Hydrogen Exchange Equilibria in Glutathione Radicals: Rate Constants

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The reduction of oxidized glutathione GSSG by hydrated electrons and hydrogen atoms to form $GSSG^{\bullet-}$ is quantitative. The radical anion dissociates into $GS^{\bullet}$ and GS^{-}, and the S-centered radical subsequently abstracts a hydrogen intramolecularly. We observe sequential development of UV absorbance signatures that indicate the formation of both α- and β-carbon-centered radicals. From experiments performed at pH 2 and pH 11.8, we determined forward and reverse rate constants for the overall equilibrium between sulfur-centered and carbon-centered radicals: $k_{forward} = 3{\cdot}10^5\ s^{-1}$, $k_{reverse} = 7{\cdot}10^5\ s^{-1}$, and $K = 0.4$. Furthermore, on the basis of the differences between the kinetics traces at 240 and 280 nm, we estimate that α- and β-carbon-centered radicals are formed at a surprising ratio of 1:3. The ratios found at pH 2 also apply to pH 7, with the conclusion that the equilibrium ratio of S-centered:β-centered:α-centered radicals is, very approximately, 8:3:1. The formation of carbon-centered radicals could lead to irreversible damage in proteins via the formation of carbon−carbon bonds or backbone fragmentation.

Introduction

Glutathione (GSH[1]) has many physiological functions. It is a substrate for the enzyme family of glutathione *S*-transferases, which catalyze the formation of glutathione conjugates of xenobiotics to facilitate their transport and detoxification (*2*). Stores of GSH are also a significant source of reduction equivalents, e.g., for reactions of the glutathione peroxidases (GPx) to convert H_2O_2 to H_2O, with the generation of oxidized glutathione (GSSG) (*3*):

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O \quad (1)$$

In turn, the GSSG is rereduced to GSH by glutathione reductase (GSR), with reducing equivalents from NADPH (*4*):

$$GSSG + NADPH + H^+ \xrightarrow{GSR} 2GSH + NADP^+ \quad (2)$$

GSH, which is rather abundant in the cytosol (5−10 mM) (*5*), is believed to react to some extent with partially reduced oxygen species and partially oxidized nitrogen species to form the glutathione radical ($GS^{\bullet}$). GSH reacts sufficiently rapidly with the hydroxyl radical ($HO^{\bullet}$) (*6*) to consider it a possible scavenger; rate constants for the reaction of GSH with H_2O_2 or $O_2^{\bullet-}$ are much smaller, 15 $M^{-1}\ s^{-1}$ (*7*) and 10^{-2}−$10^{-3}\ M^{-1}\ s^{-1}$ (*8*), respectively, such that these species are not scavenged. Peroxynitrite ($ONOO^-$) reacts more readily with carbon dioxide (CO_2) than with GSH: the products of rate constants and concentrations are approximately 39 s^{-1} and 1 s^{-1} for CO_2 (*9, 10*) and GSH (*11*), respectively. However, the nitrogen dioxide ($NO_2^{\bullet}$) and trioxidocarbonate(•1−) ($CO_3^{\bullet-}$) radicals produced in the reaction of $ONOO^-$ with CO_2 (*12, 13*) may react with GSH.

A considerable proportion of $HO^{\bullet}$, $NO_2^{\bullet}$, $CO_3^{\bullet-}$, and $ONOO^-$ reacts with proteins and membrane lipids to produce protein and lipid radicals. Although lipid radicals can be repaired by vitamin E (*14, 15*), protein radical repair by GSH, though not very efficient (*16*), would generate additional $GS^{\bullet}$ radicals.

