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Sensitized photo-oxidation of sulfur-containing amino acids and peptides in aqueous solution

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Abstract

Qualitative and quantitative studies of the photo-oxidation of sulfur-containing amino acids and methionine-containing dipeptides and tripeptides in aqueous solution sensitized by 4-carboxybenzophenone (CB) are reviewed. The mechanism of the photo-oxidation reaction was investigated using the techniques of flash photolysis, steady state photolysis and pulse radiolysis. The rate constants for quenching of the CB triplet by twelve sulfur-containing amino acids and six methionine-containing peptides were determined to be in the range 10^8–10^9 M^{-1} s^{-1} for both neutral and alkaline solutions. The amino acids varied in structure, having different numbers of COOH and NH_2 terminal groups and their sulfur atom at different locations relative to the terminal groups. The methionine-containing peptides were Met-Gly, Gly-Met, Met-Met, Met-Gly-Gly, Gly-Met-Gly and Gly-Gly-Met. Time-resolved transient spectra accompanying the quenching events were assigned to the triplet states of CB, ketyl radicals of CB, radical anions of CB and radical cations derived from the amino acids and peptides. The radical cations identified were intermolecularly $(S.\therefore S)^+$-bonded cations, intramolecularly $(S.\therefore N)^+$-bonded cations and an intramolecularly $(S.\therefore S)^+$-bonded radical cation that was observed in experiments with Met-Met. The quantum yields of the transients and their kinetics of formation and decay were measured by flash photolysis. The quantum yields of CO_2 formation were determined by steady state photolysis.

Electron transfer from the sulfur atom to the triplet state of the ketone was found to be a primary photochemical step. A detailed mechanism of the CB-sensitized photo-oxidation of sulfur-containing amino acids and methionine-containing peptides, including primary and secondary photoreactions, is proposed and discussed. Within the mechanism, contrasting behavior between the peptides and amino acids as quenchers is emphasized

Keywords: 4-Carboxybenzophenone; Sensitized photo-oxidation; Sulfur- containing amino acids; Methionine-containing peptides

1. Introduction

Carbonyl triplets can be potent oxidizing agents in biological materials. These triplets have been produced in biological systems [1,2], by both enzymatic [3] and non-enzymatic [4] methods. Recently, the benzophenone molecule has been proposed as a photoprobe in proteins and other macromolecules [5]. One of the components of biological systems most susceptible to oxidizing agents is the sulfur atom in the side-chains of certain amino acid residues of proteins [6,7]. For instance, oxidation of methionine has been implicated in the pathogenesis of some diseased states [8,9], such as cataracts, in the biological inactivation of hormones [8–10] and in stabilization problems with proteins of pharmaceutical importance [11,12].

In an attempt to characterize how these mechanisms arise in biological systems, we have studied the simpler systems of sulfur-containing amino acids [13–15] and methionine-containing dipeptides and tripeptides [16]. This paper reviews some of the most salient features of the primary photochemistry observed. In addition, it covers some of the more unusual facets of the secondary reactions that follow quenching of the triplet states.

2. Evidence for electron transfer; primary photoreactions

The quenching rate constants of triplet 4-carboxybenzophenone (CB) by sulfur-containing amino acids and peptides were measured using nanosecond laser flash photolysis. These rate constants are summarized in Table 1. It can be seen that the rate constants generally approach a diffusion-controlled rate constant for aqueous solutions. Comparison can be made with alanine since alanine is an amino acid that

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Table 1
Rate constants for quenching of the CB triplet state by sulfur-containing amino acids, alanine and methionine-containing peptides

Quenchers	$k_q \times 10^{-9}$ ($M^{-1} s^{-1}$)	
	pH neutral	pH basic
Thiaproline	2.1 [a]	2.6 [b]
S-Methylcysteine	1.8 [a]	
S-Ethylcysteine	2.1 [a]	
S-(Carboxymethyl)cysteine	0.81 [a]	0.75 [b]
S-(2-Carboxyethyl)cysteine	0.97 [a]	
Lanthionine	0.18 [a]	
Methionine	2.5 [c]	2.3 [c]
Ethionine	2.9 [a]	
Buthionine	2.7 [a]	
α-Methylmethionine	2.6 [a]	2.9 [b]
N-Acetylmethionine	1.6 [a]	
Homomethionine	2.9 [a]	2.6 [b]
Alanine	<0.0005 [a]	0.18 [b]
Gly-Met	2.0 [c]	2.0 [c]
Met-Gly	2.1 [c]	2.3 [c]
L-Met-L-Met	2.9 [d]	1.8 [d]
Gly-Gly-Met	1.8 [c]	1.9 [c]
Met-Gly-Gly	2.3 [c]	2.2 [c]
Gly-Met-Gly	2.1 [c]	2.3 [c]

[a] Ref. [13].
[b] Ref. [15].
[c] Ref. [16].
[d] This work.

