatively assign the observed UV spectrum to this radical.

At pH 3 considerably less acetaldehyde was formed during the γ-irradiation of Thr-Met, and consequently we do not expect to observe high yields of such α-amino-substituted radicals at this particular pH. This was confirmed by pulse irradiation of an N_2O-saturated aqueous solution, pH 2.9, containing 2.0×10^{-4} M Thr-Met. In this system a distinct 285-nm band (Figure 5d) is observed which indicates the presence of α-(alkylthio)alkyl radicals, formed at the expense of α-amino-substituted radicals. The α-(alkylthio)alkyl radicals subsequently disappear with second-order kinetics with a first half-life of $t_{1/2}$ > 180 μs.

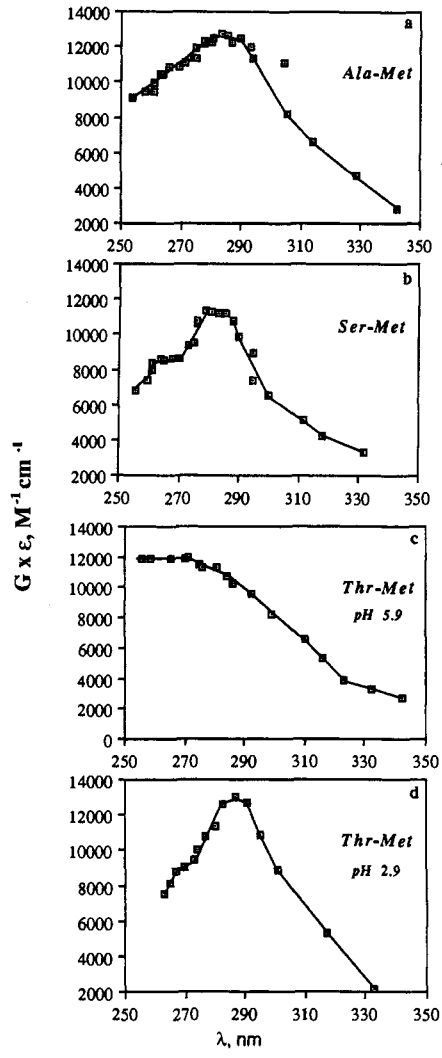


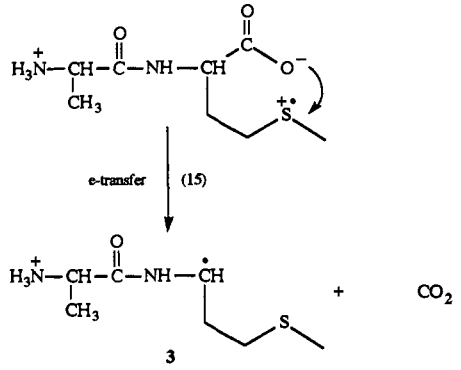
Figure 5. Optical spectra derived 60 μs after pulse irradiation of N_2O-saturated aqueous solutions containing (a) 2.0×10^{-4} M Ala-Met at pH 5.5, (b) 2.0×10^{-4} M Ser-Met at pH 5.8, (c) 2.0×10^{-4} M Thr-Met at pH 5.9, and (d) 2.0×10^{-4} M Thr-Met at pH 2.9.

Since the hydroxyl radical induced oxidation of 2-(methylthio)-ethanol leads to the exclusive formation of α-(alkylthio)alkyl radicals,[11] this thioether derivative can serve as a reference for the spectra of the α-(alkylthio)alkyl radicals formed in the peptide solutions. The UV spectrum obtained in the 2-(methylthio)-ethanol system exhibits the characteristic 285-nm band which sharply decreases on both sides of the maximum, showing a ratio of $r = (G\epsilon_{285})/(G\epsilon_{260}) = 2.0$. A similar ratio of $r = (G\epsilon_{285})/(G\epsilon_{260}) = 2.0$ is also obtained upon pulse irradiation of Thr-Met at pH 2.9, revealing that here the α-(alkylthio)alkyl radical constitutes the only abundant species after the pulse. In contrast, these ratios appear much lower for the pulse irradiation of Ala-Met at pH 5.5 (r = 1.28; Figure 5a) and Ser-Met at pH 5.9 (r = 1.33; Figure 5b), indicating the presence of an additional shoulder in the wavelength region between 255 and 280 nm. A comparison with the Thr-Met system at pH 5.9 (Figure 5c) suggests that this shoulder might be caused by the presence of α-amino-substituted radicals.

B. Quantification of Transients. At first we shall rationalize the presence of α-amino-substituted radicals in the Ala-Met system. It has been shown earlier[45] that the hydroxyl radical induced oxidation of Ala-Met yields sulfur-centered radical cations which subsequently decompose via deprotonation into α-(alkyl-

(38) Clark, K. B.; Culshaw, P. N.; Griller, D.; Lossing, F. P.; Simoes, J. A. M.; Walton, J. C. *J. Org. Chem.* **1991**, *56*, 5535–5539.
(39) (a) Schöneich, Ch.; Asmus, K.-D.; Dillinger, U.; von Bruchhausen, F. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 113–120. (b) Schöneich, Ch.; Dillinger, U.; von Bruchhausen, F.; Asmus, K.-D. *Arch. Biochem. Biophys.* **1992**, *292*, 456–467.
(40) Von Sonntag, C. In *Sulfur-centered reactive intermediates in chemistry and biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; NATO ASI Series, Series A: Life Sciences; Plenum Press: New York, 1990; Vol. 197, pp 359–366.
(41) Akhlaq, M. S.; Al-Baghdadi, S.; von Sonntag, C. *Carbohydr. Res.* **1987**, *164*, 71–83.
(42) Hiller, K.-O.; Asmus, K.-D. *J. Phys. Chem.* **1983**, *87*, 3682–3688.
(43) Hiller, K.-O.; Asmus, K.-D. *Int. J. Radiat. Biol.* **1981**, *40*, 597–604.
(44) Bobrowski, K.; Pogocki, D.; Schöneich, Ch. *J. Phys. Chem.* **1993**, 97, 13677–13684.
(45) Bobrowski, K.; Schöneich, Ch.; Holcman, J.; Asmus, K.-D. *J. Chem. Soc., Perkin Trans. 2* **1991**, 353–362.