Thus, several pathways lead to the formation of the $GS^{\bullet}$ radical, which is generally assumed to react under *in vivo* conditions with the glutathione thiolate anion (GS^-) to form the glutathione disulfide radical anion ($GSSG^{\bullet-}$) (*17, 18*), and the subsequent reaction of $GSSG^{\bullet-}$ with O_2 and disposal of $O_2^{\bullet-}$ by superoxide dismutase (SOD) terminates this radical chain reaction (*19*). The formation and reactivity of both $GS^{\bullet}$ and $GSSG^{\bullet-}$ radical types have been studied for nearly a half a century (*20*−*23*). In the 1980s, it was reported that not only S-centered radicals but also C-centered radicals are part of the chemistry of the $GS^{\bullet}$ radical (*24*). Ten years later, it was established that the S-centered and $^{\alpha}$C-centered radicals are in intramolecular equilibrium (*25*):

$$HGS^{\bullet} \rightleftarrows {}^{\bullet}GSH \quad (3)$$

Details of this reaction have been established for cysteine (Cys), GSH, and other oligopeptides (*18, 24, 26*). Calculations predict that β-radicals of cysteine are higher in energy than the corresponding α-radicals and are, therefore, likely not formed (*27*). However, hydrogen abstraction reactions have been shown to occur at γ and ε carbons in methionine, adjacent to sulfur (*28*). Such reactions are described in organic chemistry as polarity reversal catalyses (*29, 30*).

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[1] Abbreviations and chemical formulae: GSH, (reduced) glutathione; GSSG, oxidized glutathione; $GS^{\bullet}$, glutathione thiyl radical; $GSSG^{\bullet-}$, glutathione disulfide radical anion; $GS(H)SG^{\bullet}$, glutathione disulfide radical; GST, glutathione *S*-transferase; GPx, glutathione peroxidase; GSR, glutathione reductase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; H_2O_2, hydrogen peroxide; $HO^{\bullet}$, hydridooxygen(•) or hydroxyl radical; $ONOO^-$, (dioxido)oxidonitrate(1−) or peroxynitrate; CO_2, dioxidocarbon or carbon dioxide; $NO_2^{\bullet}$, dioxidonitrogen(•) or nitrogen dioxide; $CO_3^{\bullet-}$, trioxidocarbonate(•1−); O_2, dioxygen or oxygen; $O_2^{\bullet-}$, dioxide(•1−) or superoxide; SOD, superoxide dismutase; mAbs, 10^{-3} absorption units. The locants ε, β, γ, etc. are relative to the peptide carbonyl.

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It has been demonstrated that irradiated lysozyme undergoes fragmentation (*31*), probably after the migration of an electron from an α-carbon to the initially formed Cys thiyl radical. In spite of these reports, the notion that RS$^\bullet$ radicals are innocuous is persistent.

We reduced GSSG by radiolytically generated H$^\bullet$ and e^-_{aq} under acidic and basic conditions to determine rate constants and equilibrium constants for the reversible intramolecular hydrogen exchange between S-centered and C-centered glutathione radicals. We reinterpret the published pulse radiolysis data of glutathione and show that the formation of C-centered radicals takes place extremely rapidly, but also that more than one C-centered radical species participates in reaction 3. While the fast formation of $^{\alpha}$C-radicals from S-radicals is reported in the literature (*23*), we propose the additional formation of $^{\beta}$C (or $^{\gamma}$C-)-radicals. These reactions have been investigated under both alkaline and acidic conditions and will, therefore, also take place at neutral pH.

Experimental Procedures

Materials. All chemicals were used as delivered. GSH (97%) was from ABCR (Karlsruhe, Germany), GSSG was from AppliChem (98%, Darmstadt, Germany), and Sigma (99%, St. Louis, MO, USA), recrystallized 2-methylpropan-2-ol (*tert*-butanol) was from Merck (Darmstadt, Germany), KSCN (99%) was from Fluka (Buchs, Switzerland), H_2SO_4 (>95%) was from Merck (Darmstadt, Germany), and analytical grade KOH from Brenntag Schweizerhall (Basel, Switzerland). All solutions were prepared with Millipore Q filtered water (18.2 MΩ), in glassware that had been cleaned by immersion in concentrated HNO_3 followed by rinsing with pure water, and deaerated by at least 3-fold evacuation with subsequent shaking under argon at atmospheric pressure. The solution was transferred via a Gastight syringe from Hamilton (Bonaduz, Switzerland) to a syringe pump for sample delivery. Each sample was irradiated and analyzed only once.