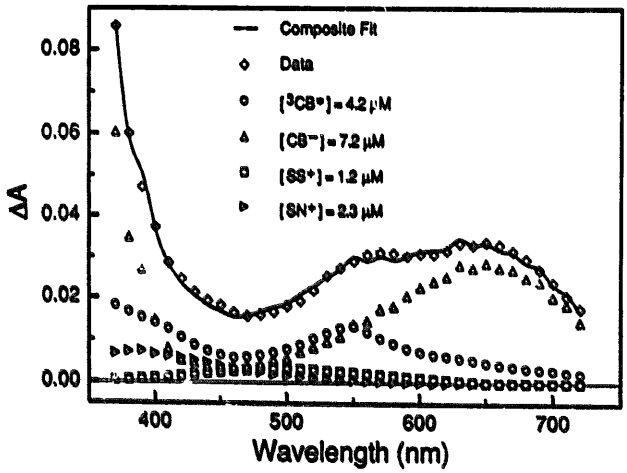


Fig. 1. Spectral resolution of the transient spectrum taken 80 ns after the flash at 337 nm. Aqueous solution of 2 mM CB and 5 mM L-Met-L-Met at pH 10.

contains no sulfur atoms. In neutral solution $k_q < 5 \times 10^5 M^{-1} s^{-1}$ [13], and in basic solution $k_q = 1.8 \times 10^8 M^{-1} s^{-1}$ [15]. This suggests that the sulfur atom is responsible for the magnitude of the quenching rate constants in Table 1.

The rapid quenching of the carbonyl triplets by sulfur-containing amino acids, which have no accessible energy levels to facilitate energy transfer quenching, leaves the possibility of electron transfer as a good candidate for the rapid quenching mechanism. One of the best ways of confirming an electron transfer mechanism is by observing transients or stable products that can plausibly arise from such a reaction. Fig. 1 shows the spectral resolution of the transient absorption taken at 80 ns after the flash of an aqueous solution of 2 mM CB and 5 mM L-Met-L-Met at pH 10. As a contrast, Fig. 2 shows the spectral resolution (at 160 ns delay) of the same system as in Fig. 1 but at pH 6.3 and a lower (0.4 mM) concentration of L-Met-L-Met. The component spectra are either spectra taken on our nanosecond laser system (with molar absorption coefficients from Ref. [17]) or literature spectra [18] taken under conditions that allowed only the isolated transients in pulse radiolysis experiments. The technique of resolving the spectra in this fashion involves a linear multiple regression [19] that has been described previously [15]. Many other similar resolutions have been performed on these systems with analogous results [14–16].

In alkaline solutions, it is relatively easy to detect the radical anion ($CB^{\bullet -}$) of CB following triplet quenching by sulfur-containing amino acids and peptides. The ketyl radical (CBH) of CB has a pK_a value of 8.2 [20], and $CB^{\bullet -}$ is easily

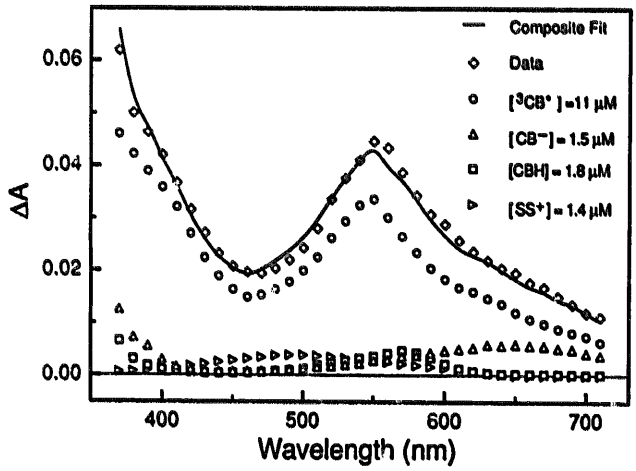


Fig. 2. Spectral resolution of the transient spectrum taken 160 ns after the end of the 337 nm flash. Aqueous solution of 2 mM CB and 0.4 mM L-Met-L-Met at pH 5.9.

