

Formation and Reduction Reactions of α -Amino Radicals Derived from Methionine and Its Derivatives in Aqueous Solutions

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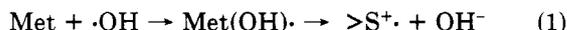
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(Received: November 15, 1982; In Final Form: February 2, 1983)

Absolute rate constants and yields have been measured for the reaction of α -amino radicals derived via an oxidative decarboxylation process from various sulfur-containing amino acids with suitable electron acceptors. The measurements were carried out with aqueous solutions and by using optical and conductivity pulse radiolysis methods. Bimolecular rate constants have been measured for the reactions of $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NH}_2$ with $\text{Fe}(\text{CN})_6^{3-}$ ($k = (3.5 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$), $\text{C}(\text{NO}_2)_4$ ($4.2 \pm 0.5 \times 10^9$), cytochrome(III) *c* ($(6.6 \pm 0.6) \times 10^8$), 4-hydroxy-2,2,6,6-tetramethylpiperidiny-1-oxy (TMPN), ($(5.4 \pm 0.5) \times 10^8$), lipoate ($(1-2) \times 10^8$), O_2 ($(1.8 \pm 0.4) \times 10^9$), *p*-nitroacetophenone (PNAP) ($(3.9 \pm 0.4) \times 10^9$), methylviologen (MV^{2+}) ($(3.6 \pm 0.3) \times 10^9$), and nicotinamide adenine dinucleotide (NAD^+) ($(8.5 \pm 3) \times 10^8$). The reduction potential of this α -amino radical is more negative than -0.94 V . The protonated form $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NH}_3^+$ reacts more slowly and $k = (1.0 \pm 0.2) \times 10^7$ and $(2.3 \pm 0.2) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ have been evaluated for the respective electron transfer reactions with MV^{2+} and TMPN. The pK of the equilibrium $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NH}_3^+ \rightleftharpoons \text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NH}_2 + \text{H}_{\text{aq}}^+$ has been determined to be 3.85. Further rate constants have been determined for hydrogen atom abstraction from cysteine by $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NH}_2$ ($k \geq (1-2) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$), and for the electron transfer processes $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NHCOCH}_3 + \text{MV}^{2+}$ ($k \approx (1-2) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$), and $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{H}\text{NH}_2 + \text{PNAP}$ ($k = 4.1 \pm 0.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$).

Introduction

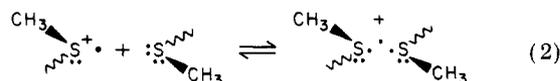
The sulfur-containing amino acid methionine, $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CHNH}_2(\text{COOH})$ (Met), has been found to be easily oxidized by free radicals.¹⁻⁴ It has been shown by radiation chemical studies, for example, that the radical-induced oxidation occurs with absolute rate constants which are practically only controlled by the diffusion of the reactants, e.g., $k = 2.3 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for the reaction $\cdot\text{OH} + \text{Met}$.² Reductive degradation is also possible, but takes place with much lower rate constants (e.g., $k = 4.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for $\text{Met} + e_{\text{aq}}^{-5}$). The high reactivity toward oxidizing radicals is a possible reason for the efficiency of methionine as a radioprotector.

Our previous radiation chemical study on the $\cdot\text{OH}$ radical induced oxidation mechanism² elaborated in particular on the effects of the amino and carboxyl functional groups ($\text{pK} = 9.27$ and 2.22 , respectively) in comparison with the corresponding oxidation of simple aliphatic sulfides.⁶ It has been found that at $\text{pH} \leq 2.5$ methionine behaves like an ordinary sulfide, and a dimer three-electron-bonded radical cation of the type $\text{R}_2\text{S}\cdot\text{SR}_2^+$ is identified as a characteristic intermediate. At $\text{pH} \geq 3$, i.e., where the carboxyl group is ionized, the oxidative mechanism is changed, the most important feature now being a decarboxylation associated with the formation of 3-methylthiopropylamino radicals (in the following generally referred to as " α -amino radicals"). It has been suggested that the sulfur-centered radical cation, $>\text{S}^+$, which is formed via the $\cdot\text{OH}$ radical adduct

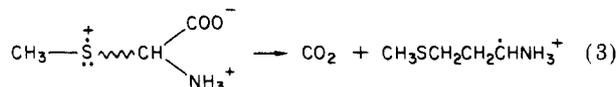


can no longer be stabilized through complexation with a

second methionine molecule



but suffers irreversible CO_2 loss in a competitive fast intramolecular process ($k_3 \geq 3.7 \times 10^6 \text{ s}^{-1}$)



The actual mechanism by which this decarboxylation occurs is still a matter for debate. Transfer of the oxidation site from sulfur to the amino group² as well as to the carboxyl group^{1,7} has been suggested, possibly following primary $\cdot\text{OH}$ addition to the sulfur function. It seems that the process leading to decarboxylation is not a simple and straightforward electron transfer but involves a cyclic structure with an interaction between the initially oxidized sulfur center and the other end of the methionine molecule.^{1,2,7} There is, however, no ambiguity about the formation of transient α -amino radicals which in fact are the predominant radical species already about $1 \mu\text{s}$ after initiation of the methionine oxidation. These α -amino radicals have been detected through time-resolved optical measurements² and also by ESR.⁷ The formation of α -amino radicals has also been suggested already in earlier studies on the photooxidation of methionine by excited flavin mononucleotide (FMN)⁸ and 4-carboxybenzophenone.⁹

