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Radical-Based Damage of Sulfur-Containing Amino Acid Residues

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1 INTRODUCTION

Sulfur (S)- and selenium (Se)-containing amino acids serve a variety of important functions in peptides and proteins such as the maintenance of structure (e.g., through disulfide bonds),[1] the binding of metals,[2] as sites for redox regulation[3] or mediators of redox processes,[4] as target sites for electrophiles,[5] and as constituents of active sites. Inherent to S- and Se-containing amino acids is their efficient reaction with free radicals and reactive oxygen species, which may lead to conformational changes and/or both activation and inactivation of enzymes dependent on the extent of modification and the nature and location of the reaction products. Such radical- and/or oxidation/reduction-based modifications are of significance for an increasing number of cellular signal transduction processes[3,5] and also for protein damage, which is frequently observed under biologic conditions of oxidative stress (see **Oxidative Damage to Proteins**, Volume 3).[6] Importantly, a radical reaction with a primary target amino acid will lead to secondary radicals. These may engage in radical- and/or electron-transfer processes with additional protein amino acids such that the ultimate location of a radical damage may evolve potentially far from the site of initial radical attack. Such processes have been documented for radicals derived from S-containing amino acids.[7] Hence, the reaction of radicals with S- and Se-containing amino acids has the potential for generating a wide array of reaction products and a thorough understanding of such processes would ultimately be of great benefit for biomedical studies attempting to correlate the oxidative damage of specific proteins under conditions of oxidative stress with physiologic observations. For this purpose, this article will cover both the reactions of free radicals with S- and Se-containing amino acids and the radical chemistry of the product radicals from these amino acids. The scope of reactions has mostly been limited to radicals of biological relevance.

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This article focuses predominantly on radical reactions of specific S- and Se-containing amino acids important for mammalian proteins and physiology, which are introduced in the following (the respective structures are displayed in Figure 1). In native mammalian proteins, S- and Se-containing L-amino acids are present as cysteine (Cys, **1**), selenocysteine (SeCys, **2**), methionine (Met, **3**), and selenomethionine (SeMet, **4**), where the number of SeCys- and SeMet-containing proteins is very small relative to the total number of proteins.[8] Cys is also key to the activity of the tripeptide glutathione (GSH, **5**), which is present in tissue at concentrations up to millimolar range. The amino acid homocysteine (Hcys, **6**) does not represent a building block of proteins, but can bind to proteins through the formation of mixed disulfides or reaction of its thiolactone form with protein nucleophiles.[9,10] Hcys can form through a free radical reaction of Met[11]

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(*vide infra*). Hcys displays pro-inflammatory activity and its physiologic levels represent an important risk factor for several pathologies, including cardiovascular disease and stroke.[9,12]

2 THIOLS AND SELENOLS

On the basis of the relatively low bond dissociation energies (BDEs) of RS-H (BDE = 367 kJ mol^{-1} [13]) and RSe-H (BDE = 310 kJ mol^{-1} [14]), thiols and selenols are excellent hydrogen donors for hydrogen transfer reactions to radicals. These reactions generate thiyl (X=S) and selenyl (X=Se) radicals, respectively (reaction 1):

$$Y^{\bullet} + R{-}X{-}H \rightarrow Y{-}H + R{-}X^{\bullet} \qquad (1)$$

Rate constants for such hydrogen transfer processes depend on the nature of the radical Y$^{\bullet}$ and, representatively measured for RXH = dithiothreitol (DTT), can be as low as 7.4×10^7 M^{-1} s^{-1} for Y$^{\bullet}$ = $^{\bullet}CH_3$[15] or as high as 1.4×10^{10} M^{-1} s^{-1} for Y$^{\bullet}$ = HO$^{\bullet}$.[16] The reaction of heteroatom (Z)-substituted carbon-centered radicals from alcohols, ethers, and amines with thiols is reversible[17–21] (equilibrium 2; for more details on such reversible reactions of Cys, Hcys, and GSH, *vide infra*):

$$-Z_n - C^{\bullet} + RS - H \rightleftharpoons -Z_n - CH + RS^{\bullet} \qquad (2)$$

For synthetic organic reactions, the reverse reactions, that is, hydrogen transfer from C–H bonds to thiyl radicals, have been explored in epimerization processes, and thiols have been referred to as "polarity reversal catalysts."[22] Thiyl radicals also play an important role in enzymatic processes such as that catalyzed by the ribonucleotide reductases,[23–25] pyruvate formate lyase,[23,25–28] benzylsuccinate synthase,[29,30] and glycerol dehydratase.[31] Here, intermediary Cys thiyl radicals abstract hydrogen atoms from substrate during the catalytic cycle of ribonucleotide reductase, benzylsuccinate synthase, and glycerol dehydratase, while in pyruvate formate lyase, an intermediary Cys thiyl radical adds to the carbonyl group of the substrate pyruvate (see **Radical Enzymes**, Volume 3).

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2.1 Cysteine, Homocysteine, and Glutathione

2.1.1 Formation and Properties of Thiyl Radicals

The mercapto group of the free amino acids Cys and Hcys ionizes with pK_a values of 8.15 and 8.7,[32] respectively (measured at 30 °C, $\mu = 0.3$) (isothermal titration microcalorimetry of Cys confirms a thiol pK_a of 8.22 ± 0.16[33]), while the thiol pK_a of GSH is 8.93 ± 0.04 (at 25 °C).[34] A significantly wider pK_a range is observed for protein Cys residues, which display pK_a values as low as 5[35] and as high as ca. 10.75.[33] Thiol ionization has a profound effect on the reaction of thiol/thiolate with free radicals as well as the potential stabilization of thiyl radicals. For example, nonoxidizing carbon-centered radicals react preferentially with the protonated thiol form (reaction 1), while oxidizing radicals prefer reaction with the deprotonated, thiolate form (reaction 3).[36]:

$$Ox^{\bullet} + R - S^{-} \rightarrow Ox^{-} + R{-}S^{\bullet} \qquad (3)$$

The resulting thiyl radicals associate with a second, nonoxidized thiolate to produce a $2\sigma/1\sigma^*$ three-electron-bonded disulfide radical anion [RS $\therefore$ SR]$^-$ (equilibrium 4).[37]:

$$RS^{\bullet} + RS^{-} \rightleftharpoons [RS \therefore SR]^{-} \qquad (4)$$

Equilibrium constants for thiyl radical/thiolate complex formation (equilibrium 4) have been measured for Cys and Hcys and are of the order of 706 and 600 M^{-1}, respectively.[38] In contrast, *inter*molecular complexes between thiyl radical and protonated thiol display low stability and either dissociate (reaction 5) or eliminate a proton to yield the disulfide radical anion (equilibrium 6):

$$[RSH \therefore SR] \rightarrow RS + RSH \qquad (5)$$

$$[RSH \therefore SR] \rightleftharpoons H^{+} + [RS \therefore SR]^{-} \qquad (6)$$

This situation can change for proteins, where Cys residues may stay in close contact through conformational restrictions. In fact, both the disulfide radical anion and its protonated form have been detected on one-electron reduction of bovine IgG

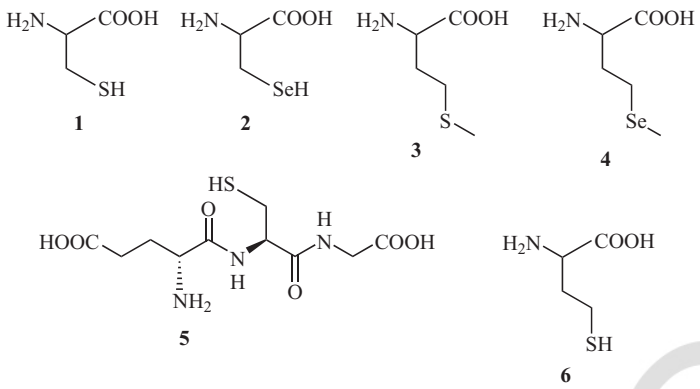


Figure 1 Structures of S- and Se-containing amino acids and GSH.

