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Mechanism of the OH· Radical Induced Oxidation of Methionine in Aqueous Solution

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Abstract: The reaction mechanism of the OH· radical induced oxidation of methionine in aqueous solutions has been investigated by radiation chemical methods, mainly ns–ms pulse radiolysis. The initial step is a competitive process between addition of the OH· radical to the sulfur atom and hydrogen abstraction at a 4:1 ratio in favor of the former. In strong acid solutions (pH ≲3) the subsequent mechanism is practically identical with the oxidation mechanism of aliphatic thioethers. The intermediate of most interest in this case is a sulfur-centered radical cation complex with an absorption maximum at 480 nm. At higher pH in slightly acid, neutral, and basic solutions the mechanism is more complex, essentially involving a fast ($k > 4 \times 10^6$ s^{-1}) intramolecular and probably sterically assisted oxidation of the amino group by the primarily oxidized sulfur function. This process includes irreversible decarboxylation of the amino acid. Both the CO_2 and the remaining α-amino radicals have been identified and their yields determined. The N-oxidation depends on the reduction potential of the amino group and takes place with both the basic and the zwitterionic form of methionine—in the latter case on account of the rapid proton exchange between the NH_3^+ and COO^- groups. Complete protonation of the carboxyl group in very acid solutions, or by the addition of powerful proton donors, e.g., $H_2PO_4^-$, or the introduction of an electron-withdrawing substituent such as in *N*-acetylmethionine lowers the reduction potential to such an extent that the oxidation site may effectively remain at the sulfur. A detailed mechanism with the associated reaction kinetics and some physicochemical properties of the intermediates are presented and discussed.

The oxidation mechanism of the sulfur-containing amino acid L-methionine (2-amino-4-(methylthio)butanoic acid) and some derivatives, induced by radicals and excited states, has been the subject of several radiation and photochemical investigations recently.[1-20]

In the course of these studies a substantial amount of data has been gathered which, however, has not so far been assembled to form a coherent picture. The only exception is provided by an elaborate photochemical investigation of the $^1O_2$ induced oxidation of aqueous methionine solutions by Foote and co-workers,[16] who found different, pH-dependent reaction pathways based on an initial addition of the singlet oxygen to the sulfur atom.

The other investigations have also provided valuable information on specific intermediates, stable reaction products, and reactivities. With the use of ESR techniques,[7,10,13,15,25] several sulfur- and carbon-centered radicals and radical ions have been identified. Only some of them are, however, likely to be formed in the oxidative attack of the OH· radical since analogous species have been identified for the early events of the oxidation of simple thioethers.[13,21-23] Other radicals also encountered in these investigations may, however, result from secondary reactions since they are typical products of the reaction of OH· radicals with the corresponding sulfoxide.[7,10,24]

A sulfur-centered radical cation was also identified by ESR in γ-irradiated single crystals of *N*-acetylmethionine,[9,11,17] and in the light of later findings on the oxidation of thioethers[20-24,26] and detailed analysis of the ^{33}S satellite spectrum, these signals were assigned to a bimolecular complex $(R_2S)_2^{+}\cdot$. This radical cation complex showed an optical absorption with a maximum in the 600–700-nm region in the solid state,[11,17,20] which is correlated with the typical absorption maximum of the simple $(R_2S)_2^{+}\cdot$ species in the 460–500-nm region in aqueous solutions.[22,23] A similarly absorbing species attributable to a radical cation complex $(R_2Se)_2^{+}\cdot$ has been found in the oxidation of aqueous seleno-methionine solutions at pH 1.[27] A $(R_2S)_2^{+}\cdot$ complex derived from methionine has been identified by combined ESR and ENDOR data in the hydrogen peroxide compound of yeast cytochrome *c* peroxidase.[28]

Pulse-radiolysis studies of aqueous methionine solutions indicated the formation of a very short lived (≤1 μs), unidentified transient absorbing in the 400-nm region[8] and revealed an almost quantitative reduction of cytochrome(III) *c* through one of the reaction intermediates in the methionine oxidation.[29] In

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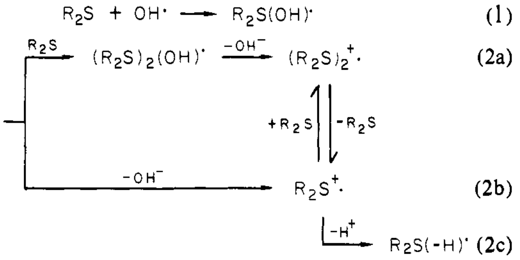
steady-state γ-radiolysis experiments, CO_2,[12,18] NH_3, and methionine sulfoxide,[12] among other compounds, have been detected as stable reaction products. Photochemical studies[5,14,19] finally suggest that the methionine carboxyl or amino group plays an important role in the oxidation mechanism initiated by electrophilic agents, possibly through an interaction of these groups with the sulfur atom.

These various findings suggest that methionine mainly reacts with the OH· radical as a thioether, i.e., oxidative attack primarily seems to occur at the sulfur atom. This is also implied by electronic structure calculations, which reveal a high HOMO and a low LUMO energy level for methionine, compared to those of nonsulfur-containing amino acids.[30] The significant features of the thioether oxidation are outlined in the scheme[22,31] of eq 1 and 2a–c. Accordingly, the OH· radical first adds to the sulfur atom.

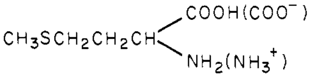


Then OH^- elimination followed by fast deprotonation—effectively elimination of water—leads to a radical, $R_2S(-H)\cdot$, which is best described by a resonance form between a carbon- and sulfur-centered radical[13,22]

This radical is a possible precursor of the corresponding sulfoxide. With increasing solute concentration (>5 × 10^{-4} M), a competing complexation reaction (eq 2a) sets in to yield a short-lived $(R_2S)_2(OH)\cdot$ radical which then dissociates into a relatively stable $(R_2S)_2^{+}\cdot$ complex radical cation. Eventually the latter also decays via equilibration to the molecular radical cation $R_2S^{+}\cdot$. The $(R_2S)_2^{+}\cdot$ species is characterized by a sulfur–sulfur three-electron bond ($2\sigma/1\sigma^*$) from the interaction of the unpaired p electron in $R_2S^{+}\cdot$ with the intact sulfur p-electron pair of a second molecule.[20–24,32–34]

The aim of the present work was a detailed study of the primary steps of the OH· radical induced oxidation of methionine in aqueous solutions in order to establish a mechanism which could possibly interrelate together the various and sometimes seemingly contradictory observations cited above. This includes the questions as to what extent methionine behaves as a thioether and what possible influence the functional groups and their respective pK_a values (2.22 for COOH and 9.27 for NH_3^+)[35] may have. The techniques employed were optical and conductivity pulse radiolysis for the detection of short-lived intermediates in the ns–ms time range and gas chromatography for the analysis of CO_2.

Where appropriate, methionine



$$CH_3SCH_2CH_2CH\begin{cases}COOH(COO^-)\\NH_2(NH_3^+)\end{cases}$$

is abbreviated to Met with the feature of interest shown in square brackets. For example, Met[COOH] and $Met^{+}\cdot$[S] represent methionine with undissociated carboxyl group and the sulfur-centered molecular radical cation, respectively.

Experimental Section

Solutions were prepared from compounds of the highest commercially available purity. The S-containing amino acids and derivatives were L-methionine (Merck >99%), *N*-acetyl-L-methionine (EGA >99%), α-methyl-DL-methionine (Sigma), *S*-methyl-L-cysteine (Aldrich), and 4-(methylthio)butanoic acid (Aldrich >99%). DL-homomethionine (5-(methylthio)norvaline) was prepared according to a procedure given in the literature.[36] The solvent was deionized, "Millipore" filtered water, the quality of which corresponds to triply quartz distilled H_2O (κ > 18 M Ω cm). pH values were adjusted by addition of appropriate amounts of $HClO_4$ and NaOH solutions. The samples were generally deoxygenated by bubbling with Ar or N_2 (Linde, $[O_2]$ <2 ppm and <3 × 10^{-4} vol %, respectively). Subsequent to deoxygenation, solutions were usually saturated with N_2O (Hoechst). The latter was passed through "Oxisorb" (Messer Griesheim) and a column containing a catalyst to remove any last possible traces of O_2.

Hydroxyl radicals were generated by irradiating the aqueous solutions with high-energy electrons or ^{60}Co γ-rays. The radiation energy is practically all absorbed by the solvent since solute concentrations were usually kept between 10^{-5} and 10^{-1} M. The three most reactive species produced and homogeneously distributed in the irradiated aqueous system are e_{aq}^-, H·, and OH·. In neutral solutions they are formed with yields of 2.8, 0.6, and 2.8 species per 100 eV absorbed energy (*G* values), respectively. The added N_2O converts the reducing hydrated electrons into oxidizing OH· radicals via $N_2O + e_{aq}^- \rightarrow N_2 + OH^- + OH\cdot$. In N_2O-saturated solutions this reaction takes place prior to any possible reaction of the hydrated electron with the S-amino acids, and thus 90% of all primary reactive species are OH· radicals.[37]

The pulse-radiolysis experiments were carried out by applying short pulses of high-energy electrons from two different Van de Graaff accelerators (0.5–5 μs, 1.55 MeV, and 5–50 ns, 3.8 MeV, respectively). The applied dose per pulse ranged from ca. 1 to 20 Gy (100–2000 rads), corresponding to ca. (0.02–1.0) × 10^{-5} M OH· radicals/pulse. The experimental arrangement and the evaluation of optical and conductivity data from pulse radiolysis experiments have already been described.[38] Reduction of tetranitromethane by e_{aq}^- and $(CH_3)_2\dot{C}OH$ radicals was used as standard chemical dosimetry system taking ϵ^{350} = 15 000 M^{-1} cm^{-1} for the nitroform anion and $G\Delta\Lambda$ = 360 S cm^2 $equiv^{-1}$. For small signals the signal/noise ratio was improved by electronic averaging of up to 32 individual signals.