The yield of α -amino radicals from methionine is rather high and accounts for ca. 80% of the available $\cdot\text{OH}$ radicals.² The yield of α -amino radicals from the $\cdot\text{OH}$ induced oxidation of simple aliphatic amines is expected to be lower by comparison owing to the deactivating effect of the $-\text{NH}_3^+$ group (at $\text{pH} < \text{pK}$ of the amino group). In this

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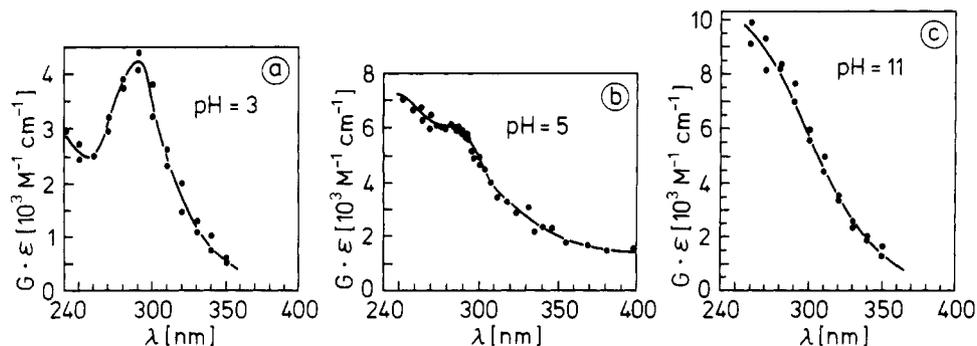


Figure 1. Optical absorption spectra recorded 5 μ s after a 1- μ s pulse in irradiated, N_2O -saturated aqueous solutions of 2×10^{-3} M methionine at pH 3.0 (a), pH 5 (b), and pH 11 (c).

case (β - ω)-amino radicals are likely to be formed as well. Corresponding findings have been reported for the $\cdot OH$ radical induced oxidation of aliphatic alcohols.¹⁰ It is of great chemical significance that only the α -hydroxy alkyl radicals formed in the latter reaction exhibit reducing properties while the β - ω species are relatively inert with respect to redox reactions.¹⁰ The same may be expected for the various amino radicals.

Reducing properties of α -amino alkyl radicals could, in fact, be deduced from ESR studies,¹¹ pulse radiolysis optical¹² and polarography studies,¹³ and more recently even directly proven by time-resolved photochemical studies.¹⁴ Theoretical considerations also predict α -amino radicals to be strong reductants.^{15,16}

In the present paper we give a more detailed report on the formation and some properties of the α -amino radical, $CH_3SCH_2CH_2\dot{C}HNH_2/NH_3^+$, derived from the oxidative decarboxylation of methionine and referred to briefly already in one of our previous papers.² We shall also extend our investigations to the corresponding α -amino radicals from some methionine derivatives. Our data and conclusions are mainly based on direct observations of the transient radicals and their reaction kinetics by using time-resolved pulse radiolysis techniques with optical and conductivity detection systems.

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), and S-methyl-L-cysteine (Aldrich). DL-Homomethionine (5-(methylthio)norvaline) was prepared according to a procedure given in the literature.¹⁷ The solvent was deionized, Millipore filtered water, the quality of which corresponds to triply quartz distilled H_2O ($\kappa > 18$ M Ω cm). pH values were adjusted by the 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 $< 3 \times 10^{-4}$ 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. 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}^- , $H\cdot$, and $\cdot OH$. 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 $\cdot OH$ radicals via $N_2O + e_{aq}^- \rightarrow N_2 + OH^- + \cdot OH$. In N_2O -saturated solutions this reaction takes place prior to any other possible reaction of the hydrated electron, and thus 90% of all primary reactive species are $\cdot OH$ radicals.¹⁸

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 μ s, 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 rd), corresponding to ca. $(0.02\text{--}1.0) \times 10^{-5}$ M $\cdot OH$ radicals/pulse. The experimental arrangement and the evaluation of optical and conductivity data from pulse radiolysis experiments have already been described.¹⁹ Reduction of tetranitromethane by e_{aq}^- and $(CH_3)_2\dot{C}OH$ radicals were used as standard chemical dosimetry system by taking $\epsilon^{350} = 15000$ $M^{-1} cm^{-1}$ for the nitroform anion and $G\Delta\lambda = 360$ $\Omega^{-1} cm^2 equiv^{-1}$. For small signals the signal/noise ratio was improved by electronic averaging of up to 32 individual signals. Data analysis and data storage was based on a Biomation 8100 transient recorder and a PDP 11/40 computer.

All experiments have been carried out at room temperature.

Results and Discussion

Formation and Some Physicochemical Properties of $CH_3SCH_2CH_2\dot{C}HNH_2/NH_3^+$ Radicals. Pulse irradiation of N_2O saturated, aqueous solutions of methionine (2×10^{-4} – 5×10^{-3} M) at pH ≥ 3 yields relatively long-lived optical absorptions in the UV which can be observed on the microsecond–millisecond time scale. Under these conditions the underlying event is the $\cdot OH$ radical attack on methionine. The spectra which are recorded at ca. 5 μ s after application of a 1- μ s pulse, i.e., after completion of some primary events, vary with pH. This is demonstrated in Figure 1a–c. At pH 3 (Figure 1a) the spectrum is dominated by an absorption band peaking at 290 nm.

