

Intramolecular Sulfur–Oxygen Bond Formation in Radical Cations of *N*-Acetylmethionine Amide

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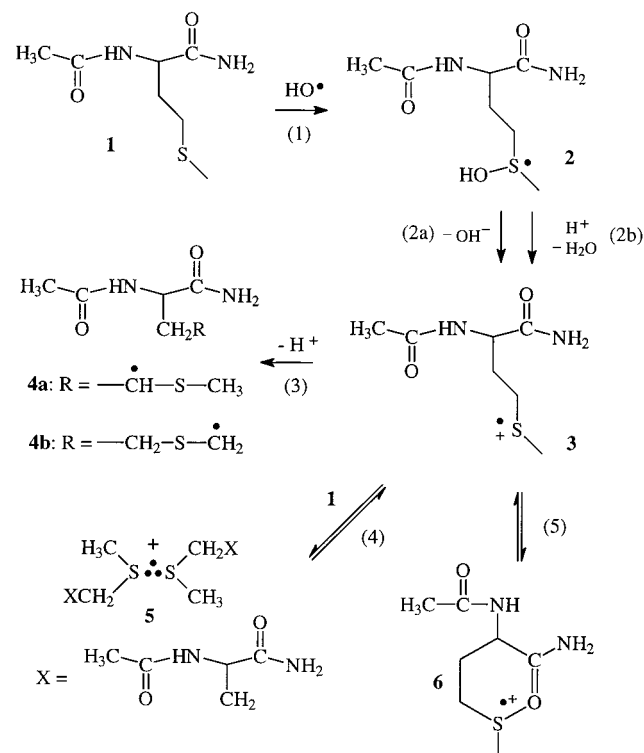
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There has been considerable interest in neighboring group effects by heteroatoms supporting the oxidation of organic sulfides.^{1,2} For small methionine (Met)-containing peptides, the one-electron (1e) oxidation of N- or C-terminal Met yields sulfide radical cations which can stabilize through the respective formation of sulfur–nitrogen³ or sulfur–oxygen⁴ $2\sigma/1\sigma^*$ three-electron bonds.^{2,5,6} Met oxidation is biologically important during conditions of oxidative stress.⁷ Specifically the oxidation of Met³⁵ in β -amyloid peptides (β AP) 1–40 or 1–42, the major constituents of senile plaques in Alzheimer's disease, has been associated with part of the neurotoxicity of these sequences.^{8,9} In the helical C-terminus of β AP1-40, AIIGLM³⁵VGGVV, radical cations of Met³⁵ have little opportunity to interact with heteroatoms from amino acid side chains or the N-terminal amino or C-terminal carboxylate group.¹⁰ However, association with the C=O group of the peptide bond C-terminal to Ile³¹ may be possible, as the ca. 3.6 Å average S–O distance between Met³⁵ and Ile³¹–C=O in the native sequence¹⁰ is close to the sum of the van der Waals radii of the two atoms.

Here, we report, for the first time, experimental evidence for sulfide radical cation–amide association during the 1e-oxidation of Met in the model compound *N*-acetylmethionine amide (**1**) (pulse radiolytically via reaction with hydroxyl radicals, HO[•];¹¹ see Scheme 1).

Figure 1, parts A and B, open circles, displays the optical spectra recorded 2 and 8 μ s after pulse irradiation of an N₂O-saturated aqueous solution, pH 4.0, containing 2×10^{-4} M **1** (pH 4.0 was selected to support complementary conductivity experiments). Under these conditions, pulse radiolysis provides HO[•] with an initial yield of $G_{HO^{\bullet},i} = 5.35$.¹² The spectra show

Scheme 1



two maxima at ca. 290 and 480 nm, respectively. These maxima represent the sulfur–sulfur bonded radical cation **5** (480 nm)¹³ and the α -(alkylthio)alkyl radicals **4** (290 nm).¹⁴ Despite relatively constant yields of the two maxima at both time points, significant time-dependent differences appear in the 300–400 nm region, indicating the presence of additional species. Potential candidates are the hydroxysulfuranyl radical **2**^{15–17} and/or the sulfur–oxygen bonded species **6**. Hydroxysulfuranyl radicals absorb with $\lambda_{\text{max}} = 340$ nm.^{16,17} The optical spectrum of **6** is not known. However, most $2\sigma/1\sigma^*$ -type sulfur–oxygen bonds absorb with $\lambda_{\text{max}} = 390 \pm 20$ nm,^{4,18–22} more or less independent of the source of the oxygen. Hence, the following analysis was based on the approximation that the optical spectrum of **6** is similar to that of a Met sulfide radical cation–carboxylate complex.⁴ The experimental spectra were deconvoluted into contributions from components **2**, **4**, **5**, and, tentatively, **6**, shown in Figure 1A,B, by using a linear regression technique of the form:^{23,24}

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

While the spectral fit in Figure 1A requires the presence of all absorbing components, **2** and **4–6**, the fit in Figure 1B requires

(12) Calculated according to the following: Schuler, R. H.; Hartzell, A. L.; Behar, B. *J. Phys. Chem.* **1981**, *85*, 192–199. The G -value denotes the amount of generated/consumed species per 100 eV absorbed energy; $G = 1.0$ corresponds to 0.1036 $\mu\text{mol/J}$ absorbed energy.