γ-radiolysis experiments were carried out by exposing the solutions to a 15 000 Curie radiation field of a ^{60}Co source at an absorbed dose rate of ca. 2 × 10^3 Gy/h or 5 × 10^2 Gy/h, respectively.

The quantitative CO_2 analysis was achieved with the use of a reliable gas-chromatographic headspace technique with a modified Varian 2800 gas chromatograph. CO_2 is separated from the N_2O matrix on a Tenax GC column. With helium as carrier gas and with a thermal conductivity detector, typical concentrations of CO_2 in the aqueous solutions between 10^{-5} M and 2 × 10^{-4} M give a response that can be quantitatively determined with a standard deviation of ≤5%.

Further details on the analytical aspect of these determinations will be published separately.[39]

All experimental work was performed at room temperature, and the limit of error for the work was estimated as ±10%, unless otherwise stated.

Results and Discussion

(1) Contributions of Hydrogen Atom Reactions. Since H· atoms are formed in irradiated aqueous solutions and cannot conveniently be converted into OH· radicals (as can e_{aq}^- via reaction with N_2O), pulse radiolysis experiments were carried out to assess possible contributions of the overall reaction

$$H\cdot + Met \rightarrow products \qquad (3)$$

and its significance for the interpretation of the OH· radical reaction data. The solutions investigated were of low pH (1–4), 5 × 10^{-4} M methionine, and 5 × 10^{-1} M *tert*-butyl alcohol. Under these conditions, OH· radicals are scavenged by the *tert*-butyl

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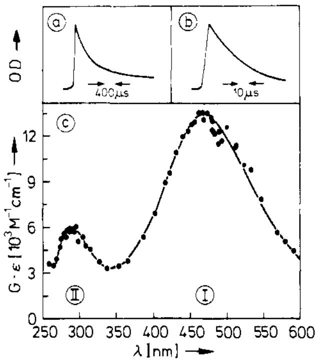


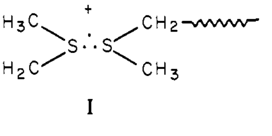
Figure 1. Absorption–time curves in pH 1.0 solutions of 5×10^{-3} M methionine at 290 nm (a) and 480 nm (b); spectrum c was taken immediately at the end of a 1-μs pulse (pH 1.1, 5×10^{-3} M methionine).

alcohol to produce $\dot{C}H_2C(CH_3)_2OH$ radicals (considered inert toward methionine), hydrated electrons are converted into hydrogen atoms via $H_{aq}^+ + e_{aq}^- \rightarrow H\cdot$, and hydrogen atoms can react quantitatively with methionine since reaction 3 is fast ($k_3 \approx 10^9$ M^{-1} s^{-1})[40] compared with H· + H· or H· + *tert*-butyl alcohol. Optical measurements showed only weak absorption below 300 nm with $\epsilon^{280} = 200$ M^{-1} cm^{-1} (based on $G(H\cdot) = 3.4$ in these acid solutions). Similar results have been reported for the reaction of H· atoms with *N*-acetylmethionine.[41] Conductivity experiments (carried out in the pH range 3–4 for sensitivity reasons) showed no conductivity change attributable to reaction 3, thus excluding formation of any radical ion. In conclusion, hydrogen atom reaction products do not interfere with most of the optical and all of the conductivity signals observable in the reaction of methionine with OH· radicals.

(2) Optical Absorptions at Low pH ($\lesssim$3). The OH· radical induced oxidation mechanism of methionine depends markedly on the pH of the solution, the most significant feature being a strong optical absorption in the visible region, appearing in acid solutions below pH 3, and the complete lack of this absorption at pH above 3. As will be seen from our results, and also is apparent from earlier rate constant measurements,[42] OH· radicals react with methionine in a diffusion-controlled reaction independent of the pH, i.e., the above effect is not the result of pH dependent differences in reactivity.

Pulse-irradiated solutions of methionine at low pH show essentially the same characteristics as simple thioether solutions. Thus, two transient absorbing species can be identified on the microsecond time scale. Figure 1a,b shows plots of the optical absorption as a function of time recorded at 290 and 480 nm, respectively, from a pulsed solution of 5×10^{-3} M methionine at pH 1.0. The overall absorption spectrum from the maximum signal of the transient absorptions is reproduced in Figure 1c.

The shorter lived species absorbing in the visible, with λ_{max} = 480 nm, is attributed to the $(R_2S)_2^+\cdot$ type radical cation complex $Met_2^+\cdot[S]$, by analogy with the thioether oxidation.[22,23] It is formed according to the mechanism given in eq 2a, i.e., via an OH· addition, complexation with a second molecule, and OH^- ion elimination. The orbital absorption spectrum is typically structureless and very broad with a half-bandwidth of 0.86 eV, and is attributed to a $\sigma \rightarrow \sigma^*$ transition of one of the bonding electrons in the three-electron bond[34] in I.



I

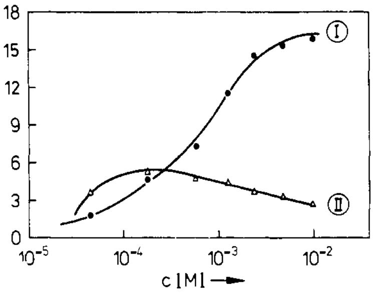


Figure 2. Yield of $Met_2^+\cdot[S]$ (I) and Met(-H)· (II) as a function of methionine concentration (pH 1.0) derived from the optical absorptions at 480 and 290 nm, respectively. (The 290-nm absorption has been corrected for the contribution of I at this wavelength.[43])

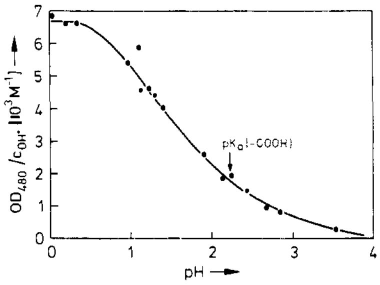
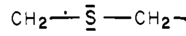
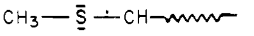


Figure 3. Yield of $Met_2^+\cdot[S]$ normalized to OH· radical concentration, as a function of pH, recorded at 480 nm (methionine concentration 5×10^{-3} M; pulse 1 μs).

Also by analogy with the thioether oxidation,[22] the longer lived UV absorption band with λ_{max} = 290 nm can be assigned to the $R_2S(-H)\cdot$ type radical Met(-H)· formed via reaction route 2b, 2c, or possibly by direct H atom abstraction. (For quantitative purposes, the UV band has to be corrected for the contribution of the strong $Met_2^+\cdot[S]$ band in this wavelength range.[43]) Two sulfur–carbon resonance stabilized radicals are possible: IIa and IIb. ESR measurements have shown a 1.5/1 ratio for the concentration of radicals IIb/IIa at pH 2.5.[13] As in the case of the corresponding radicals from simple thioethers,[13,22] the spin is assumed to be distributed between the sulfur and the respective carbon atom.



IIa



IIb

Figure 2 shows the yields of I and IIa and IIb expressed as $G\epsilon$ at 480 and 290 nm, respectively, as a function of methionine concentration measured in N_2O-saturated solutions of pH 1.0 at the end of a 1-μs pulse. The yield of I, i.e., of $Met_2^+\cdot[S]$ is seen to increase with solute concentration in a way which has been found to be typical for $(R_2S)_2^+\cdot$ radical cation complexes in thioether solutions[22] and reaches a maximum plateau value only at very high concentrations around 10^{-2} M at this pH.

Curve II, representing the Met(-H)· radical species, exhibits quite a different shape. A maximum yield is attained at around 10^{-4} M. The falloff on the low-concentration side results from incomplete OH· radical scavenging by the methionine owing to competing reactions such as mutual OH· combination. The decrease on the high-concentration side practically parallels the $Met_2^+\cdot[S]$ increase, i.e., the stabilization of the radical cation complex seems to take place at the expense of the Met(-H)· radical yield. As will be discussed later in terms of kinetic and thermodynamic data (still to be evaluated), the two curves essentially reflect the equilibrium

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[43] Met(-H)· is known to decay in a bimolecular radical–radical reaction with $2k$ depending on the pH and to have an extinction coefficient of $\epsilon = 3000$ M^{-1} cm^{-1} at 290 nm.[46] Since its yield has been determined as 20% of the OH· radicals, the contribution of $Met_2^+\cdot[S]$ to the initial 290-nm absorption can be calculated. For another derivation of this parameter based only on the Met(-H)· decay kinetics, see ref 44.

[44] K. -O. Hiller, Ph.D. Thesis, Technical University, Berlin D 83, 1979.

$$\mathrm{Met_2^{+}\cdot[S] \rightleftharpoons Met^{+}\cdot[S] + Met} \qquad (4)$$

and the respective kinetic contributions of reactions 2a and 2b,c.