Scheme 2



thio)alkyl radicals (vide infra) and via intramolecular electron transfer into CO_2 and α-N-acyl-substituted radicals, R—C(=O)NH—•CH—R′ (**3**; Scheme 2; reaction 15). The latter pathway occurs to an extent of ca. 18% (G_{CO_2} = 1.0),[45] yielding an α-amino-type radical which most probably causes the shoulder between 255 and 280 nm, observed upon pulse radiolysis of Ala-Met. A quantitative evaluation of the respective product yields in the Ala-Met system has to take into account an initial yield of hydroxyl radicals of $G°'(HO•)$ = 5.5 and an initial yield of hydrogen atoms of $G°(H•)$ = 0.6. In their reaction with thioethers hydrogen atoms yield α-(alkylthio)alkyl radicals (reaction 7). Subtraction of the initial yield of hydrogen atoms, $G(H•)$ = 0.6, from the total yield of α-(alkylthio)alkyl radicals obtained upon pulse radiolysis of Ala-Met, G = 5.2, yields a radiation chemical yield of G = 4.6 for the formation of α-(alkylthio)alkyl radicals through the reaction of hydroxyl radicals. Further subtraction of G = 4.6 from $G°'(HO•)$ = 5.5 yields G = 0.9 for the amount of hydroxyl radicals which do not react under formation of α-(alkylthio)alkyl radicals. This value corresponds well to the radiation chemical yield of CO_2 (G = 1.0) formed according to reaction 15.[45]

The C-terminal decarboxylation pathway has been shown to occur with much lower efficiency for Ser-Met (G_{CO_2} = 0.5).[45] Nevertheless, the shoulder between 255 and 280 nm is formed in almost similar quantities for Ser-Met (Figure 5b) as for Ala-Met (Figure 5a). Consequently, α-amino-substituted radicals, derived from the Ser-Met system, must originate from a different source and it seems reasonable that in the Ser-Met system the shoulder between 255 and 280 nm indicates the presence of radical **4**. The total yield of α-(alkylthio)alkyl radicals formed in the Ser-Met system amounts to G = 4.6. Subtraction of $G°(H•)$ = 0.6 gives the radiation chemical yield of G = 4.0, representative for the formation of α-(alkylthio)alkyl radicals through the reaction of hydroxyl radicals with Ser-Met. Further subtraction of G = 4.0 from $G°'(HO•)$ = 5.5 leaves a residual amount of G = 1.5 for hydroxyl radicals which do not react with Ser-Met to yield α-(alkylthio)alkyl radicals. This value, G = 1.5, corresponds well to the combined radiation chemical yields of the C-terminal decarboxylation (G_{CO_2} = 0.5)[45] and the formation of formaldehyde (G_{H_2CO} = 1.1) (vide supra).

For the quantification of α-amino-substituted radicals derived from Thr-Met, we shall compare the oxidation of Thr-Met with the hydroxyl radical induced oxidation of γ-Glu-Met.[16] A final spectrum identical to that shown for Thr-Met in Figure 5c was obtained for γ-Glu-Met[16] with the radiation chemical yield of $(G\epsilon_{260})_{\gamma\text{-Glu-Met}}$ = 11 000 M^{-1} cm^{-1}. In the latter system decarboxylation yielding α-amino-type radicals occurred both from the N-terminus and the C-terminus with the combined radiation chemical yield of G_{CO_2} = 4.3 ± 0.3.[16] Dividing $(G\epsilon_{260})_{\gamma\text{-Glu-Met}}$ by G_{CO_2} yields an extinction coefficient for α-amino-type radicals of ϵ_{260} = 2560 M^{-1} cm^{-1}. The radiation chemical yield of α-amino-type radicals derived from the oxidation of Thr-Met can accordingly be calculated to $G_{\alpha\text{-amino,Thr-Met}}$ = 4.7 by division of $(G\epsilon_{260})_{\text{Thr-Met}}$ = 12 000 M^{-1} cm^{-1} (Figure 5c) by ϵ_{260} = 2560 M^{-1} cm^{-1} [in both systems the minor contribution of H-atom-derived α-(alkylthio)alkyl radicals (G = 0.6) contributes to the same extent to the final spectra and can, therefore, be neglected]. For Thr-Met the C-terminal decarboxylation analogous to reaction 15 was shown to occur with G = 1.3.[45] Subtraction of G = 1.3 from G = 4.7 leaves a residual radiation chemical yield of G = 3.4 for the formation of α-amino-type radicals via other processes. This value corresponds well to the radiation chemical yield of $G_{\text{acetaldehyde}}$ = 3.5 ± 0.2 (for 1.5 × 10^{-4} M Thr-Met, pH 5.5).

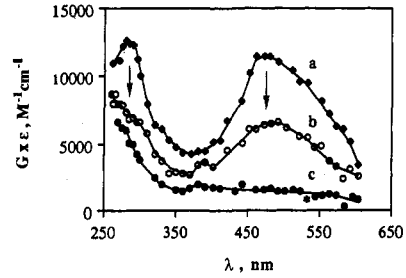


Figure 6. Optical spectra obtained (a) 2.5 μs, (b) 17.5 μs, and (c) 69.5 μs after pulse irradiation of an N_2O-saturated aqueous solution, pH 5.9, containing 2.0 × 10^{-3} M Thr-Met.

Thus, we have accounted for 100% of the products related to the initial yield of hydroxyl radicals. The radiation chemical yield of consumed peptides in all these experiments was determined by HPLC to be G = 6.1 ± 1.5. This value corresponds well to the combined amount of hydroxyl radicals and hydrogen atoms reacting with the respective peptides. The relatively high error results from the fact that, at maximum, 10–15% of the peptide was consumed at all (see above). Thus, any relation of radiation chemical products to the amount of consumed peptide must be reported with this error. However, we prefer to relate all observed products to the initial amount of hydroxyl radicals, since only such material balance gives the true yield of products per initiating radical species.

C. Formation of Dimeric Sulfur Radical Cations via Hydroxyl Radical Attack. The one-electron oxidation of thioethers leads to the formation of sulfur-centered radical cations.[5,9] At higher thioether concentrations (>5.0 × 10^{-4} M), these monomeric radical cations associate with nonoxidized thioether molecules to yield three-electron-bonded dimeric sulfur radical cations of the general structure $[>S.\therefore S<]^+$ [5,46-48] which absorb at wavelengths around $\lambda_{max} \approx$ 480 nm.

The pulse irradiation of an N_2O-saturated aqueous solution, pH 5.9, containing 2.0 × 10^{-3} M Thr-Met leads to the spectrum shown in Figure 6, curve a, observed 2.5 μs after the pulse. It consists of two distinct bands with λ_{max} = 285 nm ($G\epsilon_{285}$ = 20 480 M^{-1} cm^{-1}) and λ_{max} = 480 nm ($G\epsilon_{480}$ = 11 780 M^{-1} cm^{-1}). The 480-nm band is assigned to the $[>S.\therefore S<]^+$-type adduct formed between an oxidized and an unoxidized Thr-Met peptide. Taking $\epsilon_{480,\text{Thr-Met}}$ = 6540 M^{-1} cm^{-1} (vide infra), the dimeric radical cation is formed with G = 1.80. Upon decreasing the Thr-Met concentrations, keeping otherwise similar experimental conditions, the $[>S.\therefore S<]^+$-type adducts are formed with the decreasing yields of G = 1.22 (1.0 × 10^{-3} M Thr-Met), G = 0.93 (5.6 × 10^{-4} M Thr-Met), and 0.65 (2.8 × 10^{-4} M Thr-Met) (see Figure 2b). Thus, the formation of $[>S.\therefore S<]^+$-type adducts shows a quite opposite trend than that of acetaldehyde. At present, it might be concluded that both products are formed competitively.