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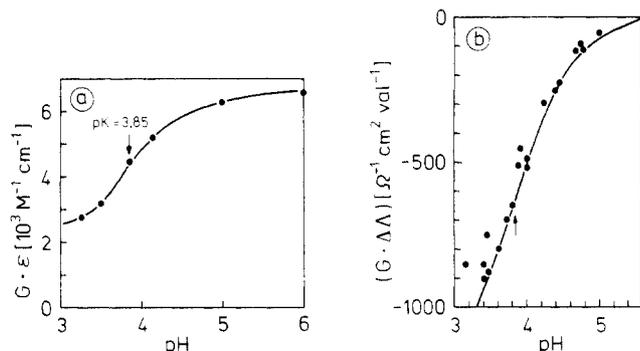
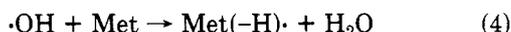


Figure 2. (a) Change in optical absorption (expressed in terms of $G\epsilon$) recorded at 260 nm 5 μ s after a 1- μ s pulse in irradiated, N_2O -saturated aqueous solutions of 5×10^{-3} M methionine as a function of pH. (b) Change in conductivity (expressed in terms of $G\Delta\Lambda$) as a function of pH in same system.

The latter has been assigned to radicals which result from hydrogen atom abstraction in the α -position to sulfur by $\cdot OH$ radicals



with $Met(-H)\cdot = \dot{C}H_2SCH_2$ or $CH_3S\dot{C}H$.⁴ The yield of reaction 4 accounts for ca. 20% of the $\cdot OH$ radicals which corresponds to $G \approx 0.2 \times 5.6 = 1.1$ species per 100 eV absorbed energy ($0.12 \mu M J^{-1}$).² The extinction coefficient of these species has been determined to $\epsilon = 3000 (\pm 600) M^{-1} cm^{-1}$ at 290 nm.⁴ Since the total yield of absorption at this wavelength amounts to $G\epsilon = 4300$ and the above α -thio radicals account only for $G\epsilon = 3300$ the remainder of $G\epsilon = 1000$ must be contributed by still another absorbing species (units of $G\epsilon$ are species $(100 eV)^{-1} M^{-1} cm^{-1}$). The only other radical present at this time scale, and formed through the reaction sequence 1–3 from the other 80% of the $\cdot OH$ radicals are the α -amino radicals. The remaining absorption is therefore attributed to these latter species.

At higher pH the overall absorption is increased at all wavelengths. The 290-nm peak shows up still as a small shoulder at pH 5 (Figure 1b) but at pH 11 (Figure 1c) just a steady increase in absorption toward the UV can be seen. Since there is no evidence that the yield of reactions 3 and 4 (decarboxylation and $Met(-H)\cdot$ formation) and ϵ of $Met(-H)\cdot$ change over this pH range to any such extent the observed pH dependence in absorption is attributed to the two acid/base forms of the α -amino radical in the equilibrium

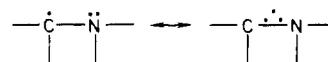


This assumption is confirmed by optical and conductivity measurements at varying pH. Figure 2a shows the change in optical absorption recorded at 260 nm ca. 5 μ s after the pulse as a function of pH. From the point of inflection of the typical pK-type curve, a pK = 3.85 is derived for the protonation of the α -amino radical.

The corresponding conductivity picture is shown in Figure 2b. At the high pH side essentially no change in conductivity is observed. This is to be expected if the $CH_3SCH_2CH_2\dot{C}HNH_3^+$ radical formed via reactions 1 and 3 is in fact deprotonated according to the forward reaction in eq 5 (H_{aq}^+ and OH^- from reactions 5 and 1, respectively, will of course immediately undergo neutralization). Shifting of equilibrium 5 to the left by decreasing the pH will then result in conversion of the free and highly conducting proton ($\Lambda = 315 \Omega^{-1} cm^2$ at 18 °C) into a much less conducting "normal" cation ($\Lambda \approx 30\text{--}50 \Omega^{-1} cm^2$). Accordingly, the conductivity should decrease and eventually

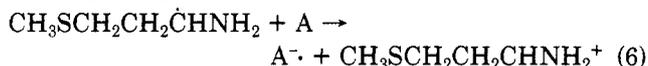
level off for the given system at $G\Delta\Lambda = 0.8 \times 5.6(30\text{--}315) = -1280$. The half-value of $G\Delta\Lambda = -640$ is again found at pH 3.85 (see arrow in Figure 2b). Also the other measurable data points (the conductivity techniques allows measurements only at $pH \geq 3$) fit well the curve which is calculated on the basis of this pK (solid line).

The relatively low pK indicates the α -amino radical to be a rather weak base in comparison with normal amines. A rationale for this is provided by the resonance between the unpaired electron at the α -carbon atom and the free electron pair at the nitrogen atom



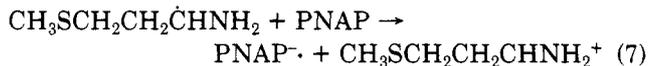
which in a theoretical consideration of simple aliphatic α -amino radicals has in fact been suggested to result in a $2\sigma/2\pi/1\pi^*$ two-center, five-electron bond between the two involved atoms.¹⁶ No resonance energy can of course be gained after protonation of the nitrogen. Resonance stabilization could also well explain the red shift in optical absorption of the neutral α -amino radical in comparison to its protonated form. The extinction coefficient of $CH_3SCH_2CH_2\dot{C}HNH_2$ is evaluated to be about 370 and 840 $M^{-1} cm^{-1}$ at 340 and 290 nm, respectively. The 340-nm value, incidentally, is very similar to $\epsilon = 510 M^{-1} cm^{-1}$ reported for the $(CH_3)_2N\dot{C}H_2$ radical.¹⁵ At 260 nm we obtained two slightly different values at pH 5 and 11, respectively, namely, $\epsilon = 1200$ and $1900 M^{-1} cm^{-1}$. This discrepancy may be due to an ionized sulfur-centered $\cdot OH$ adduct, $(>SO)^-\cdot$, which is known to be stabilized in case of simple aliphatic sulfides at high pH.²⁰ Extinction coefficients for protonated $CH_3SCH_2CH_2\dot{C}HNH_3^+$ amount to 230 and 320 $M^{-1} cm^{-1}$ at 290 and 260 nm, respectively. In any case an error limit of at least $\pm 30\%$ should be allowed for the extinction coefficients derived from our experiments.