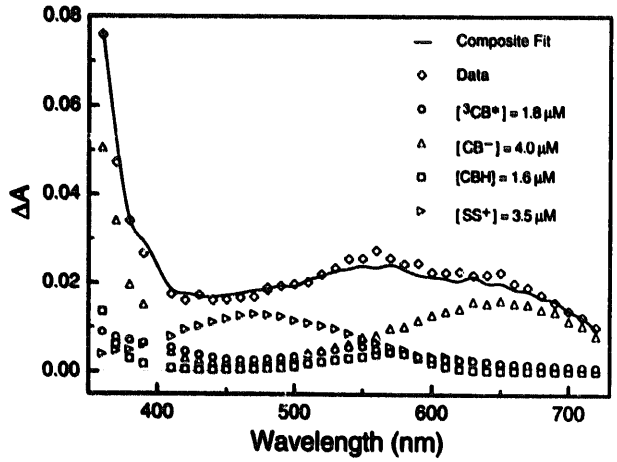


Fig. 3. Spectral resolution of the transient spectrum taken at the end of the flash at 337 nm. Aqueous solution of 2 mM CB and 20 mM L-methionine at pH 6.3.

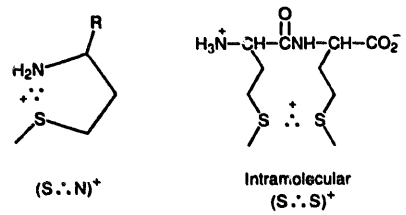


Chart 1.

seen in absorption at 660 nm where there is little interference from other possible intermediates in these systems (see Fig. 1). At pH values in the neutral range, the ketyl radical of CB is also clearly seen, although there is more interference from other products and the triplet absorption itself (see Figs. 2 and 3). In addition, following triplet quenching events with methionine in basic solution [15] or with peptides having N-terminal methionines [16], the three-electron-bonded $(S.\therefore N)^+$ radical ions have been observed; an example is presented in Fig. 1. A small amount of intramolecular $(S.\therefore S)^+$ radical cations can be seen in Fig. 2. This assignment is based on the relatively low concentration of L-Met-L-Met which might inhibit the formation of intermolecular $(S.\therefore S)^+$ radical cations. However, these intermolecular $(S.\therefore S)^+$, three-electron-bonded species [21] have been observed in other quenching events involving sulfur-containing amino acids (see, for example, Fig. 3) and methionine-containing peptides [13–16]. These three-electron-bonded species (see Chart 1) have been characterized in many systems [22].

This long list of intermediates (carbonyl radical anions, carbonyl ketyl radicals, $(S.\therefore S)^+$ radical cations and $(S.\therefore N)^+$ radical cations), identified following the quenching of triplet CB by sulfur-containing amino acids and peptides, provides strong evidence that electron transfer from the sulfur atom to the CB triplet state is the dominant quenching mechanism.

Other indirect evidence for electron transfer was observed in correlations between the quenching rate constants and the free energy of electron transfer. Plots of $\log k_q$ vs. ΔG_{el} were made where

$$\Delta G_{el} = \Delta e(E_{ox} - E_{red}) - E_T + \Delta w \quad (1)$$

is the Rehm–Weller equation [23]. In Eq. (1), ΔG_{el} is the free energy of the reaction

$$^3CB^* + \rangle S \longrightarrow CB^{\bullet -} + \rangle S^{\bullet +} \quad (2)$$

Δe is the charge transferred, E_{ox} is the oxidation potential of $\rangle S$, E_{red} is the reduction potential of CB, E_T is the triplet excitation energy of CB and Δw is the interaction energy of $CB^{\bullet -}$ and $\rangle S^{\bullet +}$. These correlations have an extensive basis in the theoretical literature. The classical basis of the theoretical models is to relate an activation free energy $\Delta G_{el}^{\ddagger}$ to the free energy of the overall reaction ΔG_{el}, given in Eq. (1). For example, this relationship in classical Marcus theory [24,25] is expressed as

$$\Delta G_{el}^{\ddagger} = (\Delta G_{el} + \lambda)/4\lambda \quad (3)$$

where λ is the reorganization energy of the medium (external and internal) associated with electron transfer. This and other electron transfer models for the activated process can be combined with either phenomenological kinetic schemes or diffusional kinetic schemes to account for the observed $\log k_q$ vs. ΔG_{el} correlations.

Several of the combinations of the theoretical electron transfer models and kinetic schemes were applied to triplets of substituted benzophenones quenched by methionine, *S*-methylcysteine and *S*-carboxymethylcysteine in the mixed solvent water–acetonitrile (3:2) at pH 6.8 [14]. The data for *S*-methylcysteine are displayed in Fig. 4. Certain physical parameters in each theory of electron transfer were used as fitting parameters in non-linear, least-squares fits to the data. The fitted values for these physical parameters gave physically reasonable numbers [14].