by $CO_2^{\bullet-}$ in a pH-dependent manner (reaction 7), and the pK_a for equilibrium (8) in bovine IgG is between 4.5 and 6.5[39]:

$$\mathrm{IgG(RSSR) + CO_2^{\bullet-} \rightarrow [IgG(RS\therefore SR)]^- + CO_2} \quad (7)$$

$$\mathrm{[IgG(RS\therefore SR)]^- + H^+ \rightleftharpoons [IgG(RSH\therefore SR)]} \quad (8)$$

The disulfide radical anion is a strongly reducing species, with $E^0 = -1.60\,\mathrm{V}$ (for the half reaction $\mathrm{e^- + RSSR = [RS\therefore SR]^-}$),[40] which efficiently reduces molecular oxygen to superoxide (reaction 9).[41] In contrast, thiyl radicals are oxidizing species with $E^0 = 1.33\,\mathrm{V}$ for the half reaction $\mathrm{RS^\bullet + e^- + H^+ = RSH}$ (determined for 2-mercaptoethanol)[40] and $E^0 = 0.74 \pm 0.01\,\mathrm{V}$ for the half reaction $\mathrm{RS^\bullet + e^- = RS^-}$[40] ($E^0 = 0.73\,\mathrm{V}$ reported for Cys thiyl radicals, $\mathrm{CysS^\bullet}$[42]). More recent electrochemical experiments on the oxidizing power of glutathione thiyl radical ($\mathrm{GS^\bullet}$) reveal $E_m = 0.92 \pm 0.03\,\mathrm{V}$ at pH 7.4[43]:

$$\mathrm{[RS\therefore SR]^- + O_2 \rightarrow RSSR + O_2^{\bullet-}} \quad (9)$$

Hence, through the association with thiolate, an oxidizing thiyl radical converts into a reducing radical anion, and the net availability of oxidizing and reducing species in a given biologic environment will be a function of thiol concentration and thiol pK_a. Thiyl radicals and disulfide radical anions can be monitored directly with time-resolved UV spectroscopy (coupled to laser flash photolysis or pulse radiolysis), where thiyl radicals have a reported absorbance maximum at 330 nm with reported absorption coefficients between 100 and $1200\,\mathrm{M^{-1}\ cm^{-1}}$ depending on the nature of the thiyl radical.[44,45] If experimentally possible, thiyl radicals should be detected complementary also through their disulfide radical anion complexes, which absorb around 420 nm with absorption coefficients of the order of $8000\,\mathrm{M^{-1}\ cm^{-1}}$ ($\mathrm{[RS\therefore SR]^-}$ from Cys and GSH)[46] (see **Radiation-Induced Radical Reactions**, Volume 1). rad005

2.1.2 The Reaction of Cys with Carbon-Centered Radicals

The forward reaction of equilibrium (2) represents a hydrogen transfer from a thiol to a carbon-centered radical. Experimental and theoretical data indicate that the kinetics of hydrogen transfer are influenced by polar effects,[15,47,48] that is, the stabilization of polar transition states through substituents on the carbon-centered radical. For example, experimental values for k_2 increase in the series $\mathrm{^\bullet CH_3 < {}^\bullet CH_2OH < {}^\bullet C(CH_3)_2OH}$, while the experimental activation energies E_a show the opposite trend.[15] Theoretical calculations (gas phase) reveal decreasing activation enthalpies for the series $\mathrm{^\bullet CH_3 > {}^\bullet CH_2OH > {}^\bullet C(CH_3)_2OH}$, consistent with the experiment[15] (in equilibrium 2, $\mathrm{^\bullet CH_3}$ is represented by $-\mathrm{Z}_n\mathrm{-C^\bullet}$ for which $n = 0$). They also reveal increasing charge separation in the transition state for the series $\mathrm{^\bullet CH_3 < {}^\bullet CH_2OH < {}^\bullet C(CH_3)_2OH}$,[15] in support of the polar effect already suggested by Roberts and Steel.[47] Polar transition states can be expressed through a series of valence-bond

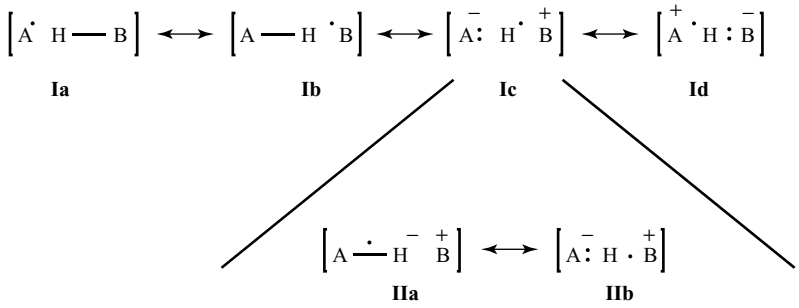
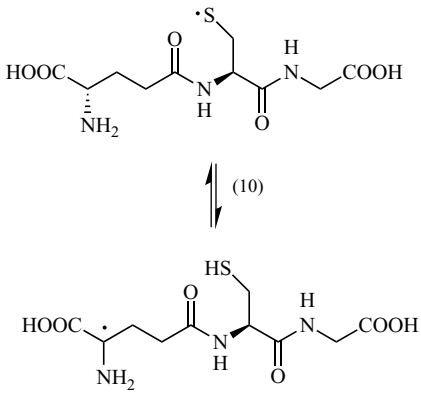


Figure 2 Valence-bond structures of transition states for hydrogen transfer reactions. [Adapted from Refs 15 and 47.]

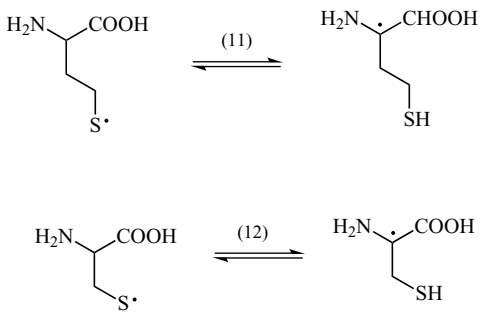
structures **Ia**–**Id**, where structure **Ic** may be better expressed as three- and one-electron bonded structures **IIa** and **IIb**, respectively, displayed in Figure 2.[15,47] With reference to equilibrium (2), A in Figure 2 corresponds to RS and B corresponds to $-Z_n$–C, that is, for the reaction of hydroxymethyl radicals with a thiol, structures **IIa** and **IIb** would translate into [RS $\therefore$ $H^{-+}CH_2OH$] and [$RS^-H{\cdot}^+CH_2OH$], respectively.

Importantly, the oxidizing carbon-centered radical $^{\bullet}CH_2CHO$ reacts only very slowly with GSH,[36] an observation that can be rationalized by the lack of stabilization of a positive charge on the methylene carbon in the transition state. In fact, oxidizing carbon-centered radicals such as $^{\bullet}CH_2CHO$ will react predominantly with thiolate, RS^-.