The transient absorption spectra obtained 17.5 and 69.5 μs after pulse irradiation of an N_2O-saturated aqueous solution, pH 5.9, containing 2.0 × 10^{-3} M Thr-Met, are shown in Figure 6, curves b and c, respectively. At 69.5 μs after the pulse, the $[>S.\therefore S<]^+$-type adduct, absorbing at 480 nm, is absent, and the residual spectrum is characterized only by the combined absorp-

(46) Asmus, K.-D. *Acc. Chem. Res.* **1979**, *12*, 436–442.
(47) Bobrowski, K.; Holcman, J. *Int. J. Radiat. Biol.* **1987**, *52*, 139–144.
(48) Bobrowski, K.; Holcman, J. *J. Phys. Chem.* **1989**, *93*, 6381–6387.

tion bands of the α-(alkylthio)alkyl radical (shoulder at 280 nm) and the α-amino-type radical. The α-(alkylthio)alkyl radicals originate from the deprotonation of initially formed [>S.∴S<]$^+$-type adducts[49] (via equilibrium with the >S$^{\bullet+}$ monomer; see Discussion). At a Thr-Met concentration of 2.0×10^{-3} M the radiation chemical yield of the α-amino-type radical at 260 nm amounts to $G\epsilon_{260}$ = 8150 M^{-1} cm^{-1}. In contrast, as shown in Figure 5c, the yield of the α-amino radical obtained 60 μs after pulse irradiation of 2.0×10^{-4} M Thr-Met amounts to $G\epsilon_{260}$ = 11 500 M^{-1} cm^{-1}. The higher yields of α-amino-type radicals at low Thr-Met concentrations parallel the higher yields of acetaldehyde formed via side-chain fragmentation of the Thr moiety.

A strong absorption band with λ_{max} = 285 nm is observed at 2.5 μs after the pulse (Figure 6, curve a) but has almost disappeared at 17.5 μs after the pulse (Figure 6, curve b). This short-lived species remains yet to be characterized.

A similar trend was observed for Ser-Met. On the basis of $\epsilon_{480,Ser\text{-}Met}$ = 7150 M^{-1} cm^{-1} (vide infra), the following yields of the [>S.∴S<]$^+$-type adducts were obtained (see Figure 2a): G = 2.70 (2.0×10^{-3} M Ser-Met), G = 2.1 (1.2×10^{-3} M Ser-Met), G = 1.5 (6.9×10^{-4} M Ser-Met), G = 0.95 (3.6×10^{-4} M Ser-Met), and G = 0.66 (2.2×10^{-4} M Ser-Met). Again, a decrease in the Ser-Met concentration results in lower radiation chemical yields of the [>S.∴S<]$^+$-type adducts. It is, however, apparent that higher yields of [>S.∴S<]$^+$-type adducts are derived in the Ser-Met system than in the Thr-Met system. Since, on the other hand, higher yields of aldehydes are formed in the Thr-Met system, this observation is consistent with the hypothesis that the [>S.∴S<]$^+$-type adducts are formed in competition to the aldehydes (or their respective precursors).

D. Extinction Coefficients of the [>S.∴S<]$^+$-Type Adducts. Hydroxy sulfuranyl radicals convert stoichiometrically into sulfur-centered radical cations at high proton concentrations[10,11,34] (see also Discussion). Furthermore, the sulfur-centered radical cations stoichiometrically associate with nonoxidized peptides at peptide concentrations of 5.0×10^{-3} M.[34,47] The pulse irradiation of N_2-saturated aqueous solutions, pH 1.0, containing 5.0×10^{-3} M Ser-Met or Thr-Met, respectively, yields [>S.∴S<]$^+$-type adducts with $G\epsilon_{480}$ = 21 450 M^{-1} cm^{-1} for Ser-Met and $G\epsilon_{480}$ = 19 620 M^{-1} cm^{-1} for Thr-Met. At this pH, with a solute concentration of 5.0×10^{-3} M (here the peptide), hydroxyl radicals are theoretically formed with the radiation chemical yield of $G^{o\prime}$(HO$^\bullet$) = 3.0.[25] Division of the measured radiation chemical yields for the [>S.∴S<]$^+$-type adducts by $G^{o\prime}$(HO$^\bullet$) = 3.0 yields $\epsilon_{480,Thr\text{-}Met}$ = 6540 M^{-1} cm^{-1} and $\epsilon_{480,Ser\text{-}Met}$ = 7150 M^{-1} cm^{-1}.

E. Reaction of $SO_4^{\bullet-}$ with Thr-Met. The pulse irradiation of an N_2-saturated aqueous solution, pH 5.5, containing 0.5 M *tert*-butyl alcohol and 5.0×10^{-3} M $S_2O_8^{2-}$ leads to the formation of a transient spectrum with λ_{max} = 390 nm ($G\epsilon_{390}$ = 5250 M^{-1} cm^{-1}) and a broad shoulder around 480 nm at 1.25 μs after the pulse (Figure 7a). A similar 390-nm band has been observed for various organic thioethers having neighboring carboxylate groups[7] and for X-Met[47] and Met-X-Met peptides[48] and has been assigned to a complex between the one-electron-oxidized sulfur and the carboxylate oxygen, >S.∴O—C=O, formed according to reaction 16. The 390-nm band is relatively stable and decays with a half-

$$>S^{\bullet+}\ {}^{-}O—C=O \rightleftharpoons >S.\therefore O—C=O \qquad (16)$$

life of $t_{1/2}$ = 93 μs (Figure 7b). The 480-nm band has been assigned to the dimeric sulfur radical cation as described above. The >S.∴O—C=O complex does not show any residual absorption at 550 nm.[7] At this wavelength a radiation chemical yield of $G\epsilon$ = 1718 M^{-1} cm^{-1} is obtained which can be solely assigned to the formation of the [>S.∴S<]$^+$-type adduct. Inspection of

(49) Mönig, J.; Goslich, R.; Asmus, K.-D. *Ber. Bunsen-Ges. Phys. Chem.* **1986**, *90*, 115–121.