In parallel work [26] on fluorescence quenching by electron transfer, some of these same combinations of electron transfer for the activated step and kinetics were used in fitting the original data from the Rehm–Weller study. In fitting the work to the Rehm–Weller data, however, one of the most powerful techniques was a variant of the two-parameter fit with a semiclassical Marcus electron transfer model [27]

$$k_{act} = \kappa_{el}\nu_n \exp(-\Delta G_{el}^{\ddagger}/k_B T) \quad (4)$$

and Tachiya–Murata diffusion kinetics [28], which allows naturally for a distance-dependent activated step. The distance dependence arises from the solvent reorganization energy

$$\lambda_s(r_{DQ}) = \frac{(\Delta e)^2}{4\pi\epsilon_0}\left(\frac{1}{2r_D} + \frac{1}{2r_Q} - \frac{1}{r_{DQ}}\right)\left(\frac{1}{n^2} - \frac{1}{\epsilon_s}\right) \quad (5)$$

and the semiclassical transmission coefficient [27,29]

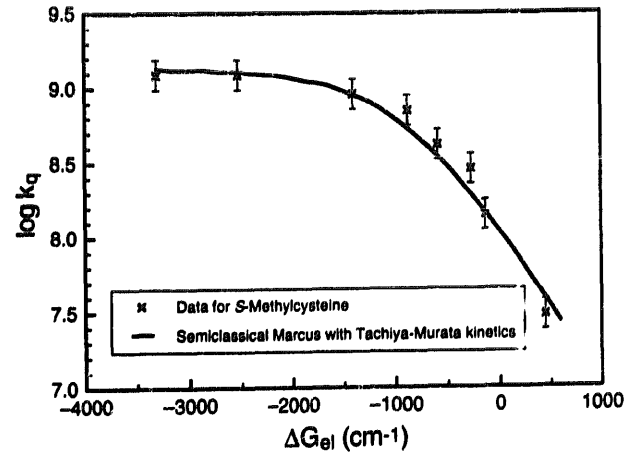
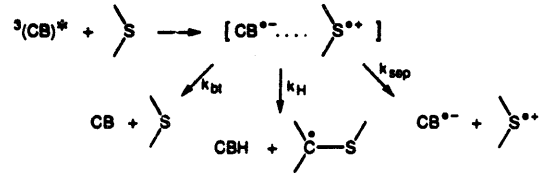


Fig. 4. Correlation of $\log k_q$ vs. ΔG_{el}; fit to the *S*-methylcysteine quenching of some substituted benzophenone triplets in water–acetonitrile (3:2) solution at pH 6.8. Semiclassical (Marcus) model of electron transfer with Tachiya–Murata kinetics. Mutual diffusion coefficient and electronic matrix element at $r_{DQ} = 7$ Å are the only two fitting parameters. $\beta = 1$ Å^{-1} and radii of quencher and triplet taken to be 3.5 Å.



Scheme 1.

$$\kappa_{el} = \frac{2\pi}{\hbar \upsilon_n} \frac{|V_{DQ}{}^0 \exp\{-\beta(r_{DQ} - r_D - r_Q)/2\}|^2}{\sqrt{4\pi k_B T \lambda}} \quad (6)$$

In Eqs. (4)–(6), $\upsilon_n = k_B T/h$, k_B is Boltzmann's constant, T is the absolute temperature, Δe is the charge transferred, r_D, r_Q and r_{DQ} are the radii of the triplet molecule and the quencher and the distance between the charge transfer centers respectively, n and ϵ_s are the refractive index and dielectric constant of the solvent, $V_{DQ}{}^0$ is the electronic interaction of the reactants at the nearest approach distance and β is the reciprocal distance that characterizes the distance dependence of the exchange interaction. In the fitting procedure which is a variant of those in Ref. [14], the two parameters are the diffusion coefficient and $V_{DQ}{}^0$. For the fluorescence data [26], this fitting scheme gave excellent fits but electronic matrix elements that were unreasonable. When applied to *S*-methylcysteine quenching of the triplet carbonyls in Fig. 4, the mutual diffusion coefficient of the reactants is 2.3×10^{-10} $m^2\ s^{-1}$ and the electronic matrix element is 390 cm^{-1}. This fit gives roughly the same goodness-of-fit [19] as the method in Ref. [14] which had the diffusion coefficient fixed, but which artificially varied the charge Δe that was transferred. Furthermore, the calculation behind Fig. 4 validates the methodology of the fitting to the fluorescence data [26].

In summary, the quenching of the aromatic carbonyl triplets appears to have a significant electron transfer component as recognized by the large rate constants of quenching and, in particular, by the observation of many electron transfer intermediates in the absorption spectra following the quenching events. In addition, the correlation of log k_q vs. ΔG_{el} also follows that expected from an electron transfer mechanism. By fitting the data to combined electron transfer models/diffusion models, molecular parameters associated with the electron transfer process can be estimated.