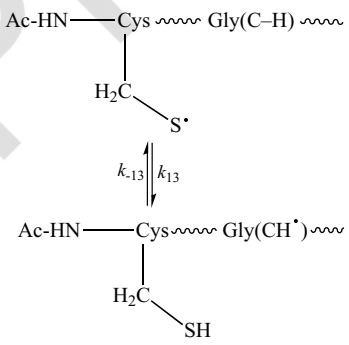
Values for k_2 and k_{-2} have been obtained through isotope exchange of C–H bonds,[49–51] radiation chemical isomerization,[17] and time-resolved pulse radiolysis experiments.[18,19,38] For alcohols and ethers, $k_2 = 10^7 - 10^8\ M^{-1}\ s^{-1}$ and $k_{-2} = 10^3 - 10^4\ M^{-1}\ s^{-1}$,[17–19] and for amino acids at pH 10.5 (i.e., the anionic form), $k_{-2} = 3.2 \times 10^5\ s^{-1}$ (Gly) and $7.7 \times 10^5\ s^{-1}$ (Ala).[38] In contrast, for amino acids within model peptide structures such as *N*-acetyl amino acid amides, and for cyclic amino acid anhydrides, diketopiperazines, $k_{-2} = 10^3 - 10^5\ M^{-1}\ s^{-1}$.[50] An important question is whether reversible hydrogen transfer reactions of $CysS^{\bullet}$ proceed *intra*molecularly in peptides and proteins, that is, whether $CysS^{\bullet}$ radicals are in equilibrium with other amino acid radicals and are potentially able to initiate the oxidation of other amino acids. For $GS^{\bullet}$, an *intra*molecular hydrogen transfer equilibrium between the $^{\alpha}$C–H group of the N-terminal γ-Glu residue and $CysS^{\bullet}$ radical had been demonstrated through electron spin resonance and pulse radiolysis experiments (equilibrium 10), where $k_{10} = 1.8 \times 10^5\ s^{-1}$.[38,52] This hydrogen transfer is facilitated through deprotonation of the N-terminal amino group of GSH ($pK_{a,NH_3^+} = 9.28 \pm 0.1$; 25 °C[34]) but proceeds well at neutral pH unless thiyl radicals are directed toward competitive pathways such as reaction with thiolate (equilibrium 4) or with oxygen (*vide infra*) (especially in physiological environment also the reaction of thiyl radicals with ascorbate has to be considered):



Analogous hydrogen transfer reactions have been documented for thiyl radicals from Hcys (equilibrium 11) and Cys (equilibrium 12),[38] where the rate constants for the forward reactions, $k_{11} = 2.2 \times 10^5\ s^{-1}$ and $k_{12} = 2.5 \times 10^4\ s^{-1}$. Reaction (12) represents a 1,3-H-shift, and generally activation energies for 1,3-H-shifts (and 1,2-H-shifts) in radicals are significantly higher than that for 1,4- and 1,5-H-shifts,[53,54] rationalizing that $k_{12} < k_{11}$:

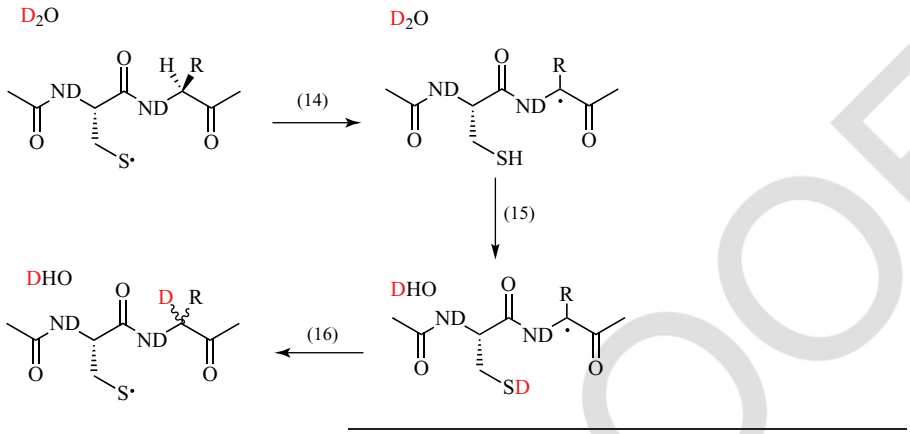


Theoretical calculations for the gas phase indicate that hydrogen transfer from the γ-Glu $^{\alpha}$C–H group in GS$^{\bullet}$ to the thiyl radical represents the preferred pathway, associated with a free energy of activation, $\Delta G^{\neq} = 40\,\mathrm{kJ\,mol^{-1}}$,[55] though thermodynamically all $^{\alpha}$C–H bonds in GS$^{\bullet}$ are expected to have a lower BDE compared to the BDE of the S–H bond ($\mathrm{BDE_{S-H}} \approx 367\,\mathrm{kJ\,mol^{-1}}$): $\mathrm{BDE_{\alpha C-H,Gln}} \leq 329\,\mathrm{kJ\,mol^{-1}}$ for the configuration H_2N–C$^{\alpha}$H(R)–CO_2H (experimental value), $\mathrm{BDE_{\alpha C-H,Cys}} = 346\,\mathrm{kJ\,mol^{-1}}$,[13] and $\mathrm{BDE_{\alpha C-H,Gly}} \leq 350\,\mathrm{kJ\,mol^{-1}}$. However, recent pulse radiolysis experiments have expanded the range of possible hydrogen transfer reactions of GS$^{\bullet}$, suggesting, based on time-resolved UV spectroscopy, that not only the $^{\alpha}$C–H but also the $^{\beta}$C–H group of the γ-Glu residue are targets for hydrogen abstraction by the thiyl radical in GS$^{\bullet}$.[56] The latter is counter-intuitive based on a comparison of BDEs, where the $^{\alpha}$C–H bond of the γ-Glu residue (experimental: $\mathrm{BDE} \leq 329\,\mathrm{kJ\,mol^{-1}}$ for the configuration H_2N–C$^{\alpha}$H(R)–CO_2H[57]) should be considerably lower than the BDE of $^{\beta}$C–H (BDE is expected to be of the order of $395\,\mathrm{kJ\,mol^{-1}}$ when compared to the secondary C–H bond in propane[58]). However, clearly the time-resolved experiments by Hofstetter *et al.*[56] suggest the presence of more than one carbon-centered radical in GS$^{\bullet}$. Some additional apparent discrepancies between theory and experiment need to be addressed: calculations for Hcys reveal an activation energy for reaction (11) of circa $50\,\mathrm{kJ\,mol^{-1}}$[59]; nevertheless, the rate constants for reactions (10) and (11) are comparable, where the experimental value of $k_{11} = 2.2 \times 10^5\,\mathrm{s^{-1}}$ was discussed as possibly being even too low.[38] On the other hand, calculations predict a high activation enthalpy for reaction (12), $\Delta H^{\neq} = 83 - 115\,\mathrm{kJ\,mol^{-1}}$.[13,55] Nevertheless, a rate constant of $k_{12} = 2.5 \times 10^4\,\mathrm{s^{-1}}$ was reported. Although it was discussed that this value may be too high, the fact remains that reaction (12) was observed for CysS$^{\bullet}$ despite the calculated high activation enthalpy. Calculations also revealed a very high free energy of activation for hydrogen transfer between the Gly residue and the thiyl radical in GS$^{\bullet}$, $\Delta G^{\neq} = 134\,\mathrm{kJ\,mol^{-1}}$ for the more stable peptide conformation,[55] consistent with the original reports of exclusive hydrogen transfer between the γ-Gln $^{\alpha}$C–H bond and the thiyl radical in GS$^{\bullet}$. However, pulse radiolysis and laser flash photolysis experiments established rate constants for reversible hydrogen transfer equilibria in radicals from several Cys-, Gly-, and Ala-containing peptides. For example, for radicals from *N*-acetyl-CysGly$_6$, equilibrium (13) is characterized by rate constants of $k_{13} = 1 \times 10^5\,\mathrm{s^{-1}}$ and $k_{-13} = 8.9 \times 10^5\,\mathrm{s^{-1}}$, that is, $k_{13} \approx 0.1$[45]:



(13)

Of course with six available Gly residues in *N*-acetyl-CysGly$_6$, the possibility exists that hydrogen transfer does not involve the residue at position $n + 1$, but Gly residues further along the model peptide chain. On the other hand, mass spectrometry experiments on products originating from thiyl radicals of several model peptides suggest that CysS$^{\bullet}$ within a model peptide can react via hydrogen transfer with amino acids at the $n + 1$ position.[60] For example, when the disulfide-containing peptide (GlyGlyCysGlyGlyLeu)$_2$ was photolyzed with 253.7-nm light in D_2O, both Gly residues in position $n + 1$ and $n - 1$ underwent covalent H/D exchange, analyzed in the final products.[60] The suggested mechanism for covalent H/D exchange is displayed in the following reactions:



Further evidence for thiyl radical-dependent $^{\alpha}C^{\bullet}$ radical formation at amino acid residue at positions $n+1$ and $n-1$ comes from photochemical studies of the disulfide-bound dimer $(LeuGlyAlaCysAlaGlyLeu)_2$, where initial thiyl radical formation resulted in the conversion of L-Ala into D-Ala.[61] Experimental mass spectrometry studies confirm the potential for $CysS^{\bullet}$ radicals to abstract hydrogen atoms from amino acid residues at positions $n+1$ and $n-1$ in the gas phase.[62] Under mass spectrometry conditions, the mobile proton model allows for the protonation of the carbonyl functions of various amide bonds, rendering the protonated amides more powerful electron-withdrawing functionalities, which can result in a higher capto-dative stabilization of some $^{\alpha}C^{\bullet}$ radicals. In calculations for solution conditions, Himo[63] showed that hydrogen bonds with both amides in the Gly model $CHO–NH–CH_2–CO–NH_2$ reduced the Gly $^{\alpha}C–H$ bond energy by circa 4.2 kJ mol^{-1}, and solvation also led to a reduction by circa 5.4–8.8 kJ mol^{-1}. However, the effects of hydrogen bonding on the free energy of activation for hydrogen transfer are currently unknown.