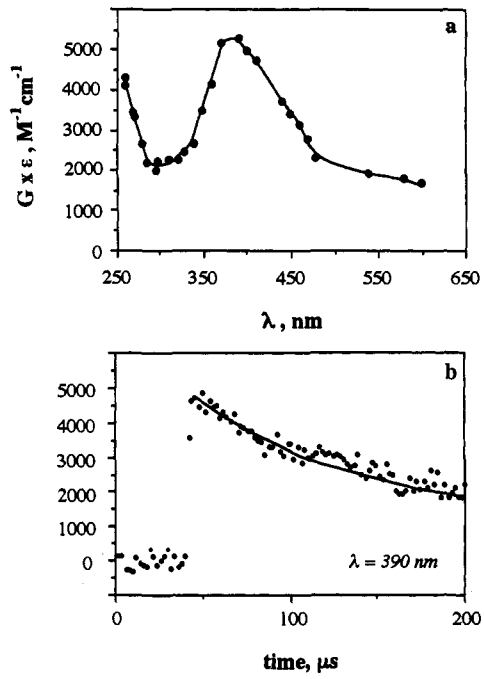


Figure 7. (a) Optical absorption spectrum obtained 1.25 μs after pulse irradiation, and (b) absorption vs time trace, recorded at 390 nm, observed after pulse irradiation of an N_2-saturated aqueous solution, pH 5.5, containing 0.5 M *tert*-butyl alcohol, 5×10^{-3} M $S_2O_8^{2-}$ and 2×10^{-3} M Thr-Met.

the spectrum in Figure 6, curve a, reveals that the extinction coefficient of the [>S.∴S<]$^+$-type adduct at this wavelength amounts to ca. 0.5 × ϵ_{480}, so that we derive $\epsilon_{550,Thr\text{-}Met}$ = 3270 M^{-1} cm^{-1}. Division of $G\epsilon_{550}$ by $\epsilon_{550,Thr\text{-}Met}$ yields G = 0.53 for the [>S.∴S<]$^+$-type adduct. On the other hand, the [>S.∴S<]$^+$-type adduct does not considerably absorb at 390 nm. Taking the extinction coefficient of ϵ_{390} = 3250 M^{-1} cm^{-1},[7b] we derive that the >S.∴O—C=O complex is formed with G = 1.6. The combination of both G-values yields G = 2.13 for the one-electron oxidation of Thr-Met by $SO_4^{\bullet-}$. This value is in a good agreement with the value predicted on the basis of the published rate constants (vide supra).

F. Short-Lived Intermediates during the Reaction of HO$^\bullet$ with Thr-Met and Ser-Met. The UV spectrum obtained 130 ns after the pulse irradiation of an N_2O-saturated aqueous solution, pH 5.9, containing 2×10^{-3} M Thr-Met is shown in Figure 8a. It exhibits one characteristic maximum around λ = 385 nm ($G\epsilon_{385}$ = 8250 M^{-1} cm^{-1}) and a second maximum around λ = 480 nm ($G\epsilon_{480}$ = 6190 M^{-1} cm^{-1}). The 480-nm band is assigned to the [>S.∴S<]$^+$-type adduct which, at this time after the pulse, is yet not completely developed (compare with Figure 6, curve a) (for >S$^{\bullet+}$ + S< → [>S.∴S<]$^+$, $k \approx 2.0 \times 10^9$ M^{-1} s^{-1} [8a]). The decay of the 385-nm band shows biphasic kinetics. A rapid decay occurs with a half-life of $t_{1/2}$ = 320 ns (Figure 8b) followed by a slower decay with $t_{1/2} \approx 100$ μs (not shown). The fast decay at 385 nm is paralleled by a fast buildup of an absorption band with λ_{max} < 260 nm which occurs with $t_{1/2}$ = 317 ns (Figure 8c). The species responsible for the slow decay with $t_{1/2} \approx 100$ μs is most reasonably the >S.∴O—C=O complex ($t_{1/2}$ = 93 μs for the decay of >S.∴O—C=O; vide supra). Consequently, the fast decay at 385 nm cannot be attributed to the reaction of such an >S.∴O—C=O adduct. It has been reported that a short-lived ($t_{1/2} \approx 220$ ns) cyclic [>S.∴NH$_2$]$^+$-type adduct **5** is formed during the hydroxyl radical induced oxidation of methionine.[6,34] This adduct absorbs at λ_{max} = 385 nm and rapidly decomposes with the parallel formation of CO_2 and α-amino-type radicals. A similar cyclic [>S.∴NH$_2$]$^+$-type adduct (**5b**; Scheme 3) is,

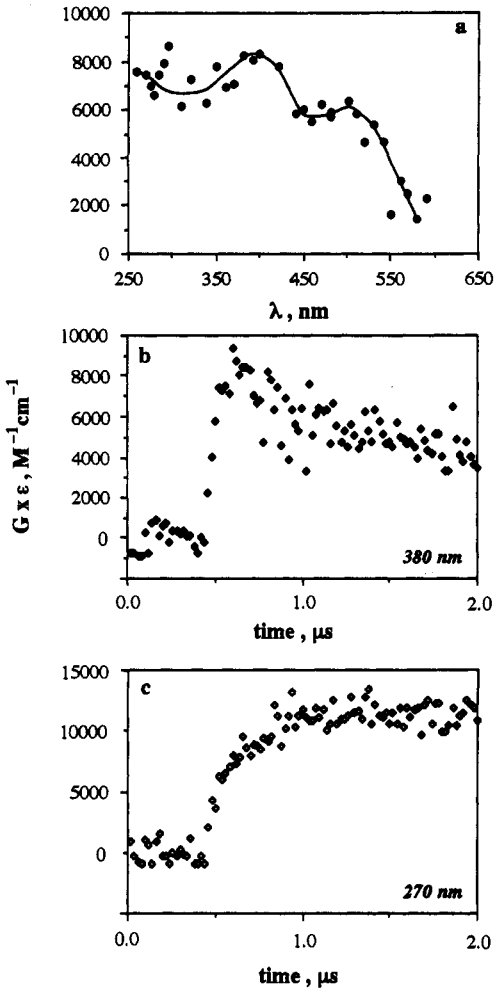
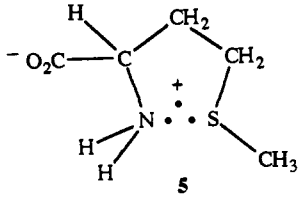


Figure 8. Pulse irradiation of an N_2O-saturated aqueous solution, pH 5.9, containing 2×10^{-3} M Thr-Met: (a) optical absorption spectrum observed 130 ns after the pulse, and absorption vs time traces recorded after the pulse at (b) 380 nm and (c) 270 nm.