Electron Transfer Reactions. The optical absorptions of the α -amino radicals decay by second-order processes with half-lives of about 300–400 μ s under our pulse radiolysis conditions, i.e., with $2k$ on the order of $10^9 M^{-1} s^{-1}$. In the presence of suitable electron acceptors A, their decay kinetics are changed to pseudo-first-order conditions indicating the general reaction



The experiments described below have generally been carried out with N_2O -saturated solutions containing $(1\text{--}5) \times 10^{-3}$ M methionine and $(1\text{--}10) \times 10^{-5}$ M electron acceptor.

a. p-Nitroacetophenone. The reaction of the deprotonated α -amino radical with *p*-nitroacetophenone (PNAP)



is best observed by the formation of the $PNAP\cdot$ radical anion absorption at 350 or 545 nm ($\epsilon = 17\,600$ and $2900 M^{-1} cm^{-1}$, respectively).²¹

Figure 3a shows, for example, a trace of the optical absorption at 350 nm as a function of time in a solution containing 5×10^{-3} M methionine and 2×10^{-5} M PNAP at pH 11. The yield of $PNAP\cdot$ amounts to 77% of the initial $\cdot OH$ radical yield which shows that reaction 7 is practically quantitative. The half-life for the $PNAP\cdot$

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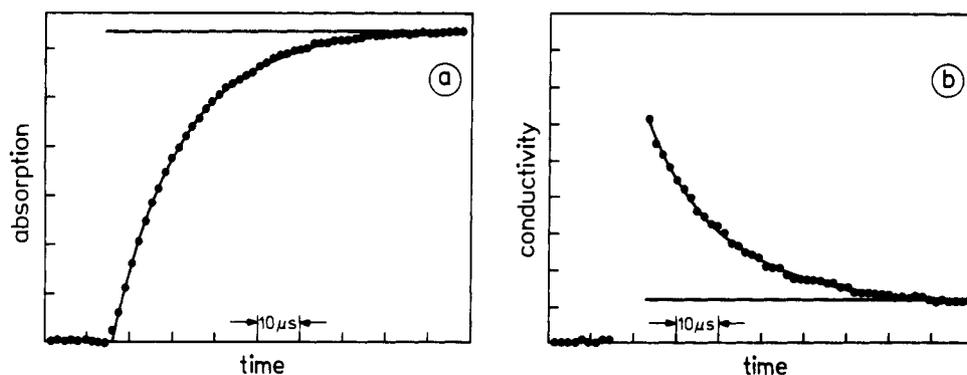
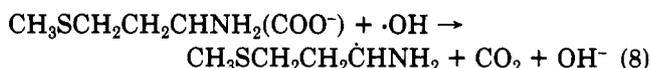


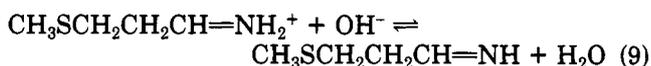
Figure 3. (a) Optical absorption at 350 nm as function of time in a 1- μ s pulse-irradiated, N_2O -saturated aqueous solution containing 5×10^{-3} M methionine and 2×10^{-5} M *p*-nitroacetophenone, at pH 11. (b) Conductivity-time curve in same solution.

formation was 12 μ s in this particular experiment. Considering also the data from solutions containing various PNAP concentrations we evaluated a bimolecular rate constant of $k_7 = (3.9 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for the electron transfer process.

Reaction 7 can also be followed by conductivity measurements. The corresponding signal is shown in Figure 3b. The initial increase in conductivity in this case is due to the formation of highly conducting OH^- ions ($\Lambda \approx 180 \Omega^{-1} \text{ cm}^2$) at the expense of the less conducting "normal" methionine anion in the overall reaction



The conductivity signal then decays exponentially with the same kinetics as for the PNAP \cdot formation. Thus the electron transfer must be accompanied by liberation of a proton to consume the OH^- ion formed in reaction 8, and this is attributed to deprotonation of the imine formed in reaction 7



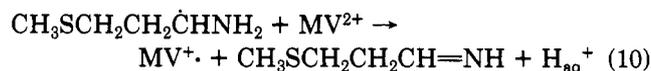
Conductivity measurements on a time scale up to a few seconds (with standing solutions) show, incidentally, that imine protonation does not occur at any pH down to 4 (limit of detection).

The reduction of PNAP can also be used as a probe for α -amino radical formation in the oxidation of methionine by electrophilic oxidants other than $\cdot\text{OH}$ radicals such as, for example, Br_2^- and $(\text{SCN})_2^-$. These radical anions have been shown to form relatively stable adducts of the type $>\text{S}\cdot\text{X}$ which are, however, in equilibrium with the radical cations $>\text{S}^+$ and $(>\text{S}\cdot\text{S})^+$.³ Oxidation through these radicals is therefore also expected to lead to decarboxylation and α -amino radical production at pH \gtrsim 3. Kinetic measurements on the reaction of α -amino radicals with PNAP are, however, only feasible in basic solutions where the lifetime of the $>\text{S}\cdot\text{Br}$ and $>\text{S}\cdot\text{SCN}$ is short enough so that reaction 7 can become the rate-determining step for the PNAP \cdot formation in the overall mechanism. Optical and conductivity curves taken under this condition (e.g., 5×10^{-3} M SCN^- , 2×10^{-4} M methionine, and 5×10^{-5} M PNAP, at pH 10.5) exhibit the same features as shown already in Figure 3 and substantiate the rate constant for reaction 7.