With the nature of the quenching process established as electron transfer, it is of interest to inquire further into the type and amount of chemical intermediates that appear immediately after the triplet quenching events. One way to quantify this investigation is to measure the primary photochemical quantum yields, namely the quantum yields immediately following the decay of the triplet state. The probable processes leading to the electron transfer intermediates, that have been observed and discussed above, are illustrated in Scheme 1. The three processes are back electron transfer (k_{bt}), escape of the radical ions (k_{sep}) and escape of the radicals formed by proton transfer within the charge transfer complex (k_H). Some of the primary quantum yields that characterize different quenchers and pH values are given in Table 2. Primary quantum yields are marked with a single prime. The results indicate that electron transfer quenching in aqueous systems of CB and sulfur-containing amino acids (or peptides) is followed mainly by charge separation (k_{sep}), resulting in efficient formation of radical anions $CB^{\bullet-}$, and by back electron transfer within the charge transfer complex (k_{bt}).

3. Kinetics of secondary reactions

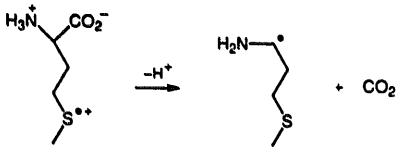
Some of the more interesting aspects of the photochemistry come in the aftermath of the quenching events [15,16]. A few of these aspects will be discussed in this section of the paper. Since the electron transfer intermediate products can be observed by overlapping absorptions which can be resolved by the multiple linear regression technique, the secondary reactions can be followed in some detail.

Table 2
Quantum yields of transients in the quenching of CB triplets by representative amino acids and sulfur-containing peptides

Compound	pH	$\Phi'_{CB^{\bullet-}}$	Φ'_{ketyl}	$\Phi'_{SS^{\bullet+}}$	$\Phi'_{SN^{\bullet+}}$
Thiaproline	6 [a]	0.55	0.25	0	0
	11 [b]	0.63	0.10	0	0
Methionine	6 [c]	0.27	0.11	0.23	0
	11 [b]	0.66	0.05	0.10	0.38
Alanine	11 [b]	0.96 [d]	–	–	–
Met-Gly	6 [e]	≈0.13	≈0.25	≈0.04	0.18 [f]
	11 [e]	0.57	0.05	<0.04	0.49
Gly-Met	6 [e]	0.22	0.15	0.22	0
	11 [e]	0.32	0.10	0.24	0

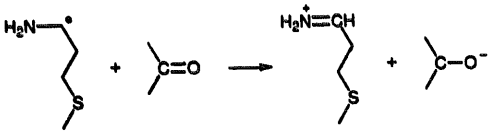
[a] From Ref. [13].
[b] From Ref. [15].
[c] This work.
[d] Sum of $\Phi'_{CB^{\bullet-}}$ and Φ'_{ketyl}.
[f] From Ref. [16].
[e] Formation of $(S\therefore N)^{\bullet+}$ is due to an additional reaction not present in Scheme 1, see Ref. [16].

One of the unusual features was a secondary growth of the radical anions of CB in basic solutions of some of the sulfur-containing amino acids, i.e. thiaproline [15], or the secondary growth of the CB ketyl radical in neutral solution [13]. These growths occurred after triplet CB had decayed and depended on the concentration of CB. The only reactive species left in this time range were radicals. With the hypothesis that the sulfur radical cations are decarboxylating agents and produce α-amino alkyl radicals



(7)

CO_2 was looked for and found in steady state photolysis experiments with gas chromatography detection [13,15]. To make this scheme more plausible, α-amino alkyl radicals were produced by pulse radiolysis methods and were seen to react with CB. The rate constants of this process

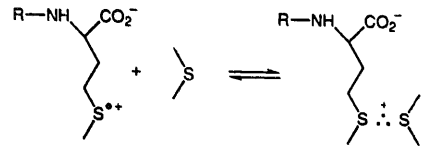


(8)

were measured [13].

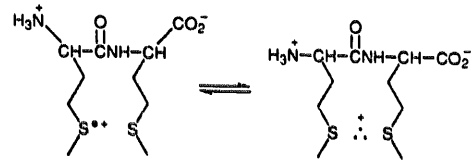
The quantum yields of CO_2 formation are listed in Table 3. There are several points of note. First, the quantum yields of α-amino alkyl radical formation Φ_α (at neutral pH) were computed [13] on the basis of the measured quantum yields of secondary ketyl radical formation, the rate constants for α-amino alkyl radicals reacting with CB from pulse radiolysis and the lifetime of α-amino alkyl radicals from pulse radiolysis. From Table 3 it can be seen that many of the Φ_α values are equal to the Φ_{CO_2} values as expected for the hypothesis that α-amino alkyl radicals (α-N) are responsible for the secondary growth of $CB^{\bullet-}$. Recently [30], it was shown that $G(CO_2)$ is larger than $G(\alpha\text{-N})$ for *S*-methylcysteine, *S*-ethylcysteine and *S*-carboxymethylcysteine (measured as the *G* value of the *p*-nitroacetophenone radical anion); this may be rationalized by β C–S bond scission [31,32] in the α-amino alkyl radicals derived from these compounds. Second, there is virtually no CO_2 formation in methionine-containing peptides [16]. It has been suggested [33] that decarboxylation in methionine takes place through a resonance structure in which the positive charge is localized on the nitrogen atom. Such a resonance structure would be destabilized by the neighboring carbonyl group of the amide bond for peptides with C-terminal methionines.