5

therefore, the likely intermediate responsible for the short-lived 385-nm band observed during the reaction of hydroxyl radicals with Thr-Met. It decomposes with the parallel formation of α-amino-type radicals, characterized by the buildup of an absorption at λ = 270 nm (Figure 8c) and as evidenced by the ESR experiments.

Discussion

Mechanism. The individual rate constants for the reaction of hydroxyl radicals with the possible target sites of the investigated model peptides besides methionine are not precisely known. They can be approximated by the rate constants for the reaction of HO• with the individual amino acids Ser ($k_{HO•+Ser} = 3.2 \times 10^8$ M^{-1} s^{-1} [26]) and Thr ($k_{HO•+Thr} = 5.1 \times 10^8$ M^{-1} s^{-1} [26]). The rate constant for the attack of hydroxyl radicals on the backbone C_α–H bonds of all dipeptides can be compared with that for Gly-Gly ($k_{HO•+C\alpha-H,Gly-Gly} = 2.4 \times 10^8$ M^{-1} s^{-1} [26]). Thus, taking a rate constant for the addition of hydroxyl radicals to the sulfur of methionine of $k_{16} = 1.0 \times 10^{10}$ M^{-1} s^{-1},[34] it follows that for methionine-containing peptides the methionine moiety is the predominant site of attack (≈93% in Thr-Met and ≈95% in Ser-Met) (reaction 17).

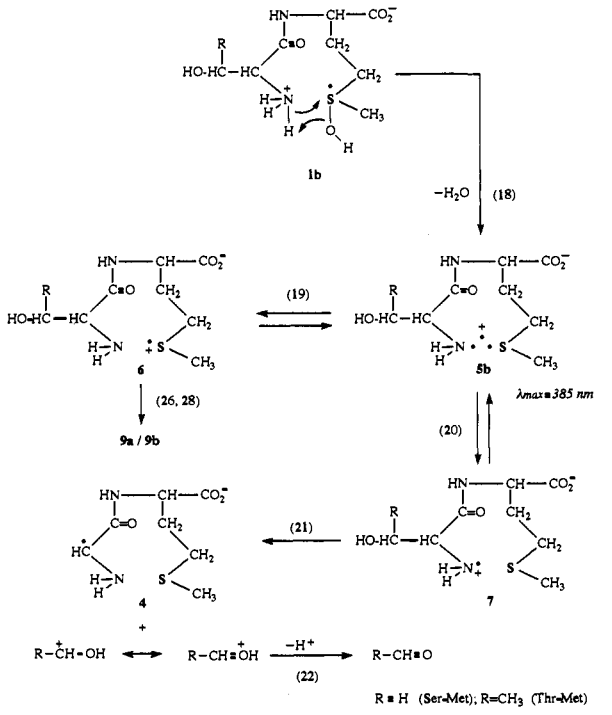
$$\underset{\textbf{1b}}{\text{HO}^\bullet + \text{X-Met} \rightarrow \text{X-Met}(>\text{S}^\bullet\text{-OH})} \qquad (17)$$

Comparing the rate constants for the hydroxyl radical attack at Gly-Leu ($k_{HO•+Gly-Leu} = 1.9 \times 10^9$ M^{-1} s^{-1} [26]) and free Ser ($k_{HO•+Ser} = 3.2 \times 10^8$ M^{-1} s^{-1} [26]), we derive that in Ser-Leu ca. 14% of the hydroxyl radicals are expected to attack the Ser side chain. On the other hand, only 3% of the hydroxyl radicals attack the Ser side chain of Ser-Met, but much higher yields of formaldehyde are formed from this peptide. Thus, a direct attack of the hydroxyl radical at the Ser side chain can be excluded as a cause of the formaldehyde formation in Ser-Met. Moreover, the comparison of the formaldehyde yields of Ser-Met, Ala-Met, and Gly-Ser-Met reveals that an efficient formaldehyde formation requires (i) the hydroxyl substituent of the side chain of Ser-Met and (ii) an N-terminal location of the Ser residue. Low yields of formaldehyde but, instead, high yields of acetaldehyde are formed during the oxidation of Thr-Met. The dipeptide Thr-Met does not contain any other structural element besides the Thr side chain which could constitute a precursor for acetaldehyde.

These results and the corresponding time-resolved measurements are rationalized by invoking reactions 18–22, displayed in Scheme 3.

It has been demonstrated that hydroxy sulfuranyl radicals can decompose via spontaneous elimination of hydroxide ion and via proton-catalyzed elimination of water to yield sulfur-centered radical cations[5,9,11,34] (vide infra). At near-neutral pH the concentration of free protons is so low that the protonated N-terminal amino group constitutes an alternative source for protons. Thus, the initially formed hydroxy sulfuranyl radical at the methionine residue, **1b**, rather undergoes an intramolecular proton transfer from the N-terminal amino group (reaction 18; Scheme 3). The elimination of water yields the short-lived $[S.\therefore N]^+$-three-electron-bonded intermediate **5b**, which has been identified by pulse radiolysis employing nanosecond time resolution (Figure 8a). Its formation suggests that the sulfur-centered radical cation and the deprotonated amino group are formed

Scheme 3



simultaneously in close vicinity, eventually even in a concerted process. This is confirmed by the fact that the formation of sulfur-centered radical cations without parallel deprotonation of the N-terminal amino group does lead neither to the formation of the $[S.\therefore N]^+$-three-electron-bonded intermediate **5b** nor to considerable yields of aldehyde products (see experiments with $SO_4^{\bullet-}$). The intermediate **5b** exists in equilibria with its acyclic derivatives **6** and **7** (eqs 19 and 20; Scheme 3). Species **7** represents a 1,2-amino alcohol radical cation, an intermediate which is particularly prone to either homolytic or heterolytic cleavage of its relatively weak[50] C_α–C_β bond, resulting in the formation of a carbon-centered radical and a cation.[31] The respective homolytic bond cleavage would yield an iminium cation and an α-hydroxy radical (reaction 23) [here the term *peptide* denotes the peptide molecule except the functional groups involved in a chemical process].

$$7 \rightarrow R{-}C^{\bullet}H{-}OH + peptide{-}CH{=}NH_2^+ \quad (23)$$

With this homolytic cleavage prevailing the only process leading to the formation of aldehydes via side-chain cleavage would be a subsequent disproportionation of the resulting α-hydroxy radicals (reaction 24). This would limit the maximum theoretical

$$2R{-}C^{\bullet}H{-}OH \rightarrow R{-}CH_2{-}OH + R{-}CH{=}O \quad (24)$$

yield of aldehydes to $G_{max} = [G°'(HO^{\bullet}) - G(CO_2)]/2$. For Thr-Met the maximum expected aldehyde yield would amount to $G_{max} = [5.5 - 1.3]/2 = 2.1$, a value clearly exceeded by the measured yield of acetaldehyde ($G = 3.5 \pm 0.2$).