The yield of PNAP \cdot corresponds to 100% of the oxidizing $(\text{SCN}^-)_2^-$ species which means that also the yield of decarboxylation now accounts for 100% of the oxidizing radical and thus is higher than for the $\cdot\text{OH}$ radical induced mechanism (80%). The same situation is practically given for the Br_2^- induced oxidation.

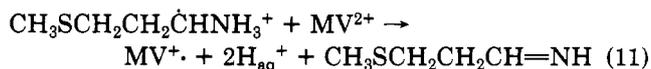
In less basic and acid solutions the stability of the $>\text{S}\cdot\text{X}$ radicals is high enough that other reactions of these species (e.g., oxidation reactions, deprotonation, second-order radical-radical reactions, etc.) could dominate over the decay route into α -amino radicals and CO_2 and accordingly much lower yields of the latter are found. Recent experiments have shown that the formation of α -amino radicals from $>\text{S}\cdot\text{X}$ is generally base catalyzed.²² Further details on the X_2^- (X = halide) induced oxidation of methionine, particularly in neutral and acid solutions, have already been reported.³

b. Methylviologen. The reaction of the α -amino radical with the dication of methylviologen, MV^{2+} , i.e.



can conveniently be monitored through the absorption of the $\text{MV}^{\cdot+}$ radical cation which has a strong absorption band peaking at 600 nm with $\epsilon = 11850 \text{ M}^{-1} \text{ cm}^{-1}$.²³ Traces of the optical absorption as a function of time recorded at this wavelength in pulse-irradiated, N_2O -saturated solutions of 5×10^{-3} M methionine and various low concentrations of MV^{2+} at pH 7 (buffered with 10^{-3} M phosphate) show again the same features as the curve in Figure 3a. The absorption increases exponentially with the half-life being inversely proportional to the MV^{2+} concentration. The bimolecular rate constant for reaction 10 derived from these experiments is $k_{10} = (3.5 \pm 0.3) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. The yield of $\text{MV}^{\cdot+}$ corresponds to the yield of the α -amino radical.² It is therefore assumed that its formation is essentially due to reaction 10. Any contribution by α -thioalkyl radicals which are also present is expected to be small ($<10\%$) since their yield is four times less than that of the α -amino radicals and furthermore their reaction rate constant with MV^{2+} appears to be comparatively low (estimated to be $\leq 10^7 \text{ M}^{-1} \text{ s}^{-1}$).

At lower pH near and below the pK of the α -amino radical the formation of $\text{MV}^{\cdot+}$ is found to occur considerably slower. Figure 4 shows the measured first-order rate constants $k' = \ln 2/t_{1/2}$ for the reduction of MV^{2+} by the α -amino radicals as a function of pH for solutions containing 5×10^{-3} M methionine and 5×10^{-5} M MV^{2+} . The results suggest that the reaction of the protonated α -amino radical



is considerably slower than the corresponding reaction of the unprotonated radical. In order to evaluate the absolute

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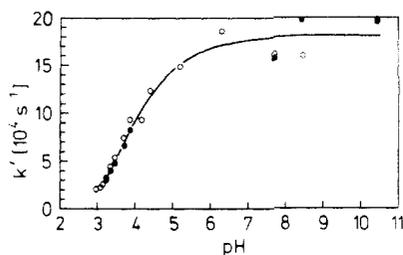


Figure 4. Measured first-order rate constants $k' = \ln 2/t_{1/2}$ as function of pH for the reduction of MV^{2+} via reactions 10 and 11 in pulse-irradiated, N_2O -saturated aqueous solutions of 5×10^{-3} M methionine and 5×10^{-5} M MV^{2+} : (O) optical, (●) conductivity measurements.

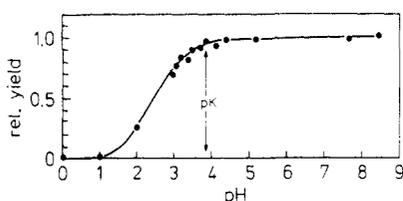


Figure 5. Yield of MV^+ from reduction of MV^{2+} by α -amino radicals (relative to $\cdot OH$ radical yield) as a function of pH in pulse-irradiated, N_2O -saturated aqueous solutions of 5×10^{-3} M methionine and 5×10^{-5} M MV^{2+} .

bimolecular rate constants for reactions 10 and 11 a computer analysis has been carried out on the basis of the data in Figure 4 and by assuming values of $k_{-5} = 1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ and $2k = 2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for the protonation and the mutual second-order decay of the α -amino radicals, respectively. Best fit of the data (solid curve in Figure 4) was obtained with $k_{10} = (3.6 \pm 0.3) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{11} = (1.0 \pm 0.2) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. The significant difference can, at least partially, be explained by the fact that in reaction 11 two species with the same sign are involved. Also, the positively charged radical would be generally expected to be weaker electron donor than the uncharged species.