Pulse Radiolysis. A Febetron 705 accelerator (Titan Systems Corp., San Leandro, CA, USA) generated electron pulses of 50 ns duration and energies of 2.0 MeV. The dose was adjusted with aluminum apertures to 7−140 Gy/pulse. The optical detection system has been described before (*32*). The dosimetry was determined by irradiation of KSCN solutions (*33*). All experiments were conducted at 295 K. The irradiation of aqueous solutions yields primary radicals, the hydrated electron (e^-_{aq}), the hydrogen atom (H$^\bullet$), and HO$^\bullet$, the dose dependent concentrations of which are known (*34*).

$$H_2O \rightsquigarrow e^-_{aq}, H^\bullet, HO^\bullet \quad (4)$$

All solutions were prepared with 1 M *tert*-butanol to scavenge HO$^\bullet$ (*35*) ($k_5 = 6.0 \cdot 10^{8 M^{-1}}$ s^{-1}), with production of the relatively innocuous 2-hydroxy-2-methylpropyl radical:

$$t\text{-BuOH} + HO^\bullet \rightarrow (CH_3)_2COHCH_2^\bullet + H_2O \quad (5)$$

Although the second-order rate constant for the reaction of GSSG with HO$^\bullet$ is 2 orders of magnitude higher ($9 \cdot 10^9$ M^{-1} s^{-1}) (*6*), the product of the rate constant and concentration (1 mM), $9 \cdot 10^6$ s^{-1}, is negligible compared to that for the reaction of 1 M *tert*-butanol with HO$^\bullet$, $6 \cdot 10^8$ s^{-1}. Thus, the reactions we observe are those of e^-_{aq} and H$^\bullet$, which react with GSSG to form GSSG$^{\bullet-}$ and its conjugate acid GS(H)SG$^\bullet$, respectively (*24, 36*):

$$e^-_{aq} + GSSG \rightarrow GSSG^{\bullet-} \quad (6)$$

$$H^\bullet + GSSG \rightarrow GS(H)SG^\bullet \quad (7)$$

with rate constants of $k_6 = 3 \cdot 10^9$ M^{-1} s^{-1} and $k_7 = 9 \cdot 10^9$ M^{-1} s^{-1}.

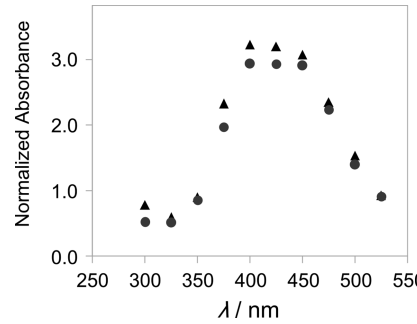

Figure 1. Spectra of an Ar-saturated 50 mM GSSG solution at pH 9.7 at 1 μs (●) and 8 μs (▲) after irradiation (36−41 Gy) in the presence of 10 mM GS$^-$/GSH and 1 M *tert*-butanol. The absorbance is normalized to a dose of 1000 Gy. No measurements below 300 nm are possible as the added thiolate blocks light transmission.

Data Treatment. Only data points obtained after 500 ns were evaluated; earlier time-points were neglected because the 50 ns pulse of electrons created lingering electromagnetic disturbances. Kinetics curves were smoothed with a low-pass filter, and the data were fit with a least-squares algorithm.

Results

In Figure 1, as proof of concept, we show the quantitative formation of GSSG$^{\bullet-}$ (*17, 37*) ($\varepsilon = 8 \cdot 10^3$ M^{-1} cm^{-1} at λ_{max} = 425 nm) (*17*) by the reaction of e^-_{aq} and H$^\bullet$ with 50 mM GSSG at pH 9.7 in the presence of 10 mM GSH/GS$^-$ and 1 M *tert*-butanol to scavenge HO$^\bullet$ quantitatively. In the presence of GSH, reactions −8 and 9 are favored, and reaction 8 is suppressed (*17, 38*):

$$GSSG^{\bullet-} \rightleftarrows GS^- + GS^\bullet \quad (8)$$

$$GS(H)SG^\bullet \rightleftharpoons GSSG^{\bullet-} + H^+ \quad (9)$$

At concentrations of an electron scavenger (GSSG in this case) as large as 50 mM, the expected radiochemical yield (*G*-value) is calculated on the basis of the Warman−Asmus−Schuler formula (*39*) and the data of Balkas et al. (*40*):

$$G(e^-_{aq}) = 2.55 + 2.23 \frac{\sqrt{k_s[S]/6 \times 10^8}}{1 + \sqrt{k_s[S]/6 \times 10^8}}$$