It is anticipated that species **7** would rather decompose via the heterolytic bond cleavage according to reaction 21 because of the formation of the highly stabilized radical **4** (compare RSE of **4** of ca. 25 ± 5 kcal/mol and RSE of α-hydroxy radical of ca 5.7 kcal/mol[36]). The resulting carbocation subsequently converts into the respective aldehyde via loss of a proton (reaction 22; Scheme 3). The unambiguous detection of radical **4** by pulse radiolysis coupled to UV and ESR spectroscopy further supports the heterolytic bond cleavage mechanism.

It should be noted that for some model 1,2-amino alcohol radical cations the heterolytic bond cleavage has been found to be sensitive to the respective substituents in the α-position to the hydroxyl substituent.[31e] For example, the formation of methyl-substitued carbocations was more efficient than that of hydrogen-substituted carbocations. This finding can be rationalized by the higher stabilization of the methyl-substituted carbocation and, in turn, would explain our observations that in the peptide systems the acetaldehyde yields from Thr-Met are higher than the formaldehyde yields from Ser-Met. However, such a rationale would imply the existence of at least one other competing mechanism leading to the decomposition of intermediate **7** besides the C_α–C_β cleavage. Two of such irreversible mechanisms can be forwarded. At first, not only the bond dissociation energy (BDE) of the C_α–C_β bond is weakened by the one-electron oxidation of the N-terminal amino group but also the BDE of the C_α–H bond.[31a] The latter can deprotonate according to reaction 25. The resulting

$$H_2N^{\bullet+}{-}CH(peptide){-}CH(R)OH \rightarrow H^+ + H_2N{-}C^{\bullet}(peptide){-}CH(R)OH \quad (25)$$
$$\mathbf{8}$$

α-amino-type radical **8** can be clearly distinguished by ESR spectroscopy from radical **4**, since it lacks the additional α-proton of the peptide backbone. Since radical **8** was not detected in the Thr-Met system, this suggests that reaction 25 does not play any significant role for this peptide.

(50) Camaioni, D. M. *J. Am. Chem. Soc.* **1990**, *112*, 9475–9483.

The second competing mechanism takes into account that species **7**, **5b**, and **6** exist in equilibrium with each other. Species **6** can undergo irreversible deprotonation in the α-position to the sulfur, yielding the α-(alkylthio)alkyl radicals **9a** and **9b**, a process which might benefit from the assistance by the proximate basic amino group (reaction 26). Such a intramolecularly general-

$$6 \rightarrow {}^+H_3N{-}(peptide){-}C^{\bullet}H{-}S{-}CH_3 \;/\; {}^+H_3N{-}(peptide){-}CH_2{-}S{-}{}^{\bullet}CH_2 \quad (26)$$
$$\mathbf{9a} \qquad\qquad \mathbf{9b}$$

$$6 + H_2O \rightarrow {}^+H_3N{-}(peptide){-}CH_2{-}S^{\bullet+}{-}CH_3 + OH^- \quad (27)$$
$$\mathbf{10}$$

base-catalyzed deprotonation in the α-position to the sulfur can, of course, only proceed if the competing protonation of the N-terminal amino group of **6** by the surrounding water medium is comparatively slow (reaction 27). The protonation of the N-terminal amino group in **6** (reaction 27) would prevent any back formation of the intermediate **5b** per se and thus represents a reaction which would compete against the overall process of side-chain fragmentation (reactions 20 and 21; Scheme 3). The further deprotonation of the sulfur-centered radical cation **10** (reaction 28) would be reasonably fast with rate constants for

$$\mathbf{10} \rightarrow \mathbf{9a}/\mathbf{9b} \quad (28)$$

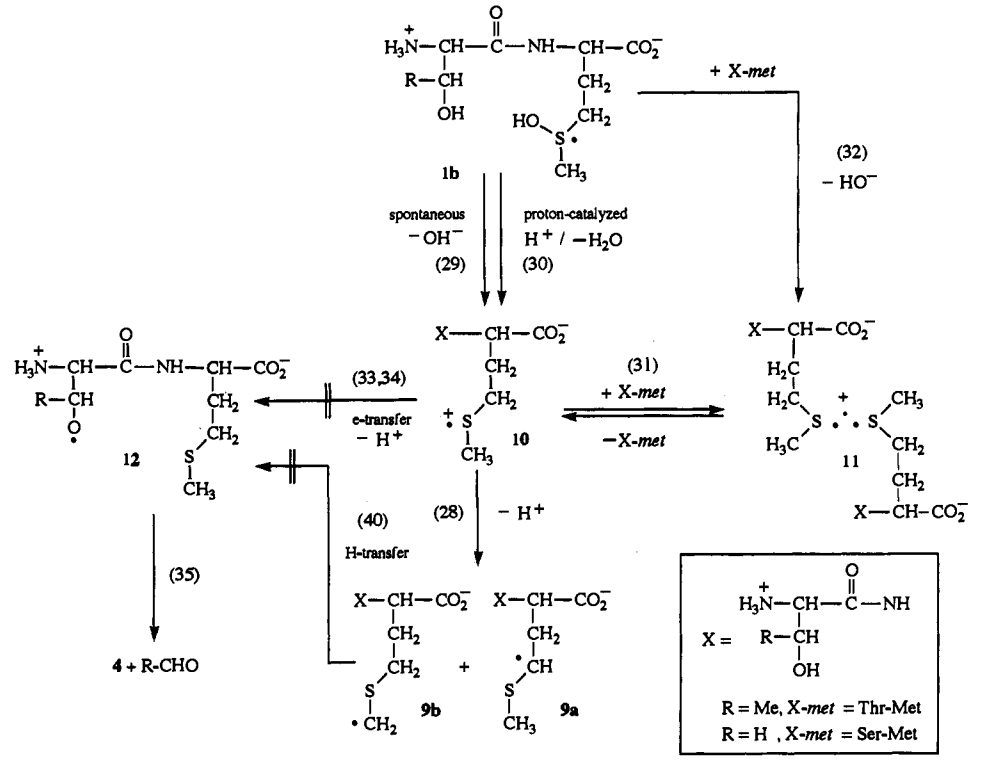
aliphatic sulfides being on the order of $k_{28} \approx 10^5$–10^6 s^{-1}.[49] Accordingly, the efficiency of aldehyde formation will be dependent on the individual rate constants of all these processes and, in particular, on how the C_α–C_β bond fragmentation pathway (reaction 21) can compete against the irreversible deprotonation reaction 25 and, through equilibria 19 and 20, against reactions 26–28, respectively.