2.1.3 The Reaction of Cys with Superoxide

Superoxide anion ($O_2^{\bullet-}$) represents the initial one-electron reduction product of molecular oxygen, and several enzymatic routes generate superoxide under conditions of oxidative stress. Superoxide serves as a precursor for various reactive oxygen species, most of which react with thiols (e.g., peroxynitrite, the product of nitrogen monoxide (NO), and superoxide, or the carbon trioxide radical anion, $CO_3^{\bullet-}$, which forms via homolysis of $ONO_2CO_2^-$, a complex of peroxynitrite and CO_2).[64] A biologically important and controversially discussed question is the *direct* reaction of superoxide with Cys (and thiols, in general). The exposure of various biologically relevant thiols to a superoxide-generating system (xanthine/xanthine oxidase) resulted in a superoxide-dependent, that is, superoxide dismutase (SOD)-inhibitable, loss of these thiols via a short chain reaction, paralleled by the consumption of oxygen.[65] Importantly, only Cys and penicillamine (Pen) promoted the formation of H_2O_2, while no H_2O_2 formation was detected for cysteamine (Cya), GSH, *N*-acetylcysteine (NAC), and DTT. These results were interpreted such that the reaction of superoxide with Cya, GSH, NAC, and DTT proceeds to generate sulfinyl radicals and HO^- (reactions 17 and 18) via an intermediary thiol/superoxide complex:

$$RSH + O_2^{\bullet-} \rightleftharpoons [RSO_2H]^{\bullet-} \quad (17)$$

$$[RSO_2H]^{\bullet-} \rightarrow RSO^{\bullet} + HO^- \quad (18)$$

$$RSH + O_2^{\bullet-} \rightarrow RS^{\bullet} + HOO^- \quad (19)$$

$$RSO^{\bullet} + RSH \rightarrow RSOH + RS^{\bullet} \quad (20)$$

Theoretical calculations confirmed the possibility for a three-electron-bonded superoxide-thiol complex and the likelihood for decomposition into sulfinyl radical and hydroxide.[66,67] However, the hydrogen peroxide formation observed for the

reaction of superoxide with Cys and Pen suggests that these two thiols may additionally react via hydrogen/electron transfer, directly leading to hydrogen peroxide and thiyl radicals (reaction 19). This reaction could require a proton transfer before electron transfer, that is, conversion of the couple $RSH/O_2^{\bullet -}$ to $RS^-/HO_2^\bullet$ before electron transfer. Interestingly, Jones *et al*.[68,69] detected $GS^\bullet$ through spin trapping when GSH was exposed to superoxide, suggesting that either a small fraction of the reaction of superoxide with GSH proceeds via reaction (19) or that thiyl radicals are generated subsequently, possibly through the reaction of sulfinyl radicals with GSH (reaction 20). The reported rate constants for the reaction of superoxide with thiols vary between 10^2 and $10^5\,M^{-1}\,s^{-1}$,[68] but it appears that values between 10^2 and $10^3\,M^{-1}\,s^{-1}$ appear most reasonable. On the basis of these relatively low rate constants, thiols will not be able to compete with SOD for superoxide unless specific local conditions allow for a high effective thiol/thiolate concentration in the vicinity of superoxide generation, and/or thiols are part of a transition metal complex (e.g., iron–sulfur clusters).

2.1.4 The Reaction of Cys with NO and NO_2

The S-nitrosation of Cys is key to the regulation of various proteins and biologic pathways.[4] Nonradical mechanisms of S-nitrosation involve the reaction of thiolate with N_2O_3, the reaction product of two radicals, NO and nitrogen dioxide (NO_2), as well as *trans*-nitrosation. In addition, a free radical mechanism of S-nitrosation has been suggested, involving the reaction of a thiyl radical with NO (reaction 21)[70]; an alternative mechanism suggested the reaction of NO with thiol in the presence of an electron acceptor.[71] Recently, rate constants of $k_{21} = (2\text{–}3) \times 10^9\,M^{-1}\,s^{-1}$ have been reported for the reaction of thiyl radicals from Cys, GSH, and PenSH with NO,[72] indicating an efficient radical–radical reaction, which is in contrast to earlier studies suggesting that the reaction of $GS^\bullet$ with NO proceeds with $k < 2.8 \times 10^7\,M^{-1}\,s^{-1}$.[73] Of biologic relevance is the question whether thiols react directly with NO,[74] especially under physiologic conditions of submicromolar concentrations of NO, and [NO] ≪ [RSH]. This question was addressed by Folkes and Wardman,[75] showing that the anaerobic reaction of NO with GSH most likely proceeds via reactions (22)–(24):

$$RS^\bullet + NO \rightarrow RSNO \quad (21)$$

$$GS^- + NO(+H^+) \rightleftharpoons GSN^\bullet OH \quad (22)$$

$$GSN^\bullet OH + NO \rightarrow GSOH + N_2O \quad (23)$$

$$GSOH + GSH \rightarrow GSSG + H_2O \quad (24)$$

$$RSH/RS^- + {}^\bullet NO_2 \rightarrow RS^\bullet + (H^+)/NO_2^- \quad (25)$$

A kinetic simulation based on reactions (22)–(24) satisfactorily fits not only their results obtained under conditions of [NO] ≪ [RSH] but also the data of others, obtained under reverse conditions where [RSH] ≪ [NO]. However, the authors conclude that the reaction of NO specifically with GSH is too slow to be of physiological significance. In contrast, the reaction of Cys and GSH with NO_2 proceeds with rate constants of the order of 2×10^7 and $5 \times 10^7\,M^{-1}\,s^{-1}$, respectively, at pH 7.4 (reaction 25).[76]

2.1.5 The Reaction of Cys with Tyrosyl Radicals

The reaction of tyrosyl radicals with Cys (equilibrium 26) represents an important step in the mechanism of ribonucleotide reductase class I[23]:

$$TyrO^\bullet + CysSH \rightleftharpoons TyrOH + CysS^\bullet \quad (26)$$

$$TyrO^\bullet + GSH \rightleftharpoons TyrOH + GS^\bullet \quad (27)$$

In addition, pulse radiolysis studies have revealed the importance of equilibrium (26) in several electron-transfer cascades.[77] More recently, the nitration of Tyr by peroxynitrite has been investigated, where especially in the presence of CO_2, peroxynitrite decomposes into ${}^\bullet NO_2$ and ${}^\bullet CO_3^-$.[64] These radicals act in concert, where the reaction of ${}^\bullet CO_3^-$ with Tyr generates the tyrosyl radical, which subsequently recombines with ${}^\bullet NO_2$. Importantly, no Tyr nitration was observed for model peptides which simultaneously contained both Tyr and Cys, rationalized by the fact that initially generated tyrosyl radicals would be reduced by Cys before recombination with ${}^\bullet NO_2$.[78] The rate constant

for the forward reaction of equilibrium (27) was recently determined as $k_{27} = 2 \times 10^6\,M^{-1}\,s^{-1}$ (pH 7.15).[79]