The pH dependence of the $\mathrm{Met_2^{+}\cdot[S]}$ formation is shown in Figure 3. The points were taken from the optical absorption signals at 480 nm in pulse-irradiated, N_2O-saturated solutions of 5×10^{-3} M methionine and normalized to the actual OH· radical concentration at each pH value. The yield of $\mathrm{Met_2^{+}\cdot[S]}$ is seen to decrease from a plateau value at pH <0.5 to almost 0 at pH 4. The half-value is obtained at pH 1.7.

This curve indicates a highly significant difference between methionine and normal thioethers. For the latter, the $(R_2S)_2^{+}\cdot$ yield stays constant in acid, neutral, and even slightly basic solutions,[22,32] and only at higher OH^- ion concentrations does their yield decrease via reactions of $R_2S^{+}\cdot$ with OH^-/H_2O. The half-value of the curve in Figure 3 is close to the pK_a (=2.2) of the methionine carboxyl group, and this is a first indication that the ionization of this functional group may play an important role in the OH· radical induced oxidation mechanism of methionine.

Before presenting further experimental data at higher pH, where $\mathrm{Met_2^{+}\cdot[S]}$ is no longer observed, it seems appropriate to evaluate curve II in Figure 2 somewhat more quantitatively. It is noted that the 290-nm absorption does not decrease to 0 at the highest experimental methionine concentrations although the $\mathrm{Met_2^{+}\cdot[S]}$ yield has reached its maximum value. Even after correction for the $\mathrm{Met_2^{+}\cdot[S]}$ contribution at this wavelength,[43] a $G\epsilon \approx 2500$ remains. There is, of course, a contribution of hydrogen atom reaction products which are formed with $G = 3.4$ at pH 1.0. Taking $\epsilon = 200$ M^{-1} cm^{-1} as an upper limit for hydrogen atom products (see section 1) and subtraction of $G\epsilon$(H products) = 3.4 × 200 = 680 leaves $G\epsilon = 1820$. Inspection of Figure 3 shows that $\mathrm{Met_2^{+}\cdot[S]}$ is formed with only about 80% efficiency at this pH. As will be shown later, the remaining 20% produces an α-amino radical which contributes with $\epsilon \approx 200$ at this pH and wavelength.[65] The yield of this species is G(OH·) × 0.2 = 0.56, at most, accounting for up to another $G\epsilon \approx 110$, but leaving a residual $G\epsilon \approx 1680$, which indicates that the reaction of OH· radicals with methionine does not quantitatively yield $\mathrm{Met_2^{+}\cdot[S]}$ radical cation complexes. We suggest that the residual absorption, exhibiting the spectral characteristics of Met(-H)· is due to this radical formed directly by the hydrogen atom abstraction reaction

$$\mathrm{Met + OH\cdot \rightarrow Met(\text{-}H)\cdot + H_2O} \qquad (5)$$

Hydrogen atom abstraction can, of course, in principle occur at any carbon atom and therefore C-2 and C-3 radicals may also be formed besides the C-4 (IIb) and C-5 (IIa) radicals.[45] However, in view of the strong deactivating effect of the NH_3^+ group, α, and β abstraction seems less likely. With the extinction coefficient taken as $\epsilon = 3000$ M^{-1} cm^{-1} for the Met(-H)· radical,[46] the yield of reaction 5 is calculated as $G = 1680/3000 = 0.56$, thus accounting for about 20% of the totally available OH· radicals. In view of the complex determination of this value, a relatively large limit of error should be allowed for, probably within the range 10–30%. In conclusion, the experimental results do strongly indicate some abstraction in competition to addition of the OH· radical to methionine.

(3) Optical Absorption and Conductivity Measurements at Higher pH (>3). The loss of the $\mathrm{Met_2^{+}\cdot[S]}$ absorption is the most significant change on going from low pH solutions to less acidic systems. The Met(-H)· absorption, however, remains unaffected, and, in addition, a longer lived underlying UV absorption as well as a very short-lived absorption around 400 nm now become apparent. Also, changes in conductivity can be detected in the pulse-irradiated solutions.

The longer lived UV absorption will be dealt with in section 5. The short-lived optical absorption, which is probably identical with that mentioned by van Hemmen and Adams,[8] exhibits characteristics typical of the $(R_2S)_2(OH)\cdot$ complex radical formed during thioether oxidation.[22,31,32] Its formation and decay kinetics are shown in Figure 4a. The plot of the optical absorption was recorded at 400 nm with an N_2O-saturated solution of 2.5×10^{-4} M methionine at pH 3.45 irradiated with a 5-ns pulse. Both the formation and the decay occur exponentially. The spectrum derived from the maximum signal change, about 100 ns after the pulse, in a 5×10^{-3} M methionine solution is recorded in Figure 4c. The broad and structureless band has a maximum at 400 nm and a half-bandwidth of 1.2 eV.

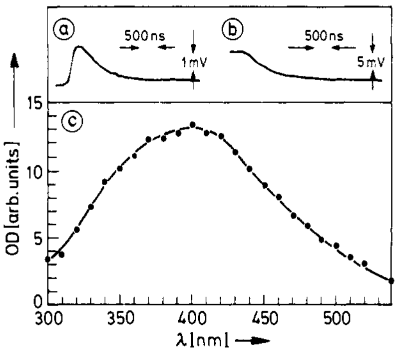

Figure 4. Optical absorption at 400 nm (a) and conductivity (b) as a function of time in N_2O-saturated solutions of 2.5×10^{-4} M methionine at pH 3.45, pulse 5 ns, and about 7 Gy. (c) Absorption spectrum of $\mathrm{Met_2(OH)\cdot[S]}$, recorded 100 ns after the pulse (methionine concentration 5×10^{-3} M).

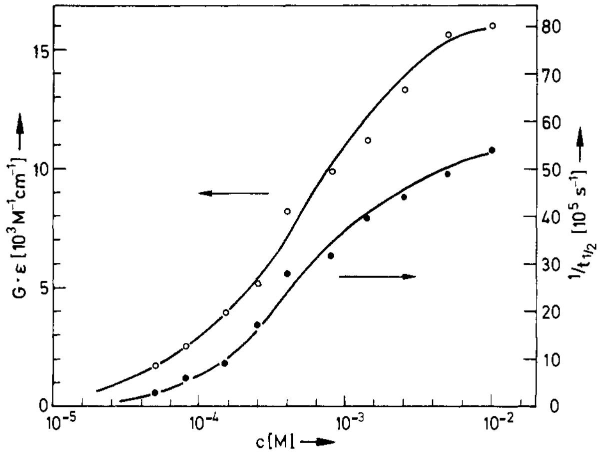

Figure 5. Yield ($G\epsilon$) (open circles) and reciprocal half-lives for the 400-nm decay (full circles) of $\mathrm{Met_2(OH)\cdot[S]}$ as a function of methionine concentration (pH 3.45; N_2O saturated solutions).

The neutral character of this short-lived species is deduced from the conductivity shown in Figure 4b. The signal decreases exponentially and practically parallels the decay of the optical absorption in Figure 4a. A decrease in conductivity in acid solutions is generally indicative of the formation of OH^- ions[22,31,47] (which are immediately neutralized) plus an associated positive counter ion, the net result being the replacement of a highly conducting proton by a less conducting normal cation. Clearly, OH^- is formed only on decay of the optically absorbing species.

The optical absorption is thus assigned to the $\mathrm{Met_2(OH)\cdot[S]}$ complex radical formed in the reaction of the molecular OH· radical adduct with a second methionine molecule and in which the OH· interacts with both sulfur atoms.

$$\mathrm{Met(OH)\cdot[S] + Met \rightarrow Met_2(OH)\cdot[S]} \qquad (6)$$

The shift in the position of λ_{max} from 350 nm in aliphatic model compounds[22,32] to 400 nm in the case of methionine is explained by a through-space interaction of the ionized carboxyl group with the chromophoric system. A similar red shift of the absorption maximum due to an ionized COO^- group has been reported in

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[45] The carbon atom of the carboxyl group is numbered C-1.

[46] K. -O. Hiller and K. -D. Asmus, *Int. J. Radiat. Biol.*, submitted.

[47] K. -D. Asmus, "Fast Processes in Radiation Chemistry and Biology", G. E. Adams, E. M. Fielden, and B. D. Michael, Eds., The Institute of Physics, Wiley, New York, 1973, pp 40–59.

the literature.[48] As for all other simple organic sulfides,[22,31,32] the molecular radical Met(OH)·[S] exhibits no detectable optical absorption.

The yield of $Met_2(OH)\cdot[S]$, determined from its absorption when this attains a maximum after the pulse, increases with methionine concentration (again similar to the thioether systems[22,31,32] and the curve is shown in Figure 5 (open circles) on a semilog plot. A plateau value is obtained only at relatively high concentrations of about 10^{-2} M. Both the rate of formation and decay of $Met_2(OH)\cdot[S]$ increase with methionine concentration. The latter levels off at a maximum value of $k_7 = 3.7 \times 10^6\ s^{-1}$, corresponding to $t_{1/2} = 185$ ns, for the reaction

$$Met_2(OH)\cdot[S] \rightarrow OH^- + \text{radical cation} \qquad (7)$$

The decay rate expressed in terms of reciprocal half-lives (full-circle curve in Figure 5) exhibits the same concentration dependence as the yield-concentration plot. A complete kinetic analysis is given in section 8.