Competing Processes Which Do Not Lead to the Formation of the [S.∴N]-Bonded Intermediate 5b. Hydroxy sulfuranyl radicals **1b** can also decompose via several additional routes which have been previously characterized for various unsubstituted and substituted thioether derivatives,[5,9] and several X-Met peptides.[45] The occurrence of these processes, in competition to the proposed proton-transfer route (reaction 18), provides the basis for the observed dependence of the aldehyde yields on both the pH and the peptide concentration and renders further support for the proposed proton-transfer mechanism. These reactions are summarized in Scheme 4.

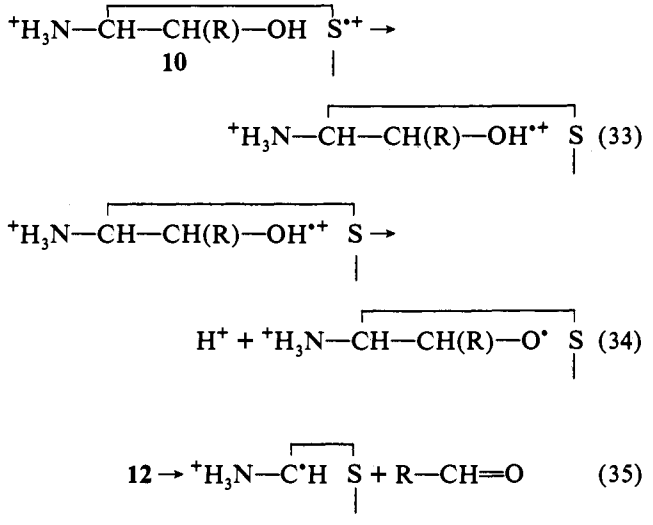
At neutral pH the hydroxy sulfuranyl radical **1b** undergoes spontaneous elimination of hydroxide ion, yielding the monomeric sulfur-centered radical cation **10** (reaction 29; Scheme 4), which cannot associate with the protonated N-terminal amino group, resulting in an $[S.\therefore N]^+$-three-electron-bonded intermediate. However, depending on the actual peptide concentration, the monomeric radical cation **10** associates with a second nonoxidized methionine moiety, according to equilibrium 31, to form the dimeric derivative **11**. Generally the rate constants for unimolecular hydroxide elimination processes are on the order of $k_{29} = (2–6) \times 10^6$ s^{-1}.[5,9,11,44] At pH values > 5 and at low peptide concentrations (vide infra), the unimolecular hydroxide elimination represents the main competitive pathway against the intramolecular proton transfer from the amino group.

At pH values < 5, external proton catalysis competes against both the intramolecular proton catalysis (reaction 18) and the unimolecular hydroxide elimination (reaction 29), leading to the formation of monomeric sulfur-centered radical cation **10** (reaction 30) or, subsequently, its dimeric form **11** (reaction 31). Typical rate constants for the external proton-catalyzed decay of hydroxy sulfuranyl radicals are on the order of 2.0×10^{10}

Scheme 4



$M^{-1}s^{-1}$.[10,11] Consequently, the overall decreased yields of aldehydes derived from Ser-Met and Thr-Met on going from neutral to acidic pH values can be rationalized in terms of the competition between reactions 30 and 18. These results again confirm that the monomeric radical cation **10** or the respective dimer **11**, particularly under acidic conditions, do not induce the C_α–C_β fragmentation, for example via a potential one-electron oxidation of the Ser or Thr side chain and the intermediate formation of alkoxyl radical **12** (reactions 33–35).



We have to rationalize, however, that still some aldehyde formation is observed after one-electron oxidation of the methionine residue at near-neutral pH (e.g., compare the reaction of $SO_4^{\bullet-}$ with Thr-Met). Recently, it has been shown that, particularly at neutral and alkaline pH values, hydroxide ions can react with monomeric and dimeric sulfur-centered radical cations, essentially in the reverse of reactions 29 and 32[51-53] [rate constants for the reaction of HO^- with the intramolecular dimeric radical cation from cyclo-Met-Met and the intermolecular dimeric radical cation from Met were determined to $k = 2.6 \times 10^9$ [52] and 4.3×10^9 M^{-1} s^{-1},[53] respectively]. Thus, the residual yields of acetaldehyde observed in the Thr-Met/$SO_4^{\bullet-}$ system, at pH 5.5, might be caused by some addition of HO^- (or H_2O, followed by liberation of a proton) to **10** or **11**, yielding **1b**, which then can undergo reaction 18. The respective yields of aldehyde formed via this process would then depend on the concentration of hydroxide ion, the rate constant for addition of HO^- to $>S^{\bullet+}$ or $[>S\therefore S<]^+$, and the rate constants for the competitive deprotonation pathways, in particular reaction 28, leading to the irreversible removal of sulfur-centered radical cation **10**. The reaction of hydroxide ions with sulfur-centered radical cations is hampered in acidic solutions.[51,52] Consequently, hardly any residual formation of aldehydes is observed at acidic pH although the proton-catalyzed decay of **1b** yields high amounts of **10**. In accord with this are the experimental observations made during the pulse irradiation of Thr-Met at pH 2.9: The initially formed sulfur-centered radical cations decay solely via deprotonation into α-(alkylthio)alkyl radicals (reaction 28; Figure 5d).

Hydroxy sulfuranyl radicals do not only decay via spontaneous or proton-catalyzed elimination of the hydroxide ions. In addition, a hydroxide ion can also be displaced through the attack of a second unoxidized thioether molecule on **1b**, directly leading to the dimeric sulfur radical cation **11** (reaction 32; Scheme 4).[5,9-11] Typical rate constants for such displacement processes are on the order of $k_{32} \approx 2.0 \times 10^8$ M^{-1} s^{-1}.[11,44] At pH 5.5–5.9, i.e. under conditions when the proton-catalyzed decay of **1b** is negligible (reaction 30), an increase of the starting concentrations of X-Met peptides leads to an increase of the overall yield of dimeric radical cations **11** for both Ser-Met and Thr-Met, respectively. The absolute yields of **11** at a given peptide concentration are higher in the Ser-Met system than in the Thr-Met system. On the other hand, an increase of the yields of **11** is accompanied by a decrease of the overall aldehyde yields in both systems. Furthermore, lower yields of α-amino-type radicals are formed at higher

(51) Schöneich, Ch.; Aced, A.; Asmus, K.-D. *J. Am. Chem. Soc.* **1993**, *115*, 11376–11383.

(52) Holcman, J.; Bobrowski, K.; Schöneich, Ch.; Asmus, K.-D. *Radiat. Phys. Chem.* **1991**, *37*, 473–478.