2.1.6 The Reaction of Cys Thiyl Radicals with O_2

In aerobic environment, thiyl radicals can convert into oxy acids, RSO_nH ($n = 1-3$), via multiple pathways. Thiyl radicals react reversibly with molecular oxygen (equilibrium 28), where $k_{28} = (2.0-2.2) \times 10^9\,M^{-1}\,s^{-1}$ and $k_{-28} = 6.2 \times 10^5\,s^{-1}$.[80,81] The electronic structure of the thiyl peroxyl radical can be partially described by a charge-transfer state, $RS^+OO^{\bullet -}$,[82] and this species is characterized through a weak absorbance with $\lambda_{max} = 540$ nm.[80] The chemistry of thiyl peroxyl radicals in aerobic solution is complex, and various radical intermediates and products have been characterized for thiyl radicals from GSH, cysteine, penicillamine, and cysteamine through ESR studies in frozen matrices, while kinetic information mostly originates from pulse radiolysis of "simpler" organic thiols, for example, 2-mercaptoethanol. The unimolecular rearrangement (reaction 29) to sulfonyl radicals, $RSO_2^{\bullet}$, proceeds with $k_{29} \approx 10^3\,s^{-1}$ [81] and is associated with a high energy of activation (circa 45 kcal mol^{-1} [83]):

$$RS^{\bullet} + O_2 \rightleftharpoons RSOO^{\bullet} \quad (28)$$

$$RSOO^{\bullet} \rightarrow RSO_2^{\bullet} \quad (29)$$

The sulfonyl radical can further react with oxygen, to yield sulfonyl peroxyl radicals (equilibrium 30), where, measured representatively for methyl sulfonyl radicals, $k_{30} = 1.1 \times 10^9\,M^{-1}\,s^{-1}$ and $k_{-30} < 10^5\,s^{-1}$[84]:

$$RSO_2^{\bullet} + O_2 \rightleftharpoons RSO_2OO^{\bullet} \quad (30)$$

Excess thiols serve as hydrogen donors to thiyl peroxyl radicals, sulfonyl radicals, and sulfonyl peroxyl radicals, yielding sulfenic acid, sulfinate acid, and sulfonate (reactions 31–34).[85,86]:

$$RSOO^{\bullet} + RSH \rightarrow RSO^{\bullet} + RSOH \quad (31)$$

$$RSO_2^{\bullet} + RSH \rightarrow RSO_2^{-} + H^+ + RS^{\bullet} \quad (32)$$

$$RSO_2OO^{\bullet} + RSH \rightarrow RSO_2OOH + RS^{\bullet} \quad (33)$$

Reaction (33) leads to peroxysulfonic acid, which may be reduced through excess thiol to sulfonic acid (reaction 34):

$$RSO_2OOH + RSH \rightarrow RSO_3^{-} + H^+ + RSOH \quad (34)$$

Sulfinate and sulfonate have been observed as stable oxidation products of Cys in peptides and proteins. In contrast, sulfenic acid is unstable and rapidly reacts with excess thiol to form disulfide unless such a reaction is prevented through the location of the sulfenic acid in a protein domain that does not permit access of thiol.[87]

2.2 Selenocysteine

The low BDE of the Se–H bond (310 kJ mol^{-1}),[14] the low standard reduction potential $E^0(RSe^{\bullet} + e^- + H^+) = +0.77$ V (compare with $E^0(RS^{\bullet} + e^- + H^+) = +1.33$ V), and the selenol $pK_a \approx 5.2$ render selenocysteine (CysSeH) a powerful antioxidant, which has been observed to catalyze the reduction of benzyl viologen by DTT.[14] As for thiols, hydrogen or electron transfer from selenocysteine generates selenyl radicals, $CysSe^{\bullet}$:

$$Y^{\bullet} + CysSeH/CysSe^{-} \rightarrow Y-H/Y^{-} + CysSe^{\bullet} \quad (35)$$

On the basis of the thermodynamic properties listed above, selenyl radicals are expected to be significantly weaker oxidants compared to thiyl radicals. However, $CysSe^{\bullet}$ radical chemistry has not been explored to the same extent as has been for $CysS^{\bullet}$ radicals. Critical to the time-resolved detection of $CysSe^{\bullet}$ are its UV properties, where some open questions remain to be addressed. When Kolano *et al.*[88] photolyzed (266 nm) dimethyl bis(*N*-*tert*-butoxycarbonyl)-L-selenocystine in acetonitrile, they observed two transients absorbing with $\lambda_{max} = 335$ and 450 nm. In contrast, no 450-nm absorbance was detected on photolysis of [(fluorenylideneamino)oxycarbonyl]methyl(*N*-*tert*-butoxycarbonyl)-L-selenocysteine. Tamba and Badiello[89] used pulse radiolysis to generate $CysSe^{\bullet}$ via one-electron reduction of low concentrations of selenocystine, and observed a 460-nm absorbance,

assigned to CysSe$^{\bullet}$, an observation also made by Nauser *et al*.[14] when they oxidized CysSeH with azide radicals ($N_3^{\bullet}$) in acidic solution. Both groups also described the spectral properties of a diselenide radical anion, for which they reported an absorption with $\lambda_{max} = 440$ and 455 nm, respectively. Especially the latter value is very close to the reported absorption for CysSe$^{\bullet}$ so that both species may not be easily distinguishable. Complicating matters, Mishra *et al*.[90,91] oxidized various concentrations of selenocystine with HO$^{\bullet}$ radicals and other one-electron oxidants and observed that especially at high concentrations of selenocystine and pH > 4, an initial selenocystine radical cation, $[CysSeSeCys]^{+\bullet}$ (absorbing with $\lambda_{max} = 560$ nm), converted into a species absorbing with $\lambda_{max} = 460$ nm, assigned to a complex of CysSe$^{\bullet}$ with nonoxidized selenocystine. Analogous complexes had been observed for thiyl radicals in the presence of high concentrations of disulfides.[92] Hence, to date, the formation of CysSe$^{\bullet}$ has been associated with at least three transient species absorbing around 460 nm, CysSe$^{\bullet}$ itself, its diselenide radical anion complex with $CysSe^-$, and a complex of CysSe$^{\bullet}$ with selenocystine. This manifold of transients will require careful experimental optimization when reactions of CysSe$^{\bullet}$ need to be studied.

2.3 Protein Cysteine

The reactions outlined above for Cys, GSH, and CysSeH will proceed to various extents for protein Cys and CysSeH, where radical and product yields will depend largely on the respective pK_a values (note that Cys pK_a values can vary between 5.0 and ca. 10.75),[33,35] conformational properties, and the accessibility to reactant sites. For example, a Cys thiyl radical generated in the vicinity of a second, nonoxidized thiol/thiolate will efficiently form disulfide radical anions in an *intra*molecular reaction. The reaction of the disulfide radical anion with oxygen will then yield disulfide and superoxide, that is, the initial formation of a thiyl radical in the presence of oxygen will lead to the two-electron oxidation product (disulfide). Protein Cys thiyl radicals will also have the opportunity to abstract hydrogen atoms from suitably located amino acids within the three-dimensional structure, and evidence for such hydrogen transfer reactions was provided for human insulin.[7]

For proteins containing both Cys and CysSeH in an appropriate conformation, such as mammalian thioredoxin reductase (TRR), there is the possibility for the formation of selenium–sulfur bonds, R-Se-S-R, and selenium–sulfur bond radical anions, $[R\text{-}Se\text{-}S\text{-}R]^{\bullet-}$. On the basis of model studies on the one-electron reduction of DTT, diselenothreitol, and selenothioldthreitol, Nauser *et al*.[93] have forwarded the hypothesis that the single CysSeH residue in TRR, located in the sequence Gly-Cys-CysSeH-Gly, may protect TRR from deleterious reactions of a thiyl radical by providing a one-electron redox equivalent such that a thiyl radical, Gly-CysS$^{\bullet}$-CysSeH-Gly, would react via *intra*molecular electron transfer with the deprotonated CysSeH residue (p$K_a = 5.2$) to yield a selenyl radical, Gly-Cys-CysSe$^{\bullet}$-Gly. Because of the low Se–H BDE (310 kJ mol^{-1}), the selenyl radicals are not expected to react with protein C–H bonds such as described for thiyl radicals.