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(15) By analogy to the reaction of HO[•] with organic sulfides.^{16,17}

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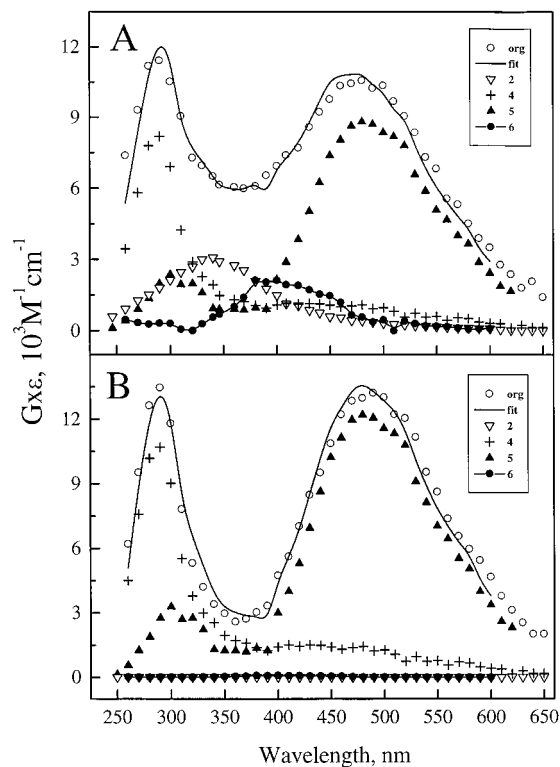


Figure 1. Optical spectra recorded (A) 2 and (B) 8 μs after pulse irradiation of an N_2O -saturated aqueous solution, pH 4.0, containing 2×10^{-4} M N-Ac-Met- NH_2 (**1**). The open circles (○) represent the experimental spectra, and the lines (—) the fits, obtained through summation of the component spectra obtained after spectral deconvolution.

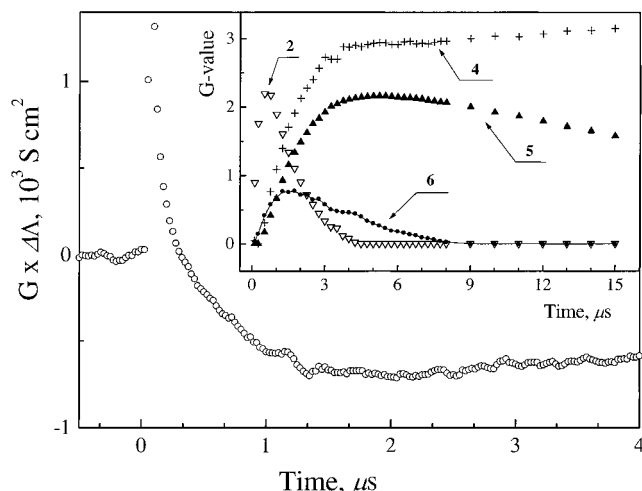


Figure 2. Conductivity vs time profile obtained after pulse irradiation of an N_2O -saturated aqueous solution, pH 4.0, of 2×10^{-4} M N-Ac-Met- NH_2 (**1**). Insert: Yield vs time profiles for the individual components in Figure 1.

4 and **5** only. The sum over all component spectra yields an excellent fit of the experimental spectra. The insert in Figure 2 displays profiles of the yields (G) vs time for the respective species, obtained by deconvolution of the experimental spectra at various time points, and division of $G \times \epsilon$ by the extinction

coefficients of the respective component spectra.²⁵ Radical **2** converts into species **4–6** (via **3**) with $t_{1/2} = 1 \mu\text{s}$ ($k = 7 \times 10^5 \text{ s}^{-1}$). Species **6** decomposes within 8 μs whereas **5** is more stable. Ultimately, **5** and **6** convert into **4** (via **3**). The yield of **2** and **4–6** after complete reaction between HO^\bullet and **1**, i.e. at $t \geq 2 \mu\text{s}$, amounts to $G = 5.35$, corresponding to $G_{\text{HO}^\bullet, i} = 5.35$. This agreement validates our spectral deconvolution. At 2 μs after the pulse, radical cations **5** and **6** are present with yields of $G_5 = 1.7$ and $G_6 = 0.7$, i.e., $G_{5+6} = 2.4$.