(53) Bobrowski, K.; Hug, G. L.; Marciniak, B.; Kozubek, H. *J. Phys. Chem.* **1994**, *98*, 537–544.

concentrations of peptide. All these results are in accord with the hypothesis that the reactions leading to the one-electron oxidation of the methionine sulfur at pH < 6 contribute considerably less to the side-chain fragmentation of Ser and Thr. Higher yields of dimeric sulfur radical cations for Ser-Met as compared to Thr-Met correlate well with the general lower efficiency of side-chain fragmentation observed for Ser-Met.

The deprotonation of the monomeric radical cation **10** (reaction 28) can theoretically lead to two different α-(alkylthio)alkyl radicals **9a** and **9b**. Both species can subsequently, on a longer time scale, either dimerize or disproportionate. The disproportionation of **9b** (reaction 36), followed by addition of H^+ and HO^- to the respective product ions (reactions 37 and 38), would hereby lead to a hemithioacetal (reaction 38) which further decomposes into free thiol and formaldehyde (reaction 39). This pathway does most probably account for the small yields of formaldehyde found for all the X-Met peptides which do not contain N-terminal Ser or Thr.

$$2R{-}CH_2{-}S{-}CH_2^{\bullet} \rightarrow R{-}CH_2{-}S{-}CH_2^{+} + R{-}CH_2{-}S{-}CH_2^{-} \quad (36)$$

$$R{-}CH_2{-}S{-}CH_2^{-} + H^+ \rightarrow R{-}CH_2{-}S{-}CH_3 \quad (37)$$

$$R{-}CH_2{-}S{-}CH_2^{+} + OH^- \rightarrow R{-}CH_2{-}S{-}CH_2{-}OH \quad (38)$$

$$R{-}CH_2{-}S{-}CH_2{-}OH \rightarrow R{-}CH_2{-}SH + H_2C{=}O \quad (39)$$

The possibility that either species **9a** or **9b** undergoes hydrogen transfer with the Ser or Thr side chain to yield an alkoxyl radical **12** (reaction 40; Scheme 4) can be excluded on the basis of the bond dissociation energies of the α-(R–S)–C–H bond (BDE = 93 kcal/mol[36]) and the RO–H bond (BDE ca. 103 kcal/mol[54]). Experimentally, such a pathway can be excluded on the basis of the pH dependence of the aldehyde yields. Under acidic conditions, sulfur-centered radical cations **10** are formed which subsequently decompose via deprotonation (reaction 28).

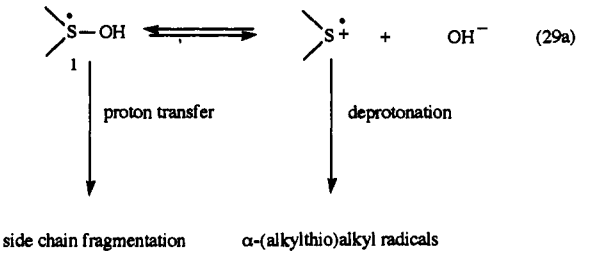
In the presence of oxygen the reaction of hydroxyl radicals with Thr-Met (5×10^{-4} M, pH 6.0) yields acetaldehyde with $G = 1.6 \pm 0.3$, i.e. 50% of the yield derived in deoxygenated solutions, corresponding to an efficiency of 0.29 based on $G^{o\prime}(HO^{\bullet}) = 5.5$. Thus, the side-chain cleavage mechanism is of importance at physiological oxygen concentrations. The reduced yield though may originate from the fact that molecular oxygen can react with some of the involved intermediates. It has been demonstrated, for example, that molecular oxygen can add to the hydroxy sulfuranyl radical[10,55] (reaction 41; $k_{41} = 1.0 \times 10^8$ M^{-1} s^{-1}) and,

$$>S^{\bullet}{-}OH + O_2 \rightarrow HO{-}\underset{|}{\overset{|}{S}}{-}OO^{\bullet} \quad (41)$$

$$\mathbf{11} + HO^- + O_2 \rightarrow S< + O{=}S< + H^+ + O_2^{\bullet -} \quad (42)$$

in the presence of HO^-, react with the dimeric sulfur radical cation **11** (reaction 42).[51,56] Both for methionine model peptides[56] and simple aliphatic sulfides,[51] the latter process was shown to yield sulfoxides with the respective yields depending on the thioether concentration, oxygen concentration, and pH. On the other hand, only negligible sulfoxide yields were obtained when the respective model peptides[56] and aliphatic sulfides[51] were reacted with hydroxyl radicals in the absence of molecular oxygen.

Scheme 5



It should be pointed out that ethylene, one biologically important product of methionine oxidation, may be produced by subsequent reactions of the product radicals **3**, **4**, **9a**, or **9b**. However, these potential processes do not interfere with the side-chain cleavage mechanism under investigation, since this pathway is already completed before or with the formation of these intermediates.

Conclusion

Hydroxy sulfuranyl radicals of X-Met dipeptides can induce the fragmentation of neighboring N-terminal Ser and Thr moieties. So far, this mechanism has been examined for model dipeptides, but it might equally well occur in larger peptides or proteins provided (i) they contain N-terminal Ser or Thr residues and (ii) they are flexible enough to allow these residues to interact with Met residues within the peptide/protein sequence.

We shall stress that the presented proton-transfer mechanism is not restricted only to an initiation via the addition of hydroxyl radicals to the Met moiety. More generally, an initially formed sulfur-centered radical cation might add hydroxide ion, yielding the same key intermediate, the hydroxy sulfuranyl radical **1**, which subsequently undergoes the proton-transfer process. The efficacy of this process will depend on the extent to which hydroxy sulfuranyl radicals **1** can exist in the general equilibrium 29a (Scheme 5), with a lifetime long enough to undergo intramolecular proton transfer.

In water, sulfur-centered radical cations are expected to exist in a hydrated form. The bond dissociation energy for the $(CH_3)_2S^{\bullet +}{---}OH_2$ complex was calculated to be around 16.8 kcal/mol.[57] Therefore, mechanistically it seems not even to be necessary that a hydroxide ion *adds* to a sulfur-centered radical cation. A proton-transfer from the hydrated sulfur-centered radical cation to any appropriate base B might be sufficient to generate hydroxy sulfuranyl radicals as outlined in reaction 43. Further studies are

$$R_2S^{\bullet +}{---}OH_2 + B \rightarrow R_2S^{\bullet}{-}OH + BH^+ \quad (43)$$

now in progress to investigate the influence of primary and secondary structure of specifically designed peptides on these fragmentation processes, induced by intramolecular proton transfer, and whether adducts of hydroxyl radicals at other amino acids such as Phe, Tyr, Trp, or His might be involved in similar mechanisms.

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