With $G(e^-_{aq}) = 3.6$ so calculated and $G(H^\bullet) = 0.6$, we expect the formation of 17.3 μM (GS$^\bullet$ + GSSG$^{\bullet-}$) at a dose of 42 Gy. Given that $K_8 = 5 \times 10^{-4}$ M (*41*), we expect [GSSG$^{\bullet-}$]/[GS$^\bullet$] = 100. The amount of GSSG$^{\bullet-}$ found corresponds to a quantitative yield of reactions −8 and 9 according to $c = \rho \cdot D \cdot G \cdot 0.1036$ μM, in which *c* is the concentration of primary radicals interacting with GSSG, ρ is the density (1 kg/l), *D* is the dose, and *G* is the sum of $G(e^-_{aq})$ and $G(H^\bullet)$ (*34*). The radical GS(H)SG$^\bullet$ is too short-lived to be observed because the equilibrium of reaction 9 lies to the right ($pK_a = 5.9$) and is attained nearly instantaneously (*17*). We expect an initial absorbance at 425 nm of 137 mAbs, in excellent agreement with the measured 131 ± 4 mAbs.

All other experiments were carried out in the absence of GSH. Because only 1 mM GSSG was present, we used the standard value for $G(e^-_{aq})$, 2.75. Spectral changes at pH 11.8 (Figure 2) show the conversion of the GSSG$^{\bullet-}$ at 420 nm to one or more species that have large, increasing absorptivities at wavelengths lower than 300 nm. The spectrum at $\lambda > 430$ nm at 0.75 μs appears to show a contribution from e^-_{aq}. The increases in absorbance at 240 nm and at 280 nm (Figure 3) proceed with

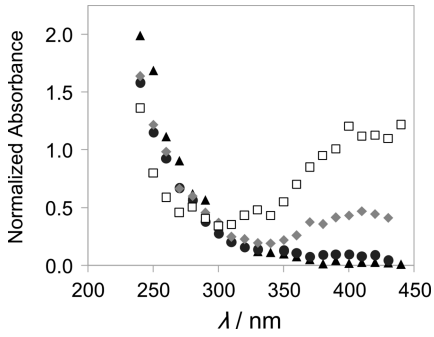

Figure 2. Spectra of Ar-saturated 1 mM GSSG solution at pH 11.8 after irradiation (56−66 Gy) in the presence of 1 M *tert*-butanol. □, 0.75 μs; gray diamond, 4 μs; ●, 15 μs; and ▲, 25 μs. The absorbance at 0.75 μs is due to $GSSG^{\bullet-}$. The absorbance is normalized to a dose of 1000 Gy.

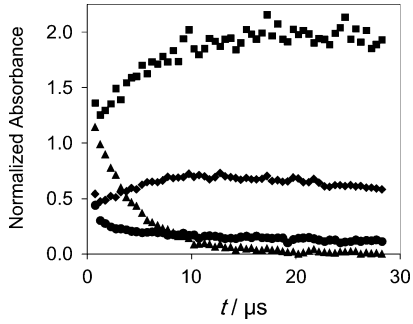

Figure 3. Time-dependent changes in absorbance at ■, 240 nm; ◆, 280 nm; ●, 330 nm; and ▲, 420 nm of an Ar-saturated 1 mM GSSG solution at pH 11.8 after irradiation (56−66 Gy) in the presence of 1 M *tert*-butanol. The absorbance is normalized to a dose of 1000 Gy.