The rate of conductivity change (Figure 4b) is also seen to increase with increasing methionine concentration. Within the concentration range accessible for conductivity experiments with use of the DC method in the nanosecond time range ($\leq 2.4 \times 10^{-3}$ M[31]), it follows only qualitatively the pattern of the optical decay curve in Figure 5. On an absolute basis, the conductivity signals decrease slightly faster than the optical $Met_2(OH)\cdot[S]$ signal—particularly noticeable at lower methionine concentrations. For example, in solutions containing 1.5×10^{-4} M, 2.5×10^{-4} M, and 10^{-3} M methionine, the conductivity signal decreases with $t_{1/2}$ = 800, 535, and 295 ns, while the optical signal decays with $t_{1/2}$ = 1070, 610, and 330 ns, respectively. Further, the change in conductivity attains a maximum at low methionine concentrations of around 10^{-4} M, where $Met_2(OH)\cdot[S]$ is formed only with ca. 20% efficiency, and remains constant at higher concentrations. These observations suggest that not only $Met_2(OH)\cdot[S]$ but also its immediate precursor, the molecular radical Met(OH)·[S], already yield OH^- ions in the reaction

$$Met(OH)\cdot[S] \rightarrow OH^- + \text{radical cation} \qquad (8)$$

Accordingly, the kinetics of the conductivity signals reflect the sum of the rate constants of reactions 7 and 8 whereas the optical decay at 400 nm depends solely on reaction 7. Neither reaction 7 nor 8 reveal the nature of the radical cation, although this does not seem to be a sulfur-centered radical species.

The yield of reactions 7 and 8 measured by conductivity at pH 3 in a 5×10^{-3} M methionine solution accounts for 70% of the totally available OH· radicals.[49,50] Contribution of the low pH mechanism (see section 2) at this pH is of the order of 10%, again leaving 20% of the OH· radicals for H abstraction. Owing to the increasing influence of acid–base equilibria, it is not possible to evaluate the yields of reactions 7 and 8 from the conductivity signals in the more neutral pH range. However, this becomes possible in more strongly basic solutions, beyond the pK for deprotonation of the methionine amino group, where the conductivity signals shown in Figure 6a are obtained. In this pH range, the oxidation

$$OH\cdot + Met^-[NH_2,COO^-] \rightarrow OH^- + Met^{\pm}\cdot \text{ or } Met\cdot \qquad (9)$$

of the overall negatively charged methionine leads to a radical zwitterion which may also internally rearrange to form one or more neutral radicals. Since neither of these species would be detectable by the conductivity technique, the net effect of reaction 9 is the replacement of the ordinary anion by a much more conducting OH^- ion, leading to an overall increase in conductivity.

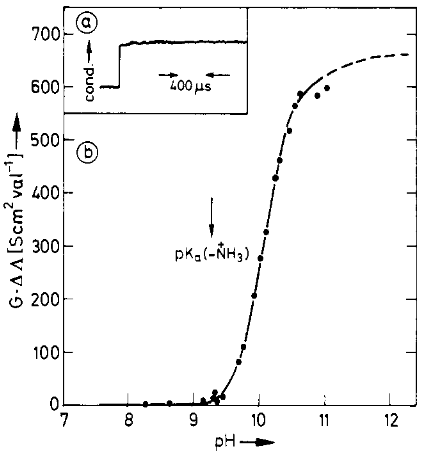

Figure 6. (a) Conductivity–time curve from N_2O-saturated solution of 10^{-3} M methionine at pH 10.5. (b) pH dependence of the conductivity change ($G\Delta\Lambda$) 20 μs after the pulse in the same solution.

The yield of this reaction can be evaluated from the pH dependence of the observed $G\Delta\Lambda$ values, which are plotted in Figure 6b for a methionine concentration of 10^{-3} M. A plateau value is seen to be reached at about pH 11. For a quantitative interpretation of the curve, the buffering action of the amino group has to be considered, which is significant even in millimolar solutions where the concentration of produced OH^- ions is in the order of 10^{-6} M. The conductivity signal is practically unaffected only at high pH (>pK_a + 1.5 pH units). The steep low-pH side of the curve in Figure 6b can be quantitatively accounted for by this buffering effect.[44,51,52]

The extrapolated maximum change in conductivity (indicated by the dotted curve) comes to $G\Delta\Lambda = 660\ (\pm 60)$.[53] With $\Delta\Lambda = -(Met^-) + (OH^-) = -35 + 180 = 145\ S\ cm^2\ equiv^{-1}$,[50] the yield of reaction 9 is $G = 4.55$, again accounting for ca. 80% of the OH· radicals.

These results, therefore, also suggest that ca. 20% of the hydroxyl radicals undergo a hydrogen atom abstraction reaction (reaction 5) and that the remaining 80% of the OH· radicals add primarily to, i.e., oxidize, the sulfur atom which then complexes to form the $Met_2(OH)\cdot[S]$ radical irrespective of pH. Subsequently, either the sulfur-centered radical cation complex $Met_2{}^{+}\cdot[S]$ is stabilized or a competitive reaction mechanism leads to entirely different oxidation products, depending on pH.

(4) Sulfur-Nitrogen Electron Transfer and Decarboxylation. One of the predominant stable reaction products in the pH >3 range is CO_2.[12,18,54] At high methionine concentrations in neutral and basic solutions, a limiting value of around $G(CO_2) = 4.85$ (±0.2) is obtained, accounting for about 85% of the OH· radicals, i.e., again apparently related to the fraction of OH· radicals which add primarily to the sulfur atom. In acid solutions below pH 3, the yield of CO_2 decreases and is practically 0 at pH 0, i.e., when the maximum yield of the sulfur-centered radical cation complex $Met_2{}^{+}\cdot[S]$ is formed. The respective $G(CO_2)$ values are listed in Table I.

The question now arises as to the exact nature of the oxidized methionine intermediates formed in reactions 7 and 8 at pH >3, i.e., which other site in methionine could react with the primarily oxidized sulfur to release CO_2. From the thioether oxidation it is known that both radical cations $R_2S^{+}\cdot$ and $(R_2S)_2{}^{+}\cdot$ are good oxidants,[22] and two possible sites in the methionine molecule,

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[48] M. Sheves, K. Nakanishi, and B. Honig, J. Am. Chem. Soc., **101**, 7086 (1979).

[49] This value has been adjusted to a 15% contribution of an acid–base equilibrium of an α-amino radical (see sections 4 and 5).

[50] l (positive ion) is taken to be 35 S cm² equiv⁻¹ in analogy to similarly sized and charged species. The uncertainty, however, is rather large and could be as much as ±15. Since the proton and OH^- ion conductances, however, are much larger (315 and 180 S cm² equiv⁻¹, respectively), the overall uncertainty for $\Delta\Lambda$ is still relatively small.

[51] K. -O. Hiller and K. -D. Asmus, to be submitted for publication.

[52] (a) D. D. Perrin and B. Dempsey, "Buffers for pH and Metal Ion Control", Chapman and Hall, London, 1974, p 12; (b) ibid., p 163.

[53] The extrapolation takes into account the acid–base equilibrium $OH\cdot + OH^- \rightleftharpoons O^-\cdot + H_2O$ ($pK = 11.9$) and the fact that H· atom abstraction from $Met^-[NH_2,COO^-]$ by $O^-\cdot$ or addition of $O^-\cdot$ does not lead to a change in conductivity.

[54] B. Masloch, H. Möckel, K. -O. Hiller, and K. -D. Asmus, to be submitted for publication.

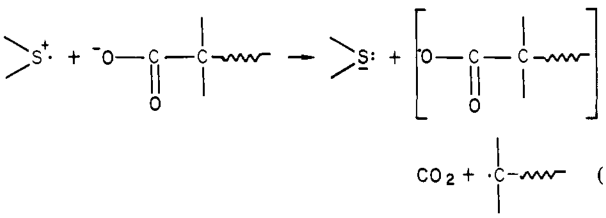
Table I. CO_2 Yields from γ-Irradiated Solutions[a]

compd (conditions)	pH	$G(CO_2)$	% of OH· leading to N-ox
methionine (5×10^{-3} M)	0.0	0	0
	1.0	0.8	26
	1.4	1.4	44
	1.7	2.0	55
	2.0	2.6	63
	2.3	3.5	77
	2.6	4.4	89
	2.8	5.0	97
	6.1	5.0	89
	8.2	4.7	85
	10.2	4.9	89
plus phosphate (0.1 M)	1.7	1.6	44
	2.0	2.2	54
	2.3	2.7	59
	2.6	3.2	65
	2.8	3.5	68
N-acetylmethionine	5.2		
(5×10^{-3} M)		2.6	47
(2×10^{-3} M)		2.7	49
(5×10^{-4} M)		2.5	45
4-(methylthio)butanoic acid (10^{-3} M)	3.9	<0.3	
2-aminobutanoic acid (10^{-3} M)	6.8	<0.1	
plus $(CH_3)_2S$ (10^{-2} M)	6.8	1.5	

[a] All solutions were saturated with N_2O.

namely, the carboxyl and the amino group, may be considered for an oxidative reaction.