In addition to being the source of α-amino alkyl radicals, the sulfur-centered radical cations play other roles in the secondary reactions following the quenching of triplet carbonyls by sulfur-containing amino acids and methionine-containing peptides. At sufficiently high concentrations of the sulfur-containing compound, it is possible to form intermolecularly bonded $(S.\therefore S)^+$ radical cations



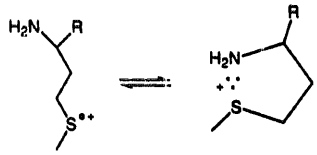
(9)

The intramolecularly $(S.\therefore S)^+$-bonded species appear to be formed in neutral aqueous solutions of low concentration in L-Met-L-Met (see Fig. 2)



(10)

The sulfur-centered radical cations also appear to form intramolecular $(S.\therefore N)^+$ radical cations (see Fig. 1) at high pH in some amino acids and peptides with N-terminal methionine residues [16].



(11)

The five-membered structure shown in Chart 1 seems to be particularly favored

There are some additional features of the secondary kinetics that at first sight appear to be anomalous: (1) the yield of secondary $CB^{\bullet-}$ is pH dependent [15]; it appears to decrease above pH 11; (2) the secondary growth rate constant of $CB^{\bullet-}$ with methionine (and alanine) as quencher is not linearly dependent on the CB concentration [15]; (3) the decays of $(S.\therefore N)^+$ radical cations of methionine-containing peptides are biexponential and depend on the pH [16].

Detailed mechanistic schemes have been given for the secondary reactions following the quenching events for triplet CB with sulfur-containing amino acids [13,15] and methionine-containing peptides [16] as quenchers. Rather than repeat these here, we will introduce some simplified schemes in order to explain the anomalies (listed above) in the secondary reactions.

Some quantitative insight can be obtained by considering alanine [15], which shows some of the features of the sulfur-containing amino acids in alkaline solution, but which cannot undergo the reactions connected with the sulfur atom in the lower part of scheme 1 of Ref. [15]. For instance, the secondary growth of $CB^{\bullet-}$ with alanine as electron donor shows a less dramatic decrease in $CB^{\bullet-}$ yield with increasing pH. The kinetic mechanism in Scheme 2 is designed to rationalize anomalies (1) and (2) above. This mechanism leaves out some of the features of scheme 1 of Ref. [15], but uses only processes that should be relevant to alanine. An attempt is

made to explain the sublinear k_{obs} vs. [CB] dependence of curves b, c and e in Fig. 2 of Ref. [15] and the correct alanine curve in Fig. 7 of Ref. [15]. It should be noted that the top curve (for alanine) in Fig. 7 (open squares) was incorrectly labeled in our original figure caption [15]. In Scheme 2, α-N is the α-amino alkyl radical formed from the decarboxylation of alanine, $N^{\bullet+}$ is the radical cation of alanine and N$^{\bullet}$-OH is the neutralized OH^- adduct of the radical cation. The kinetic equations for Scheme 2 are

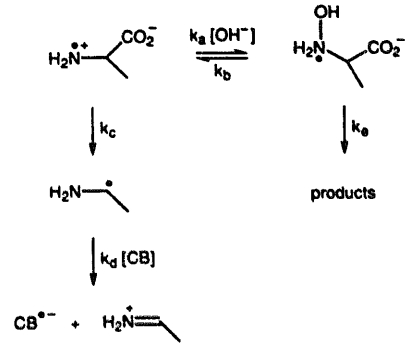
$$\frac{d[CB^{\bullet-}]}{dt} = k_d[\alpha N][CB] \quad (12)$$

$$\frac{d[\alpha N]}{dt} = k_c[N^{\bullet+}] - k_d[\alpha N][CB] \quad (13)$$

$$\frac{d[N^{\bullet+}]}{dt} = k_b[N^{\bullet}\text{-OH}] - k_c[N^{\bullet+}] - k_a[N^{\bullet+}][OH^-] \quad (14)$$

$$\frac{d[N^{\bullet}\text{-OH}]}{dt} = k_a[N^{\bullet+}][OH^-] - (k_b + k_e)[N^{\bullet}\text{-OH}] \quad (15)$$

Using the steady state approximation only for [N$^{\bullet}$-OH], the solution is



Scheme 2.