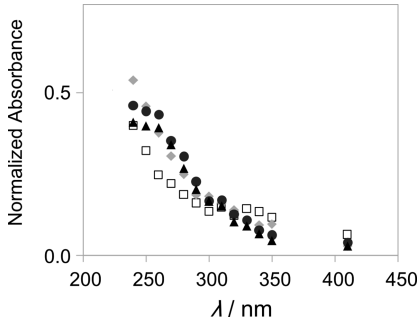

Figure 4. Spectra of an Ar-saturated 1 mM GSSG solution at pH 2 after irradiation (23−30 Gy) in the presence of 1 M *tert*-butanol. □, 0.75 μs; gray diamond, 4 μs; ●, 15 μs; and ▲, 25 μs. The absorbance is normalized to a dose of 1000 Gy.

different half-lives, 7 and 4 μs, respectively, and the maxima are reached at ca. 20 and 10 μs, respectively. This is evidence for at least two concurrent processes. Importantly, the spectral signatures of these processes are consistent with the formation of both $^{\alpha}$C- and $^{\beta}$C-type radicals (*42*).

Figures 4 and 5 show the results of pulse radiolysis of GSSG at pH 2; at wavelengths <300 nm, the initially increasing absorbance at lower wavelengths is transformed to a shoulder at ca. 260 nm (Figure 4). The spectra in Figure 4 could indicate that a fast equilibrium is established between radical species that are subject to further reaction. If so, isosbestic points could be imagined at ca. 250 and 310 nm. While the kinetics traces at 260 nm (not shown, but see Figure 4) and 280 nm are similar, they are clearly different from that at 240 nm (Figure 5). The absorbance of the RS• radicals at 330 nm (*26*) seen at 0.75 μs decays to a residual absorbance, presumably of the species that has an absorbance maximum at ca. 265 nm. At 410 nm, the absorbance maximum of $GSSG^{\bullet-}$, the decay is complete in 2

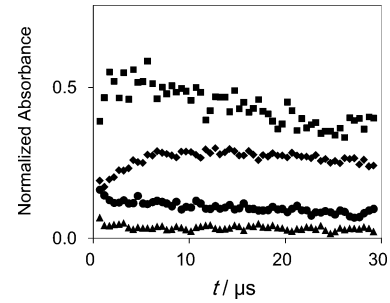

Figure 5. Time-dependent changes in absorbance at ■, 240 nm; ◆, 280 nm; ●, 330 nm; and ▲, 420 nm of an Ar-saturated 1 mM GSSG solution at pH 2 after irradiation (56−66 Gy) in the presence of 1 M *tert*-butanol. The absorbance is normalized to a dose of 1000 Gy.

μs. Thus, under acidic conditions, we also find evidence for the formation of both $^{\alpha}$C- and $^{\beta}$C-type radicals.

We determined rate constants both at 260 and 280 nm and found them to be identical, but because the signal-to-noise ratio is superior at 260 nm, we rely on measurements taken at that wavelength. The rate constant for product formation at pH 2 is $k_{obs} = (1.0 \pm 0.5) \cdot 10^6\ s^{-1}$ and at pH 11.8 is $k_{obs} = (2.6 \pm 0.3) \cdot 10^5\ s^{-1}$.

Discussion

The main conclusion that we can draw from the work we present here is that, after initial formation of the GS• radical, hydrogen transfer leads to the formation of both $^{\alpha}$C- and $^{\beta}$C-centered radicals in alkaline and in acidic solution. Whereas, in alkaline solutions, the formation of the $^{\alpha}$C-radical of Glu with subsequent deamination has been reported (*21*), this process is expected to be slower in acidic solution because the protonated ammonium group deactivates the $^{\alpha}$C-hydrogen. β-Elimination from the Cys $^{\alpha}$C-radical could lead to desulfurization (*43*), a process that takes place at a rate that is at least 1 order of magnitude slower than the processes discussed here (Nauser, T., unpublished work).

The reaction of e^-_{aq} and H• with GSSG to produce $GSSG^{\bullet-}$ is quantitative, and the subsequent generation of GS• is straightforward. We chose this method because of literature reports that the reaction of HO• with GSH produces both S- and C-centered radicals by H•-abstraction (*37, 44*). The clean formation of GS• from $GSSG^{\bullet-}$ implies that any other species observed must be a product of GS•. Thus, any C-centered radical on GSH originates from intramolecular H• transfer to GS•. We observed no long-lived ($t_{1/2} > 10^{-4}$ s) adduct at 380 nm, which excludes any contribution of the bimolecular reaction of GS• with GSSG (*45*).