To account for the observed CO_2 elimination and the change in reaction mechanism near the pK_a of the carboxyl group, a reaction such as reaction 10 has been proposed (or a corresponding reaction of the Met(OH)· radical including OH^- ion elimination) as a possible mechanism.[18] This scheme would also explain why the neutral, undissociated COOH group, which is not sufficiently reactive, is unable to compete with the stabilization of the sulfur-centered complex $Met_2^{+}\cdot[S]$ in strong acid solutions.


(10)

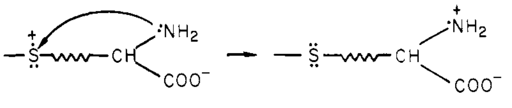
However, a number of experimental observations with methionine derivatives do not agree with this interpretation. For example, the OH· radical induced oxidation of *N*-acetylmethionine (p$K \approx 3.65$ by comparison with *N*-acetylglycine[55]) leads to a sulfur-centered radical cation complex (*N*-acetyl $Met)_2^{+}\cdot[S]$ even in neutral solutions, where the carboxyl group is present in its anionic form (absorption spectrum similar to that of $Met_2^{+}\cdot[S]$ shown in Figure 1c, $\lambda_{max} = 480$ nm). At the same time, the CO_2 yield is decreased by a factor of about 2 as compared with Met. Also, practically no decarboxylation is observed in the reaction of OH· radicals with 4-(methylthio)butanoic acid, i.e., the deaminated methionine (for comparative CO_2 yields see Table 1). This agrees well with the results of Neta and co-workers who found only negligible CO_2 yields after OH· induced oxidation of several aliphatic carboxylic acids at neutral pH.[56]

These findings suggest the amino group as the secondary site of oxidation with transfer of the unpaired spin from sulfur to nitrogen. This is in agreement with the thermodynamic consideration that the amino group should undergo oxidation more readily than a carboxyl group. It is thus postulated that the transfer of charge between the sulfur and the nitrogen atom takes place according to reactions 11 and 12. Reactions 11 and 12 must

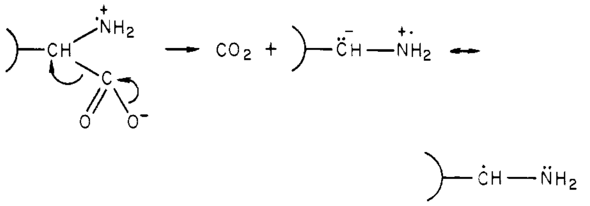
$$Met^{+}\cdot[S] \rightarrow Met^{+}\cdot[N] \qquad (11)$$

$$Met_2^{+}\cdot[S] \rightarrow Met^{+}\cdot[N] + Met \qquad (12)$$

be fast compared with reactions 7 and 8 since neither of the sulfur-centered radical cations are observed after OH^- elimination of their immediate precursors. With a look at the respective rate constants given in Table III and by considering that reactions 11 and 12 occur quantitatively even below 10^{-4} M methionine, a bimolecular process can be ruled out at least at lower solute concentrations since it would require a rate constant well above the diffusion limit. The N-oxidation is therefore assumed to be an *intra*molecular process, e.g., reaction 13, associated with a repair of the sulfur function. The N-centered aminium radical cation is likely to interact with the carboxyl group leading directly to CO_2 and an α-amino radical. Both these products have been identified as will be discussed later. Reaction 14 is expected to be fast since it leads to energetically much more stable species. Fast decarboxylation processes are, in fact, known with reported rate constants of 2×10^{-7} s^{-1} (lower limit)[57] and 1.6×10^{9} s^{-1} (at 60 °C)[58] for the decarboxylation of acetoxyl radicals, for example.

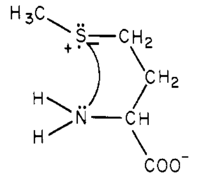

(13)

$Met^{+}\cdot[S]$ $\qquad$ $Met^{+}\cdot[N]$


(14)

From the kinetic point of view, reactions 13 and 14 seem to occur simultaneously. Mechanistically, however, the N-oxidation and decarboxylation should be viewed as two independent, consecutive processes. If it was only one single concerted step it would practically mean a direct oxidation of COO^- by $>S^{+}\cdot$, which can be ruled out on the basis of the experimental findings. The mechanism given in eq 13 and 14, incidentally, is in principle very similar to that proposed by Cohen[19] in a photooxidation study of methionine.

The following pieces of experimental evidence suggest that the N-oxidation is sterically assisted with the participating sulfur and nitrogen centers adopting a favorable five-membered ring configuration (III).


III

In solutions of pH 4.6, containing 5×10^{-3}/M methionine, α-methylmethionine, homomethionine, and S-methylcysteine,

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(55) J. T. Edsall and J. Wyman, Eds., "Biophysical Chemistry", Vol. I, Academic Press, New York, 1958, p 466.

(56) V. Madhavan, H. Levanon, and P. Neta, *Radiat. Res.*, **76**, 15 (1978).

(57) B. C. Gilbert, R. G. Holmes, P. D. R. Marshall, and R. O. C. Norman, *J. Chem. Res., Miniprint*, 1949 (1977).

(58) W. Braun, L. Rajbenbach, and F. R. Eirich, *J. Phys. Chem.*, **66**, 1591 (1962).

respectively, reducing radicals are formed with 100%, 95%, 67%, and 4% efficiency relative to the yield of OH· radicals. At least about 80% of these reducing radicals are α-amino radicals, i.e., the above yields are practically a quantitative measure for the yield of N-oxidation. (A possible but at most 20% relative contribution may be accounted for by the hydrogen abstraction products which also exhibit slight reducing properties.[46]) For chemical determination of the α-amino radicals and interdistinction see section 6.

Among these methionine derivatives, α-methylmethionine can also arrange in a five-membered ring with regard to the sulfur–nitrogen interaction, and homomethionine can do so in an almost equally favorable six-membered ring configuration. With s-methylcysteine, however, the molecule would have to adopt a much more strained four-membered configuration which satisfactorily explains the low yield of N-oxidation in this case.

Direct evidence for an oxidation of an amine by sulfur-centered radical cations can be derived from the observed formation of the corresponding amine radical cation (λ_{max} = 475 and 325 nm) in solutions containing 5×10^{-3} M $(CH_3)_2S$ and 6.7×10^{-5} M *p*-phenylenediamine (pH 8). It is also evident from solutions containing 10^{-3} M 2-aminobutanoic acid and 10^{-2} M $(CH_3)_2S$ at pH 6.8, where >99% OH· radicals are scavenged by the thioether and $G(CO_2)$ = 1.5. Without the thioether $G(CO_2)$ is <0.1. This shows that the oxidized sulfur function can indeed oxidize an amino group. The relatively low CO_2 yield compared with methionine solutions shows, however, that the intermolecular and sterically unassisted process is far less efficient.

The N-oxidation is, of course, only efficient if the reduction potential of the amino group is high enough to transfer an electron to the oxidized sulfur atom, and the rate constant of this process must be higher than for other possible competing reactions. Lowering the reduction potential by means of electron-withdrawing substituents, as in the case of *N*-acetylmethionine, decreases the yield of N-oxidation and decarboxylation, as expected. For example, in N_2O-saturated, pH 5.2 solutions of *N*-acetylmethionine, the CO_2 yield drops to G = 2.6 and stays constant over the concentration range 5×10^{4}–5×10^{-3} M. This is about half the value of the yield from a corresponding methionine solution. At the same time, the S-centered radical cation complex (*N*-acetyl $Met)_2{}^{+}\cdot[S]$, as already mentioned, is stabilized and can be identified from its characteristic optical absorption—observable even in basic solutions at pH 10. Its yield, in contrast to the CO_2 yield, increases however with increasing solute concentration. For example, at pH 2.95 ϵ^{480} is equal to 2100, 3700, and 5600 M^{-1} cm^{-1}, on the basis of G = 5.5, in solutions containing 4.9×10^{-4}, 1.3×10^{-3}, and 10^{-2} M *N*-acetylmethionine, respectively. In the same systems the respective half-life of the 480-nm absorption increases from 8.8 over 12.6 to 55 μs. Both observations are typical for the equilibrium between the sulfur-centered molecular and complex radical cation. Furthermore, the yield of (*N*-acetyl $Met)_2{}^{+}\cdot[S]$ at high solute concentration practically equals the yield of OH· radicals which undergo addition to the sulfur atom. These results therefore conclusively demonstrate that, in contrast to the methionine system, dissociation of the OH· radical adduct (at least reaction 7) to the sulfur-centered radical cations must now be faster than the electron transfer leading to N-oxidation (reactions 11–13). The lower and concentration-independent CO_2 yield consequently would result from a competition between N-oxidation and deprotonation (in analogy to reaction 22), both of which apparently occur with similar rates in the *N*-acetylmethionine system. Preliminary conductivity results indicate, in fact, a partial deprotonation together with the formation of α-amino radicals. In general, the *N*-acetylmethionine results are considered to be relevant to biological systems since this compound represents the simplest model for peptides containing a thioether linkage.

Although it is well established that the protonated amino group, $NH_3{}^{+}$, has a lower reduction potential than the unprotonated NH_2 group, we have to assume, however, that also in the zwitterionic form of methionine (pH 2–10) the reduction potential of the amino group is still high enough to transfer an electron to the oxidized sulfur atom. This is clearly reasonable since $NH_3{}^{+}$ and COO^{-} are exceptional proton-donor and -acceptor groups, respectively[59]), and the fast proton exchange of eq 15 can provide enough concentration of an unprotonated amino group.