3 THIOETHERS AND SELENOTHIOETHERS

Free radicals can react with thio- and selenoethers via homolytic substitution[94,95](see **Intramolecular Homolytic Substitutions in Synthesis**, Volume 2) or electron transfer.[90,96,97] Electron-transfer reactions have been studied in quite detail for the thioether- and selenoether-containing amino acids methionine (Met) and selenomethionine (SeMet), and these have been reviewed in detail below. More recent reports also focused on the reaction of Met with hydrogen atoms (H$^{\bullet}$).

rad018

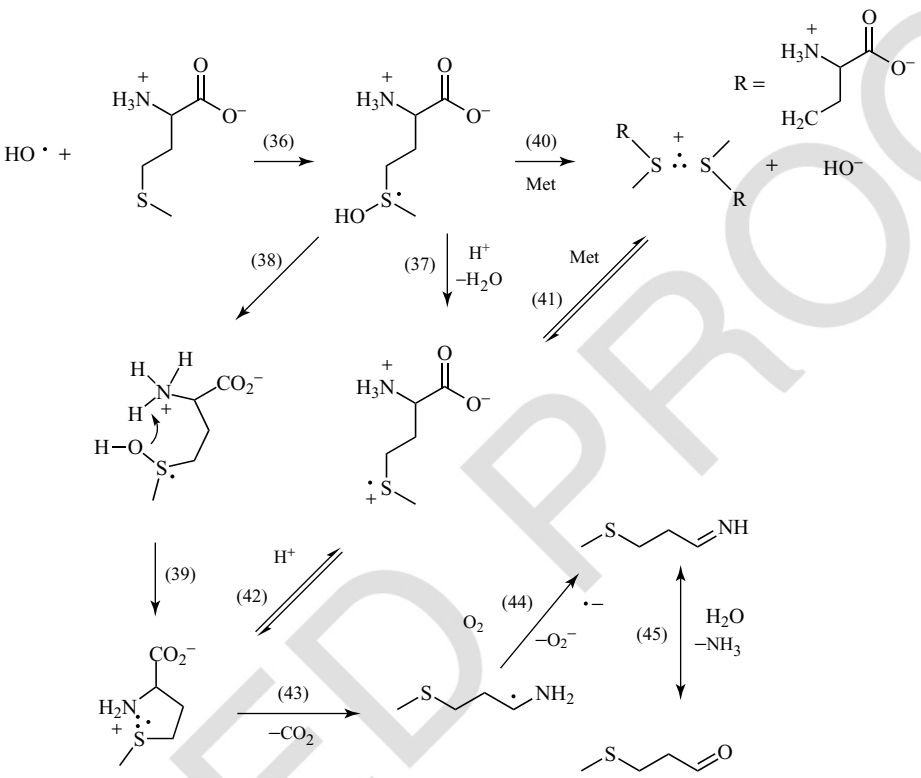
3.1 Methionine

3.1.1 Reaction of Methionine with Oxidizing Radicals

Reaction of Met with the Hydroxyl Radical

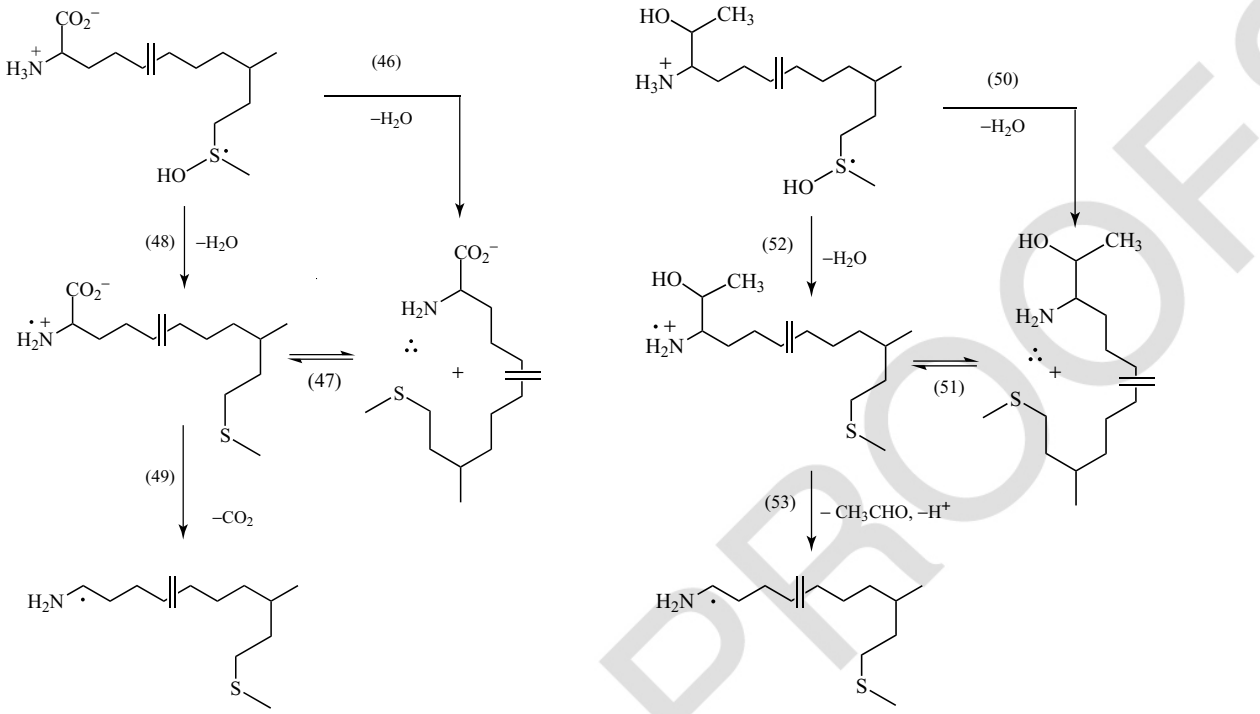
The reaction of Met with hydroxyl radicals (HO$^{\bullet}$) was characterized in great detail through radiation chemical methods such as pulse radiolysis and γ-radiolysis.[98] This reaction proceeds via addition of HO$^{\bullet}$ to the sulfur (reaction 36), resulting in a hydroxysulfuranyl radical. Subsequent reactions of this hydroxysulfuranyl radical depend on various parameters such as the nature of the

Met-containing substrate (i.e., whether the reaction involves the free amino acid Met, peptide-bound Met, or protein-bound Met), the concentration of the Met-containing substrate, and pH:



Hydroxysulfuranyl radicals of free Met decompose via three competitive pathways: protonation followed by the elimination of water, where the proton is either derived from the solvent (pH < 3; reaction 37) or the N-terminal amino group (pH > 3; reactions 38 and 39), and reaction with a second Met (reaction 40). Reactions (38) and (39) lead to a very short-lived sulfur–nitrogen three-electron-bonded intermediate $(>S\therefore NH_2R)^+$, which formally exists in equilibrium with a Met sulfur radical cation. Ultimately, this reaction sequence results in the formation of CO_2 and an α-amino-substituted carbon-centered radical (reaction 43). The latter reacts in a diffusion-controlled manner with molecular oxygen, ultimately forming superoxide and 3-(methylthio)propanal (methional) (reactions 44 and 45).[11] Evidence for a sulfur–nitrogen three-electron-bonded intermediate was also obtained for the reaction of HO• with peptides containing an N-terminal Met residue.[99] An important feature of water elimination from the hydroxysulfuranyl radical at pH > 3 is the ability of the protonated N-terminal amino group to function as a proton donor. The question arises whether such proton transfer is also possible over longer distances, that is, when Met is incorporated into peptides. Experiments with γ-Glu-Met and γ-Glu-Gly-Met-Gly revealed a circa 50% efficiency for decarboxylation according to reactions (46)–(49) for both peptides at pH 5.9–6.4,[100] suggesting that proton transfer, followed by formation of a sulfur–nitrogen three-electron-bonded intermediate, can occur when Met is not the N-terminal amino acid:

Pulse radiolysis experiments on the nanosecond timescale revealed formation of a sulfur–nitrogen three-electron-bonded intermediate also for *S*-methylglutathione.[101] Additional support for



the interaction of Met hydroxysulfuranyl radicals with the N-terminus is derived from experiments with Thr-$(X)_n$-Met,[102,103] where a fraction of the sulfur–nitrogen three-electron-bonded intermediate suffers C_α–C_β cleavage of the Thr residue, generating acetaldehyde (CH_3CHO) (reactions 50–53). No significant difference in the efficiency of acetaldehyde formation was observed between Thr-Met and Thr-Gly-Met, while only a circa 30% reduction was observed when comparing Thr-Met with Thr-$(Gly)_4$-Met.[103] Hence, a flexible Gly-containing sequence permits the interaction of Met hydroxysulfuranyl radicals with the N-terminus. In contrast, incorporation of a single Pro residue between Thr and Met (i.e., in Thr-Pro-Met) results in a significant reduction (by >70%) in the efficiency of acetaldehyde formation, suggesting that the Pro residue provides a barrier for *intra*molecular proton transfer and formation of the sulfur–nitrogen three-electron-bonded intermediate:

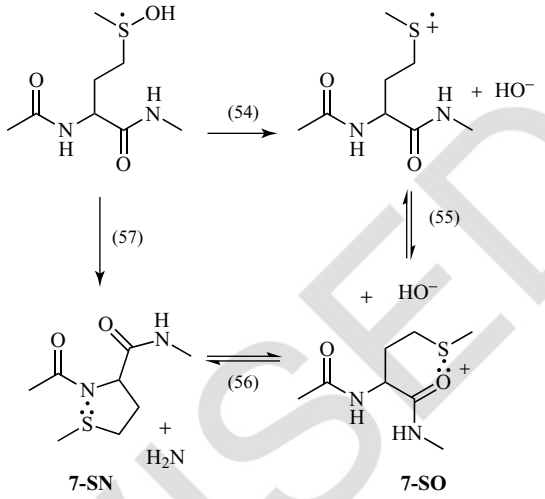
It remains to be shown to what extent proton transfer reactions between Met hydroxysulfuranyl radicals and proton donors (His, Lys, and Arg) may promote sulfide radical cation formation in proteins. In this respect, we note that $HO^\bullet$ radicals can not only originate from the exposure of aqueous solutions to ionizing radiation, but that $HO^\bullet$ (and/or their metal-bound equivalents) will be generated by Fenton reactions. In fact, acetaldehyde was observed on exposure of Thr-Met to a Fenton system consisting of $[Fe(II)(EDTA)]^{2-}$ and H_2O_2, though control experiments with a Met-deficient peptide, Thr-Leu, suggest that under these experimental conditions, a fraction of acetaldehyde could result from a direct oxidation of the N-terminal amino group.[104]

Theoretical calculations predict that the sulfur–nitrogen three-electron-bonded intermediate with the N-terminal amino group represents the most stable structure of a one-electron oxidized Met in aqueous solution.[105] This is also reflected in a calculated lower one-electron reduction potential for sulfide oxidation of an N-terminal Met residue as compared to a non-N-terminal Met residue within a peptide.[105] These calculations are *not* a contradiction to the experimental observations presented above, where an interaction between a non-N-terminal Met residue and the N-terminal amino group (e.g., in γ-Glu-Gly-Met-Gly and Thr-Gly-Met) was proposed based on a reaction product (i.e., formation of CO_2 and acetaldehyde). The formation of these reaction products indicates

that the interaction between a Met residue and the N-terminal amino group is kinetically possible but does not allow to quantify an equilibrium constant for the formation of the sulfur–nitrogen three-electron-bonded intermediate.

Interestingly, theoretical calculations predict that sulfur radical cations are not significantly stabilized by interaction with either the oxygen or the nitrogen of an amide bond.[105] These calculations are in contrast to experimental data with a series of *N*-acetyl (N-Ac)-substituted peptides (which do not contain a free N-terminal amino group) and with specific organic model compounds of Met, in which Met is embedded into a norbornane frame.

Pulse radiolysis experiments with *N*-Ac-methionyl amide, *N*-Ac-$(Gly)_3$Met$(Gly)_3$,[106] and cyclo-Met-Met[107] provided evidence for the formation of both sulfur–nitrogen and sulfur–oxygen three-electron-bonded intermediates with the peptide bond (such as that shown in the following reactions:



In addition, sulfur–oxygen three-electron-bonded intermediates were identified for the reaction of HO$^\bullet$ radicals with (1*R*, 2*S*, 4*R*, 6*R*)-6-(methylthio)bicyclo[2.2.1]heptane-2-carboxamide (**8**-*endo*) but not (1*R*, 2*R*, 4*R*, 6*R*)-6-(methylthio) bicyclo[2.2.1]heptane-2-carboxamide (**8**-*exo*) (Figure 3).[108]

Moreover, when HO$^\bullet$ radicals were reacted with the diketopiperazine-substituted Met analog (1 R,2 R,4 S,6 R)-6′-methylene-6-(methylthio) spiro[bicyclo[2.2.1]heptane-2,2′-piperazin]-3′-one hydrate (**9**) (Figure 3), time-resolved UV

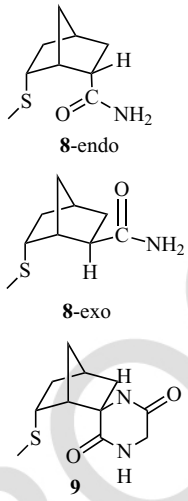
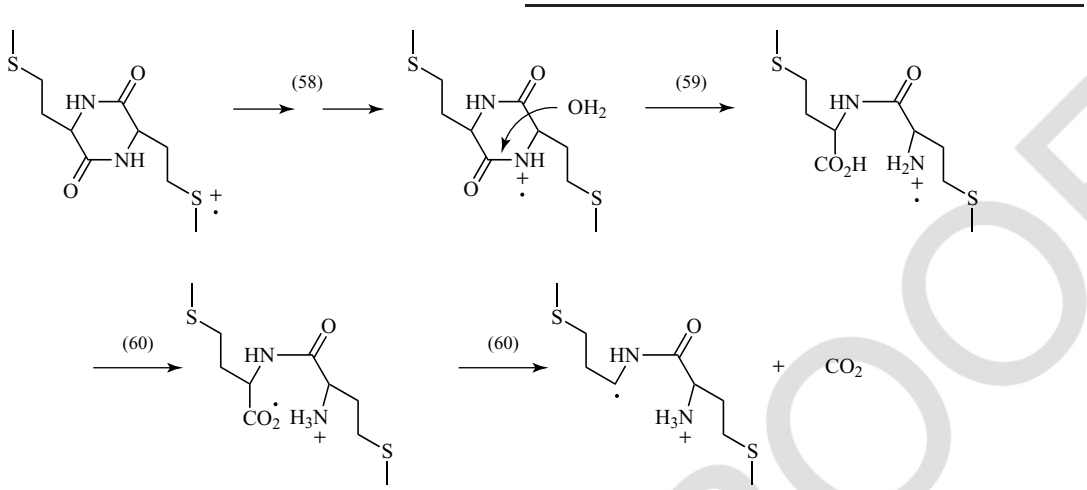


Figure 3 Structures containing Met embedded into a norbornane frame.

spectroscopy indicated the formation of a sulfur–oxygen three-electron-bonded intermediate, though the width of the UV peak with λ_{max} around 400 nm was significantly smaller compared to analogous UV peaks observed for the Met-containing peptides and **8**-*endo*.[108] Parallel electrochemical experiments in acetonitrile revealed a significantly lower peak potential for the oxidation of **8**-*endo* (1.07 V vs $Ag/AgNO_3$) compared to **8**-*exo* (1.40 V vs $Ag/AgNO_3$), consistent with the potential for stabilization of intermediary sulfur radical cations by interaction with the amide bond.