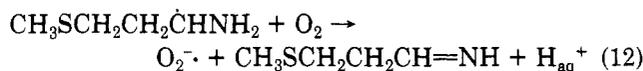
Figure 5 finally shows the pH dependence of the yield of MV^{2+} reduction by α -amino radicals (relative to the yield of $\cdot OH$ radicals) in the same solutions as for Figure 4. It can be seen that high yields of MV^+ are still found at and even below the pK of the α -amino radical clearly showing that reaction 11 does indeed occur. The decrease in yield at low $pH < 3$ is due to the overall reduction of α -amino radical yield and reflects the competition between the decarboxylation process and the stabilization of the oxidized sulfur center as $(>S\cdot S<)^+$ radical cation.^{1,2}

c. Other Electron Acceptors. A similar picture as for methylviologen was obtained for the reduction of tetramethylpiperidiny *N*-oxide (TMPN) by the α -amino radicals. Kinetic data were in this case obtained from time-resolved conductivity curves since reduction of TMPN is associated with an uptake of protons, i.e., with a change in conductance. Bimolecular rate constants of $k = (5.4 \pm 0.5) \times 10^8$ and $(2.3 \pm 0.3) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ have been evaluated for the reactions of TMPN with the neutral and the protonated form of the α -amino radical, respectively. These rate constants are of the same order of magnitude as were obtained for the reduction of TMPN by α -hydroxy alkyl radicals.²⁴

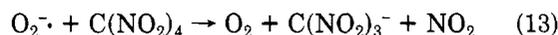
The reduction of cytochrome(III) *c* by radical intermediates from the $\cdot OH$ induced oxidation of methionine has already been reported and has been attributed to a hydroxyl adduct.²⁵ Since, however, both yield and lifetime

of any of the precursors of the α -amino radical, e.g., a species absorbing at 400 nm, are not affected by the presence of electron acceptors^{2,26} and furthermore the yield of cytochrome(III) *c* reduction is identical with the yield of α -amino radicals we consider in fact the latter to be the reducing agent.

The rate constant for the reaction



was determined by kinetic analysis of the α -amino radical decay at 290 nm to $k_{12} = (1.8 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. Although it was not possible to identify O_2^- unambiguously and directly by its own absorption spectrum (due to overlapping absorptions) the following indirect experiment provides evidence for the superoxide anion formation. On pulse radiolysis of an N_2O -saturated solution ($pH 6.5$) containing 5×10^{-3} M methionine, 10^{-4} M O_2 , and 2.0×10^{-5} M $C(NO_2)_4$ the formation of a high yield of stable nitroform anions, $C(NO_2)_3^-$, can be observed. Kinetic analysis of the formation of the $C(NO_2)_3^-$ absorption at 350 nm yielded a rate constant of $k_{13} = 1.95 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for the electron transfer reaction

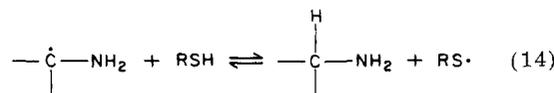


in excellent agreement with literature values of $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for this reaction.²⁷ Alternatively, O_2 could add to the α -amino radical, and the resulting peroxy radical could act as a reductant toward tetranitromethane. It can be expected, however, that such a reaction would probably occur with a lower rate constant or possibly be preceded by superoxide elimination from the peroxy radicals.

Further compounds which are easily reduced by the α -amino radicals are $Fe(CN)_6^{3-}$, lipoate, and nicotinamide adenine dinucleotide (NAD^+). The bimolecular rate constants for these and all the other electron transfer reactions presented above are listed in Table I, together with the one-electron redox potentials of the various electron acceptors. It can be seen that $CH_3SCH_2CH_2\dot{C}HNH_2$ reduces all our compounds with high rate constants close to the limits set by the diffusion of the reactants. From our data it can be derived that the reduction potential of the α -amino radical must be higher than that of NAD^+ , i.e., more negative than -0.94 V.

The reactivity of the α -amino radicals with respect to reduction reactions may be associated with the electronic structure of these species. In case of the neutral $\dot{C}HNH_2$ radicals the carbon–nitrogen bond seemingly assumes a partial π -character with some electron density in the antibonding π^* orbital.¹⁶ The protonated species $\dot{C}HNH_3^+$, on the other hand, is essentially a carbon-centered radical with the unpaired electron in a *p* orbital, since now nitrogen cannot provide a free electron pair anymore. Generally, it seems reasonable to assume that antibonding electrons are particularly easy to transfer and therefore the electronic situation would not only be in favor of a fast electron transfer but also explain the lower rate constants for the reactions of the protonated species.

H· Atom Transfer with Cysteine. Recently, an equilibrium



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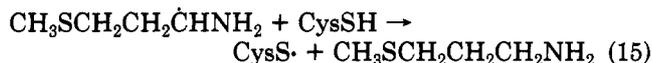
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TABLE I: Rate Constants for the Electron Transfer Reactions from Various α -Amino Radicals to Various Substrates S

α -amino radical	substrate S	k , $M^{-1} s^{-1}$	pH	$E_7^{1}(S/S^{\cdot-})$, V
$CH_3SCH_2CH_2\dot{C}HNH_2$	$Fe(CN)_6^{3-}$	$(3.5 \pm 0.4) \times 10^9$	5.8	+0.36
	$C(NO_2)_4^a$	$(4.2 \pm 0.5) \times 10^9$	4.5	+0.31 ^g
	cytochrome (III) ^c	$(6.6 \pm 0.6) \times 10^8$	5.0	+0.254
	TMPN ^b	$(5.4 \pm 0.5) \times 10^8$	5.0	-0.18
	lipoate	$(1-2) \times 10^8$	8.2	-0.29
	O_2	$(1.8 \pm 0.4) \times 10^9$	5.5	-0.33 (1 atm)
	PNAP ^c	$(3.9 \pm 0.4) \times 10^9$	4.3-11.0	-0.358
	MV^{2+} ^d	$(3.6 \pm 0.3) \times 10^9$	7.0	-0.447
$CH_3SCH_2CH_2\dot{C}HNH_3^+$	NAD^+ ^e	$(8.5 \pm 3) \times 10^8$	6.6	-0.94
	TMPN	$(2.3 \pm 0.3) \times 10^8$ ^f		
$CH_3SCH_2CH_2\dot{C}HNHCOCH_3$	MV^{2+}	$(1.0 \pm 0.2) \times 10^7$ ^f	4.7	
	$CH_3CH_2CH_2\dot{C}HNH_2$	PNAP	$(4.1 \pm 0.4) \times 10^9$	10.8