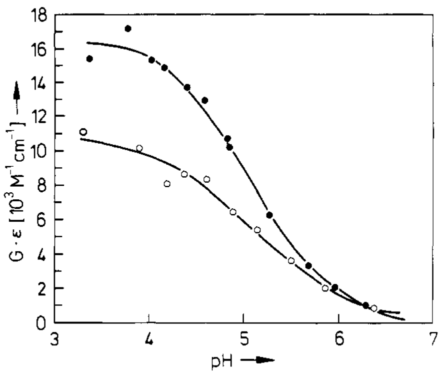
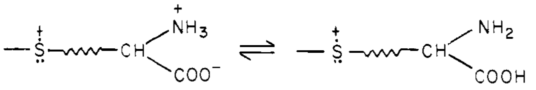


Figure 7. pH dependence of the $Met_2{}^{+}\cdot[S]$ yield (480 nm) in N_2O-saturated solutions of 5×10^{-3} M methionine and 0.2 M (open circles) and 1.0 M (full circles) phosphate.



$$-\overset{+}{\underset{..}{S}}\sim\sim CH\begin{matrix}NH_3^{+}\\COO^{-}\end{matrix} \rightleftharpoons -\overset{+}{\underset{..}{S}}\sim\sim CH\begin{matrix}NH_2\\COOH\end{matrix} \qquad (15)$$

The exchange may also occur via *intermolecular* proton donation to an intact methionine molecule, and a similar mechanism has been put forward to explain the dehydromethionine formation in the $^1O_2$ induced oxidation of methionine.[16,60] In strong acid solutions, where the carboxyl group is completely protonated, the proton-exchange reaction (15) and the N-oxidation can no longer take place efficiently. The $Met_2{}^{+}\cdot[S]$ vs. pH curve in Figure 3 thus reflects the pK_a of the carboxyl group. However, since it also reflects kinetic parameters (such as for the *intra*molecular electron-transfer reactions 11 and 12), it cannot be expected to follow the thermodynamic pK curve exactly.

If this concept is correct, not only the presence of free protons but also any other process hindering proton transfer from the carboxyl group should result in a decrease of N-oxidation in the zwitterionic pH range. This is found to be the case. Addition of high concentrations of $H_2PO_4{}^{-}$ ions, for example, to an N_2O-saturated solution of 5×10^{-3} M methionine leads to the observation of $Met_2{}^{+}\cdot[S]$ even in a pH range (3–6) where, in the absence of phosphate, such stabilization of the sulfur-centered radical cation complex is otherwise not apparent (phosphate does not interfere directly with any of the primary radiation chemical species and steps). Accordingly, N-oxidation, and thus the CO_2 yield (see Table I), decreases in the presence of the proton donor.[18] The pH dependence of the $Met_2{}^{+}\cdot[S]$ yield in such solutions for two different phosphate concentrations of 0.2 and 1.0 M is shown in Figure 7. It is noted that the $Met_2{}^{+}\cdot[S]$ yield increases with $H_2PO_4{}^{-}$ concentration. Accordingly, the CO_2 yield is also decreased with increasing phosphate concentration. No effects are observed anymore below 10^{-2} M phosphate. An important process contributing to this behavior is acid catalysis of the ionization of the OH· radical adducts. Reaction 7a, for example, is clearly

$$Met_2(OH)\cdot[S] + H_2PO_4{}^{-} \rightarrow Met_2{}^{+}\cdot[S] + H_2O + HPO_4{}^{2-} \qquad (7a)$$

evidenced in the $H_2PO_4{}^{-}$ concentration-dependent decay of the $Met_2(OH)\cdot[S]$ absorption. Also reaction 8a has an observable

$$Met(OH)\cdot[S] + H_2PO_4{}^{-} \rightarrow Met^{+}\cdot[S] + H_2O + HPO_4{}^{2-} \qquad (8a)$$

effect; its competition with reaction 6 explains the small but noticeable decrease in the measurable $Met_2(OH)\cdot[S]$ absorption at high $H_2PO_4{}^{-}$ concentrations.

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(59) S. N. Vinogradov, and R. H. Linnell, "Hydrogen Bonding", Van Nostrand-Reinhold, New York, 1971, p 231.

(60) (a) M. L. Kacher, Ph.D. Thesis, University of California, Los Angeles, 1977; (b) C. S. Foote, private communication.

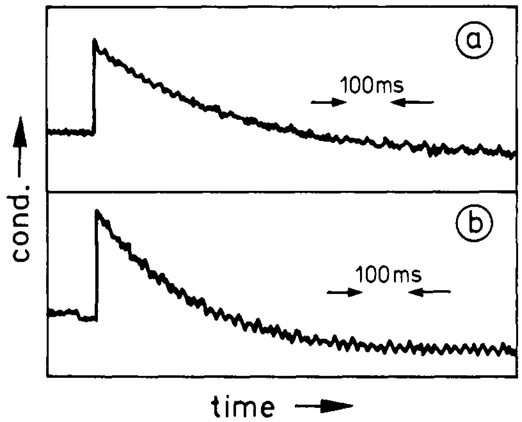


Figure 8. Conductivity–time curves in N_2O-saturated solutions of 10^{-3} M methionine at (a) pH 10.8 and (b) pH 11.03 (pulse 1 μs).

The dihydrogen phosphate does, however, not only enhance the rate of formation of the S-centered radicals but also stabilizes the latter through protonation in the forward reaction of the equilibrium of eq 16 followed by association of the completely pro-

$$\text{-}\dot{S}^{+}\sim CHNH_2(COOH) + H_2PO_4^- \rightleftharpoons \text{-}\dot{S}^{+}\sim CHNH_3^{+}(COOH) + HPO_4^{2-} \qquad (16)$$

tonated radical with a second methionine molecule to form $Met_2^{+}\cdot[S]$. Decarboxylation is of course still possible through the back-reaction of equilibrium 16 and subsequent intramolecular N-oxidation of the unprotonated radical cation. The yield of this process will, however, be decreased (see Table I) owing to competing reactions such as deprotonation or other termination reactions of the completely protonated $\text{-}\dot{S}^{+}\sim CHNH_3^{+}(COOH)$ species. The situation in $H_2PO_4^-$-containing solutions is therefore similar to that of *N*-acetylmethionine.

A comparable catalytic phosphate effect has been discussed for the enhanced formation of $Cl_2^{-}\cdot$ in weakly acid media after OH· induced oxidation of chloride solution in the presence of phosphate.[61] The falloff around pH 6 (see Figure 7) results from the significant decrease of $H_2PO_4^-$ concentration via the $H_2PO_4^-/HPO_4^{2-}$ equilibrium (pK_a in 1 M solution 6.49[62]). HPO_4^{2-} is apparently not able to catalyze reactions 7 and 8 efficiently nor reduce the equilibrium concentration of $\text{-}\dot{S}^{+}\sim CHNH_2(COOH)$.

A similar effect to that of phosphate is also obtained with other powerful proton donors such as acetic and malonic acid in their undissociated forms. Further details on the oxidation of methionine in the presence of these proton donors will be published separately.[54,63]

(5) Pulse Radiolytic Detection of CO_2 and α-Amino Radicals. The formation of CO_2 can be detected pulse radiolytically through its hydrolysis in basic solutions. Figure 8 shows two conductivity time signals obtained with N_2O-saturated solutions of 10^{-3} M methionine, at pH 10.8 and 11.03, respectively. The initial increase in both curves is accounted for by reaction 9 (see Figure 6a). At longer times the signals decrease again, owing to the hydrolysis reactions 17 and 18, i.e., conversion of the OH^- ion to the less-

$$CO_2 + OH^- \rightarrow HCO_3^- \qquad (17)$$

$$HCO_3^- + OH^- \rightleftharpoons CO_3^{2-} + H_2O \qquad (18)$$

conducting carbonate anions. α-amino radicals formed along with CO_2 are not expected to give a conductivity change in this pH range by disproportionation and subsequent hydrolysis (see section 6).

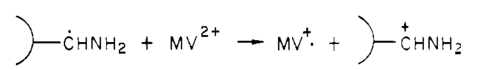
The decay curves are exponential, and the half-lives derived from these and corresponding experiments at various pH's are inversely proportional to the OH^- ion concentration. The bimolecular rate constant for the rate-determining step, reaction 17, is determined as $k_{17} = 8.8 \times 10^3\ M^{-1}\ s^{-1}$, which agrees well with the published value of $8.5 \times 10^3\ M^{-1}\ s^{-1}$.[64] The more marked contribution of reaction 18 at pH 11.03, which consumes a second OH^- ion ($pK_{18} = 10.33$[52]), is responsible for the more pronounced negative progression of the signal (below the original base line) compared to the lower pH signal. Hydrolysis is not observed in neutral or acid solutions since the $CO_2 + H_2O$ reaction is too slow[64] for measurement by pulse radiolytic techniques. These experiments do not, of course, supply any information on the rate of CO_2 liberation from the N-oxidized methionine species.