In general, sulfur radical cations decompose by deprotonation, forming α-(alkylthio)alkyl radicals.[98] However, especially for sulfur radical cations from cyclo-Met-Met, kinetic data revealed additional pathways, based on an incomplete conversion into α-(alkylthio)alkyl radicals.[107] While a detailed characterization of these additional pathways must await further experimental results, our hypothesis for the decomposition of sulfur radical cations of cyclo-Met-Met is summarized in reactions (58)–(60).

The reaction of HO$^\bullet$ with the protein calmodulin (CaM) in the presence of Ca^{2+}, investigated by pulse radiolysis, leads to the formation of an intermediate with λ_{max} circa 390 nm at circa 0.6–1.1 µs after the pulse.[109] The spectral features of this intermediate display a higher resemblance to a sulfur–nitrogen three-electron-bonded intermediate as



compared to a sulfur–oxygen three-electron-bonded intermediate. Hence, this species was assigned to a sulfur–nitrogen three-electron-bonded intermediate. Over the following 4–5 µs, this intermediate converts into a second species with λ_{max} circa 410 ± 5 nm, reminiscent of the spectrum of a tyrosyl radical.

Reaction of Met with Peroxyl Radical

Peroxyl radicals are moderately good to strong one-electron oxidants depending on their substitution. The trichloromethylperoxyl radical, $CCl_3OO^{\bullet}$ ($E^0 = 1.60$ V vs NHE for the reaction $CCl_3OO^{\bullet} + e^- + H^{+}$[110]) reacts with Met under one-electron transfer yielding sulfur–sulfur and sulfur–nitrogen three-electron-bonded species in a pH-dependent manner.[111] In contrast to the reaction of $HO^{\bullet}$ with Met,[98] where the sulfur–nitrogen three-electron-bonded intermediate is almost exclusively formed at pH >3, an exclusive formation of sulfur–nitrogen three-electron-bonded intermediates by $CCl_3OO^{\bullet}$ occurs at pH >9.[111] It has been suggested that the initial step in the reaction of peroxyl radicals with organic sulfides leads to an adduct (reaction (62) for the reaction of $CCl_3OO^{\bullet}$ with dimethyl sulfide),[112] and indirect evidence for such an adduct has been provided through the reaction of $CCl_3OO\text{-}^{\bullet}S(CH_3)_2$ with iodide[113]:

$$CCl_3OO^{\bullet} + S< \rightleftharpoons CCl_3OO - {}^{\bullet}S< \qquad (62)$$

If such adduct would also form during the reaction of $CCl_3OO^{\bullet}$ with Met, the experimentally observed pH dependence for the formation of sulfur–nitrogen three-electron-bonded intermediates would suggest that it does not decompose via *intra*molecular proton transfer such as observed during the reaction of Met with $HO^{\bullet}$.[98] Overall, the reaction of Met with $CCl_3OO^{\bullet}$ proceeds with $k = 2.9 \times 10^7\ M^{-1}\ s^{-1}$, while the reaction of Met with the less oxidizing peroxyl radical $CF_3CH(Cl)OO^{\bullet}$ proceeds with $k = 1.4 \times 10^6\ M^{-1}\ s^{-1}$.[111] For the reaction of less oxidizing peroxyl radicals with organic sulfides, an alternative reaction mechanism exists, in which formally an oxygen atom is transferred to the sulfide to yield sulfoxide. However, isotopic labeling experiments in aqueous solution ($H_2{}^{16}O$) saturated with $^{18}O_2$ indicate that the oxygen atom in the sulfoxide originates from water, leading to the mechanism proposed in reaction (63).[112]:

$$R^{18}O^{18}O-{}^{\bullet}S< + {}^{16}OH_2 \rightarrow R^{18}O^{\bullet} + H_2{}^{18}O + >S = {}^{16}O \qquad (63)$$

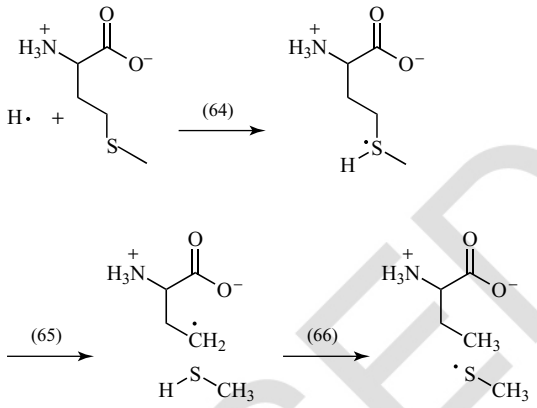
Reaction of Met with the Carbonate Radical

Of potential biological relevance is also the reaction of Met with the carbonate radical ($^{\bullet}CO_3^-$). As indicated above, this radical is generated through the reaction of peroxynitrite with CO_2, followed by homolytic dissociation into $^{\bullet}NO_2$ and $^{\bullet}CO_3^-$.[64] While $^{\bullet}NO_2$ does not react measurably with Met, a rate constant of $3.6 \times 10^7\ M^{-1}\ s^{-1}$ has been derived for the reaction of $^{\bullet}CO_3^-$ with Met.[64] On the basis of $E^0 = 1.84 \pm 0.1$ V for the reaction $^{\bullet}CO_3^- + e^- + H^+ = HCO_3^-$,[114] it can be assumed that

$^{\bullet}CO_3^-$ reacts with Met via one-electron oxidation. However, previous time-resolved experiments have not reported the formation of a sulfur–nitrogen or sulfur–sulfur three-electron-bonded species such as observed for the reaction of Met with HO$^{\bullet}$.[115]

3.1.2 Reaction of Met with H$^{\bullet}$-Atoms

The reaction of Met with H$^{\bullet}$-atoms is of potential relevance for radiation biology. This reaction involves bimolecular homolytic substitution (S_H2), via an initial addition of the H$^{\bullet}$-atom to the sulfur, followed by homolytic carbon–sulfur bond cleavage, and hydrogen transfer from the intermediary CH_3SH, ultimately generating α-aminobutyric acid and methylthiyl radicals ($CH_3S^{\bullet}$) (reactions 64–66).[11] These processes have been documented for both free and peptide- and protein-bound Met.[11,116,117]



3.2 Selenomethionine

The reactions of HO$^{\bullet}$ radicals with selenomethionine proceed analogous to the reactions with Met, except that a selenium–nitrogen three-electron-bonded intermediate, formed via water elimination from an initial HO-$^{\bullet}$Se < adduct, is significantly more stable.[118,119]

4 CONCLUSION

The reactions of free radicals with S- and Se-containing amino acids are reviewed. These reactions encompass hydrogen and electron transfer as well as homolytic substitution. Future experimental work shall focus in particular on radical transfer reactions in proteins, where sites of ultimate damage may evolve quite remote from sites of initial free radical attack. These mechanisms may rationalize some of the biological effects of free radicals.

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Abstract: The reactions of biologically relevant free radicals with S- and Se-containing amino acids are reviewed with regard to amino acid reactivity, the nature of product radicals, and ensuing radical reactions. Emphasis is placed on the formation and reactions of thiyl and selenyl radicals as well as radical cations from sulfides and selenides. The reactions of thiols with carbon-centered radicals, superoxide, nitrogen monoxide, nitrogen dioxide, and tyrosyl radicals are covered as well as the reactions of thiyl radicals with oxygen. Reversible *intra*- and *inter*molecular hydrogen transfer reactions of thiyl radicals are discussed in view of recent experimental and theoretical data. One-electron oxidation reactions of sulfides are reviewed especially with regard to the stabilization of sulfide radical cations through heteroatom-containing substituents, which are present in peptides and proteins. The relevance of these radical reactions for protein oxidation under conditions of oxidative stress is discussed.

Keywords: cysteine; selenocysteine; methionine; selenomethionine; glutathione; homocysteine; free radical; oxidation; reduction; hydrogen transfer; electron transfer; three-electron bond; radical cation; radical anion; sulfur; selenium.