$$[CB^{\bullet-}] - [CB^{\bullet-}]_0 = \frac{k_c}{k'}[N^{\bullet+}]_0\left\{1 - \frac{k_d[CB]}{k_d[CB] - k'} \times \exp(-k't) - \frac{k'}{k_d[CB] - k'}\exp(-k_d[CB]t)\right\} \quad (16)$$

where

$$k' = k_c + \frac{k_a k_c}{k_b + k_e}[OH^-] \quad (17)$$

An equation for the secondary yield of the ketyl radical anion can be derived by first letting $t \rightarrow$ infinity in Eq. (16)

Table 3
Quantum yields [a] of CO_2 formation from the quenching of triplet CB by amino acids and peptides and quantum yields of transients formed in secondary reactions

Amino acid or peptide	pH 6.8			pH ≈ 11	
	Φ_{CO_2}	Φ^{∞}_{ketyl}	Φ_a [d]	Φ_{CO_2}	$\Phi^{\infty}_{CB^{\bullet-}}$
Thiaproline	0.64 [b]	0.60 [c]	0.60	0.45 [e]	0.59 [f]
S-Methylcysteine	0.45 [b]	0.11 [c]	0.15		
S-Ethylcysteine	0.51 [b]	0.09 [c]	0.15		
S-(Carboxymethyl)cysteine	0.59 [b]	⩽0.06 [c]	⩽0.07		⩽0.03
S-(2-Carboxyethyl)cysteine	0.49 [b]	0.10 [c]	0.13		
Methionine (Met)	0.28 [b]	0.25 [c]	0.27	0.55 [g]	0.66 [f]
Ethionine	0.16 [b]	0.19 [c]	0.21		
α-Methylmethionine	0.12 [b]	≈0.13 [c]	≈0.13		0.59 [f]
N-Acetylmethionine	0.05 [b]	⩽0.20 [c]	⩽0.06		
Homomethionine	0.03 [b]	⩽0.07 [c]	⩽0.07		0.10 [f]
Alanine	[i]	[i]	[i]	0.63 [j]	0.85 [f]
Gly-Gly-Met	0.02 [k]	⩽0.03			⩽0.03
Gly-Met	0.02 [k]	⩽0.03			⩽0.03
Gly-Met-Gly	0.02 [k]	⩽0.03			⩽0.03
Met-Gly	0.01 [k]	⩽0.03			⩽0.03
Met-Gly-Gly	0.01 [k]	⩽0.03			⩽0.03

[a] From steady state photolysis with each value being an average of at least three independent measurements.
[b] This work, ±20%.
[c] Ref. [13].
[d] Efficiency of radical formation, calculated from the lifetime of the α-amino alkyl radical and the rate constants of reaction with CB [13].
[e] Ref. [15], ±10%.
[f] Ref. [15].
[g] Ref. [15], ±15%.
[h] This work, ±50%.
[i] No quenching of CB triplet.
[j] Ref. [15], ±13%.
[k] Ref. [16], ±50%.

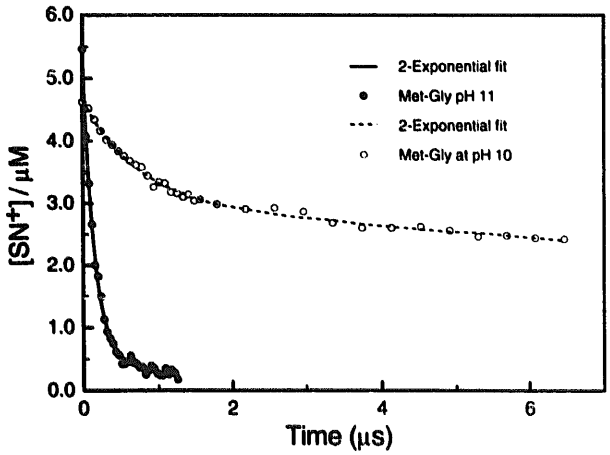


Fig. 5. Concentration profiles of $(S.^{\cdot}.N)^+$ of Met-Gly at two different pH values. Concentrations of $(S.^{\cdot}.N)^+$ were determined at each time point by a separate spectral resolution of the transient spectra following the quenching of the CB triplet by Met-Gly.