With regard to the α-amino radical formed in reaction 14, only a few essential features are to be mentioned here; a more detailed survey is to be published separately.[65] The optical absorption steadily increases from about 350 mm toward shorter wavelengths, without, however, attaining a maximum as low as 250 nm. It exists in an acid–base equilibrium (eq 19) with a $pK_a = 3.8$ derived from

$$CH_3SCH_2CH_2\dot{C}HNH_3^{+} \rightleftharpoons CH_3SCH_2CH_2\dot{C}HNH_2 + H_{aq}^{+} \qquad (19)$$

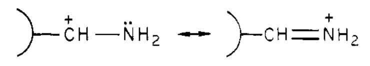
optical and conductivity measurements[44,65] as a function of pH. Thus, the relatively stronger absorption of the deprotonated radical (ε ratio at 260 nm is 3:1) contributes significantly to the longer living UV absorption observable in pulse-irradiated solutions at pH >4. Equilibrium 19 is also responsible for the fact that no changes in conductivity are to be seen in neutral solutions since the OH^- ions from reactions 7 and 8 are quantitatively neutralized by the protons released from the methionine zwitterions in the reaction sequence (11)–(14) and (19).

(6) Some Chemical Reactions of the Radical Intermediates. The α-amino radical exhibits reducing properties, as would be expected from its structure. For example, it readily reacts with the dication of methylviologen (MV^{2+}, $E_7^1 = -0.449$ V[66]) (eq 20). This

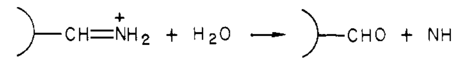

(20)

reaction can easily be followed via the formation of the well-known visible absorption of the $MV^{+}\cdot$ radical cation ($\epsilon^{600} = 11\,850\ M^{-1}\ cm^{-1}$[67]) in solutions containing high concentrations of methionine and comparatively low concentrations of MV^{2+}. The rate constant for the above reaction was determined from these experiments as $k_{20} = (3.7 \pm 0.3) \times 10^9\ M^{-1}\ s^{-1}$ at pH 7 (1 mM phosphate buffer). The reaction occurs quantitatively (based on internal dosimetry with N_2O-saturated 0.2 M *i*-PrOH/0.5 mM MV^{2+} system) and at pH >3 accounts for practically 100% of the N-oxidation, i.e., corresponding to at least the yield of the initial OH· radical adduct to the sulfur atom. Reaction 20 has also been used to determine the yields of reducing radicals in similar systems with α-methylmethionine, homomethionine, and S-methylcysteine (see section 4).

The oxidized amino radical in reaction 20 can be regarded as a protonated imine by resonance structure formulation:



It is, of course, subject to hydrolysis forming the corresponding aldehyde and ammonia:


(20a)

The imino compound would also be formed by a disproportionation reaction of the α-amino radical in the absence of a suitable scavenger. Our mechanism thus clearly accounts for the reported methional and ammonia formation.[12,19] (Further work on more

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[61] E. Wold, T. Brustad, and M. Kongshaug, *Int. J. Radiat. Phys. Chem.*, **8**, 699 (1976).

[62] J. P. Fox and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 1436 (1974).

[63] (a) M. Göbl, K. -O. Hiller, and K. -D. Asmus, to be submitted for publication. (b) M. Göbl, part of Ph.D. Thesis, Technical University, Berlin.

[64] D. M. Kern, *J. Chem. Educ.*, **37**, 14 (1960).

[65] K. -O. Hiller and K. -D. Asmus, to be submitted for publication.

[66] E. Steckhan and T. Kuwana, *Ber. Bunsenges. Phys. Chem.*, **78**, 253 (1974).

[67] E.g., P. A. Trudinger, *Anal. Biochem.*, **36**, 222 (1970).

quantitative aspects is under way.)

The α-amino radical similarly reduces many other compounds, one of them being cytochrome(III) *c*, and it would appear that the reduction of this compound after OH· induced oxidation of methionine, reported by Simić and Taub[29] with $G = 5$, is essentially a result of this reaction. A full account of all these reduction reactions of the α-amino radicals is to be published separately.[65]

The Met(-H)· radicals IIa,b also have reducing character as can be deduced from the total yield of reduced species or by the experimental findings after more selective oxidation of the sulfur compounds by Tl^{2+}. This has been shown in particular with tetranitromethane.[46,65,68]

The $Met^{+}\cdot[S]$ and $Met_2^{+}\cdot[S]$ radical cations exhibit oxidizing character as is apparent from the N-oxidation reactions 11 and 12. It also oxidizes, e.g., hexacyanoferrate(II) readily. Thus, the $Met_2^{+}\cdot[S]$ absorption in pulse-irradiated solutions of 5×10^{-3} M methionine at pH 1 is found to decay faster with increasing hexacyanoferrate(II) concentration, and a rate constant of $k_{21} = (6.3 \pm 0.7) \times 10^9\ M^{-1}\ s^{-1}$ has been evaluated for reaction 21.

$$Met_2^{+}\cdot[S] + [Fe(CN)_6]^{4-} \rightarrow 2Met + [Fe(CN)_6]^{3-} \quad (21)$$

This rate constant is similar to that found for the corresponding reactions of thioether radical cations.[22] In fact, reaction 21 may only represent an overall reaction, and the more reactive species may be the molecular radical cation $Met^{+}\cdot[S]$ in equilibrium with the complex.

No reduction or oxidation reactions could be attributed to the OH· radical adducts Met(OH)·[S] and Met_2(OH)·[S].

(7) Some Physicochemical Properties of $Met^{+}\cdot[S]$ and $Met_2^{+}\cdot[S]$. Given that $Met_2^{+}\cdot[S]$ is formed only through OH· radicals which primarily add to the sulfur atom and which account for about 80% of the available OH· radicals, the extinction coefficient of this sulfur-centered radical cation complex can be calculated as $\epsilon^{480} = 7300\ (\pm 800)\ M^{-1}\ cm^{-1}$ from the yield at pH 0. This value is of the same order as ϵ for the $(R_2S)_2^{+}\cdot$ species from aliphatic thioethers and other thia compounds.[22,32,33]

Information on the stability of the radical cations $Met^{+}\cdot[S]$ and $Met_2^{+}\cdot[S]$ and their equilibrium (eq 4) can be obtained from the decay kinetics of the 480-nm absorption in acid solutions, a typical example of which is shown in Figure 1b. The decay generally occurs exponentially with the half-life increasing with methionine concentration. A second order contribution which is significant in the decay of corresponding thioether species[22,32] is negligible in the methionine system, and the decay can be assigned exclusively to the proton elimination from the molecular radical cation giving rise to radicals IIa,b (eq 22). The actual $Met^{+}\cdot[S]$ concentration

$$\underset{}{Met^{+}\cdot[S]} \rightarrow \underset{\text{IIa,b}}{Met(-H)\cdot} + H_{aq}^{+} \quad (22)$$

depends on the equilibrium with $Met_2^{+}\cdot[S]$, i.e., on the methionine concentration. Kinetically, the system is described by the differential equation[22,69] (23), where $c = [Met^{+}\cdot[S] + [Met_2^{+}\cdot[S]]$,

$$-dc/dt = [k_{22}K_4/(K_4 + [Met]_{COOH})]c \quad (23)$$

k_{22} = the true rate constant for the proton elimination reaction 22, and $K_4 = [Met^{+}\cdot[S]]\cdot[Met]_{COOH}/[Met_2^{+}\cdot[S]]$. The suffix COOH on the methionine concentration term takes into account that the sulfur-centered radical cations are only stabilized if the carboxyl group is protonated. At all pH values the respective concentrations are calculated on the basis of $pK_a(COOH) = 2.22$. Inspection of eq 23 shows that the measurable first-order rate constant $k' = \ln 2/t_{1/2}$ for the decay of the $Met_2^{+}\cdot[S]$ absorption is given by eq 24 or 25.

$$k' = k_{22}K_4/(K_4 + [Met]_{COOH}) \quad (24)$$

$$1/k' = 1/k_{22} + (1/k_{22}K_4)[Met]_{COOH} \quad (25)$$

A plot of $1/k'$ vs. $[Met]_{COOH}$ obtained from pH 0 solutions is reproduced in Figure 9 (full circles, concentration axis is extended by a factor 10) and shows the expected linear dependence. The rate constant $k_{22} = 2.4 \times 10^5\ s^{-1}$ and the equilibrium constant $K_4 = 1.7 \times 10^{-5}$ M, derived from intercept and slope, respectively, are listed in Table II.

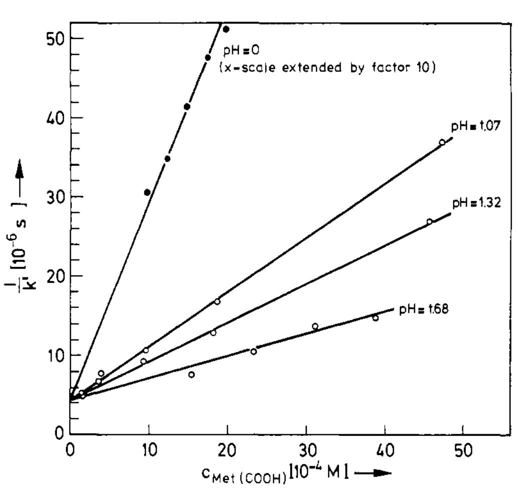

Figure 9. Plots of $1/k'$ vs. $[Met]_{COOH}$ at various pH values: full circles, pH 0.