In addition to the equilibrium between S-centered and C-centered radicals in glutathione (reaction 3), •GSH is also subject to acid−base equilibrium (*25*):

$$^{\bullet}GSH \rightleftarrows {}^{\bullet}GS^- + H^+ \qquad (10)$$

The rates of establishing all equilibria between carbon- and sulfur-centered radicals, is represented by the observed rate constant k_{obs}, i.e., reaction 3, is the sum of the forward and reverse rate constants (*46*). Notably, at pH 11, the availability of •GSH is dependent on reaction 10; thus, k_{-3} is multiplied with K_{10}. Given that the absorptivities of •GSH and $^{\bullet}GS^-$ at 260 nm are likely to be similar, as the chromophore remains unchanged, k_{obs} for the formation of ($^{\bullet}GSH + {}^{\bullet}GS^-$) may be written as follows:

$$k_{obs} = k_3 + k_{-3} \cdot K_{10}^{-1} \quad (11)$$

The protonation equilibrium of the glutathione radical, pK_{10} is expected to be very similar to pK_a (GSH), 9.2 (*47*); thus, $K_{10} =$ 400 at pH 11.8. As an electron deficient compound, the pK_a of •GSH could be even lower, which would strengthen our argument that $k_{obs} = k_3$ at pH 11.8. Since, at pH 2, the thiol is fully protonated, k_{obs} (pH 2) $= k_3 + k_{-3}$. The difference between the observed rate constants at pH 2 and pH 11.8 is, thus, $\Delta k_{obs} = k_{obs}$ (pH 2) $- k_{obs}$ (pH 11.8) $= k_{-3}$•$(1 - K_{10}^{-1}) \approx k_{-3} = 1 \cdot 10^6$ $s^{-1} - 2.6 \cdot 10^5$ $s^{-1} = 7 \cdot 10^5$ s^{-1}. These reactions must be intramolecular because intermolecular rate constants are known to be 4 orders of magnitude lower under similar conditions (*28, 48*). In this way, we calculate that $k_3 = k_{obs}$ (pH 2) $- 7 \cdot 10^5$ $s^{-1} = 3 \cdot 10^5$ s^{-1}, and $K_3 \approx 0.4$. Thus, at equilibrium, the fraction of C-centered radicals is ca. 30%, and the ratio of sulfur to carbon centered radicals is ca. 2:1.

The rate constants are of the same order of magnitude as those determined for *N*-Ac-Cys-Gly_6 and similar molecules (*26*). From the absorbance versus time curves in Figures 3 and 5, it can be seen that the strong absorbance at 240 nm is already present within microseconds after the pulse. Since GS• thiyl radicals absorb weakly in that region (*25*) and $GSSG^{\bullet -}$ would exhibit a maximum at 425 nm, we must assign this absorbance to the presence of substantial amounts of C-centered radicals. In proteins, such hydrogen abstractions could, thus, lead to irreversible damage via the formation of hydroperoxides (Gebicki, J., Nauser, T., and Koppenol, W. H., unpublished work), thioethers (*49*), or carbon−carbon bonds, or via backbone fragmentation (*31*).

Closer inspection of the spectra in Figures 2 and 4 reveals that the shapes of the absorption bands change with time, with higher absorbance toward lower wavelengths initially and later a shoulder at approximately 260 nm. Also, the maxima at 240 and 280 nm are reached at different times. According to Neta et al. (*42*), the spectral characteristics of $^{\alpha}$C- and $^{\beta}$C-type radicals are different, with $^{\alpha}$C-radicals exhibiting maximal absorbance at ca. 265 nm and $^{\beta}$C-radicals having increased absorbance at lower wavelengths. The UV−vis patterns observed allow us to attribute only the radical type, i.e., $^{\alpha}$C- or $^{\beta}$C- radicals, but not to precisely identify the residue at which the C-centered radical is located: because reference spectra are lacking, we are unable to exclude the formation of $^{\gamma}$C- radicals.

At pH 2 (Figure 4), the first recorded spectrum already shows the signature of a $^{\beta}$C-radical. Within microseconds, an absorbance with a shoulder at ca. 260 nm appears, which we ascribe to the $^{\alpha}$C-radical. A similar pattern is also found at pH 11: here, too, the $^{\beta}$C-radical is formed initially, but immediate deprotonation (reaction 10) of the thiol inhibits reaction −3 and confines the radical; no spectral signature of the $^{\alpha}$C-radical is observed.