^a Proceeds possibly via an intermediate radical adduct.³⁵ ^b 4-Hydroxy-2,2,6,6-tetramethylpiperidiny-1-oxyl. ^c *p*-Nitroacetophenone. ^d Methylviologen. ^e Nicotinamide adenine dinucleotide. ^f Computer-evaluated data. ^g Dissociative, i.e., irreversible process.

has been proposed from photochemical studies on the reduction of aromatic ketones by amines in the presence of thiols in benzene solutions.²⁸ An estimate of $k_{-14} > 10^3 M^{-1} s^{-1}$ is given for the back reaction but no equilibrium constant and rate constant for the forward reaction could be evaluated. An estimate for the latter, however, in aqueous solutions, could now be derived for the reaction of our α -amino radical with cysteine (CysSH) in pulse-irradiated, N_2O -saturated, solutions of $10^{-2} M$ methionine and $5 \times 10^{-4} M$ cysteine at pH 8.3, i.e., at the pK of the sulfhydryl group. Under these conditions the thiyl radical formed in the reaction



associates with the corresponding thiolate anion



to the three-electron-bonded cystine radical anion which shows a strong optical absorption at 410 nm ($\epsilon = 5800 M^{-1} cm^{-1}$)²⁹ and under the experimental conditions is stable enough to be detectable through time-resolved optical measurements. The rate constant for the formation of this radical anion has been evaluated to $(1-2) \times 10^9 M^{-1} s^{-1}$ and thus is similar to that of the forward reaction in eq 16.³⁰ The rate constant for reaction 15 can therefore be assumed to be at least this value. The yields of cystine radical anions formed via the reaction sequence 15 and 16, incidentally, account for ca. 50% of the initial $\cdot OH$ radicals, and increase to ca. 90% if $Br_2\cdot^-$ is the oxidizing radical. These latter data are based on $K \approx 1 \times 10^4$ for equilibrium 16.³¹

At lower pH, e.g., at pH 4, reaction 15 cannot be monitored anymore through reaction 16 since $CysS^-$ is no longer available. No $CysS\cdot:SCys^-$ formation is also observable at higher pH, e.g., 10.5, where cysteine is completely ionized to $CysS^-$ and no hydrogen atom is available at the sulfhydryl group for abstraction.

Formation of α -Amino Radicals from Methionine Derivatives. One-electron reduction reactions similar to eq 6 may also be used as a probe for the formation of reducing radicals in the oxidation of methionine derivatives. The methylviologen system is particularly suited since its redox

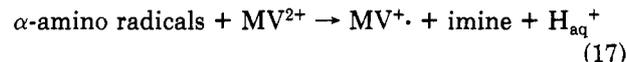
TABLE II: Relative Yields of Reducing Radicals from Oxidation of Various Compounds S Measured as $[MV^{\cdot+}]/[\cdot OH]$ in Pulse Irradiated, N_2O -Saturated Solutions of $5 \times 10^{-3} M$ S and $2.5 \times 10^{-5} M$ MV^{2+} at pH 4.7

S	$[MV^{\cdot+}]/[\cdot OH]$
methionine	1.0
α -methylmethionine	0.95
homomethionine	0.67
<i>S</i> -methylcysteine	≤ 0.05
methionine ethyl ester	0.07 ^a
<i>N</i> -acetylmethionine	0.04 at $2.5 \times 10^{-5} M$ MV^{2+} 0.16 at $1 \times 10^{-4} M$ MV^{2+}

^a At pH 5.7.

potential is negative enough to monitor only relatively good reductants, and the reduction product $MV^{\cdot+}$ exhibits an absorption band in the visible which does not interfere with other, mostly UV absorptions.

The yields of $MV^{\cdot+}$ formation from the reactions



are summarized in Table II. The experimental conditions were generally N_2O -saturated solutions (pH 4.6) of $2.5 \times 10^{-5} M$ MV^{2+} and $5 \times 10^{-3} M$ of the various methionine derivatives. As indicated in eq 3, the formation of α -amino radicals from methionine is accompanied by decarboxylation. No such process can, of course, be expected with esters and, accordingly, only a very low yield of reducing radicals is observed during the $\cdot OH$ radical induced oxidation of methionine ethyl ester. The observed yield may, in fact, even result from some ester hydrolysis during the time of preparation of the solution and the actual experiment. Alternatively, it could result from the small yield of the $H\cdot + MV^{2+}$ reaction.