Further proof for the simultaneous formation of **5** and **6** was obtained by time-resolved conductivity measurements.²⁶ Reactions **2**, **4**, and **5** generate HO^- and weakly conducting Met sulfide radical cations ($\Lambda \approx 45 \text{ S cm}^2$).²⁷ At pH 4.0, HO^- spontaneously neutralizes by reaction with highly conducting H^+ ($\Lambda = 315 \text{ S cm}^2$).²⁶ Hence, reactions **2**, **4**, and **5**, followed by the neutralization of HO^- , result in an overall loss of specific conductivity ($\Delta\Lambda = +45 - 315 = -270 \text{ S cm}^2$). Figure 2 shows that pulse irradiation of N_2O -saturated solutions of 2×10^{-4} M **1**, pH 4.0, indeed results in a negative conductivity signal with kinetics paralleling the decomposition of **2** (the initial positive spike at $t < 1 \mu\text{s}$ is caused by primary radiation chemical processes).²⁶ The negative conductivity signal reaches $G \times \Delta\Lambda = -722 \text{ S cm}^2$ at 2 μs after the pulse. An exclusive reaction of **2** via reactions **2**, **4**, and **5** would lead to $G \times \Delta\Lambda = 5.35 \times (-270 \text{ S cm}^2) = -1445 \text{ S cm}^2$. Hence, the yield of radical cations derived from **1** amounts to $0.5 \times G_{\text{HO}^\bullet} = 2.68$, in quite good agreement with $G_{5+6} = 2.4$, quantified by UV spectroscopy. This experiment confirms that species **6**, represented by the 390 nm absorbance, is indeed a radical cation. From the conductivity experiment the extinction coefficient of **5** was calculated as follows. At 8 μs after the pulse, **5** is the only radical cation observable in solution and at this point in time we obtain $\epsilon_{480}(\mathbf{5}) = G \times \epsilon_{480}(\mathbf{5}) / (G \times \Delta\Lambda / -270 \text{ S cm}^2) = 5917 \text{ M}^{-1} \text{ cm}^{-1}$.

Our data show that Met sulfide radical cations can associate with the oxygen of an amide function, representative for a peptide bond. Under our experimental conditions of 2×10^{-4} M substrate **1**, the yield of **6** represents ca. 30% of the overall radical cation yield. However, larger relative yields of **6** can be expected for lower, physiologically more relevant, concentrations of **1**, where reaction **5** will compete favorably with reaction **4**. The formation of sulfide–amide oxygen bonded intermediates is also more likely for peptides which show preexisting close sulfur–oxygen distances, such as in $\beta\text{API-40}$.¹⁰ As a potential alternative to structure **6**, sulfide radical cation **3** may also associate intramolecularly with one of the amide nitrogens. However, for amides theoretical calculations show a larger coefficient of the orbital on oxygen in n_0 compared with the coefficient of the orbital on nitrogen in π_2 .²⁸ This feature suggests that an amide oxygen interacts better with electrophiles than an amide nitrogen,²⁸ in favor of structure **6**.

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(24) Here $\Delta A(\lambda_i)$ is the observed absorbance change of the composite spectrum, $\epsilon_i(\lambda_i)$ is the molar absorption coefficient of the i -th species at the j -th wavelength of observation, and $a_i = c_i l$ where c_i is the concentration of the i -th transient and l the optical path length of the monitoring light. For details see: Marciniak, B.; Bobrowski, K.; Hug, G. L. *J. Phys. Chem.* **1993**, *97*, 11937–11944.

(25) Using $\epsilon_{340}(\mathbf{2}) = 3400 \text{ M}^{-1} \text{ cm}^{-1}$,¹⁷ $\epsilon_{290}(\mathbf{4}) = 3000 \text{ M}^{-1} \text{ cm}^{-1}$,¹⁴ $\epsilon_{480}(\mathbf{5}) = 5917 \text{ M}^{-1} \text{ cm}^{-1}$ (vide infra), and $\epsilon_{390}(\mathbf{6}) = 3250 \text{ M}^{-1} \text{ cm}^{-1}$.⁴ A small contribution of **4** generated by the reaction of pulse radiolytically generated H^\bullet atoms ($G_{\text{H}^\bullet, i} = 0.6$)¹¹ with **1** was subtracted from the component spectrum of **4**.

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