and then dividing by $[N^{\cdot+}]_0$. This gives $\Phi'_{ketyl} = k_c/k'$, or the reciprocal is

$$1/\Phi'_{ketyl} = 1 + \frac{k_d k_c [OH^-]}{k_c (k_h + k_c)} \quad (18)$$

This should be related to the top curve in Fig. 7 of Ref. [15]. It shows the fall off in the secondary yield of the ketyl radical anion as the pH increases, and thus accounts for anomaly (1) above. For the kinetics when $k' \gg k_d[CB]$, Eq. (16) becomes

$$[CB^{\cdot-}] - [CB^{\cdot-}]_0 \quad (19)$$

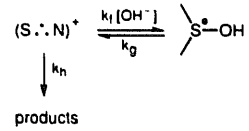
$$= \frac{k_c}{k'} [N^{\cdot+}]_0 \{1 - \exp(-k_d[CB]t)\}$$

indicating that the secondary growth should be pseudo-first-order and linear in [CB]. However, when $k_d[CB] \gg k'$, Eq. (16) becomes

$$[CB^{\cdot-}] - [CB^{\cdot-}]_0 = \frac{k_c}{k'} [N^{\cdot+}]_0 \{1 - \exp(-k't)\} \quad (20)$$

indicating that the growth rate constant becomes independent of [CB], and from the definition of k' in Eq. (17), dependent on $[OH^-]$.

It can be seen from scheme 1 of Ref. [15] that there may be an $[OH^-]$ dependence for the decay of $(S.^{\cdot}.N)^+$. This is illustrated by the two decay curves for $(S.^{\cdot}.N)^+$ of Met-Gly, one at pH 10 and one at pH 11 in Fig. 5. These curves were constructed by resolving the transient spectra at each time point after quenching events with triplet CB and Met-Gly. The decay at the lower pH appears to be biexponential, whereas at the higher pH the decay appears to be mostly single exponential. In order to simulate this behavior, we took the simple kinetics of Scheme 3 which selects out some features of the more complete scheme 1 of Ref. [15]. The pseudo-first-order rate constant in the forward direction in the equilibrium of Scheme 3 is $k_f[OH^-]$, and the reverse rate



Scheme 3.

constant is k_g. For simplicity, the top part of scheme 1 of Ref. [15] is reduced to a single irreversible channel characterized by the rate constant k_h in Scheme 3. For this simplified scheme, the general case would be a two-exponential decay of $(S.^{\cdot}.N)^+$ because of the approach to equilibrium. However, at low pH, only the reaction with rate constant k_h is operational, leading to a single exponential; at very high pH, the pseudo-first-order rate constant $k_f[OH^-]$ drives the equilibrium to the right, also making the decay of $(S.^{\cdot}.N)^+$ look first order. This behavior has been simulated qualitatively with the rate constants $k_h = 2 \times 10^4\ s^{-1}$, $k_f = 5 \times 10^9\ M^{-1}\ s^{-1}$ and $k_g = 5 \times 10^5\ s^{-1}$. The value of k_h was chosen to agree with the rate constants of $(S.^{\cdot}.N)^+$ decay at pH 6 [16].

4. Conclusions

The dominant mechanism for quenching of carbonyl triplets by sulfur-containing amino acids and methionine-containing peptides appears to be electron transfer. This is consistent with the high rate constants and is confirmed by the observation of the intermediates following the quenching events which are electron transfer products. The electron transfer appears to involve initially the sulfur atom, and not the nitrogen atom. With theoretical models of electron transfer and diffusion kinetics, it is possible to obtain reasonable estimates for the molecular parameters associated with the transition state. The primary photochemical quantum yields of some of the main radicals and radical ions were determined. They vary with the pH, which affects the balance between the ketyl radical and its anion and the availability of lone pairs of electrons to form three-electron bonds in the sulfur-centered radical cations.

The secondary reactions of the electron transfer products are very rich and varied. One prominent reaction is the decarboxylation of the amino acids. This reaction is significant because the α-amino alkyl radicals are good reducing agents which have been observed to reduce the ground state of CB. Decarboxylation of the methionine-containing peptides was not observed. A resonance structure with the radical cation site on the nitrogen atom in amino acids appears to be the precursor to decarboxylation. The electron-withdrawing substituents on the analogous nitrogen in the C-terminal of methionine-containing peptides may adversely affect the stability of this resonance structure of the radical cation, leading to little or no decarboxylation in these peptides. There are a multitude of reactions in alkaline solution due to the reaction of the various radical cations with OH^-. Certain aspects of these complicated kinetics can be simplified and explained by more elementary kinetics, e.g. the variation in the observed

growth rate constants of CB radical anions after triplet quenching by alanine (and methionine) and the secondary yields of these anions. An additional example and a similar simplification involves the switching between single and biexponential decay of the $(S.^{\cdot}.N)^+$ radical cations of Met-Gly as a function of pH.

The biological implications are that sulfur-containing amino acids and methionine-containing peptides are very susceptible to attack at the sulfur atom by carbonyl triplets. The potential for decarboxylation and the formation of good reducing α-amino alkyl radicals was demonstrated with the amino acids. However, this migration of damage through the formation of secondary radicals appears to be mitigated in methionine-containing dipeptides and tripeptides. Migration of damage in larger peptides and proteins is still possible with sulfur radical cation sites interacting with the tertiary structure of these biological systems.

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