Strong evidence has been provided that the decarboxylation reaction (eq 3) is an intramolecular process involving a cyclic structure which enables interaction between the oxidized sulfur center and the other end of the methionine molecule.^{1,2,7} Our experiments now gave high yields of reducing radicals similar to methionine also for α -methylmethionine and homomethionine, but almost negligible yields of $MV^{\cdot+}$ formation for *S*-methylcysteine solutions. The result would, therefore, suggest that interaction occurred in fact between sulfur and nitrogen, since methionine, α -methylmethionine, and homomethionine could form favorable 5- and 6-membered ring structures while *S*-methylcysteine could arrange only in a sterically strained 4-membered ring. Other experimental data,⁹ including some very recent investigation³² on the oxidation of 3-

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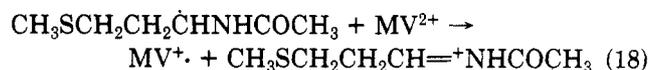
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methylmercaptopyrrolamine, 4-methylbutyric acid, and some N-substituted derivatives of methionine, also support this consideration. Our early suggestion that interaction occurred between sulfur and the carboxylate group¹ which would increase the number of participating atoms in the cyclic structures by one and thus would not discriminate the *S*-methylcysteine anymore has now been revived on the basis of some ESR experiments.⁷ Our experiments do not exclude the—and in fact reported—decarboxylation of oxidized *S*-methylcysteine. The lack of methylviologen reduction shows, however, that $\text{CH}_3\text{SCH}_2\dot{\text{C}}\text{HNH}_2$ radicals, if formed at all, would not exhibit the expected strong reducing properties. β -Scission of this radical has been suggested to occur only in basic solutions at $\text{pH} > 9.2^7$ while our experiments have been carried out at $\text{pH} 4.6$. At the moment we are not able to establish a common basis to accommodate all experimental findings and additional work on the *S*-methylcysteine and related systems seems still necessary.

The α -amino radicals from α -methylmethionine, $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}(\text{CH}_3)\text{NH}_2$, and homomethionine, $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}_2\dot{\text{C}}\text{HNH}_2$, are evidenced also through optical absorptions similar to that of $\text{CH}_3\text{SCH}_2\text{CH}_2\dot{\text{C}}\text{HNH}_2$ (see Figure 1). They all exist in acid–base equilibria as given in eq 5 for the methionine derived radical. Preliminary experiments indicate that the α -methylmethionine derived radical has a higher pK than the methionine-derived species. Higher electron donation by the methyl group relative to hydrogen provides a reasonable explanation for this. Analogous findings have also been made for the pK values of α -hydroxyalkyl radicals.^{33,34} The high yield of reducing radicals from homomethionine was also demonstrated in solutions containing O_2 and $\text{C}(\text{NO}_2)_4$ through a reaction sequence corresponding to reactions 12 and 13. The yield of tetranitromethane reduction in this case was measured to be about 85% relative to the initially available $\cdot\text{OH}$ radicals and thus is seen to correlate with the yields of MV^{2+} reduction (see Table II) within experimental limits of error.

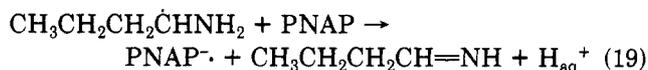
Very low yields of reducing radicals are finally found for *N*-acetylmethionine although $\cdot\text{OH}$ radical attack has been

shown to lead to decarboxylation with about 50% efficiency over the entire $\text{pH} 4$ – 11 range.² The yield of MV^{2+} reduction is, however, strongly dependent on the methylviologen concentration indicating that the reaction



is probably slow and has to compete with other reaction pathways of the α -amino radical. At 10^{-4} M MV^{2+} reaction 18 occurs with 16% efficiency and the half-life for the $\text{MV}^{\cdot+}$ formation was about $140 \mu\text{s}$. The rate constant of $5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ derived from these data is, however, not the true rate constant for reaction 18 since the observable rate of $\text{MV}^{\cdot+}$ formation reflects not only reaction 18, but all competing reactions of the α -amino radical as well. The true rate constant for the electron transfer reaction is thus estimated to only $(1\text{--}2) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. This rather low value is, of course, explained by the effect of the electron-withdrawing acetyl substituent.

The fact that these reduction reactions are indeed a good probe for α -amino radicals, in general, is finally supported by experiments carried out with a longer chain aliphatic amine. In the reaction of $\cdot\text{OH}$ radicals with *n*-butylamine, for example, hydrogen atom abstraction could in principle occur at any carbon atom but only the α -amino radical is expected to undergo electron transfer via



The observed yield of this reaction accounts for ca. 33% of the $\cdot\text{OH}$ radicals, a value which is similar to the yield of reducing α -hydroxy radicals formed in the reaction of $\cdot\text{OH}$ radicals with 1-butanol.¹⁰ The bimolecular rate constant for reaction 19 is derived to be $(4.1 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.

Acknowledgment. The authors are very grateful to H.-P. Schenck for his experimental help in the determination of some of the rate constants.

Registry No. $\text{CH}_3\text{SCH}_2\text{CH}_2\text{C}\cdot\text{HNH}_2$, 86101-37-3; $\text{CH}_3\text{SCH}_2\text{CH}_2\text{C}\cdot\text{HNH}_3^+$, 86101-38-4; $\text{CH}_3\text{SCH}_2\text{CH}_2\text{C}\cdot\text{HNHCOC}\cdot\text{H}_3$, 86101-39-5; $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}\cdot\text{HNH}_2$, 26374-10-7; $\text{Fe}(\text{CN})_6^{3-}$, 13408-62-3; $\text{C}(\text{NO}_2)_4$, 509-14-8; O_2 , 7782-44-7; TMPN, 2226-96-2; PNAP, 830-03-5; MV^{2+} , 4685-14-7; NAD^+ , 865-05-4; cytochrome *c*, 9007-43-6; lipoate, 1077-29-8; L-methionine, 63-68-3; α -methyl-DL-methionine, 2749-07-7; DL-homomethionine, 6094-76-4; *S*-methyl-L-cysteine, 1187-84-4; L-methionine ethyl ester, 3082-77-7; *N*-acetyl-L-methionine, 65-82-7.

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