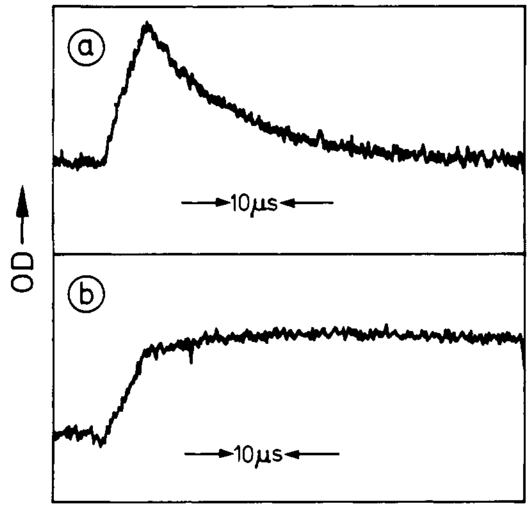

Figure 10. Absorption-time curves at 480 nm (a) and 290 nm (b) in solutions of 5×10^{-3} M methionine at pH 1.5 (pulse 5 μs).

Table II. Rate Constants k_{22} for Proton Elimination from $Met^{+}\cdot[S]$ and K_4 for Equilibrium in Equation 4

pH	$10^{-5}k_{22}$, s^{-1}	10^4K_4, M	pH	$10^{-5}k_{22}$, s^{-1}	10^4K_4, M
0.0	2.4	0.17	1.32	2.5	7.9
1.07	2.5	5.8	1.68	2.4	11.2

The decay of the $Met_2^{+}\cdot[S]$ species, however, is not only dependent on the methionine concentration but also on the pH of the solution. The corresponding $1/k'$ curves at various pH values in Figure 9 are seen to have a common intercept, and k_{22} can therefore be identified as the true rate constant for the irreversible proton elimination from $Met^{+}\cdot[S]$.

The slopes of the curves are seen to decrease with decreasing pH, indicating that K_4 is pH dependent. The values listed in Table II show significant differences even between pH 1 and 0, i.e., in a range where the yield of $Met_2^{+}\cdot[S]$ varies little. At present, we have no conclusive interpretation of this effect but tentatively consider partial dissociation of one carboxyl group in the $Met_2^{+}\cdot[S]$ complex (which could have a $pK < 2$) as a possible reason.

The $K_4 = 5.8 \times 10^{-4}$ M value obtained at pH 1.07 is reflected in the $Met_2^{+}\cdot[S]$ vs. methionine concentration in curve I of Figure 2 where the 50% yield is seen to be formed at around 5×10^{-4} M. At pH 0 the stability ($1/K_4$) of $Met_2^{+}\cdot[S]$ is approximately 1-2 orders of magnitude higher than for most of $(R_2S)_2^{+}\cdot$ radical

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[68] M. Göbl and K. -D. Asmus, to be submitted for publication.

[69] E.g., A. A. Frost and R. G. Pearson, "Kinetik und Mechanismen homogener chemischer Reaktionen", Verlag Chemie, Weinheim, 1964.

Table III. Rate Constants Evaluated by Computer Simulation[a] and Direct Kinetic Analysis[b]

$*k_5 = 4.5 \times 10^9\ M^{-1}\ s^{-1}$	$k_4 \approx 10^9\ M^{-1}\ s^{-1}$
$*k_{26} = 1.8 \times 10^{10}\ M^{-1}\ s^{-1}$	$k_{11/12} > 3.2(3.7) \times 10^6\ s^{-1}$
$*k_6 = 8.0 \times 10^9\ M^{-1}\ s^{-1}$	$k_{22} = 2.4 \times 10^5\ s^{-1}$
$k_7 = 3.2 \times 10^6\ s^{-1}$ (pH 7)	$k_{27} \approx 7.0 \times 10^8\ M^{-1}\ s^{-1}$ (pH 3.35)
$k_7 = 3.7 \times 10^6\ s^{-1}$ (pH 3.45)	$k_{28} \approx 2.0 \times 10^9\ M^{-1}\ s^{-1}$ (pH 4.5[64])
$*k_8 = 10^5\ s^{-1}$	

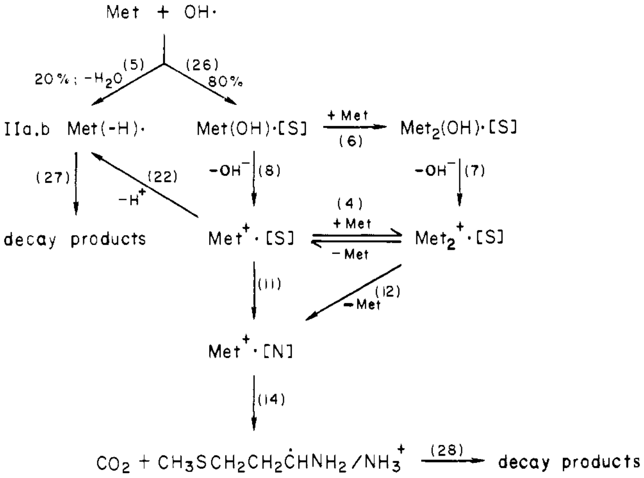
[a] Asterisk shows rate constants evaluated by computer simulation. [b] Error limit for computer-process data ±30%.

cation complexes from thioethers.[22]

If reaction 22 plays a significant role, the Met(-H)· radical formation in this deprotonation process must be in evidence. This is demonstrated in Figure 10 which shows traces of the optical absorptions at 480 and 290 nm as a function of time in solutions of 5×10^{-3} M methionine at pH 1.5. The curve obtained at 290 nm exhibits not only a fast initial step attributed to Met(-H)· formed in abstraction reaction 5, and a contribution from Met_2^{+}·[S] at this wavelength, but also a slow secondary increase which grows at the same rate as the 480-nm Met_2^{+}·[S] absorption decays.

(8) Reaction Mechanism and Rate Constants. All the various experimental observations and their interpretations are compatible with Mechanism I (the numbers in parentheses denote the respective equation numbers in the text).

Mechanism I



By applying the appropriate differential equations[44,70] together with the measurable yields of Met(-H)·, Met_2^{+}·[S], $Met_2(OH)$·[S], CO_2, and the α-amino radical, their rates of formation and decay and their concentration and pH dependences can be mathematically processed to give the optimal values listed in Table III. Reactions 11–14 and 28 and their respective rate constants are, of course, only applicable for solutions of pH ≳3, where the carboxyl group is dissociated. The overall accuracy is estimated to ±30%.

The rate constants k_5 and k_{26} reflect the 1:4 ratio between hydrogen atom abstraction and addition. It is also interesting to note that k_8 is smaller than k_{22} which explains why the molecular radical cation is not seen. Reactions 8 and 22 together constitute water elimination from the OH· radical adduct, which had been proposed as a one-step reaction for aliphatic thioethers.[22] In the light of the present and other recently published data,[31] the latter should probably be viewed as a two-step mechanism as well.

The rate constant k_7 for the dissociation of $Met_2(OH)$·[S] is found to be approximately 30–40 times (acid catalysis is indicated) higher than that of k_8 for the dissociation of the molecular OH· radical adduct. This may be explained in terms of a charge-transfer structure of the complex radical, with the positive charge localized in the vicinity of the two sulfur atoms and the negative charge at the leaving hydroxyl group. It can also be expected that a second sulfur atom in the direct proximity of the bond undergoing heterolytic fission facilitates the reaction by its negative inductive effect.

No rate constant can be given for the forward reaction of the equilibrium 4. At high methionine concentrations Met_2^{+}·[S] is formed entirely via the complex $Met_2(OH)$·[S], and at low concentrations this reaction cannot compete with the deprotonation reaction 22 and the *intra*molecular electron transfer (11)–(13). A value of about $10^9\ M^{-1}\ s^{-1}$ found for similar systems[31-33] can, however, be regarded as a reasonable estimate.

The nitrogen–sulfur electron transfer leading to the N-oxidation must occur with a rate constant greater than approximately $4 \times 10^6\ s^{-1}$. This value seems reasonable considering that this reaction is likely to be a sterically assisted process.

The kinetic scheme also fully accounts for the curves shown in Figure 5. Thus the yield of $Met_2(OH)$·[S] is determined essentially by the rate constants of its formation, k_6, and decay, k_7. The rate of decay $(1/t_{1/2})$ curve is directly related to the actual $Met_2(OH)$·[S] concentration at any given methionine concentration. Consequently, the two curves follow the same course with an identical point of inflexion ($\sim 5 \times 10^{-4}$ M).

The computer simulation analysis also permits calculation of the extinction coefficient for the $Met_2(OH)$·[S] as $\epsilon = 5800$ (±600) $M^{-1}\ cm^{-1}$. Finally, the scheme is fully in accordance with the concentration dependence and the kinetics of the conductivity signals associated with the decay of the OH· radical adduct species.

Conclusion

The OH· radical induced oxidation of methionine in aqueous solution is shown to be very complex in its primary stages and depends markedly on the pH, as well as on the solute concentration. In acidic solutions below pH 3, the mechanism is almost identical with the oxidation mechanism reported for thioethers. The oxidized sulfur function is stabilized by complexation in the absence of electron-donating groups. The mechanism becomes more extensive, however, in less acid, neutral, and basic solutions where the amino group can be oxidized by the primarily oxidized sulfur function. This sterically assisted process leads to irreversible decarboxylation of the amino acid and the formation of reducing α-amino radicals.

Acknowledgment. We wish to thank Professor S. F. Nelsen for helpful discussions as well as Professor M. Brenner for his kind gift of DL-homomethionine.

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[70] For the evaluation of the conductivity signals, no distinction was made between the molecular and complex radical cations.