A comparison of Figures 2 and 4 confirms that $K_3 \approx 0.4$; considering that the initial absorption at 260 nm is a factor of 3 higher at pH 11.8 (1.1 mAbs/Gy, Figure 2) than at pH 2 (0.37 mAbs/Gy, Figure 4) and that, at high pH, there are only C-centered radicals, we conclude, again, that, at pH 2, ca. 33% of the radicals are C-centered.

Abedinzadeh et al. (*18*) made similar observations upon the generation of GS• from GSH and HO• or $Br_2^{\bullet -}$. They determined two different rate constants for product build-up at 270 and 320 nm, and explained their findings with a reaction scheme in which the oxidant attacks GSH at different sites with different rates, with direct production of both S- and C-centered radicals. However, according to such a reaction scheme, mathematics requires that all products must be formed with the same rate constant, namely, k_{sum}, which is the sum of all rate constants involved (*50*). Intuitively, this can be rationalized because the kinetics in this case are determined by the limiting reagent, i.e., HO• or $Br_2^{\bullet -}$. This requirement is not met by either their or our experimental data: the *sequential* evolution of spectra observed by Abedinzadeh et al. (*18*) is better described by *sequential* evolution of products, e.g., initial formation of an S-centered radical ($\lambda_{max} = 330$ nm) that reacts to form and is in equilibrium with a C-centered radical ($\lambda_{max} \approx 265$ nm).

Our data also indicate that there is interconversion between $^{\alpha}$C- and $^{\beta}$C-radicals (Figure 4). The $^{\beta}$C-centered radical is kinetically favored: experiments at pH 11.8 show only the presence of $^{\beta}$C-radicals (Figure 2); since both C-centered radicals are formed at pH 2, we assume that the interconversion of the C-centered radicals at lower pH proceeds via the S-centered radical:

$$^{\beta}G^{\bullet}SH \rightleftarrows HGS^{\bullet} \rightleftarrows {}^{\alpha}G^{\bullet}SH \quad (12)$$

Using published values for the spectra of $^{\beta}$C-centered radicals derived from β-chloroalanine (*42*), Figure 2), we can make a rough estimate of the ratio of $^{\alpha}$C- and $^{\beta}$C-centered radicals: the spectra obtained at pH 2 at 20 μs are most suitable because, at that pH, the spectral signatures of both radical types are rapidly formed, and after 20 μs, no further increase in absorbance is observed. Thus, H-exchange equilibria are rapidly established. With 1650 M^{-1} cm^{-1} and 730 M^{-1} cm^{-1} for the molar absorptivities of the $^{\beta}$C-centered radical at 240 and 260 nm (34, Figure 2), respectively, and with 1400 M^{-1} cm^{-1} and 2000 M^{-1} cm^{-1} for those of the $^{\alpha}$C-centered radical at 240 and 260 nm (34, Figure 2), respectively, we obtain a value of ca. 3:1 for the ratio of $^{\beta}$C/$^{\alpha}$C-radicals directly after the decay of $GSSG^{\bullet -}$. Our findings show that, after the initial formation of the GS• radical, S-centered, $^{\alpha}$C-centered, and $^{\beta}$C-centered radicals undergo equilibrium interconversions and are present at a ratio of approximately 8:1:3, respectively. Thus, the oxidation of Cys residues in proteins could produce C-centered radicals that undergo reactions other than disulfide bridge formation, ultimately leading to fragmentation or polymerization, as has been observed after γ-radiolysis of insulin that contained disulfides (*31, 49*). Repair of a C-centered radical via H-atom transfer, intramolecularly or from an antioxidant such as ascorbate, is expected to result in partial racemization at that carbon, with potentially serious consequences (*28*).

The relative stability of $^{\alpha}$C radicals was predicted (*25, 51, 52*) and demonstrated (*48*) earlier. Here, we show that $^{\beta}$C radicals in GSH are formed intramolecularily in significant quantity and that they are surprisingly stable.

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